CENTRATE TREATMENT TO PRODUCE A NITRIFYING BIOMASS FOR BIOAUGMENTATION

By

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A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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THE UNIVERSITY OF MANITOBA

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ABSTRACT

The City of Winnipeg is currently conducting studies to minimize expansion costs for wastewater treatment when upgrading to include nitrification. One of the methods considered is centrate treatment. This study examined treatment of centrate by nitrification in a dedicated reactor. The biomass produced was used as seed for bioaugmentation of cold reactors (10°C) treating synthetic wastewater without nitrification. As a result of seeding, nitrification was initiated in the seeded reactors. The degree to which effluent ammonia nitrogen (NH_3-N) was reduced depended on the seed dose and the temperature to which the seed was acclimated. Seed acclimated to warmer temperatures experienced decreases in nitrification rates after suddenly cooling to 10°C.

Based on the results of the seeding, simulation modeling was conducted using BioWin to predict the benefits of seeding nitrifiers into treatment systems with different hydraulic and solids retention times. It was found that, when compared with conventional nitrification systems, producing seed by centrate nitrification could decrease the volume requirements by up to 20%.

Microbial analysis using fluorescence *in situ* hybridization (FISH) of ammonia oxidizing bacteria showed that the seed was being washed out of the seeded systems inadvertently with the effluent. This observation explained why poor NH_3-N removal was achieved when seed was added to SBRs with short hydraulic retention times. The FISH signal associated with ammonia oxidizers correlated well with effluent NH_3-N and nitrate-nitrogen (NO_3-N) concentrations and the nitrification rate.

Centrate was found to be a suitable substrate for the production and harvest of nitrifying seed. Seed produced at the same temperature as the reactor into which it is to be added provided the greatest benefit.

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ABBREVIATIONS

AMO	ammonia monooxygenase
ANAMMOX	anaerobic ammonium oxidation
AOB	ammonia oxidizing bacteria
Apparent SRT	(the proportion of solids removed from the reactor daily)$^{-1}$. Does not include solids entering the reactor with the influent stream. (days)
ASM	Activated Sludge Model
ATP	adenosine triphosphate
BABE	bioaugmentation batch enhanced
BNR	biological nutrient removal
BOD	biological oxygen demand
C/N	carbon to nitrogen ratio
$CaCO_3$	calcium carbonate
COD	chemical oxygen demand
DAPI	4',6-diamidino-2-phenylindole dihydrocholoride hydrate
DNA	deoxyribonucleic acid
DO	dissolved oxygen
EBPR	enhaced biological phosphorus removal
FISH	fluorescence *in situ* hybridization
H^+	proton
HNO_2	unionized nitrous acid
HPOAS	high purity oxygen activated sludge
HRT	hydraulic retention time
IAWQ	Internation Association for Water Quality
MBR	membrane bioreactor
$MLSS_{conv}$	concentration of mixed liquor suspended solids in a conventional nitrification system (mg/L)

$MLSS_{new}$	concentration of mixed liquor suspended solids in a nitrification system with novel solids management (mg/L)
mRNA	messenger RNA
N	nitrogen
$NaHCO_3$	sodium bicarbonate
Na_2CO_3	sodium carbonate
NaOH	sodium hydroxide
NB10	nitrifying bacteria acclimated to 10°C
NB20	nitrifying bacteria acclimated to 20°C
NB25	nitrifying bacteria acclimated to 25°C
NB30	nitrifying bacteria acclimated to 30°C
NEWPCC	North End Water Pollution Control Centre
NH_3-N	ammonia nitrogen
NH_4^+	ammonium ion
NO_2-N	nitrite nitrogen
NO_3-N	nitrate nitrogen
NOB	nitrite oxidizing bacteria
P	phosphorus
PO_4-P	phosphate phosphorus
RAS	return activated sludge
SBR	sequencing batch reactor
SCOD	soluble chemical oxygen demand
SEWPCC	South End Water Pollution Control Centre
SHARON	single reactor system for high rate ammonium removal over nitrite
SRT	solids retention time
SRT_{min}, θ_x^{min}	minimum SRT for nitrification (d)
TCOD	total chemical oxygen demand
TKN	totak Kjeldahl nitrogen
tRNA	transfer RNA

TS	total solids
UV	ulraviolet
VS	volume savings (%)WWTPwastewater treatment plant
WAS	waste activated sludge

SYMBOLS

Alk	Concentration of alkalinity (mmol/L)
b	decay rate of ammonia oxidizers (d^{-1})
b_T	decay rate of ammonia oxidizers at a given temperature (d^{-1})
b_{10}	decay rate of ammonia oxidizers at 10°C (d^{-1})
Fac	Fraction of readily biodegradable COD which is VFA's
Fbs	Fraction of total influent COD which is readily biodegradable
Fna	Fraction of influent TKN which is ammonia
Fnox	Fraction of influent organic nitrogen which is particulate
Fnus	Fraction of influent TKN which is soluble unbiodegradable
FPO4	Fraction of influent TP which is phosphate
Fup	Fraction of total influent COD which is particulate unbiodegradable
FupN	The N:COD ratio for the influent particulate unbiodegradable COD
FupP	The P:COD ratio for the influent particulate unbiodegradable COD
Fus	Fraction of total influent COD which is soluble unbiodegradable
Fxsp	Fraction of slowly biodegradable influent COD which is particulate

FZba	Fraction of total influent COD which is autotrophic organisms.
FZbam	Fraction of total influent COD which is acetoclastic methanogen organisms.
FZbh	Fraction of total influent COD which is non-polyP heterotrophic organisms
FZbhm	Fraction of total influent COD which is H_2-utilizing methanogen organisms
FZbp	Fraction of total influent COD which is polyP heterotrophic organisms
FZbpa	Fraction of total influent COD which is propionic acid acetogen organisms.
f	coefficient for normalizing FISH signal in an environment with variable solids concentration
ISS	Concentration of inert suspended solids (mg SS/L)
K_N	half saturation coefficient for ammonia oxidation (mg NH_3-N/L)
Mg	Magnesium (mg Mg/L)
Nos	Soluble biodegradable organic nitrogen (mg N/L)
Nus	Soluble unbiodegradable organic nitrogen (mg N/L)
P	the proportion of ammonia oxidizers in the effluent compared to that in the reactor (g/g)
PP-hi	Fixed stored polyphosphate (mg P/L)
PP-lo	Releasable stored polyphosphate (mg P/L)
Q^i	influent flow rate (volume/time)
Q^s	seed source flow rate (volume/time)
Q^w	waste biomass flow rate (volume/time)
S	NH_3-N concentration in the effluent (mg/L)
S^o	NH_3-N concentration in the influent stream (mg/L)
SbH2	Dissolved H_2 COD (mg COD/L)
Sphb	Stored VFA (mg COD/L)
Sbsa	Acetic acid COD (mg COD/L)

Sbsc	Soluble readily biodegradable complex COD (non-VFA)
Sbsp	Propionic acid COD (mg COD/L)
Sus	Soluble unbiodegradable COD (mg COD/L)
T	temperature (°C)
T_o	initial temperature (°C)
t	time
U	specific nitrification rate (mg N/mg*d)
VSS_{main}	volatile suspended solids concentration of the main-stream tank (mg/L)
$VSS_{nitrifiers}$	volatile suspended solids concentration of the seed (mg/L)
V_r	reactor volume (L)
VSS_{seed}	volatile suspended solids concentration of the seed (mg/L)
VSS_{total}, $VSS_{reactor}$	volatile suspended solids concentration of all solids in the reactor (mg/L)
X_a	concentration of ammonia oxidizers in the reactor (mg/L)
X_a^e	concentration of ammonia oxidizers in the effluent (mg/L)
X_a^o	concentration of ammonia oxidizers in the influent stream (mg/L)
X_a^s	concentration of ammonia oxidizers in the seed source (mg/L)
X_e	concentration of VSS in the effluent, mg VSS/L
Xi	Particulate unbiodegradable COD (mg COD/L)
Xon	Particulate biodegradable organic nitrogen (mg N/L)
Xop	Particulate biodegradable organic phosphorus (mg P/L)
X_r	VSS concentration in the reactor (mg/L)
Xsc	Slowly biodegradable colloidal COD (mg COD/L)

Xsp	Slowly biodegradable particulate COD (mg COD/L)
XStru	Precipitated struvite (mg struvite/L)
X_w	concentration of VSS in the WAS, mg VSS/L
Y	yield coefficient of ammonia oxidizers, mg /mg NH_3-N
Zba	Autotrophic organism mass (mg COD/L)
Zbam	Acetoclastic methanogen organism mass (mg COD/L)
Zbh	Non-polyP heterotrophic organism mass (mg COD/L)
Zbhm	Hydrogenotrophic methanogen organism mass (mg COD/L)
Zbpa	Propionic acid acetogen organism mass (mg COD/L)
Ze	Endogenous residue from organism decay (mg COD/L)
$\Delta N/\Delta t_T$	ammonia oxidation rate at temperature, T°C (mg/L*h)
$\Delta N/\Delta t_{10C}$	ammonia oxidation rate at 10°C (mg/L*h)
k_T	rate factor for temperature dependency of nitrification
μ	growth rate of ammonia oxidizing bacteria (d^{-1})
μ_{10C}	growth rate of ammonia oxidizers at 10°C (d^{-1})
μ_{max}	maximum growth rate of ammonia oxidizers at temperature, T (d^{-1})
$\mu_{max\ after\ seeding}$	the maximum growth rate after seeding into a new environment (d^{-1})
μ_T	growth rate of ammonia oxidizers at temperature, T (d^{-1})
θ	hydraulic retention time (HRT) (d)
θ_x^s	seeded SRT (d)
τ_N	temperature dependency factor for nitrification

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1. INTRODUCTION

The North End Water Pollution Control Centre (NEWPCC) in Winnipeg, Manitoba, Canada operates for the purpose of carbon or chemical oxygen demand (COD) removal without the intentional removal of other nutrients like nitrogen (N) and phosphorus (P). Nutrient loading with N and P compounds into receiving waters can result in algal blooms, odour and visual problems and a poor environment for the survival of higher aquatic organisms. As environmental regulations become more stringent the NEWPCC will be required to include nutrient removal, namely, N in their treatment process (Appendix A).

Nitrification is generally accepted as the rate limiting step in wastewater treatment due to slow growth rates of nitrifying bacteria. The slow growth rates require designs with long solids retention times (SRTs) to maintain nitrifiers in the system. However, longer SRTs also increase the solids inventory in the system and can lead to overloading the final clarifiers with solids. Using conventional design practices this means expanding the volume of a COD-removing facility by 2 to 3 times its existing volume.

One of the most significant and concentrated sources of ammonia (NH_3) entering a wastewater treatment plant (WWTP) is actually generated within the treatment system itself from dewatering anaerobically digested primary and waste activated sludges (WAS). Centrifugation generates liquor that is high in ammonia (up to 1200 mg NH_3-N/L) and solids (up to 2700 mg TS/L).

Centrate is usually recycled back to the front of a WWTP where it is combined with the influent stream. It contributes as much as 20 to 25% of the NH_3-N load into NEWPCC but constitutes less than 1% of the total influent flow.

Current trends to build central sludge processing facilities often lead to centrate nutrients loads that are much higher than they would be for a WWTP treating "its own" sludge. Such regionalization is found in Winnipeg, New York, San Diego, and there are several separate sludge processing facilities serving large regions in the United Kingdom (Barnes, 2000; Jeavons *et al.*, 1998), and South Africa (Pitman, 1999).

It has been suggested that centrate should be treated as a separate stream. It is thought that this will ease the treatment requirement of the main stream and prevent shock NH_3-N loads from decreasing the overall effluent quality. Some methods that have been used to treat centrate include the BABE (**B**io-**A**ugmentation **B**atch **E**nhanced) process (Berends *et al.*, 2003), the SHARON® process (**S**ingle reactor system for **H**igh rate **A**mmonium **R**emoval **O**ver **N**itrite) and ANAMMOX (**An**aerobic **Amm**onium **Ox**idation). The proposed method of centrate treatment is nitrification with the added benefit of producing a concentrated source of nitrifying bacteria that could be used as seed for the main-stream tanks. The SHARON® process requires heating to temperatures of 30 to 40°C which would eliminate the possibility of using the biomass produced as a nitrifying seed source for the main-stream. The

ANAMMOX process requires the addition of NO_2-N and is usually combined with a SHARON® reactor. While these options eliminate the NH_3 load associated with centrate, they do not offer any additional benefit as seed to the main-stream. The BABE process, however, does offer additional benefit by producing nitrifying biomass at a cooler temperature that results from a small input of return activated sludge (RAS) to the side-stream reactor. This research will examine the feasibility of nitrifying centrate from the NEWPCC in a dedicated side-stream reactor. The warm temperature and high NH_3 concentration will be utilized to produce an enriched nitrifying biomass. The biomass produced will be examined for its nitrification potential upon addition (seeding) into a cold environment similar to that found in the main-stream tanks of the NEWPCC. Both biological reactions and microbiological characteristics of seeded systems will be studied and the results will be used to model seeding using an existing wastewater treatment simulation model.

2 LITERATURE REVIEW

2.1 Upgrading a WWTP to include nitrification

Because the growth rate of nitrifying bacteria is much slower than heterotrophic bacteria, the solids retention time (SRT) must be long enough to permit the growth and reproduction of nitrifiers. When all of the requirements for nitrification are met then heterotrophic growth needs are also satisfied. Nitrifier growth rate is highly dependent on temperature, dissolved oxygen concentration and pH (Equation 1). The minimum SRT (SRT_{min}) necessary to maintain nitrification taking all of these environmental factors into consideration is often approximated by Equation 2 (U.S.EPA, 1975).

$$\mu = 0.47 \times e^{0.098(T-15)}\left[\frac{DO}{DO+1.3}\right]\left[1-0.0833(7.2-pH)\right] \quad [1]$$

$$\frac{1}{SRT_{\min}} = \frac{\mu_{\max} S^o}{K_N + S^o} - b \quad [2]$$

These equations hold true for a nitrifying biomass that is acclimated to its environment. A safety factor is usually applied to ensure nitrification is maintained should adverse conditions occur such as shock loads, toxins or cold temperatures. Applying a safety factor of 2, Figure 2.1 was generated from Equations 1 and 2. The graph shows the minimum SRT necessary to maintain nitrification at various temperatures assuming the effects of DO and pH are negligible.

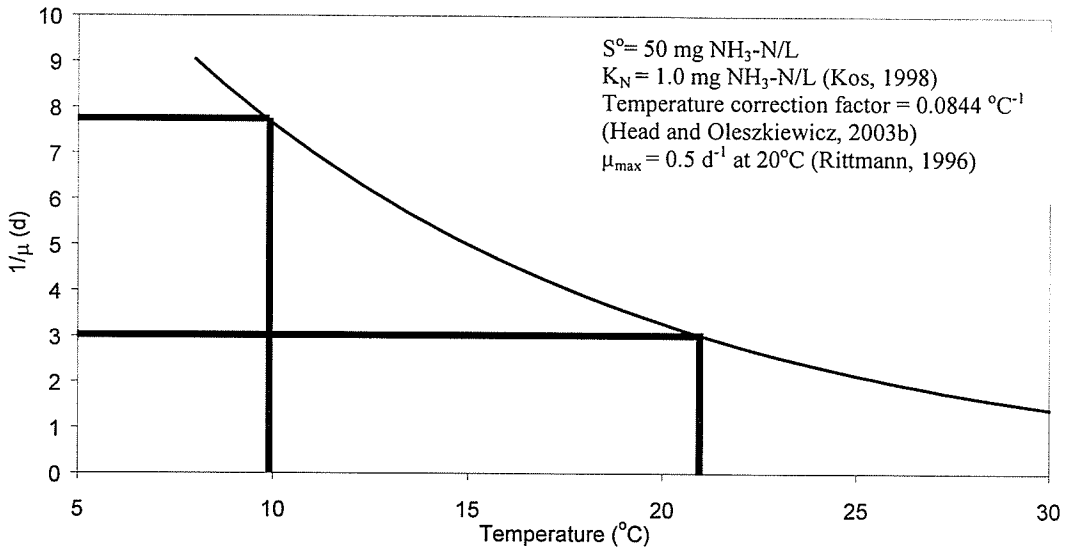


Figure 2.1 Minimum SRT required for nitrification as a function of temperature.

For a typical WWTP operating with an SRT of 3 days, at all temperatures below about 20°C, nitrification will not be present due to nitrifying bacteria being wasted from the system faster than they can reproduce (Figure 2.1). To upgrade the system in this example for nitrification in winter months, the SRT must be increase to approximately 8 days.

Unfortunately, increasing the SRT also means increasing the mass of inert solids in the system. Yuan *et al.* (2000) provides a comprehensive example of how solids concentrations increase with increased SRT (Figure 2.2). The mass of solids increases at a much faster rate than the increase in the desired nitrifying biomass. For example, when the SRT is 5 days the TSS concentration in the reactor is 2200 mg/L and the concentration of nitrifiers is almost nil. If the SRT is increased to 12 days without increasing the volume of the tank, the TSS concentration increases to 5800 mg/L while the concentration of nitrifiers only increases to 300 mg/L. As the mass of solids

increases, the activate sludge tank volume must also increase proportionally to maintain the same solids concentration in the reactor. Clarifier surface area must also be enlarged so they do not become overloaded.

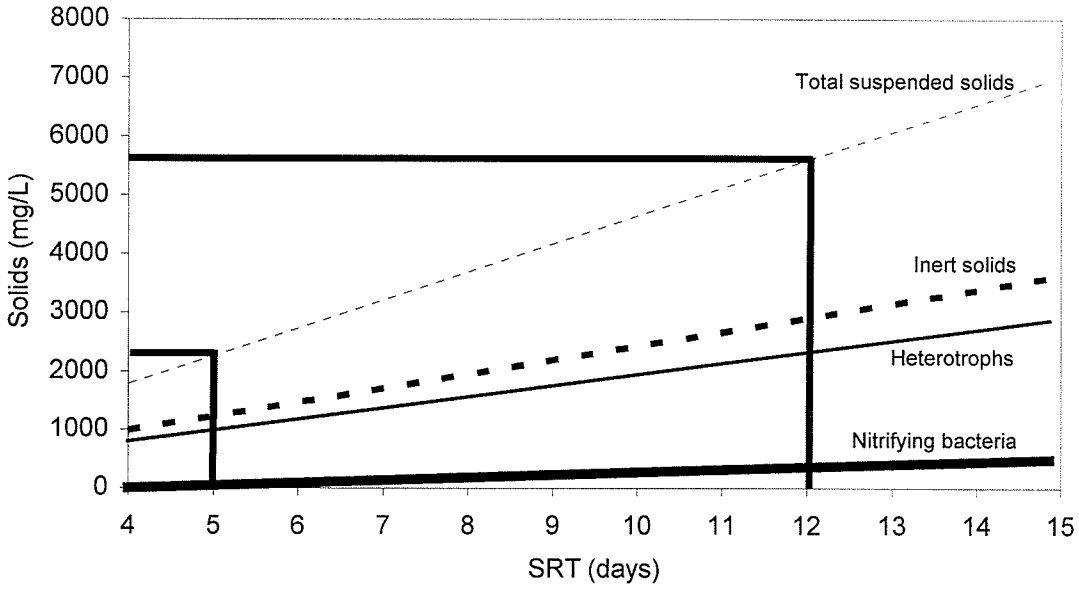


Figure 2.2 An example of the concentration of different components of an activated sludge system at various SRTs as per parameters in Figure 1 (adapted from Yuan *et al.*, 2000).

Any method that can increase the concentration of nitrifying bacteria without increasing the concentration of other components in the treatment system is desirable. The system must then be thought of as two separate entities: 1) the retention time of the nitrifying biomass and 2) the retention time of all other solids. Increasing the retention time of the nitrifying bacteria without increasing the retention time of the rest of the solids is referred to as *short-SRT nitrification* (Kos, 1998).

2.2 Volume savings and nitrification

Increasing the SRT by wasting less sludge causes a large increase in the concentration of solids in a bioreactor treating wastewater. If the flow rate is unchanged, the increase solids loading rate to final clarifiers can cause the clarifiers to fail. But there are a variety of techniques that can be applied to alleviate the increased load to the final clarifiers while still increasing the nitrification efficiency of a treatment plant. The increased nitrification efficiency can be defined in two ways: 1) a decrease in effluent NH_3 or 2) the ability to achieve the same effluent NH_3 concentration in a smaller tank.

Volume savings for nitrification systems can only be accomplished through decreasing the solids load entering the final clarifier while maintaining effluent quality. For the purpose of this review, volume savings VS (%) will be expressed as the percent decrease in mixed liquor suspended solids (MLSS) load to the final clarifier that can maintain the same effluent NH_3 concentration as a conventional nitrification system. The volume savings is calculated by Equation 3:

$$VS(\%) = \frac{MLSS_{conv} - MLSS_{new}}{MLSS_{conv}} \qquad [3]$$

where "conv" denotes a conventional nitrification configuration and "new" denotes the configuration with novel solids management. Thus, if we can achieve the same effluent quality at a lower solids concentration, we can

increase the capacity of the reactor by increasing the flow, without overloading the final clarifier.

One method for increasing the solids inventory in a wastewater treatment system without increasing the solids concentration entering the final clarifiers is by a process called "step-feeding" or "RAS re-aeration". This process includes maintaining a high concentration of biomass at the front of the reactor and diluting it with influent as it passes through the system. Fillos *et al.* (1996) have used this process in full-scale and achieved partial nitrification without increasing the volume of the tanks. The reactor configuration is shown in Figure 2.3.

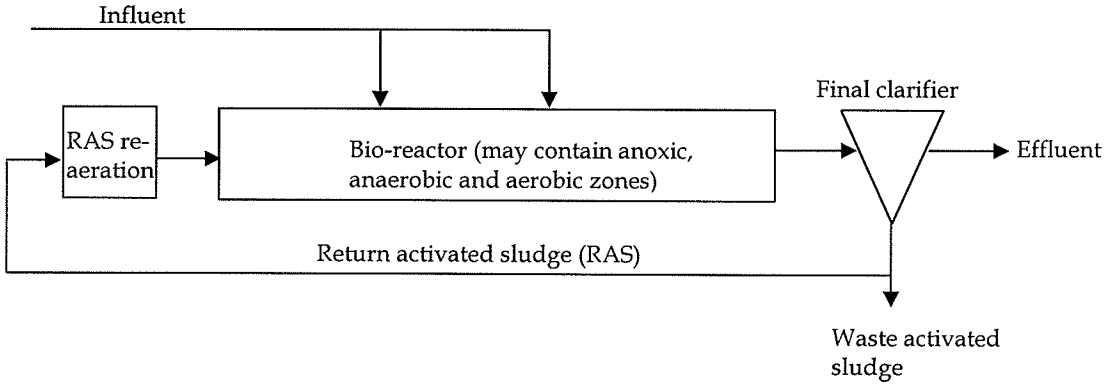


Figure 2.3 Schematic of reactor configuration for RAS re-aeration.

Another method for achieving nitrification goals without increasing the solids concentration is to make the retention time of nitrifying bacteria longer than that of other solids in the treatment system. This can include the

manipulation of solids "in house" or the creation or purchase of more specialized nitrifying biomasses.

2.2.1 Centrate input to the main-stream process

A major source of NH_3 entering a WWTP is actually generated within the treatment system. Centrate from the dewatering of anaerobically digested sludges is a concentrated source of NH_3 and, in the case of enhanced biological phosphorus removal (EBPR) plants, a source of dissolved phosphorus (Table 2.1). This high strength liquor is usually recycled untreated to the front of a wastewater treatment plant where it contributes significantly to nutrient loading and suspended solids loading (up to 71%) to the main-stream (Lawler and Singer, 1984). In many treatment plants centrate is added to the influent only while the centrifuges are in operation and in some cases, the additional loads from the side-stream can overload the BNR system. When the nutrient load from the centrate corresponds with the high NH_3 load of the influent, effluent NH_3 and PO_4^- limits can be exceeded.

Table 2.1 Dewatering liquor characteristics from anaerobically digested biosolids.

Temperature (°C)	TSS (mg/L)	Nitrogen (mg/L)	TKN (mg/L)	PO_4-P (mg/L)	Alkalinity (mg/L $CaCO_3$)	Reference
30-32	1000-3000	600-1200	800-1500	75-150†	1500-4000	Ali *et al.*, 1998
-	460	427-931	-	-	2190	Gordon *et al.*, 2000
-	-	1000	-	144†	-	Pitman *et al.*, 1991
-	468-498	244	293-305	22-34	-	Ghyoot *et al.*, 1999
-	408	635	-	-	650-2200	Carrio *et al.*, NY City
28-32	<400	600-700	-	15	-	Arnold *et al.*, 2000

†Enhanced biological phosphorus removal plant

Because centrate is an important source of NH_3, it is important to consider how it affects effluent quality. At the NEWPCC in Winnipeg, centrate is

currently recycled to the primary influent line where it is diluted and passes through primary treatment which included grit removal and primary sedimentation. The centrate then passes in diluted form through the treatment plant. The NH_3 load from the centrate is not removed because NEWPCC does not practice nitrification.

One method suggested for centrate management for the NEWPCC upgrade includes feeding centrate into the RAS re-aeration tank (Figure 2.3). The concentration of biomass in the RAS re-aeration tank can be very large, making nitrification rates rapid. The NO_3^- produced can then be denitrified in anoxic tanks using the influent as a degradable carbon source. Simulation modeling by Head and Oleszkiewicz (2000) identified that high concentrations of NO_3- produced from centrate nitrification in a RAS re-aeration tank could potentially compromise phosphorus release in the anaerobic zone of phosphorus removing facilities.

2.2.2 Methods of achieving short-SRT nitrification

2.2.2.1 WAS storage

Yuan *et al.* (1998) suggested waste activated sludge (WAS) storage with aeration to achieve short-SRT nitrification. During aerated storage without substrate addition the heterotrophic organisms have a higher decay rate than the nitrifying biomass. As the heterotrophs decay, they release nitrogen that becomes substrate for the nitrifying organisms in the liquor. Over time the

composition of the biomass in the storage tank changes such that the concentration and proportion of nitrifiers is larger than originally found in the WAS. For the storage tank to be beneficial, the stored sludge can only be used occasionally, such as in the case of shock nitrogen loads or toxicity. The main stream is operated without an SRT safety factor; thus upon addition of the stored sludge, the concentration of nitrifying bacteria in the main reactor is the same as would occur if the plant was operated with a longer SRT.

Table 2.2 depicts how the SRT of the nitrifying biomass can be increased by using a relatively small WAS storage tank. The nitrifiers alone have an SRT the same as a conventional system operating at a longer SRT while the inert solids have an SRT that is less than a conventional system with a longer SRT but longer than the main-stream tanks. The main-stream tank is operated at an SRT shorter than that of the nitrifiers (10 d for both examples). Assuming that the conventional and seeded aeration tanks have the same sludge concentrations, the volume savings for the examples in Table 2.2 are about 10 to 20% as shown by the mixed liquor suspended solids (MLSS) ratios. If the solids levels are allowed to exceed those of the conventional tank, then the volume savings are as great as 20 to 26%.

Table 2.2 Design and properties of plants with a WAS storage tank (Yuan *et al.*, 2000).

	SRT, conventional 15 d Main-stream SRT 10 d	SRT, conventional 20 d Main-stream SRT 10 d
SRT, storage tank	2.5 d	5 d
V, storage tank	$0.08V_{main}$	$0.17V_{main}$
SRT, nitrifiers	15 d	20 d
SRT, heterotrophs	17.5 d	25 d
SRT, inerts	12.5 d	15 d
Volume savings	10%	20%
$1-(V_{main}+V_{st})/V_{Conv}$	20%	26%

SRT conventional = the SRT that the plant would have to be operated at to achieve nitrification
SRT, nitrifiers = SRT conventional
SRT, heterotrophs = SRT conventional + SRT, storage tank
SRT, inerts = SRT main-stream + SRT, storage tank

2.2.2.2 One train operated with nitrification

Others have proposed maintaining nitrification in only one train of a WWTP. The biomass produced in that train can be used as seed for other trains that are operating under conditions that would preclude nitrification (*i.e.* the SRT is too short) or where nitrification is incomplete. For example, Randall and Cokgor (2001) describe a system where 100% of the WAS from a nitrifying MUCT train was added to a pure oxygen BNR. More complete nitrification was achieved but the hydraulic load had to be decreased to achieve full nitrification. With a similar configuration Neethling *et al.* (1998) found that adding WAS from dissolved air activated sludge system at a rate of 35% (VSS_{seed}/VSS_{main}) to a pure oxygen activated sludge system was enough to achieve full nitrification in the seeded reactor.

2.2.2.3 *Seeding with nitrifying bacteria*

Seeding nitrifying bacteria from an external source can also be used to achieve short-SRT nitrification. Theoretically, continuous seeding of nitrifiers into an activated sludge tank will supplement the population and allow nitrification to take place even when the SRT is too short. When seeding is occurring nitrification will occur to some degree at all SRTs (Kos, 1998).

Loss of nitrification can be recovered by seeding biomass from another nitrifying system (Andersson and Rosen, 1990), and seeding a non-nitrifying system with nitrifiers from a similar system (i.e. temperature, pH etc.) can initiate nitrification where none existed before (Neethling *et al.*, 1998). To be effective, the amount of nitrifying biomass added must be enough to achieve the desired effluent NH_3 concentration. That is to say, the activity of the added nitrifiers has to be at least equal to or greater than the mass that would be maintained in a conventional nitrification system (Yuan *et al.* 1998).

Nitrifying bacteria for seeding can be grown and harvested in-house or purchased from commercial vendors such as ONDEO-NALCO Chemicals (Naperville, IL) (Abeysinghe *et al.*, 2002; de Silva *et al.*, 2000), or the General Environmental Science company (Hung et al., 1987). Bio-augmentation can also be unintentional as in the case described by Daigger *et al.* (1993) where sloughing of nitrifying bacteria from an upstream trickling filter served as a source of nitrifying bacteria that improved the nitrification capabilities of a downstream suspended growth reactor.

Nitrifying bacteria for the purpose of seeding can be generated from the nitrification of centrate and have been shown, through modeling, to be extremely beneficial in decreasing the SRT required for nitrification. Kos (1998) showed with modeling that a WWTP that receives 33% of its N load from centrate can reduce the volume required for nitrification by 40% by nitrifying centrate and recycling the biomass back into the main-stream tanks (Figure 2.4). Rittmann (1996) and Kos (1998) also demonstrated with modeling that increasing the seed dose of nitrifying bacteria increases the benefit.

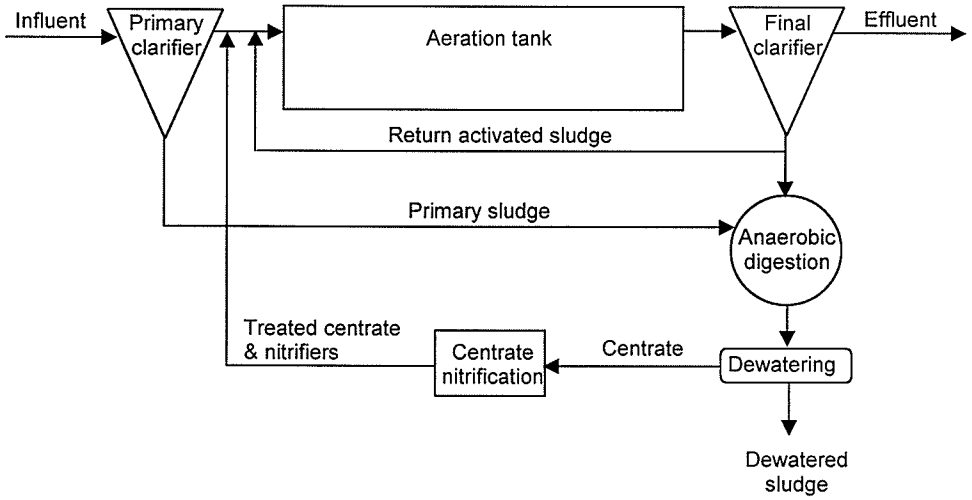


Figure 2.4 Simplified schematic of centrate nitrification for the purpose of seeding nitrifying bacteria into the main-stream (adapted from Kos, 1998). This configuration has been patented as the InNitri® process.

There are a number of examples where centrate has been used as a source of NH_3 for the growth of nitrifying bacteria. Salem *et al.* (2003) used a configuration where a percentage of RAS was kept in a separate aerated tank and centrate was added as an NH_3 source. The SRT of the side-stream reactor

was longer than that of the main-stream tanks, and the nitrifying biomass produced was fed back into the main-stream where it contributed to nitrification in that reactor. The process configuration has been dubbed the BABE process (Berends *et al.*, 2003). In contrast Katehis *et al.* (2002) showed limited improvement in effluent quality while seeding nitrifying bacteria grown on centrate into a full-scale wastewater treatment plant.

2.3 Producing nitrifying bacteria from centrate

2.3.1 Treating high-ammonia liquors in a biological reactor

There are a number of studies showing successful application of biological treatment of liquors containing high concentrations of NH_3. Reactor configurations range from complete mix continuous feed reactors, membrane bioreactors (MBR), and sequencing batch reactors (SBRs).

2.3.1.1 Ammonium oxidation to nitrate

The most common type of biological treatment for high ammonia liquors is the full oxidation of NH_4^+ to NO_3^-. Likely, the reason for its popularity is due to the wide range of readily available literature for its use in the treatment of wastewater. Table 2.3 provides a summary of several studies where successful nitrification of concentrated wastes has been achieved.

Table 2.3 Summary of successful nitrification of high NH_3 liquors using various activated sludge configurations.

	Configuration	Temperature (°C)	SRT (d)	HRT (h)	Influent NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	Reference
Coal gasification wastewater (P)	Complete mix	26	12 -37	72	>500	<3.1% NH_3in	Gallagher *et al.*, 1986
Landfill leachate (B)	MLE†	20	20	6.5	1200	<50	Shiskowski & Mavinic, 1998a
	4 Stage Bardenpho	20	40	13.7	1200	<50	
Sludge liquor (P)	MBR	35	--	2.2	244	total removal	Ghyoot *et al.*, 1999
Synthetic wastewater (B)	3 Stage plug-flow	--	--	24-48	1000	<10	Sumino *et al.*, 1997
Industrial wastewater	3 Stage plug-flow	--	--	24-48	840-960	<10	
Sludge liquor (F)	Complete mix	>15	--	15	500	<25	Jeavons *et al.*, 1997
Sludge liquor (P)	Complete mix	<32	5-10	13	1000	<10	Smith *et al.*, 1999
Sludge liquor (F)	Complete mix	25	--	15	800	<20	Philip *et al.*, 1999

†Modified Ludzack-Ettinger
P=pilot-scale; B=bench-scale; F=full-scale

2.3.1.2 Ammonium oxidation to nitrite

Recently, significant research has been conducted on the partial oxidation of NH_4^+ to NO_2^- (*eg.* van Kempen *et al.*, 2001; Hao *et al.*, 2002; Mulder *et al.*, 2001). The process named SHARON® operates with a short SRT without solids retention at a high temperature. The term SHARON® (Single reactor system for High Activity Ammonia Removal Over Nitrite) has been used to describe two different reactor configurations; 1) a reactor operating only for the partial oxidation of NH_4^+ to NO_2^- (van Dongen *et al.*, 2001; Hao *et al.*, 2002) or 2) a reactor operating for partial oxidation of NH_4^+ to NO_2^- with simultaneous denitrification with NO_2^- as the electron acceptor (Mulder *et al.*, 2001; van Kempen *et al.*, 2001).

At high temperatures (30°C to 40°C), the growth rate of the ammonia oxidizing bacteria is greater than the nitrite oxidizing bacteria. If the reactor

is operated with a short enough SRT, the nitrite oxidizers are washed out of the system (Mulder *et al.*, 2001). The SHARON® process is favoured over full oxidation of NH_4^+ to NO_3^- because when operated with denitrification it does not require alkalinity, it requires 25% less aeration energy and the tank can be smaller due to the shorter SRT requirements (Mulder *et al.*, 2001).

2.3.1.3 Advantages to biological treatment of centrate

Treating centrate in a side-stream with a small nitrifying reactor may prove to be a viable alternative to full expansion to accommodate nitrification at existing NH_3 loads. Centrate is particularly suited to biological nitrification in a dedicated side-stream because:

- The warm temperature of the centrate allows faster growth rates of nitrifying bacteria. The growth rate of nitrifying bacteria is highly sensitive to temperature (U.S. EPA, 1975). Therefore, maintaining the warm temperature allows the side-stream tank to be operated with a short SRT and have a small volume.
- Low available organic carbon allows more NH_3 to be converted to nitrifying bacteria mass rather than being diverted to heterotrophic bacteria that uptake NH_3 through assimilation (de Silva and Rittmann, 1999).
- As proposed by Kos (1998) and Berends *et al.* (2003), the use of side-stream liquors can be used for the production and harvest of nitrifying

bacteria. The nitrifiers produced can be used as seed to protect against loss of nitrification in the main-stream activated sludge tanks or to prevent instances of poor effluent quality due to shock NH_3 loads in the influent (Rittmann, 1996).

- Centrate nitrification can reduce variability in NH_3 loads. Treatment plant influent is subject to diurnal and seasonal flow, temperature and strength variability, while centrate flows are relatively constant. Removing the NH_3 load from centrate prevents the compounding effect that can occur when centrate NH_3 load corresponds with high influent NH_3 loads (Jeavons *et al.*, 1998; Arnold *et al.*, 2000).
- It is more efficient kinetically and economically to treat a small concentrated stream than a large dilute stream that would result from recycling centrate untreated. Mossakowska *et al.* (1997) reported that centrate nitrification can be accomplished in a reactor volume that is as small as 2% of the main-stream aeration basin volume.
- The side-stream treatment method can be designed based on the particular characteristics of the dewatering liquor to meet specific treatment requirements. Full N removal from the centrate increases the C/N ratio of the influent to the main-stream. This increases the denitrification capacity of the main-stream, thereby improving its efficiency (Wett *et al.*, 1998).

2.3.2 Centrate nitrification in a sequencing batch reactor (SBR)

Using an SBR (and variations thereof) for centrate treatment has a number of advantages over other types of reactor configurations. SBRs have certain advantages kinetically since the initial concentration of NH_3 in the reactor is allowed to rise much higher than would normally be seen in a complete-mix reactor system. In a batch reactor the aeration cycle length can also be altered until the desired level of treatment is obtained.

Table 2.4 provides a few examples of highly concentrated NH_3 liquors being treated in SBRs. The initial concentration of NH_3-N in the reactor is very high according to conventional activated sludge standards. With time, the biomass acclimates to these high concentrations and is able to achieve high nitrification rates. In a municipal wastewater treatment system where the proportion of nitrifying bacteria in the mixed liquor is usually less than 10% ($VSS_{nitrifiers}/VSS_{total}$), typical nitrification rates are between 0.1 and 0.42 mgN/mgVSS*d (U.S. EPA, 1975).

Table 2.4 Nitrification rates in batch fed reactors treating high ammonia liquor.

Temperature	SRT	S^o	MLSS	Nitrification Rate			Reference
°C	d	mg N/L	mg/L	mgN/L*d	mgN/mg SS*d	mgN/mgVSS*d	
32	-	125	5600	600-800	0.11-0.14	-	Arnold *et al.*, 2000
30	4-20	150	4000-9000	1000	0.11-0.25	1.08	Mossakowska *et al.*, 1997
20-25	50	200	-	1200-1400	-	-	Wett *et al.*, 1998
20	-	125		400	0.6	-	Henderson *et al.*, 1997

SBRs treating high concentrations of NH_3 in the influent can be controlled automatically by on-line measurements and control of oxygen concentration, airflow and pH making analysis of N fractions unnecessary (Mossakowska *et al.*, 1997; Wett *et al.*, 1998). Careful calibration of on-line sensors is required and correlation of instruments to N fractions is necessary before automatic control can be employed. Mossakowska *et al.* (1997), for example, found that there was a direct relationship between the NH_3 concentration, dissolved oxygen and the air flow rate required to maintain the desired dissolved oxygen concentration (Figure 2.5). Oxygen demand and airflow was highest as NH_3 was oxidized to NO_2^- (t=4 h), airflow remained elevated as NO_2^- was oxidized to NO_3^- (t=4 to 6.5 h) and once all NO_2^- was oxidized, dissolved oxygen levels remained elevated despite low airflow. When the air supply is turned off during settling and decanting, the oxygen concentration dropped quickly (t=10.5 to 12 h).

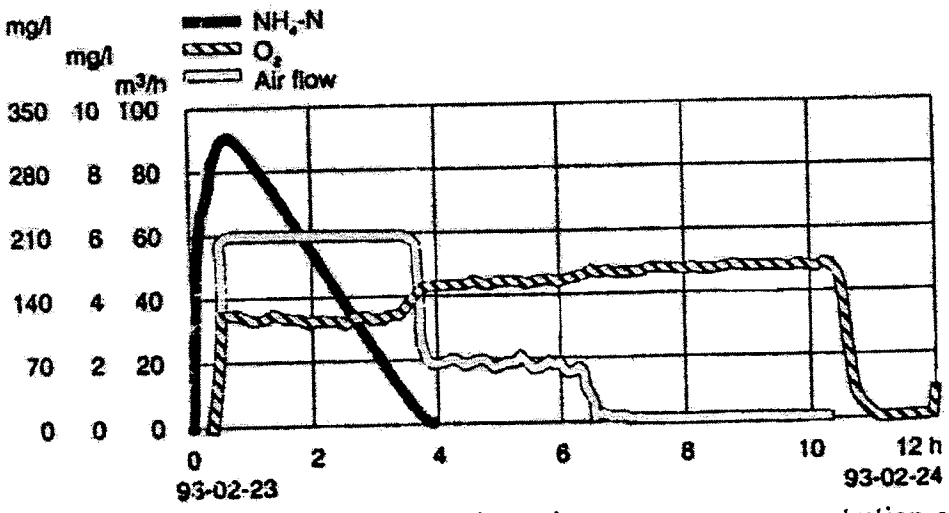


Figure 2.5 On-line measurements of ammonium nitrogen, oxygen concentration and air flow during nitrification of centrate in a sequencing batch reactor (Mossakowska *et al.*, 1997).

Finally, in an SBR a settling phase can be implemented to maintain a desired solids concentration or to keep an SRT sufficiently long to achieve full nitrification. Because the supernatant is recycled back to the front of the treatment plant there is no real concern about effluent solids. In cases where SRT control is of concern the settling phase can be eliminated making the SRT and HRT equal, or solids wasting after settling can be discontinued with solids removal only with the supernatant (Henderson *et al.*, 1997).

2.3.3 Obstacles to centrate nitrification

Due to the chemical nature of centrate, there are a number of obstacles that make centrate treatment difficult or undesirable. Following is a description of these characteristics.

2.3.3.1 Free ammonia toxicity

The free ammonia concentration is highly dependent on pH and temperature and is in equilibrium with ammonium (NH_4^+) under the following relationship:

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad [4]$$

For each unit of increase in pH, the concentration of free ammonia increases by 10 fold (Table 2.5).

Table 2.5 Changes in free ammonia concentration with changes in pH with a constant total ammonia concentration at 20°C.

pH	Total NH_3 (mg/L)	Free NH_3 (mg/L)
6	1000	0.5
7	1000	5
8	1000	50

If present in high concentrations, free ammonia can inhibit both NH_3 and NO_2^- oxidizing bacteria (AOBs and NOBs, respectively). Free ammonia toxicity to AOBs can occur between 10 and 150 mg/L (Anthonisen *et al.*, 1976). At a neutral pH, the total NH_3 concentrations would have to be greater than 1000 mg/L to be inhibitory. NO_2^- oxidizers, however, are more sensitive with toxicity occurring at concentrations as low as 0.1 to 1 mg/L free NH_3. Through gradual increases in NH_3 concentration with biomass acclimation, nitrifying bacteria are capable of completely oxidizing NH_3 to NO_3^- with total NH_3 concentrations as high as 3000 mg N/L (Mahne *et al.*, 1996).

Free NH_3 toxicity to NOBs can cause NO_2^- accumulation during nitrification of high NH_3 liquors. The AOBs continue to nitrify NH_3 to NO_2^- with NO_2^- accumulating until the concentration of free NH_3 is below the toxic limit to the NO_2^- oxidizers. Once the concentration is below the toxic threshold, NO_2^- is oxidized to NO_3^- (Anthonisen *et al.*, 1976). Ammonia toxicity can be an advantage for the SHARON® process where the goal is to select for the accumulation of NO_2^- and eliminate the production of NO_3^-.

2.3.3.2 *Unionized nitrous acid toxicity*

Excessive NO_2^- accumulation can lead to unionized nitrous acid (HNO_2) formation by the following relationship:

$$H^+ + NO_2^- \leftrightarrow HNO_2 \qquad [5]$$

The dependence of unionized nitrous acid concentration on the pH is presented in Table 2.6. As nitrification proceeds, alkalinity is consumed and the pH decreases. For every unit of pH decrease, the concentration of nitrous acid increases 10 fold. This further illustrates the importance of controlling pH during nitrification. The decrease in pH associated with nitrification can enhance the toxicity of nitrous acid and possibly lead to system failure.

Table 2.6 Changes in free nitrous acid concentration with changes in pH with a constant NO_2^- concentration at 20°C.

pH	NO_2^- (mg/L)	HNO_2 (mg/L)
6	100	0.8
7	100	0.08
8	100	0.008

Anthonisen *et al.* (1976) found that nitrous acid was inhibitory to nitrifying organisms between 0.22 to 2.8 mg/L. Nitrous acid toxicity is particularly a problem in low pH conditions even at low nitrite concentrations. HNO_2 toxicity is a result of the following reaction:

$$(HNO_2)_{extracellular} \leftrightarrow (NO_2^- + H^+)_{intracellular} \qquad [6]$$

The proton interferes with the transmembrane pH gradient required for adenosine triphosphate (ATP) synthesis (Glass *et al.*, 1997). However, research by Sears *et al.* (1998) on low pH nitrification (pH 5.5 to 6.5) showed that nitrifying bacteria can adjust to low pH and that the system will eventually resolve the toxicity problem through acclimation.

2.3.3.3 Need for addition of alkalinity

Centrate does not contain enough alkalinity to achieve full nitrification without alkalinity addition (ex. Ali *et al.*, 1998; Arnold *et al.*, 2000; Barnes, 2000; Ghyoot *et al.*, 1999; Mossakowska *et al.*, 1997). Alkalinity addition is required to meet the inorganic carbon demands of nitrification (7.14 mg $CaCO_3$/mg N oxidized) (U.S. EPA, 1975), compensating for CO_2 stripping during aeration, as well as buffering the pH. As the alkalinity is consumed during nitrification the pH of the mixed liquor decreases and can contribute to low pH stress and the possibility of free nitrous acid toxicity (Anthonisen *et al.*, 1976). Various alkali agents can be used to control the pH including $NaHCO_3$, Na_2CO_3, or lime.

2.3.3.4 COD demand for denitrification

Centrate nitrification without complete nitrogen removal exerts a COD demand for denitrification in the main-stream into which it is added (Salem *et al.*, 2003). Full N removal from centrate by denitrification in the side-stream

would eliminate this problem. One possible solution suggested by Barnard (pers. comm. 2000) is to recycle the nitrified centrate into a gravity thickener for denitrification. However, this may cause floating sludge and consumes carbon that could otherwise be used for P release in phosphorus removing facilities. Full N removal also has an additional benefit of preserving P release in the main-stream tanks through the elimination of high NO_3^- inputs into P release zones.

Side-stream denitrification with methanol or some other source of readily available carbon is possible but requires continuous carbon inputs. Approximately 50% of the alkalinity consumed in nitrification could be recovered through denitrification (U.S. EPA, 1975) but the need for alkali addition would not be completely eliminated.

2.3.3.5 Poor settlability of biomass

A reactor treating centrate does not develop a highly concentrated biomass, even with a long SRT. The COD/NH_4 ratio of centrate can be less than 0.5:1 as compared to a ratio of 10:1 in municipal wastewater. Due to low available carbon, heterotrophic growth is poor, creating conditions for poor sludge flocculation and settlability.

Henderson *et al.* (1997) proposes supplemental carbon addition to increase the solids concentration to improve settling. This, however, would result in diverting NH_3 away from nitrifying bacteria to meet the N requirements of

heterotrophic bacteria and requires continual inputs of degradable organic carbon. Gupta and Sharma (1996) found that maintaining a COD/TKN ratio near 1.0 creates a biomass with good settling properties and also yields a nitrifier fraction of about 20 to 24% of the total biomass. Addition of readily degradable carbon could be in the form of raw sewage or primary sludge and this carbon can also be used for full N removal by denitrification. The COD/TKN ratio should be managed to obtain the maximum number of nitrifiers while maintaining sludge settlability. The BABE process uses RAS to increase the solids concentration in the side-stream reactor allowing the nitrifiers to be captured in the sludge flow during settling (Berends *et al.*, 2003).

Depending on the configuration, sludge settlability in the side-stream may not be imperative since the biomass will be recycled into the main stream. Settling is only used to maintain a sludge concentration and sludge age sufficient for side-stream nitrification.

2.4 Obstacles to seeding nitrifying bacteria

A major problem associated with seeding is that the environment under which the seed is grown is often different than the environment into which it is to be seeded. To have a very small side-stream nitrification tank, the seed must be grown under optimum conditions for high growth and nitrification rates. On the one hand, the purpose of seed production is to decrease the

total volume of the WWTP by improving nitrification efficiency. On the other hand, it is desirable to produce a seed that has the highest potential for nitrification upon addition to the main-stream. To have the highest nitrification potential in the seeded main-stream the seed has to produced in conditions identical to the stream into which they are to be added (sub-optimal for nitrification) and therefore requires a larger volume than if the seed was grown under optimum conditions. Following are the major obstacles to seeding nitrifying bacteria.

2.4.1 Temperature shock and seeding

2.4.1.1 Temperature dependency of nitrification

Previously, centrate was cited as being an excellent source of NH_3 for the production of nitrifying seed. However, centrate nitrification tanks are expected to be 10 to 20°C warmer than the main-stream tanks into which the nitrifying bacteria are to be seeded. If the temperature decrease is large enough, the nitrifying bacteria could be rendered incapable of nitrification and the side-stream could not serve as a source of seed. However, the process may still prove to be useful since nitrification of the side-stream would continue to be a method for decreasing the NH_3 load to the main-stream.

It is widely known that nitrification is highly sensitive to temperature and it is likely that nitrification will cease or continue at a much decreased rate. Many researchers have attempted to quantify the temperature dependency of different types of nitrifying biomasses and have resulted in a relatively narrow range of temperature dependency factors and growth rates (Table 2.7). It is not clear in most cases whether or not the correction factors were determined from a nitrifying biomass that experienced a rapid change in temperature or a biomass that was acclimated to the new temperature for a long period of time. Despite this lack of information, the temperature correction factors all lie between 1.072 and 1.127.

Table 2.7 Temperature dependence of nitrifying bacteria growth rates.

Equation for growth rate, μ (d^{-1})	Temperature Correction factor	Reference
$(0.18)e^{0.12(T-15)}$	1.127	Downing and Hopwood, 1964
$(0.47)e^{0.09(T-15)}$	1.103	U.S. EPA, 1975
$(0.33)1.27^{(T-15)}$	1.127	Barnard, 1975
$(0.18)\ e^{\ 0.0729(T-15)}$	1.0756	Painter and Loveless, 1983
$(0.5)\ e^{\ 0.0917(T-20)}$	1.096	Biowin Default
$\mu_{max}e^{\ 0.0695(T-To)}$	1.072	Jones, 2002

Temperature effects can be minimized by producing the nitrifying seed at the same temperature as the reactor into which they are to be seeded. WAS storage (Yuan *et al.*, 1998; 2000) with centrate nitrification also reduces the effect of temperature because the WAS is approximately the same temperature as the main-stream. Similarly, the BABE process adds RAS to

the side-stream tank causing a decrease in temperature of the side-stream tank (Berends *et al.*, 2003).

2.4.1.2 Cold shock mechanisms

The growth rates of microorganisms are strongly affected by temperature and nitrifying bacteria are no exception. Most organisms can grow within a temperature range of about 30°C with a minimum, optimum and maximum temperature for growth within this range (Figure 2.6) (Brock, 2000). Nitrification has been observed over a range of 2°C (Oleszkiewicz and Berquist, 1988) to 44°C (Lubkowitz-Bailey and Steidel, 1999) with maximum nitrification rates occurring at 30 to 35°C (U.S. EPA, 1975; Lubkowitz-Bailey and Steidel, 1999). The temperature drop of interest in this research is expected to be less than 20°C; *i.e.*, from a maximum of 30°C down to a minimum of 10°C.

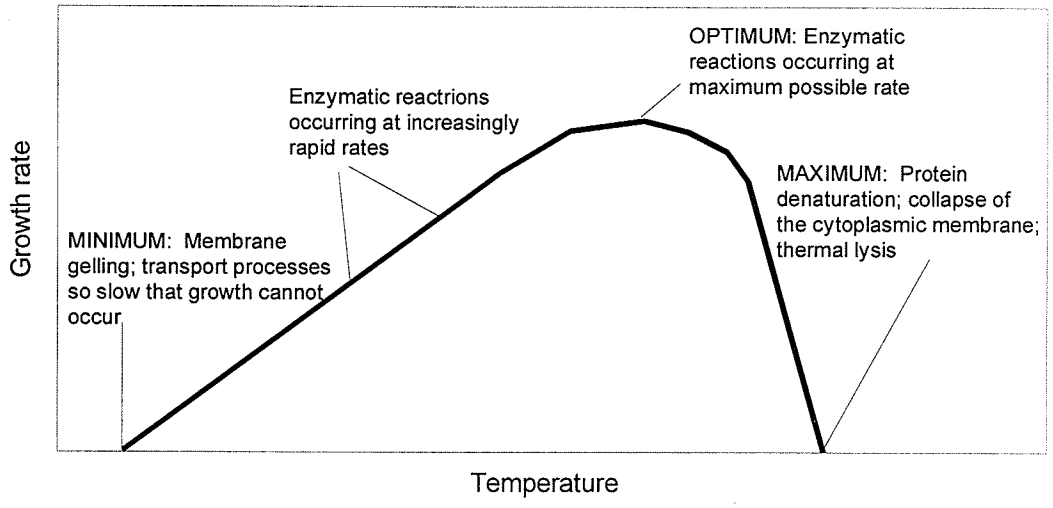


Figure 2.6 Effect of temperature on growth rate and the molecular consequences for the cell (adapted from Madigan *et al.*, 2000).

Most research on microbial growth and temperature has been conducted on maximum temperatures for growth while the mechanisms behind minimum growth temperatures are not well defined. A rapid decrease in temperature leads to physiological changes in bacteria with the degree of cold-shock response being dependent on the degree of decrease in temperature; *i.e.* "the larger the range of the temperature shift, the more pronounced the response" (Jones and Inouye, 1994). The physiological changes that occur in bacteria include:

- A decrease in cellular membrane fluidity. Membrane fluidity is increased at cold temperature by altering the fatty acid composition of the cellular membrane at cold temperatures. If the temperature is low enough, the membrane no longer functions properly in nutrient transport or proton gradient formation (Madigan *et al.*, 2000).

- The production of cold shock proteins has a role in cold-shock adaptation (Jones and Inouye, 1994; Graumann and Marahiel, 1996). In fast growing organisms, such as, *E.coli* where most of the research on cold-shock has been done, protein synthesis is resumed in as little as 4 h after a decrease in temperature from 37°C to 10°C (Graumann and Marahiel, 1996). This period of time is called the acclimation phase, during which time cold shock proteins accumulate. In slow growing organisms, like AOB, the time to recovery is expected to be longer and in extreme cases the organism may never fully recover from the cold-shock.
- The inhibition of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein synthesis. The production of many cellular proteins is halted while cold shock proteins accumulate (O'Connell *et al.*, 2000). Cold shock proteins have been shown to allow ribosomes to translate messenger RNA (mRNA) at low temperatures (Thieringer *et al.*, 1998). In ammonia oxidizing bacteria (AOB), NH_3 is oxidized by the membrane protein and enzyme, ammonia monooxygenase (AMO). This enzyme is affected by temperature in the usual way with increased reaction rates with increased temperature over a defined temperature range (Madigan *et al.* 2000). It is likely that in addition to the reduction in reaction rate with temperature, the cold-shock

response can contribute to a decline in NH_3 oxidation rate by interfering with AMO production.

2.4.2 Grazing of seeded biomass by protozoa

Protozoa and other higher organisms survive by consuming microorganisms. There have been several cases where bio-augmentation failure has been attributed to this grazing. For example, Lee and Welander (1994) found that in nitrifying biofilms, the suppression of rotifers and nematodes resulted in an increase in nitrification to twice the level of a reference reactor without predator suppression. Lee and Welander (1996) also found that dispersed bacteria are readily consumed by protozoa and metazoa. Bouchez *et al.* (2000) attributed nitrification failure due to increased grazing pressure on nitrifying bacteria that was created by increased growth of bacterivorous organisms. Verhagen and Laanbroek (1992) found that, due to their large cell size, nitrifying bacteria are selectively preyed upon by flagellates. However, recent work by Lee and Oleszkiewicz (2002) showed that grazing was not occurring at a significant rate in reactors operating under similar conditions to those used in this research.

Predation is equivalent to decay in that they both result in the loss of nitrifying bacteria. This loss causes a net decrease in the SRT of nitrifying organisms and must be accounted for in the calculation of seed dose required to achieve the desired level of treatment (Lee and Welander, 1994).

2.4.3 Poor settling properties of seeded biomass

Nitrifying bacteria that settle poorly in side-stream nitrification tanks may not settle well in the environment into which they are added. Nitrifying bacteria that fail to be incorporated into the main-stream sludge floc may not settle, thus resulting in inadvertent solids wasting with the effluent. Head and Oleszkiewicz (2003a) showed that AOB were being preferentially wasted from seeded reactors. The proportion of AOB in the effluent solids was found to be higher than the proportion in the reactor mixed liquor. The use of carrier materials such as floating polyurethane foam particles (Parker *et al.*, 2000) or weighting agents (Li and Hultman, 1997) might be used to retain seeded nitrifying bacteria.

2.5 Determining the seeded SRT

The determination of seeded SRT treats nitrifying bacteria as a separate entity from the other solids in the treatment system. Through seeding, the retention time of nitrifying bacteria can be different than the retention time of the other solids in the system. For example, maintaining a nitrifying biomass in a side-stream tank operating at an independent SRT can be an effective means of decreasing the overall system SRT needed to maintain a suitable effluent NH_3 concentration (*i.e.*, short-SRT nitrification).

Rittmann (1996) showed with modeling that the residence time of nitrifying bacteria increased when nitrifiers were seeded. In effect, the time needed to

double the nitrifier population decreases, making the observed retention time of nitrifiers longer than that which would be calculated from the mass of sludge wasted daily.

Development of equations for the estimation of seeded SRT of seeded systems has been done elsewhere (ex. Daigger *et al.*, 1993; Rittmann, 1996). The seeded SRT is calculated by first estimating the concentration of ammonia oxidizers in the influent stream of the system to be seeded (X_a^o) (Equation 7). In this case S^o is the NH_3-N concentration of the centrate and S is the effluent NH_3-N concentration of the treated centrate from the seed source reactor. The seeded SRT of the seeded reactor can then be determined from Equation 8 by accounting for nitrifying bacteria (specifically, ammonia oxidizers) entering and leaving the system.

$$X_a^o = \frac{Q^s}{Q^i} \bullet \frac{\theta_x}{\theta}\left[\frac{Y(S^o - S)}{1 + b\theta_x}\right] \qquad [7]$$

$$\theta_x^s = \frac{X_a V}{Q^w X_a + Q^e X_a^e - Q^i X_a^o} \qquad [8]$$

The concentration of ammonia oxidizers in the seeded SBR (X_a) can then be estimated by Equation 9. In this case S^o is the NH_3-N concentration of the wastewater fed to the seeded reactor and S is the final achievable steady-state NH_3-N concentration in the effluent from these reactors. Simultaneous calculation of Equations 8 and 9 determines the seeded SRT (θ_x^s).

$$X_a = \frac{\theta_x^s}{\theta}\left[\frac{Y(S^o - S)}{1 + b\theta_x^s}\right] \qquad [9]$$

If S is unknown, it can also be calculated with Equation 10 simultaneously with Equations 8 and 9.

$$S = K_N \frac{1 + b\theta_x^s}{YU\theta_x^s - (1 + b\theta_x^s)} \qquad [10]$$

An example of how seeding nitrifying bacteria affects chemostat systems is given by Rittmann (1996) and is reiterated here. Using Equations 8, 9 and 10 and the parameters in Figure 2.1, Figure 2.7 was re-created and shows the impact of seed concentration on 2 seeded systems; one where 67% of the reactor mixed liquor is wasted daily ($\theta_x = \theta = 1.5$ d) and another where 33% is wasted daily ($\theta_x = \theta = 3$ d).

As the dose of seed increases, the effluent quality improves and the net observed growth rate of the nitrifying bacteria decreases; *i.e.* the retention time of the nitrifying bacteria (θ_x^s) increases. The system operating with θ_x = 1.5 d does not contain any X_a before seeding is started but the system operating slightly above θ_x^{min} does contain some nitrifiers before seed is added.

Although the formulas for determination of the seeded SRT of the nitrifying bacteria are quite simple in their calculation, problems arise in estimating exactly how many nitrifying bacteria are needed to achieve full nitrification in the seeded system. The activity of the seed source may change upon addition

to their new environment and this is touted as one of the main reasons for bio-augmentation failure (Abeysinghe *et al.*, 2002). Changes in growth rate, nitrification rate, or decay all have an impact on the mass of seed required to reach the desired treatment level. Figure 2.8 provides an example of the seed dose required to achieve a desired effluent quality depending on the specific nitrification rate of the seed (U) and the kinetic parameters listed in Figure 2.1. As U decreases, the mass of seed required increases. Similarly, as the decay rate increases the mass of seed required increases, but the impact of decay rate has a much weaker influence on the required dose.

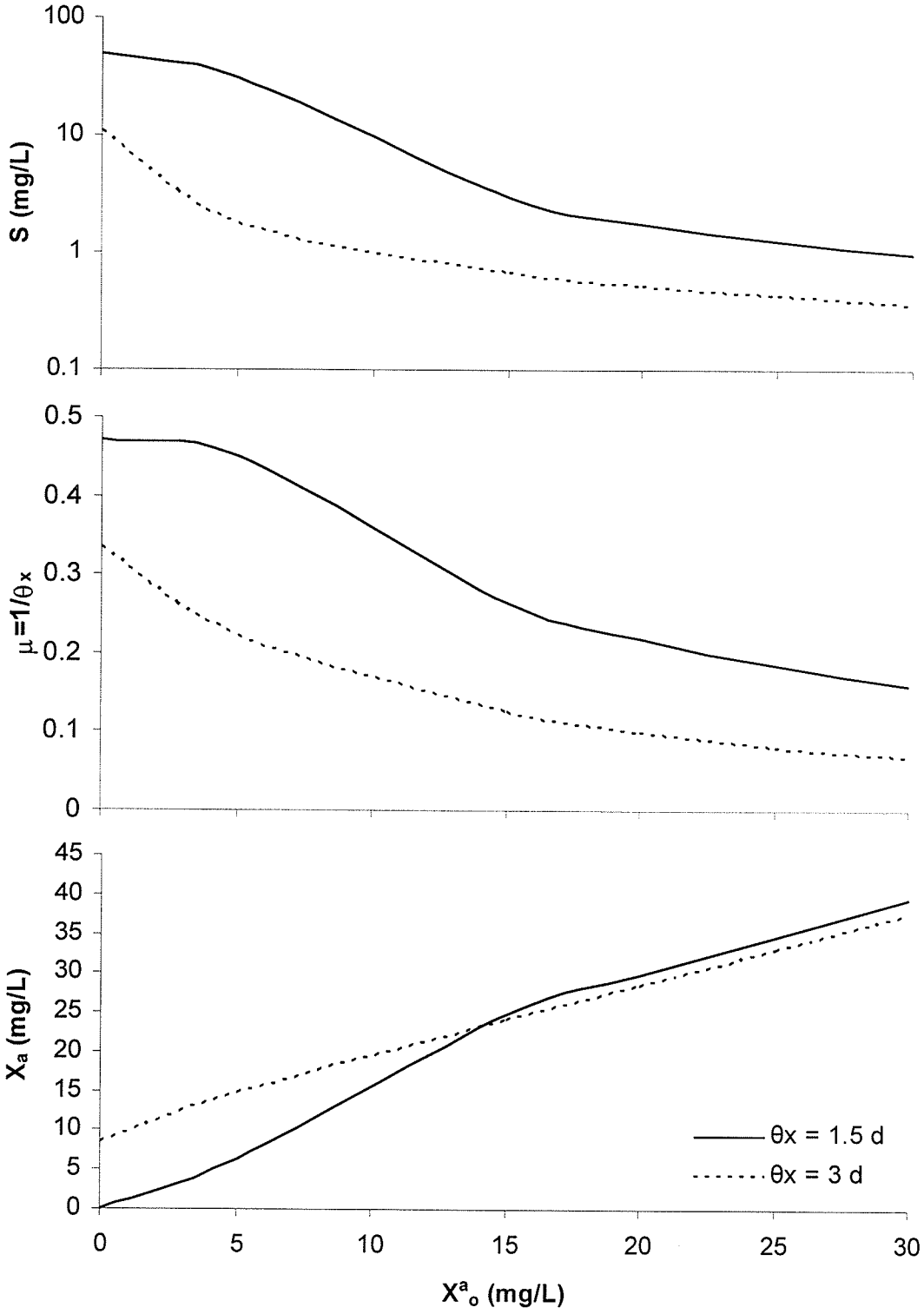


Figure 2.7 Impact of nitrifying seed dose on effluent quality, growth rate and nitrifier concentration in a seeded chemostat as per the parameters listed in Figure 2.1. (adapted from Rittmann, 1996). U = 1.7 mg NH_3-N/mg nitrifiers*d

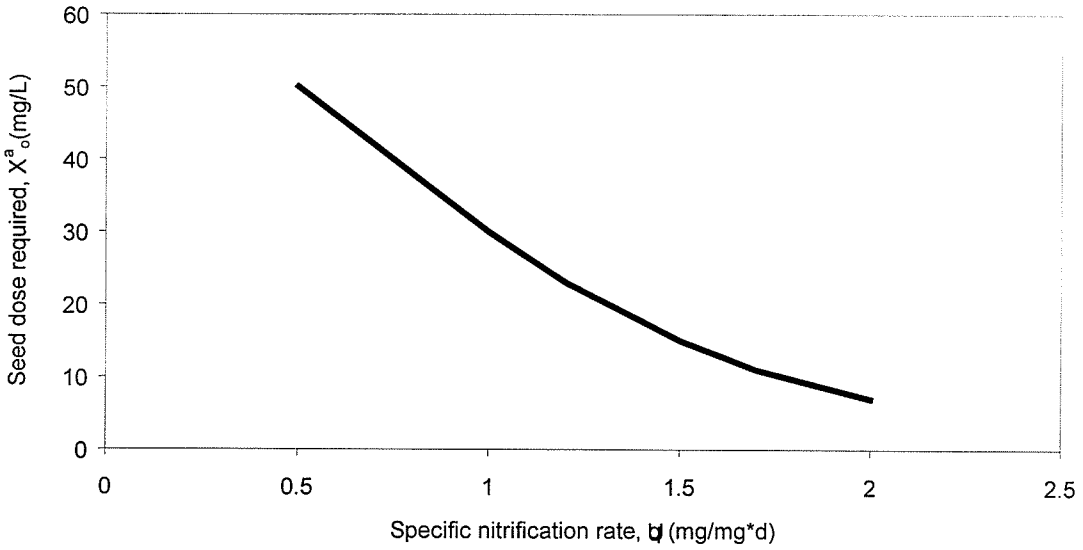


Figure 2.8 Seed dose required to achieve an effluent NH_3-N concentration of 2 mg NH_3-N/L when the specific nitrification rate varies. Kinetic parameters are listed in Figure 2.1.

Because wet chemistry of seeded systems does not thoroughly depict the fate of the seeded biomass, it is desirable to use microbial techniques to observe the seed *in situ*. Section 2.7 will describe how fluorescence *in situ* hybridization can be used to determine the fate of seeded biomass.

2.6 Modeling nitrification using the Activated Sludge Models (ASM)

2.6.1 The ASM models

Modeling is becoming common-place for the design, upgrade and optimization of wastewater treatment facilities. The activated sludge models (ASM) (developed by the IAWQ task group for Mathematical Modeling for Design and Operation of Biological Wastewater Treatment) are based on a "matrix" format where chemical and biological transformations are represented by a series of interrelated equations. The ASM models are

constantly improved as new research into the kinetic and stoichiometric values of wastewater treatment systems are conducted. Gujer *et al.* (1999) and Henze *et al.* (1999) provide a good summary of the stoichiometric and composition matrix and kinetic rate expressions for ASM3 - the most recent version of the models.

The ASM models have been successfully applied to predicting effluent COD, P and N fractions in wastewater effluent after considerable calibration and wastewater characterization (Koch *et al.*, 2000; Koch *et al.*, 2001; Wichern *et al.*, 2001). The model, BioWin (EnviroSim, 2002), which uses ASM defined equations, is currently being used to optimize and upgrade the wastewater treatment facilities in Winnipeg to include N and possibly phosphorus removal.

2.6.2 Wastewater characteristics

Modeling nitrification requires input parameters for wastewater and biomass characteristics. Every wastewater is different and varies from plant to plant depending on socio-economic factors, water use, infiltration/inflow, the use of garbage disposals, industry, and the storage capacity of the collection system (Barker and Dold, 1997).

Nitrogen fractions in the influent wastewater stream depict the amount of N that is actually available for nitrification. The N fractions are expressed as a proportion of the total TKN in the influent stream (N_{Ti}). The N_{Ti} is first split

into two major categories; ammonia-N and organically bound-N. The organic-N is considered as "biodegradable" or "unbiodegradable" and "soluble" or "particulate" (Figure 2.9). BioWin, a wastewater treatment simulation model, allows the user to define the fraction of each type of TKN in the influent stream or the model can provide default values. The N fractions in BioWin are listed in Table 2.8.

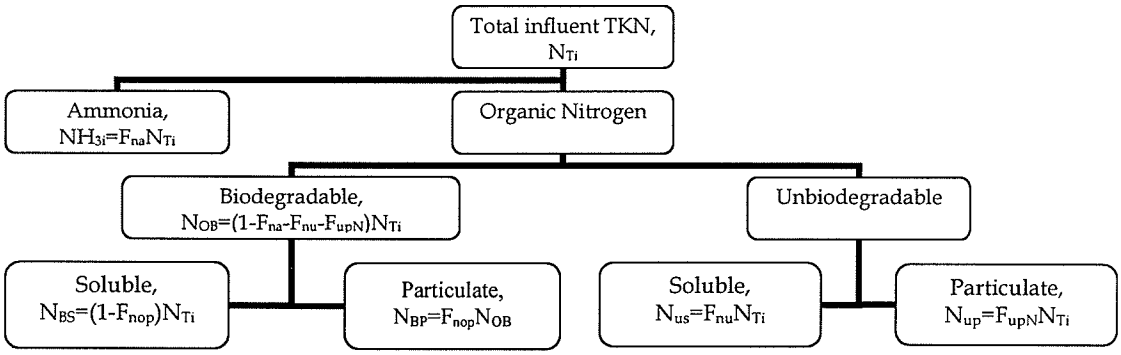


Figure 2.9 Division of municipal wastewater TKN into constituent N fractions (Barker and Dold, 1997).

Table 2.8 Fractions of TKN in the influent stream.

Symbol	Definition
F_{na}	Fraction of influent TKN which is ammonia
F_{nox}	Fraction of influent organic nitrogen which is particulate
F_{nu}	Fraction of influent TKN which is soluble unbiodegradable
F_{upN}	The N:COD ratio for the influent particulate unbiodegradable COD
F_{Zba}	Fraction of total influent COD which is autotrophic organisms
F_{nop}	Fraction of biodegradable organic TKN which is particulate

Similarly, the biomass that treats the wastewater differs depending on the chemical composition of the wastewater, solids and hydraulic retention times, temperature, reactor configuration, method and type of aeration system in addition to many other environmental factors. The kinetic and stoichiometric characteristics of the biomass can be manipulated in the model or default

values can be used. Table 2.9 lists the nitrification parameters that can be changed with the provided default values.

Table 2.9 Default values for nitrification kinetics and stoichiometry in BioWin.

Parameters	Default value	New* default values	Arrhenius temperature correction factor
umax	0.500 d^{-1}	0.9 d^{-1}	1.096
K_N NH_4^+	1.000 mg/L	0.70 mg/L	1.000
b_A	0.04 d^{-1}	0.17 d^{-1}	1.029
Yield	0.150		-
N in biomass	0.680		-
N in inerts	0.680		-
Endogenous residue	0.080		-
COD:VSS ratio	1.420		-

*In the near future, Envirosim will be releasing a new version of BioWin with different default values than the version used in this research (Jones, pers. comm., 2003).

Although several studies have found that the stoichiometric and kinetic parameters do not change appreciably for domestic wastewaters, the same does not hold true for the growth rate of nitrifying bacteria (Barker and Dold, 1997). The most important input parameters for modeling nitrification are the growth and decay rates. Barker and Dold (1997) suggest that these parameters are specific to every wastewater and can actually be considered a wastewater characteristic.

2.6.3 Using the model

Generally, the BioWin model can be utilized in 2 ways:

2.6.3.1 Predicting the effluent quality

This process might be used to upgrade an existing WWTP or to optimize operation of an existing plant. The kinetic parameters of the biomass and the

characteristics of the influent must be well known. The known parameters are input into the model and then the reactor sizes and operation can be manipulated such that the desired level of treatment is achieved. Such is the procedure behind the current upgrades to the NEWPCC in Winnipeg where land area for expansion is limited and it is desirable to minimize expansion costs.

2.6.3.2 Estimating the kinetic parameters of the biomass

In this case the kinetic parameters of the model are manipulated until the modeled effluent output values match the observed effluent quality from a WWTP or laboratory reactor. This requires knowledge of the wastewater characteristics and the operating conditions of the reactor. Dold (2002) used this procedure to determine the nitrification kinetics, specifically growth and decay rates in a laboratory reactor.

2.7 Theory of fluorescence *in situ* hybridization

There are three kinds of RNA including messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). The rRNA is integral to ribosome structure and is particularly suited for studying microbial evolution because it is found in all organisms. Closely and distantly related microorganisms can be compared by examining the variable and stable sequences of rRNA (Prescott *et al.*, 1999). The 16S and 23S rRNAs contain sequences that are

highly conserved but also have regions of sufficient variability to adequately differentiate between related organisms (Madigan *et al.*, 2000).

Phylogenetic groups of organisms have oligonucleotide signature sequences that are specific to most or all members of the group. The sequences are not present in other groups, even if the groups are closely related. Signature sequences have been identified for eubacteria, archaebacteria, eucaryotes as well as other major bacterial groups (Prescott *et al.*, 1999) and there are several data bases available of ribosome sequences for comparative analysis. Two examples of such databases are the Ribosomal Database Project (http://rdp.cme.msu.edu/html/) and the ARB database (http://www.arb-home.de/).

In situ hybridization is a procedure by which specific types of microorganisms can be observed by annealing a fragment of DNA or RNA (oligonucleotide probe) onto a target sequence of RNA inside a cell. The target nucleic acid is retained *in situ* and, under the right conditions, is accessible for hybridization to a probe. Probes are typically 20 to 30 bases in length and can be synthesized in the lab which allows specific probes to be designed. They are labeled by incorporating a reporter molecule or fluorescent label during synthesis. Preserved cells are incubated with the labeled probe under well defined temperature and salt conditions. The probe hybridizes to the target gene sequence with excess probe being removed in a subsequent washing step. The labeled cell can then be detected using a

fluorescent microscope using the appropriate wavelength of light specific to the label.

The first step in conducting FISH is the collection and fixation of cells. Fixation can be done by crosslinkage using formaldehyde-based fixatives. The crosslinking fixatives give greater accessibility and stable retention of cellular RNA and create chemical bonds between nucleic acids and proteins (Du Sart and Choo, 1998).

The target sequence must then be denatured and hybridized. Hybridization is carried out under optimal conditions for the annealing of the probe to the target nucleic acid in the cell. This can be achieved by the use of a dilution of deionized formamide in a salt solution or by heat, or a combination of the two (Du Sart and Choo, 1998).

Hybridization depends on the ability of the probe nucleic acid to anneal with its complementary strand of target nucleic acid under environmental conditions where the nucleic acid is present in single-stranded form. The form of the nucleic acid is dependent on:

- The nature of the probe and the target nucleic acid: RNA/RNA hybrids are more stable than RNA/DNA hybrids, which are more stable than DNA/DNA hybrids (Du Sart and Choo, 1998).
- The length of the probe: Longer probes form more stable hybrids however, short probes are required for in situ hybridization because

the probe has to diffuse into the dense matrix of cells or chromosomes. (Du Sart and Choo, 1998).

- The extent of sequence matching between the probe and target: Labeled probes can hybridize non-specifically to sequences that are similar but are not entirely homologous to the probe sequence. The degree of non-specific binding can be manipulated by varying the stringency of the hybridization reaction. Non-homologous hybrids are less stable than the perfectly matched hybrids. They can be dissociated by performing washes at specific stringencies (Du Sart and Choo, 1998).
- The composition of the hybridization solution: Four parameters influence the denaturation and renaturation of nucleic acids in the hybridization solution (Du Sart and Choo, 1998):

  - *Temperature*: The stringency of hybridization can be manipulated by changing the temperature, or the temperature for hybridization can be manipulated either by the addition of denaturing agents such as formamide or dimethylsulfoxide, or by varying the concentration of salt (Amann and Schleifer, 2001). Hybridization for the analysis of wastewater microorganisms is usually done at 46°C with a probe-specific percentage of formamide and salt.

- *pH*: In the pH range 5.0 to 9.0 the rate of renaturation is independent of pH. Higher pH can be used to produce more stringent hybridization conditions (Du Sart and Choo, 1998).

-*Monovalent cations*: Monovalent cations (*i.e.*, sodium ions) interact electrostatically with phosphate groups of nucleic acids, so that electrostatic repulsion between the two strands decreases with increasing salt concentration. Therefore, higher salt concentrations increase the stability of the hybrid (Du Sart and Choo, 1998).

-*Organic solvents*: Formamide addition reduces the thermal stability of double-stranded polynucleotides so that hybridization can be performed at a lower temperature (*i.e.*, 46°C). Without formamide, hybridization must take place at much higher temperatures which can affect the morphology of the cells being targeted (Du Sart and Choo, 1998; Amann and Schliefer, 2001).

Hybridization is then followed by a more stringent washing step at 48°C. The stringency of the wash buffer is usually adjusted by lowering the salt to a probe-specific concentration rather than by the addition of formamide (Amann and Schliefer, 2001). Washing of the hybridized sections is carried out to remove probe that has bound to sequences different from the intended target or non-specifically to other cell components.

Labeled nucleotides can be observed with a fluorescent microscope and specific filters that allow visualization of the wavelength emitted by the

fluorescent dye. Some fluorescent markers fade quickly as the emitted wavelengths become exhausted from exposure to UV light. Antifading reagents can be added before analysis. Image capture software and digital photography can minimize the problem of fading signals by minimizing the light exposure time to the hybridized sample (Du Sart and Choo, 1998).

2.8 Limitations of FISH for identifying specific organisms

2.8.1 Physical conservation of rRNA

Most probes developed for ammonia oxidizing bacteria (AOB) target 16S rRNA (*eg.* Mobarry *et al.*, 1996; Wagner *et al.*, 1996; Guschin, 1997). Oligonucleotide probes that bind to 16S rRNA rely on the presence of large quantities of rRNA. Ribosome synthesis is energetically costly to the cell and it is likely that bacteria maintain ribosomes during periods of starvation of up to several months. Wagner *et al.* (1995) found that AOB conserve rRNA even in the presence of a nitrification inhibitor. Gieske *et al.* (2001) also found evidence of AOB maintaining their ribosome content during periods of inactivity. Therefore, FISH cannot be used to estimate growth rates of AOB but can indicate the potential of the cell to synthesize protein, like ammonia monoxygenase (AMO), the enzyme responsible for ammonia oxidation.

2.8.2 Genetic conservation of rRNA

Phylogenetically distant organisms may have almost identical 16S rRNA sequences (Amman and Ludwig, 2000). In some cases the similarity can limit the applicability of FISH analysis by making it difficult to discriminate between closely related populations. For example, an 18mer probe targeting a region of an rRNA molecule has a 1:418 chance of an unrelated target cell being detected (Head *et al.*, 1998). However, because even in variable regions of rRNA there may be only a few positions that vary between taxa, the probability of detecting an unrelated cell is considerably increased (1:45, if only 5 positions are variable).

Where probe specificity is a problem, targeting the 23S rRNA may be more successful. The 23S rRNA is approximately twice as long and contains several highly variable regions (Amann and Ludwig, 2000). It has also been suggested that this problem can be overcome by using multiple specific oligonuclotide probes targeting several different sites on the rRNA molecule and labeling them with different fluorochromes.

2.8.3 Presence of unknown organisms

Hybridization may occur with unknown organisms or unknown organisms may be phylogenetically members of the target group but do not contain a matching target set of genes. Many phylogenetically defined groups do not have identifiable common target sites (Amann and Ludwig, 2000). In this

research, AOBs are the target organisms. There may exist AOBs that are not labeled with commonly used oligonucleotide probes for AOBs or they may exist other organisms that contain the target sequence but do not perform ammonia oxidation.

2.8.4 Detection limit

Cell counts of individually labeled cells may also underestimate the number of cells present where rRNA contents are below the detection limit (Amann and Ludwig, 2000). Some organisms have highly variable rRNA content that can be correlated to cellular activity. The detection limit of probes that target rRNA is sensitive to changes in cellular rRNA content (Amann and Ludwig, 2000).

2.9 FISH analysis for detecting ammonia oxidizing bacteria (AOB)

2.9.1 Types of AOB

The lack of phenotypic differences between AOB and the difficulties in isolating them in pure culture from environmental samples make them particularly suited to rRNA based studies. Most studies on AOB have been done using *Nitrosomonas europaea* because it can be grown in pure culture more easily than other AOB (Head *et al.*, 1998).

There are 2 phylogenetically distinct groups of autotrophic AOB: one within the Beta (β) sub-class Proteobacteria while the other is within the Gamma (γ)

sub-class. The major species of AOB under each sub-class are shown in Figure 2.10.

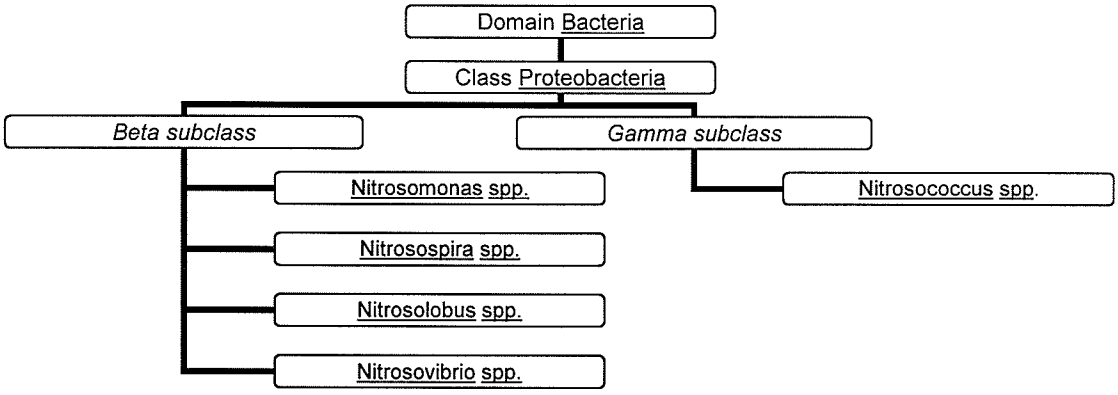


Figure 2.10 Ammonia oxidizing bacteria of the Beta and Gamma subclasses.

The oligonucleotide probes for targeting specific sequences of 16S rRNA in AOB are well documented. Table 2.10 is a list of some of the commercially available probes for identifying AOB *in situ* - ranging from general to very specific. These probes can be used individually or in combination with other probes to detect AOB in natural and engineered environments.

Table 2.10 Probe sequence for fluorescence *in situ* hybridization of 16S rRNA.

Specificity	Probe name	Sequence	Reference
Universal, almost all life	Univ1390	GACGGGCGGTGTGTACAA	Guschin *et al.*, 1997
Eubacteria	Eub338	GCTGCCTCCCGTCGGCGT	Amann *et al.*, 1990
β-subclass of Proteobacteria	BET42a	GCCTTCCCACTTCGTTT	Manz *et al.*, 1992
Ammonia oxidizing β proteobacteria	Nso190	CGATCCCCTGCTTTTCTCC	Mobarry *et al.*, 1996
Ammonia oxidizing β Proteobacteria	Nso1225	CGCCATTGTATTACGTGTGA	Schramm, 1999; Ballinger, 1998; Guschin, 1997
Nitrosomonas spp., N. europaea, N. eutropha, Nitrosococcus mobilis	Nsm156	TATTAGCACATCTTTCGAT	Mobarry *et al.*, 1996
Nitrosolobus multiformis, Nitrosospira briensis, Nitrosovibrio tenuis	Nsv443	CCGTGACCGTTTCGTTCCG	Mobarry *et al.*, 1996

2.9.2 Quantification of AOB using FISH

FISH has been widely used to identify AOB in activated sludge samples (*e.g.* Biesterfeld *et al.*, 2001; Juretschko *et al.*, 1998; Mobarry *et al.*, 1996). Quantification of AOB using FISH can be done in 2 ways: 1) direct cell counts or 2) relative area quantification. Either method depends on labeling the target AOBs plus the entire biomass present in the system. The total biomass present is usually quantified using 4',6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) (Biesterfeld *et al.*, 2001) or a general oligonucleotide probe like EUB 338 (Morgenroth *et al.*, 2000), Univ 1390 (Frigon *et al.*, 2002) or Univ 1392 (Raskin *et al.*, 1996). DAPI targets all organisms that contain DNA but does not distinguish between living and dead cells. As an alternative, Yuan and Blackall (2002) suggest using Lissamine green to identify only the viable organisms. Lissamine green is a selective stain for the cytoplasm of degenerating and degenerated cells. In contrast, oligonucleotide probes only target organisms with corresponding sequences. Using the probe EUB 338 has limitations in that it does not bind to eukaryotic organisms like stalked ciliates, fungi, filamentous organisms or rotifers that are very commonly found in activated sludge systems.

2.9.2.1 Direct cell counts

Direct cell counts involve counting the number of cells labeled by a fluorescent probe and expressing that number as a percentage of the total

number of cells present. Silyn-Roberts and Lewis (2001), for example, used DAPI staining to count the total number cells and then presented the probe-labeled cells as a percentage of the total cells stained by DAPI. Direct cell counts are time consuming thus limiting counts to a few thousand cells. Automated counting using image analysis software also has limitations since only very sophisticated software can distinguish between individual cells and cells in very close proximity to each other or in aggregates. Confocal laser scanning microscopy can eliminate the problem of counting densely aggregated cells by examining optical sections, but its single-cell resolution requires many images to obtain a representative sample of the population in question.

Direct cell counts can be converted to ratios of target cells per mass of total solids if the relationship between cell numbers and biomass concentration has been determined for the population of interest (Frigon *et al.*, 2002). Translating the number of cells to a concentration term requires the cultivation of the target cells in pure culture, which is not always possible.

2.9.2.2 Relative area counts

Relative area counts express the total area of targeted cells against the total area of biomass photographed. This procedure has been widely used in examining activated sludge samples (*e.g.*, Mudaly *et al.*, 2000, 2001; Morgenroth *et al.*, 2000; Biesterfeld *et al.*, 2001). This method of quantification

can account for cells that are in close proximity in two dimensions but cannot differentiate between cells that are overlapping. Area is more readily translated to concentration if it is assumed that the density of cellular contents in all cells is the same and overlapping of cells is minimized.

Relative area determination alone does not take into account inevitable changes in biomass concentration that occur in biological wastewater treatment systems. Beisterfeld and Figueroa (2002) found no correlation between nitrification efficiency and the relative area of Nso 190 against EUB 338 in a nitrifying trickling filter. While the absolute area of AOB might remain constant, the relative value would decrease if the absolute area labeled by EUB338 increased. An additional function (*f*) could be included to account for changes in total biomass concentration such that a comparison can be made between sampling times and sampling locations. The equation for correcting for differences in biomass concentration might take the form of Equation 11. The term *f* could represent the TSS, VSS, total cell numbers or some other expression of total biomass.

$$Corrected\ AOB\ Concentration = f \times \frac{Area\ Labeled\ by\ Nso190}{Total\ Area\ of\ Biomass} \qquad [11]$$

Daims *et al.* (2001) calculated biovolume based on cell area to approximate the biochemical reaction space occupied by a target population of ammonia and nitrite oxidizing bacteria. Raskin *et al.* (1996) correlated DAPI stained area with the VSS concentration in anaerobic bioreactors while Biesterfeld *et al.*

(2001) were successful in correlating AOB area (labeled with Nso 190) with ammonia removal rates in a nitrifying trickling filter. Others have not been as successful in correlating nitrification rates with AOB area. For example, Daims *et al.* (2001) found that the presence of high quantities of AOBs was not indicative of ammonia oxidizing activity. Konuma *et al.* (2001) also had difficulty using FISH for observing AOBs in low NH_3 loaded wastewater treatment systems due low signal intensity.

2.10 Summary

Recycled dewatering liquors (centrate) are a significant source of NH_3-N entering a WWTP but have shown to be a suitable substrate for high-rate nitrification in a dedicated side-stream reactor. The nitrifying biomass produced can be recycled to the main-stream bio-reactors of a WWTP where it can continue nitrification. Formulae have been developed to estimate the seeded sludge age of the treatment system when these nitrifiers are added as seed. The obstacles and benefits to centrate nitrification and seeding have been discussed. Wastewater simulation modeling and microbial analysis can both aid in tracking the seeded biomass through the system.

3. OBJECTIVES

3.1 Determine conditions under which centrate can be successfully nitrified

- Develop a nitrifying biomass capable of consistently treating centrate
- Determine suitable solids retention time, temperature, NH_3-N loading rates, pH, and aeration conditions for consistent removal

3.2 Determine the nitrifying capability of the biomass generated by nitrification of centrate

- Determine effect of NH_3-N concentration on nitrification rate
- Determine the kinetic coefficients of the nitrifying biomass
- Determine effect of sudden decrease in temperature on nitrification rate
- Determine potential for nitrification after seeding into a new environment (chemical analysis) at various HRTs

3.3 Determine the fate of the nitrifying bacteria after seeding

- Identify and quantify the seeded nitrifiers
- Determine potential for nitrification after seeding into a new environment (microbial analysis)

3.4 Determine whether BioWin can accurately model the observed laboratory data

- Model different centrate management practices
- Model the impacts of seeding
- Compare the observed data and the model output

4. MATERIALS AND METHODS

4.1 Centrate nitrification - Reactor start-up

The primary objective was to develop a nitrifying biomass acclimated to high NH_3 centrate at 10°C, 20°C, 25°C and 30°C. The biomass produced was used for all subsequent tests.

4.1.1 Source of biomass

The original source of biomass was obtained from the return activated sludge line at the South End Water Pollution Control Centre (SEWPCC) located in Winnipeg, Manitoba, Canada. The SEWPCC is a HPOAS non-nitrifying plant (ADWF 60 ML/d; SRT 3.5 d).

4.1.2 Source of centrate

The centrate used throughout this study was obtained from the North End Water Pollution Control Centre (NEWPCC) in Winnipeg. The NEWPCC receives sludge from two other plants in the City: the SEWPCC and the West End which is a non-nitrifying, coarse bubble air activated sludge plant (ADF 30 ML/d; SRT = 3.5 d). The NEWPCC is a HPOAS and treats 230 ML/d (ADWF) with approximately 40% of the drainage area served by combined sewers; with some food and garment industry wastes. The two smaller plants are serviced by separate sewer system and carry mainly domestic wastewater. Sludge treatment at NEWPCC consists of blending of primary

and waste activated sludges, gravity co-thickening, anaerobic digestion at 38°C for 17 d, with dewatering of digested solids by centrifugation.

Centrate was delivered to the laboratory from the plant in 3 X 20 L batches. This was stored for up to 4 weeks at 4°C in closed containers.

4.1.3 Establishment of nitrifying biomass at 27°C

Three-3 L reactors with a working volume of 2.4 L each were seeded with biomass from the SEWPCC. For 42 days the reactors were fed 1:1 centrate diluted with tap water. During this time, the reactors were operated at 27°C on a cycle of fill (2 min, 800 mL), react (6 h 45 min), settle (1h), decant (3 min, 800 mL) and idle (10 min). Fill and decant were controlled by peristaltic pumps. In order to build up nitrifying biomass, solids were only removed with the decant liquors. Air Cadet pumps provided air through diffuser stones that were placed on the bottom of each reactor. The aeration rate was maintained such that all of the biomass was in suspension. Dissolved oxygen (DO) measurements showed that this was sufficient to maintain a DO level above 2 mg/L. After 21 days, sodium bicarbonate ($NaHCO_3$) was added to supply alkalinity and control the pH. pH controllers with peristaltic pumps were used to feed $NaHCO_3$ such that the pH was maintained above 7.2. Upper pH was not controlled.

After 42 days the reactors were fed full strength centrate as collected from the NEWPCC. The reactors were then operated with an apparent SRT and HRT

of 5 d with continuous aeration. With SRT and HRT equal, complete control over SRT was possible. Wasting of excess biomass occurred once per day by removing one fifth of the mixed liquor volume. Feeding occurred 3 times per day. Because aeration was continuous at this point, air was supplied from a laboratory air supply line from an air compressor. The pH control was as described previously. The reactor configuration is shown in Figure 4.1.

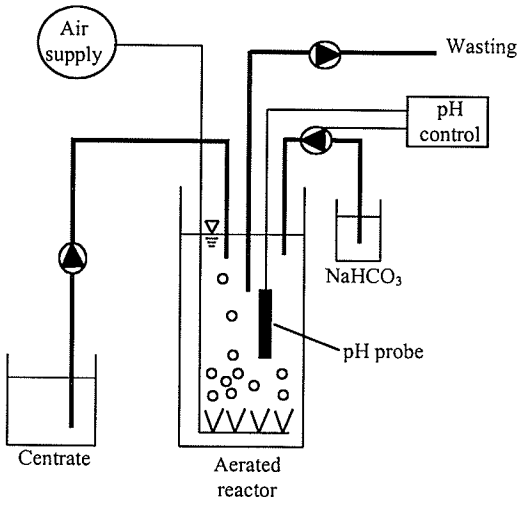


Figure 4.1 Reactor configuration for treatment of centrate.

4.1.4 Operation of seed source reactors at 20, 25 and 30°C (NB20, NB25, NB30)

After 75 days at 27°C with SRT and HRT of 5 days, the reactors were changed to 20°C (NB20), 25°C (NB25) and 30°C (NB30) to cover the temperature range typically found in centrate. Water baths were employed to maintain the proper temperatures. Feeding, wasting, aeration and pH control continued as previously described in *4.1.3*.

4.1.5 Operation of nitrifying reactor at 10°C (NB10)

Nitrifying seed was also generated from centrate at 10°C. The biomass used for reactor start-up was taken from NB20. Initially, this 2.5 L reactor was operated for 33 days with an apparent SRT and HRT of 10 days with continuous aeration. However, the reactor failed to fully remove the NH_3-N and often resulted in massive accumulations of NH_3-N. The apparent SRT and HRT of this reactor were increased to 12 days which resulted in more stable NH_3-N removal. Feeding and wasting was once per day. The pH was monitored continuously and adjusted manually once per day immediately before feeding by adding a volume of concentrated $NaHCO_3$ such that the pH was raised to at least 8.0.

4.2 Effect of NH_3-N concentration on nitrification rate

The purpose of this study was to determine how nitrification activity varied with the initial NH_3-N concentration in the reactor. Biomass was removed from NB20 and split into 100 mL portions. Then, 100 mL dilutions of centrate (to make a wide range of NH_3-N concentrations) were added to the biomass and tap water was added to make a final volume of 450 mL. Aeration was provided by diffuser stones with an aeration rate great enough to keep the biomass in suspension. A control reactor containing tap water and the highest NH_3 dose was included to monitor for NH_3 loss due to volatilization. The temperature was maintained at 20°C. Concentrated $NaHCO_3$ (1.0 mL)

was added to each reactor to provide alkalinity and prevent the pH from dropping below 7.2. The mixture was then aerated and the NH_3-N removal rate determined over a period of at least 2 hours. The VSS concentration of the biomass added was determined prior to feeding.

4.3 Effect of sudden decrease in temperature on nitrification rates

The objective of this study was to determine the impact of a sudden decrease in temperature on a nitrifying biomass grown on centrate. This study quantified nitrification rates before and after exposure to 10°C for nitrifying biomass acclimated to 20°C, 25 °C and 30°C.

4.3.1 Operation of seed source reactors

Three 2.4 L reactors were operated at 20, 25, and 30°C as described in ***4.1.4.***

4.3.2 Operation of batch reactors at 10°C

Waste biomass (480 mL) from the seed source reactors (i.e. NB20, NB25 and NB30) was cooled quickly to 10°C in an ice water bath. Stirring was provided to ensure even cooling throughout the liquor. The temperature of the reactors was maintained at 10°C by conducting the experiment in an environmental chamber at 10°C. Centrate (35 mL) was added to the cooled biomass and the mixture was aerated with a diffuser stone. The temperature of the mixed liquor was monitored during the course of the experiment to

ensure that the air supply was not changing the temperature of the reactor contents. NH_3–N removal rates were determined by sampling directly from the reactors over a period of at least 6 hours. A schematic of the reactor configuration is shown in Figure 4.2.

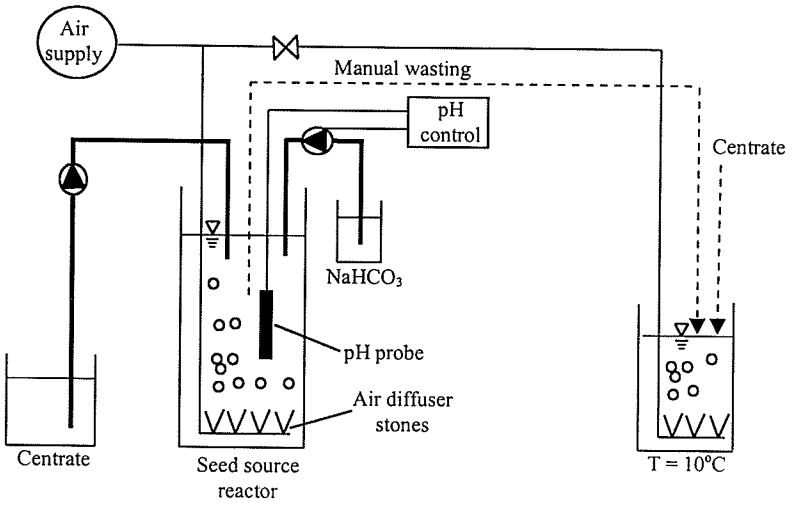


Figure 4.2 Reactor configuration for the determination of cold shock in a batch test.

4.3.3 Determination of temperature correction factor

The decrease in nitrification rate for each temperature range was determined by Equation 12. The percent decrease in nitrification rate is the same as the percent decrease in growth rate as shown by the relationship in Equation 13. The values for X_a and Y need not be known since they are eliminated as Equation 12 is calculated. It was assumed that Y did not change with temperature (Abeysinghe *et al.*, 2002).

$$\textit{Decrease in Nitrification Rate}\,(\%) = \frac{\Delta N/\Delta t_T - \Delta N/\Delta t_{10C}}{\Delta N/\Delta t_T} \times 100\% = \frac{\mu_T - \mu_{10C}}{\mu_T} \times 100\% \qquad [12]$$

$$\mu_{\max} = \frac{Y \bullet -\Delta N / \Delta t}{X_a} \qquad [13]$$

The growth rate of ammonia oxidizers can be determined at any temperature by Equation 14 and the temperature dependence factor (Γ_N) can be expressed by an exponential expression (Equation 15) and is often referred to as the Arrhenius factor for temperature.

$$\mu_T = \mu_{\max} e^{k_t(t-20)} \qquad [14]$$

$$\tau_N = e^{k_t} \qquad [15]$$

The rate factor, k_t, can be solved for directly by rearranging Equation 16.

$$1 - \left[\frac{dN/dt_{10^\circ C}}{dN/dt_T} \right] = \frac{e^{k_t(10-20)}}{e^{k_t(T-20)}} \qquad [16]$$

4.4 Seeding nitrifying biomass into a continuous flow reactor at 10°C

The purpose of this study was to determine if nitrification could be induced by seeding a continuous flow system at 10°C operating with an apparent SRT too short for nitrification to occur.

4.4.1 Synthetic wastewater feed

Synthetic wastewater was used as a substrate for the following tests to minimize variability in substrate characteristics that is often seen in raw wastewater collected from a treatment plant. The wastewater composition is shown in Table 4.1. This particular recipe was deemed appropriate for the analysis to be conducted in this research because it contained significant quantities of ammonia nitrogen, sufficient alkalinity for nitrification, a carbon source (beef and yeast extract) in addition to microelements.

Table 4.1 Synthetic wastewater recipe for reactors at 10°C.

Ingredient	Concentration (mg/L)
Beef extract powder	150
Yeast extract powder	150
$MgSO_4 \bullet 7H_2O$	50
$MnSO_4 \bullet 7H_2O$	5.0
$FeSO_4 \bullet 7H_2O$	2.2
KCl	7.0
NH_4Cl	150
K_2HPO_4	196
$NaHCO_3$	556
$CaCl_2$	3.8

4.4.2 Operation of continuous flow reactors

Two continuous flow reactors with working volumes of 2.0 L were constructed. The biomass for reactor start-up was obtained from a non-nitrifying SBR operated at 10°C that was fed synthetic wastewater. The volume of the clarifier was 1.5 L and the clarifier underflow was $0.3*Q^i$ where the influent flow rate, Q^i, was 4.8 L/d. The reactor configuration is shown in Figure 4.3.

Wasting of biomass occurred once per day by removing mixed liquor directly from the line between the reactor and the clarifier. The reactor configurations are shown in Figures 4.3 and 4.4. The volume of solids to waste was determined by Equation 17.

$$Q^w = \frac{V_r}{apparent\ SRT} \qquad [17]$$

The reactors were operated initially with an apparent SRT of 4 days at 10°C which was later reduced to 2.5 d on day 63. After operating the reactors for 29 days, one of the systems was seeded with biomass from NB20 while the other was used as a control. The initial seeding rate was $VSS_{seed}/VSS_{reactor}$ = 2% and was increased to 3.5% on day 53. This seeding rate was thought to provide a realistic regime where the seeded biomass is a very small percentage of the activated sludge biomass.

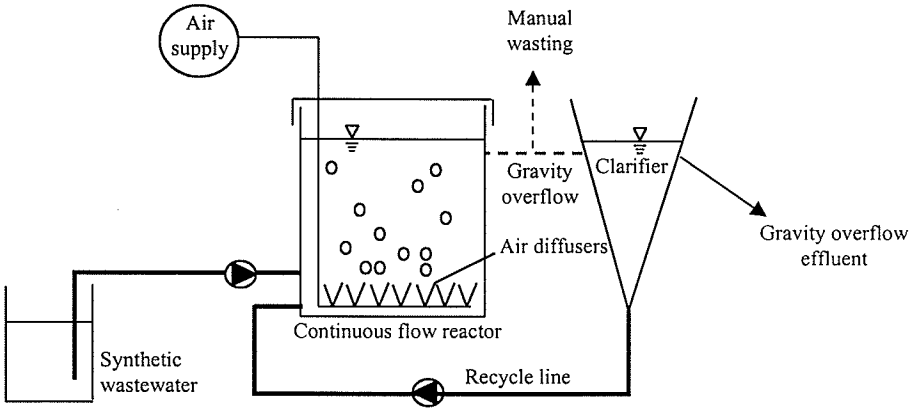


Figure 4.3 Continuous flow reactor configuration at 10°C – the control reactor.

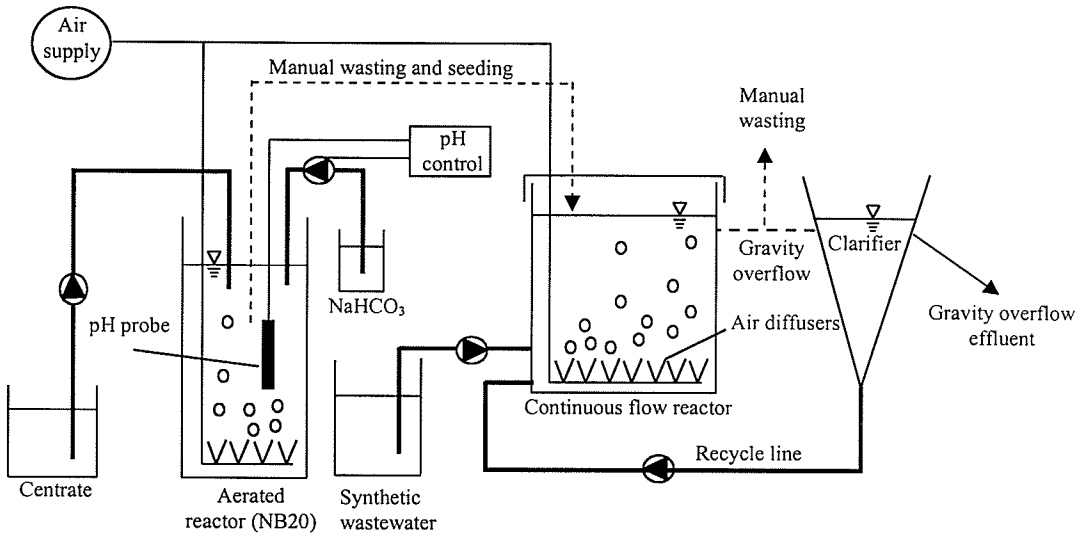


Figure 4.4 Continuous flow reactor at 10°C seeded daily from NB20.

All tubing was changed weekly to prevent the build-up of attached growth in the lines. The reactor walls were scraped daily with a soft spatula to remove attached growth. This was not sufficient to remove all attached growth so the reactors themselves were replaced on a weekly basis starting on day 101.

Effluent quality differences between two continuous flow configurations were compared. NH_3-N, NO_3-N, SCOD, TSS, and VSS concentrations were monitored for the seed source (NB20) and the continuous flow reactors.

4.5 Seeding nitrifying biomass into SBRs at 10°C

The objective of this study was to determine whether, with seeding, full nitrification could be achieved in sequencing batch reactors operating with apparent SRTs too short for nitrification to occur. The differences in nitrification rates between the nitrifying biomass from each source were

determined for HRTs ranging from 8 to 96 hours and apparent SRTs ranging from 3.5 to 12 days.

4.5.1 Seeding NB20 into SBRs with SRT 4 d and HRTs 12 to 96 h

Six SBRs (2L) were fed synthetic wastewater and operated at 10°C. The initial biomass for the start-up of these reactors was from a non-nitrifying reactor fed a similar substrate at 5°C and SRT of 10 days. Aeration was provided by diffuser stone with additional mixing by magnetic stirrer. The HRTs for the 6 reactors were 12 h, 24 h, 43.6 h, 53.3 h, 68.6 h and 96 h. Feeding, settling and decanting were three times per day for the reactors with HRT 12 and 24 h (feed - 50 min, aerate - 6 h 10 min, settle - 60 min and decant - 50 min) while these occurred once per day for the reactors with longer HRTs (feed - 50 min, aerate - 22 h 10 min, settle - 60 min and decant - 50 min). Wasting occurred once per day for all the reactors and was performed by removing one fourth of the reactor volume immediately before the final settling stage.

The reactors were operated for 2 apparent SRTs before sampling commenced. After 4 apparent SRTs, the SBRs were seeded daily with 100 mL of the nitrifying biomass produced in NB20. The reactor configuration is shown in Figure 4.5.

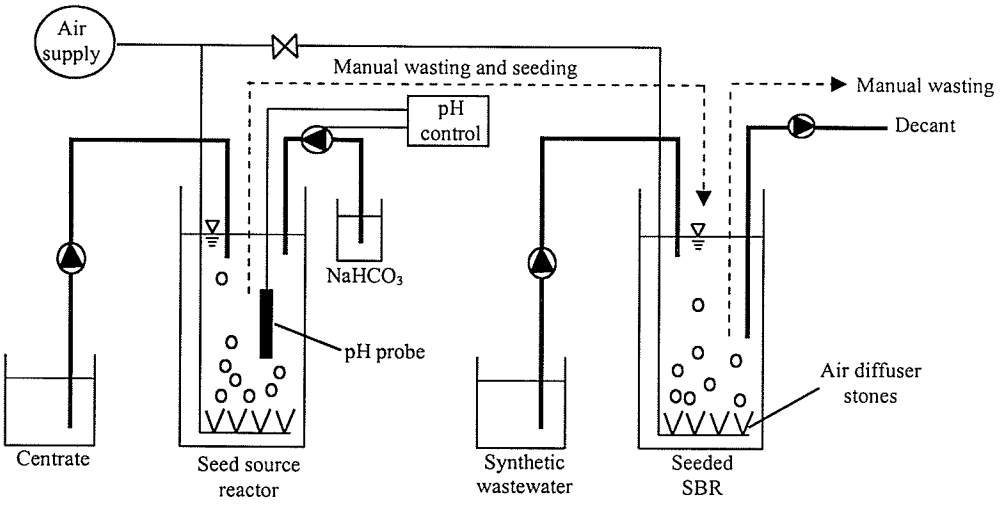


Figure 4.5 Reactor configuration for seeding nitrifying bacteria into non-nitrifying SBRs.

4.5.2 Seeding NB25 and NB30 into SBRs with SRT 4 d and HRTs 12 and 24 h

These SBRs (2L) were operated with an apparent SRT of 4 days at 10°C. The reactors were fed synthetic wastewater. Aeration was provided by diffuser stone with additional mixing by magnetic stirrer. There were three cycles per day (feed - 50 min, aerate - 6 h 10 min, settle - 60 min and decant - 50 min). Wasting occurred once per day at the end of the third cycle by wasting one fourth of the reactor volume immediately before the final settling stage. Seeding 100 mL daily with NB25 and NB30 began after running the reactors for 25 days. Seeding with NB25 and NB30 lasted for 63 days. The reactor configuration used was similar to that in Figure 4.5.

Samples for influent and effluent NH_3-N were taken at least 5 days per week from the cold SBRs. NO_3-N, TSS, VSS, and SCOD, TCOD were measured 3 times per week.

4.5.3 Seeding NB10 into SBRs with SRT 4 d and HRT 12 h

The SBR (2L) was operated with and apparent SRT of 4 days at 10°C. The reactor was fed synthetic wastewater. Aeration was provided by diffuser stone (feed - 50 min, aerate - 6 h 10 min, settle - 60 min and decant - 50 min). Wasting occurred once per day at the end of the third cycle by wasting one fourth of the reactor volume immediately before the final settling stage.

Sampling from the SBR began after 2 weeks (approximately 3.5 SRTs) of operation and daily seeding with 100 mL of nitrifying bacteria into the SBRs began after 24 days (approximately 6 SRTs). Seeding with NB10 lasted for 60 days. The reactor configuration used was similar to that in Figure 4.5.

Samples for influent and effluent NH_3-N were taken at least 5 days per week from the 6 cold SBRs. NO_3-N, TSS, VSS, and SCOD, TCOD were measured 3 times per week.

4.5.4 Seeding NB10 and NB20 into SBRs with SRT 12 d and HRT 8 h

Two - 2 L SBRs were operated with an apparent SRT of approximately 12 days and an HRT of 8 hours. The reactors were fed 1.5 L of synthetic wastewater 4 times daily (feed - 50 min, aerate - 4 h 10 min, settle - 60 min and decant - 50 min). Wasting occurred daily by removing 100 mL of mixed liquor at the end of the fourth cycle in addition to the solids lost with the decant liquors.

The reactors were run for 27 days (approximately 2.3 apparent SRTs) before sampling commenced. The reactors were sampled for 10 days to establish the baseline data before seeding with nitrifying bacteria from the seed source reactors. After these 10 days of sampling the cold SBRs were seeded once daily for 24 days with 100 mL of nitrifying bacteria - one reactor was seeded with NB10 and the other with NB20. The reactor configuration is similar to that shown in Figure 4.5.

Samples for influent and effluent NH_3-N were taken at least 5 times per week. TSS, VSS, COD, TKN and NO_3-N were also measured 3 times per week.

4.5.5 Summary of SBR seeding regime

SBRs with various SRTs and HRTs were seeded with nitrifying biomass acclimated to 10°C, 20°C, 25°C and 30°C. A summary of the seeding regimes used is listed in Table 4.2.

Table 4.2 Summary of seeding regime: Apparent SRTs and HRTs of seeded SBRs.

HRT (hours)	NB10		NB20		NB25		NB30	
	Apparent SRT (d)	Number of days seeded	Apparent SRT (d)	Number of days seeded	Apparent SRT (d)	Number of days seeded	Apparent SRT (d)	Number of days seeded
8	12	24	12	24	-	-	-	-
12	4	61	4	58	4	64	4	64
24	-	-	4	58	4	64	4	64
43.3	-	-	4	37	-	-	-	-
56	-	-	4	39	-	-	-	-
68.6	-	-	4	42	-	-	-	-
96	-	-	4	39	-	-	-	-

4.6 Determination of biomass characteristics

4.6.1 Determination of maximum nitrification rate of seed reactors, r_{su}

The purpose of this study was to determine the maximum nitrification rate of the nitrifying biomass. The maximum nitrification rate can be used to estimate the maximum growth rate of the biomass. The ammonia removal rate was determined by sampling from the seed reactors for at least 2 hours after feeding. The initial substrate concentration in the reactors was as close as possible to 40 to 50 mg/L NH_3-N during the maximum rate determination tests. The NH_3-N concentration in the reactor was plotted over time. The slope of the line is the nitrification rate. NO_3-N concentration was not used for the determination of nitrification rate because the concentration in the reactor was above the range that could be accurately measured with precision.

4.6.2 Determination of nitrifier concentration

The concentration of nitrifiers in the seed source reactors was estimated based on the mass on NH_3-N that was oxidized daily. Equation [9] was used to estimate the concentration of nitrifiers.

$$X_a = \frac{Y(S^o - S)}{1 + b\theta_x} \qquad [9]$$

The yield was assumed to be 0.24 g VSS/gNH_3-N and b was assumed to be 0.1d^{-1} at 20°C which is within the range of 0.058 to 0.153 d^{-1} found by Lee and

Oleszkiewicz (2002). The temperature correction factor determined by Equations 15 and 16 was applied to the decay rate to account for differences due to temperature.

4.6.3 Determination of nitrifier growth rates

The growth rates of the nitrifying bacteria in the seed source reactors were calculated by Equation 17.

$$u_{max} = \frac{-Y\, dN/dt_{max}}{X_a} \qquad [18]$$

The growth rates of seeded nitrifiers were determined by the reciprocal of Equation 8 (*i.e.*, 1/seeded SRT).

4.7 Chemical and physical analysis

All analyses were conducted according to Standard Methods (APHA et al. 1997). Dissolved oxygen (DO) was measured using an oxygen-sensitive membrane electrode (galvanic type) by method 4500-O G. NH_3-N was measured by the automated phenate method (4500-NH_3 G) or by the ammonia-selective electrode method (4500-NH_3 D). TKN was measured according to the Semi-Micro-Kjeldahl Method (4500-N_{org} C). NO_x-N was measured by the automated cadmium reduction method (4500-NO_3^- F). Soluble COD (SCOD) samples were prepared by filtering through a 0.45 μm glass filter and analyzed by the closed reflux, colorimetric method (5220 D).

Total suspended solids (TSS) and mixed liquor volatile suspended solids (MLVSS) were measured according to methods 2540 D and 2540 E respectively.

4.8 Simulation modeling using BioWin

The objective of simulation modeling was to determine the impacts of centrate treatment on the overall wastewater treatment process.

4.8.1 Reactor configurations used in modeling

4.8.1.1 Continuous flow reactor configurations

NH_3 levels in the effluent of nitrifying and non-nitrifying wastewater treatment plants were modeled using configurations shown in Figures 4.6 and 4.7. Figure 4.6 depicts a wastewater treatment plant equipped for biological nutrient removal (BNR) including nitrification, denitrification and phosphorus removal. The treatment plant in Figure 4.7 focuses on BOD removal and does not employ nitrification.

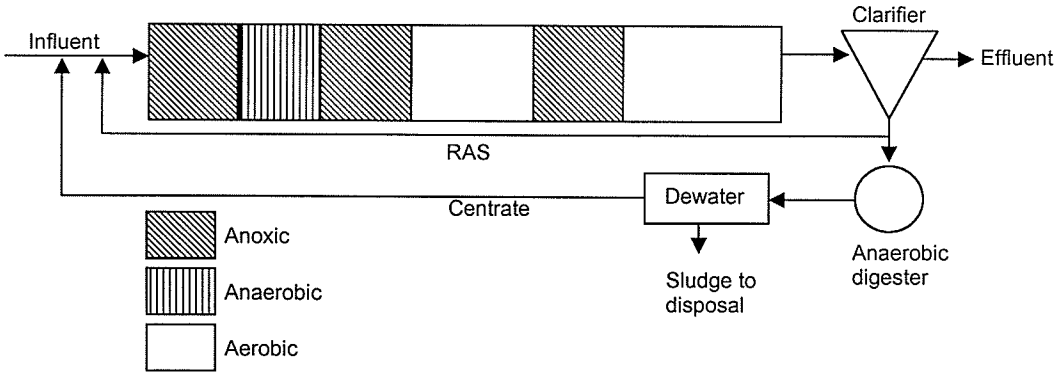


Figure 4.6 Configuration of a continuous flow Bardenpho BNR wastewater treatment plant.

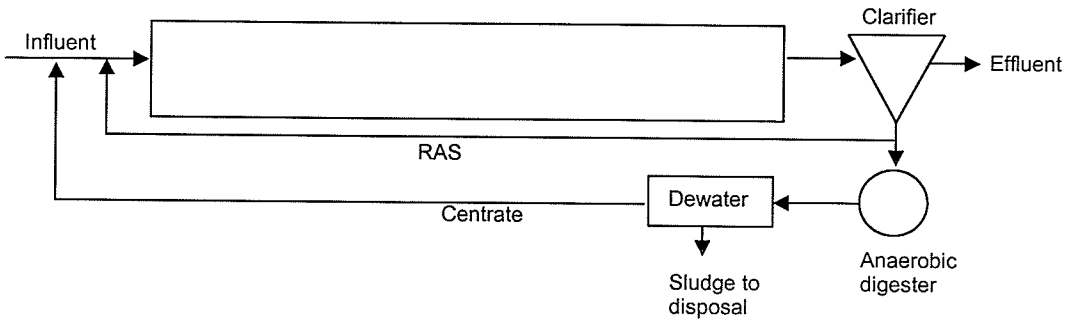


Figure 4.7 Configuration of a continuous flow, non-nitrifying, BOD removing wastewater treatment plant.

4.8.1.2 Sequencing batch reactor configuration

An SBR configuration similar to that of the seeded SBRs described in Figure 4.5 was modeled. The cycle lengths used were similar to those described in section *4.4.*

4.8.2 Wastewater input data

4.8.2.1 Wastewater input data for modeling continuous flow reactors

The daily flow pattern used for modeling was obtained from the City of Warsaw, Poland (1999). Peak flow values, daily and seasonal, were adjusted according to values given in Metcalf & Eddy (1992). A 55 day "wedding cake" wastewater flow pattern was used to mimic a seasonal flow pattern. The "wedding cake" pattern contains a peak day in terms of flow, in a peak week in a peak month with average flow before and after the peak month (Figure 4.8). The wastewater characteristics used in modeling are shown in Table 4.3.

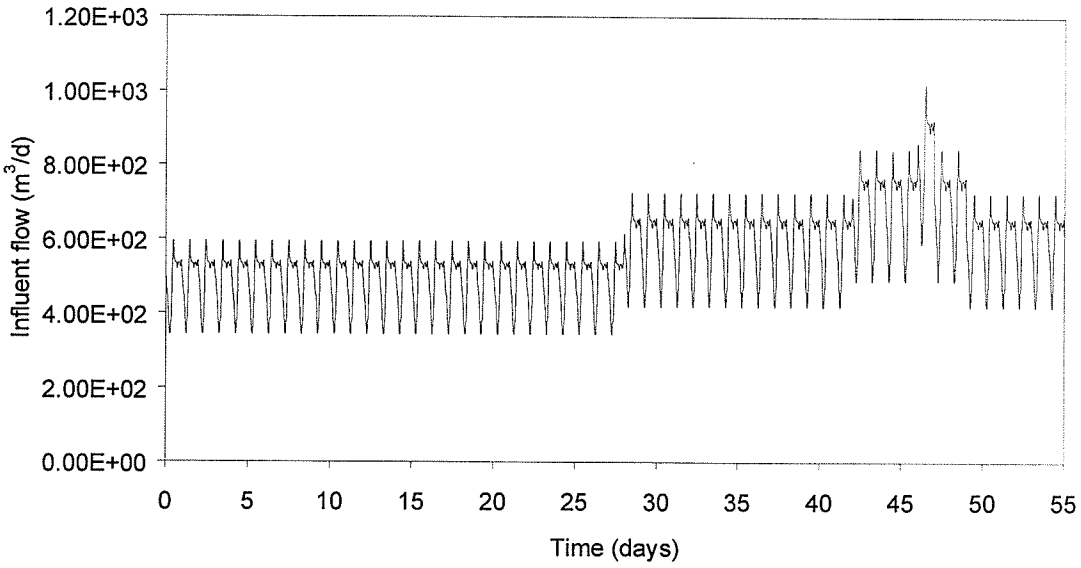


Figure 4.8 Influent flow pattern for modeling continuous flow reactors.

Table 4.3 Wastewater fractions and concentrations for influent to continuous flow systems (all values are model defaults).

Parameter	Input Value	Parameter	Input Value
Fbs	0.200	TKN	40.000 mgN/L
Fac	0.150	Total P	10 mgP/L
Fxsp	0.750	NO_3-N	0.000 mg/L
Fus	0.050	Alk	6.000 mmol
Fup	0.13	ISS	15.000 mg/L
Fna	0.075	Mg	30.000 mg/L
Fnox	0.500	DO	0.000 mg/L
Fnu	0.00		
FupN	0.068		
FupP	0.021		
FZbh	0.0001		
FZba	0.0001		
FZbp	0.0001		
FZbpa	0.0001		
FZbam	0.0001		
FZbhm	0.0001		

4.8.2.2 Wastewater input data for modeling sequencing batch reactors

Figure 4.9 is one example of a flow pattern used for modeling SBRs. In this example, the reactor is fed once daily. The first 23 days represents the days

before seeding was started and this period was used to establish a baseline of effluent NH_3-N. Then from day 24 to 53 the reactor was seeded daily with a volume of nitrifying bacteria. The influent flow of wastewater had to be decreased during this time to account for the additional stream associated with the seed.

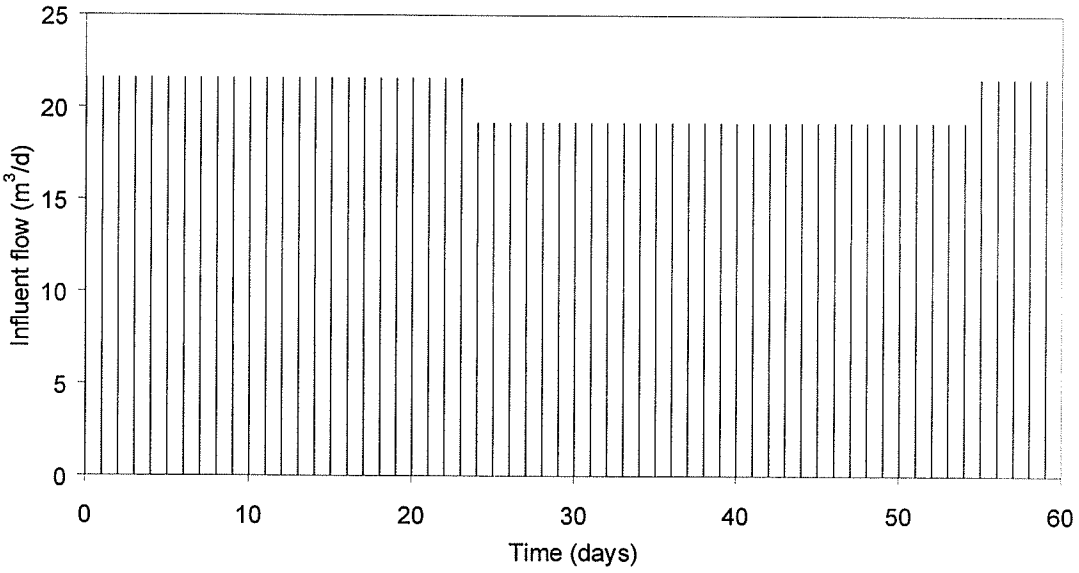


Figure 4.9 An example of an influent flow pattern for SBRs.

The volumes and flow rates used in modeling were much larger than those used in the laboratory. However, this has no effect on the model output data for NH_3-N concentration. The proportions and volumes used in the model were scaled up directly from those used in the lab.

The characteristics of wastewater fed to the modeled SBRs were based on the parameters that were measured for the synthetic wastewater used in this research (Table 4.4) while others were calculated directly from the synthetic

wastewater recipe in Table 4.1. Measured, calculated and assumed values are indicated in Table 4.4.

Table 4.4 Synthetic wastewater characteristics used for modeling.

Parameter	Input Value	Parameter	Input Value
Fbs*	0.700	CODt**	300.000 mg/L
Fac	0.0001	TKNt**	55.000 mgN/L
Fxsp*	0.000	Total P*	35.000 mgN/L
Fus*	0.290	NO_3-N**	0.000 mgN/L
Fup	0.000	Alk*	15.000 mmol/L
Fna*	0.600	ISS**	0.000 mg/L
Fnox	0.500	Mg*	10.000 mg/L
Fnus	0.15	DO	0.000 mg/L
FupN	0.068		
Fpo4	0.500		
FupP	0.021		
FZbh	0.000		
FZba	0.000		
FZbp	0.000		
FZbpa	0.000		
FZbam	0.000		
FZbhm	0.000		

*Calculated values
**Measured values
All other values are model default values and are assumed to be "typical" values for wastewater.

4.8.3 Centrate input data

Treatment and non-treatment options for centrate were modeled and compared with conventional centrate recycling practices where centrate is recycled to the front of a plant as it is produced. The options modeled included managing centrate flow rather than treatment, as well as biological and physical treatment.

4.8.3.1 Centrate characteristics

The characteristics of the centrate used in modeling are listed in Table 4.5. Some of the values are based on laboratory measurements of the centrate collected from the NEWPCC while others were estimated based on the assumptions detailed below Table 4.5.

Table 4.5 Centrate characteristics used in modeling.

Parameter	Input Value	Parameter	Input Value
Zbh	0.000 mg/L	Sbsp	0.000 mg/L
Zba	0.000 mg/L	SbH2	0.000 mg/L
Zbp	0.000mg/L	Sbsc	0.000 mg/L
Zbpa	0.000 mg/L	Sbsa	0.000 mg/L
Zbam	0.000 mg/L	NH_3-N**	650.000 mgN/L
Zbhm	0.000mg/L	Nos	2.790 mgN/L
Ze	0.000 mg/L	NO_3-N**	0.000mgN/L
Xsp**	232.500 mg/L	PO_4-P	50.00mgP/L
Xsc	77.500 mg/L	Sus	25.000 mg/L
Xi	65.000 mg/L	Nus	0.000 mgN/L
Xon	2.790 mgN/L	ISS**	200.000 mg/L
Xop	3.635 mgP/L	XStru	0.000 mg/L
Sphb	0.000 mg/L	Mg	50.000 mg/L
PP-lo	0.000 mgP/L	Alk*	100.000 mmol/L
PP-hi	0.000 mgP/L	DO**	0.000 mg/L

*Calculated values

**Measured values

Notes: All other values were assumed.

The active biomass concentration in the centrate solids (Z) was assumed to be nil due to the nature of the environment from which it came (mesophilic anaerobic digestion followed by dewatering).

The concentration of soluble degradable COD (S) in the centrate was assumed to be zero. Most degradable soluble COD would have been consumed while the liquor was in the anaerobic digester.

Because the solids in the centrate originated from an anaerobic digester, the solids in the centrate were assumed to fall within two main categories: 1) slowly degradable particulate COD (Xsp) and 2) inert suspended solids (ISS).

The alkalinity in the centrate was increased for the purpose of modeling such that alkalinity was not limiting.

All other assumed values were shown to have little impact on the results of modeling.

4.8.3.2 Management of centrate for input into continuous flow reactors

Three different flow patterns were used to model centrate flow management (Figure 4.10). The volume and characteristics of the centrate for all of the flow patterns are the same. The three patterns used were:

- 8 h/d, 5 d/wk: In this case the centrate is recycled only during the day as the centrate is produced. On the weekends, there is no centrate production and therefore no flow of days 5 and 6 of Figure 4.10.
- Centrate as an NH_3-N supplement: During the course of a day the NH_3-N loading rate entering a treatment plant varies. In this case, centrate was fed only during the night during low NH_3-N loading into the plant. This flow pattern is very nearly the opposite of feeding the centrate only during the day. This process would involve storing the centrate produced to use it as a NH_3-N supplement to equalize the source of NH_3-N available to nitrifying bacteria or to "even out" peaks and valleys in influent NH_3-N concentrations.

- Constant centrate flow: In this case, the centrate is fed to a storage tank as it is produced and bled into the main-stream treatment train of the treatment plant at a constant rate.

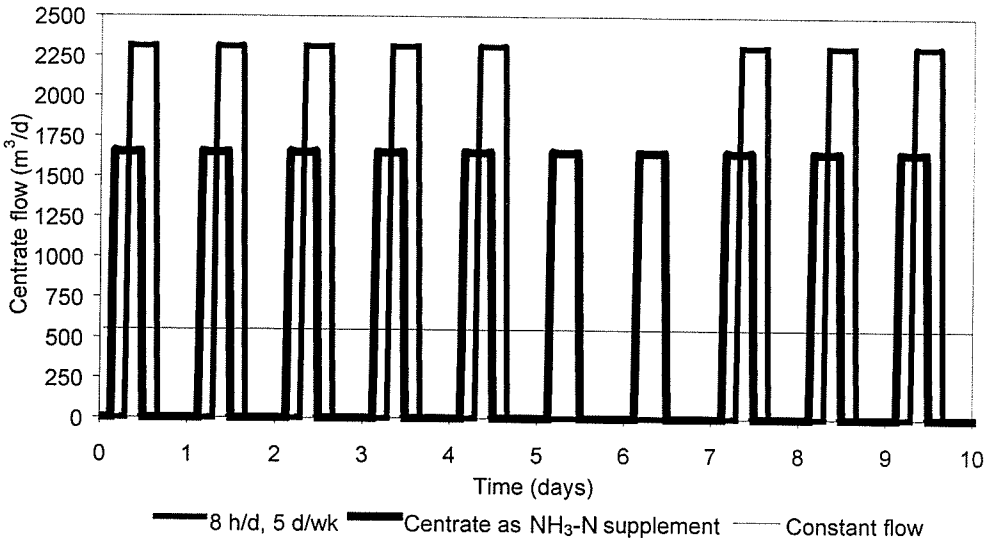


Figure 4.10 Centrate flow patterns used in modeling centrate input into continuous flow reactors.

4.8.4 Kinetic input parameters for autotrophs capable of nitrification

BioWin allows the user to input any desired kinetic or stoichiometric value for the growth of a variety of microorganisms that are involved in treating wastewater. Because nitrification is the main interest in this research, only those values that effect the growth of autotrophs were altered in the model. These included μ_{max} (temperature dependent), b (temperature dependent) and Y (0.24 g/g). All other parameters were left as the model default values. A temperature of 10°C was used in the simulation of continuous flow reactors and SBRs treating wastewater. Temperatures ranging from 10 to 30°C were used for simulating centrate treatment.

4.8.4.1 Modeling the biological treatment of centrate

Modeling was conducted to estimate the number of autotrophs (nitrifying bacteria) that could be produced from the nitrification of centrate with the characteristics listed in Table 4.5. The reactor configuration in the model was similar to that shown in Figure 4.1 and a cycling regime similar to that described in section *4.1.4*. The kinetic values were adjusted such that the effluent quality was similar to that achieved in the laboratory. The treated centrate characteristics varied depending on the kinetic and stoichiometric values that were input into the model as described in section *4.8.4*.

The autotrophic bacteria concentrations generated by the model were used to determine the benefits of using that biomass as a source of nitrifying seed for the treatment process configurations shown in Figures 4.6, 4.7 and the seeded SBRs. These numbers were also used to model the benefits of seeding nitrifying biomass into SBRs operating under conditions similar to those described in section *4.5*.

4.8.5. Management of biologically treated centrate

4.8.5.1 Treated centrate into continuous flow reactors

Biologically treated centrate was fed into the continuous flow reactors that are depicted in Figures 4.7 and 4.8. For these reactors, the treated centrate was added at a constant rate during the 55 day "wedding cake" simulation. The

main parameter that was tested here was the concentration of nitrifying bacteria (Zba) that was present in the treated centrate.

4.8.5.2 Management of biologically treated centrate for input into SBRs

Figure 4.11 provides an example of a flow pattern of biologically treated centrate fed into an SBR fed with synthetic wastewater with the flow pattern shown in Figure 4.9. In this example, no centrate is fed into the SBR for the first 23 days of the simulation. The treated centrate is then added once per day to the SBR until day 54. The same volume of treated centrate was applied to all SBR simulations.

4.9 Microbial Analysis

The objective of conducting microbial analysis of the biomass developed in this research was to monitor changes in the mixed liquor population during seeding of nitrifying bacteria.

4.9.1 Sampling of biomass and cell fixation

Grab samples of mixed liquor suspended solids were collected from the seed source reactors and the seeded reactors over the course of this research. The samples were centrifuged at 10, 000 rpm for 5 minutes and the supernatant discarded. The samples were then re-suspended in fresh 4%

paraformaldehyde in PBS and fixed overnight. The samples were then centrifuged at 10, 000 rpm for 5 minutes and the supernatant discarded. A 1:1 mixture of ethanol and PBS was added, the sample was re-suspended and then stored at -20°C.

Effluent samples from the reactors were also collected. Several tubes were filled with 1.5 mL of effluent and centrifuged for 5 minutes at 10, 000 rpm. The supernatant was discarded and the pellet of solids from each tube was combined into one tube. The sample was then fixed and stored as described previously.

4.9.2 Fluorescent *in situ* hybridization

In situ hybridization was performed as specified by Oerther *et al.* (2002) with the probes listed in Table 4.6. A 2 µL sample was applied to each well of the slide (Erie Scientific Company, Portsmouth, NH) and then dried at 46°C for 5 minutes. The sample was then dehydrated in 50, 80 and 96% ethanol for 1 minute each and dried at 46°C for 5 minutes. 8 µL of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl (pH 7.0), 0.01% sodium dodecyl sulfate (SDS), x% formamide) and 1 µL of fluorescently labeled probe (50 ng/µL) were added to each well. The sample was then hybridized at 46°C for 1 to 2 hours in a moisture chamber. The slide was then washed in pre-warmed washing solution (X nM NaCl, 20 mM Tris-HCl (pH 7.0), 0.1% SDS). Washing buffer was removed by serial washing in deionized water (3 seconds X 2). Slides

that were stained with DAPI were air dried first before staining with 40 µl of 2 µg/mL DAPI for 1 to 2 minutes. The slides were rinsed again by serial washing in deionized water and allowed to air dry.

Table 4.6 Oligonucleotide probes used for visualization of biomass with FISH.

Description	Oligonucleotide Probe Database Name	Sequence (5' to 3')	Label
Ammonia oxidizing Beta Proteobacteria[1]	S-*-Nso-1225-a-A-20	CGC CAT TGT ATT ACG TGT GA	Cy3
Genus *Nitrosomonas*[2]	S-G-Nsm-0156-a-A-20	TAT TAG CAC ATC TTT CGA T	Cy3

1) Schramm, 1999, Ballinger *et al.*, 1998, Guschin *et al.*, 1997
2) Mobarry *et al.*, 1996

4.9.3 Microscopy and image analysis

Slides were examined with a Nikon E400 microscope (Nikon Canada) at 400X magnification with Chroma filter block G-2A for Cy3 labeled probes and UV-2A for DAPI. Photomicrography was done with a digital microscopy documentation system by Kodak (MDS 290) (Mandel Scientific, Guelph, Canada) with 1792 X 1200 pixels and CCD resolution of 1901 X 1212. Exposure time was set at 8 seconds for the Cy3 labeled probe and 2.5 seconds for the DAPI stain. Ten fields were photographed for each well on the slide. The images were saved as TIFF files and processed with Adobe Photoshop Elements. Image analysis and quantification was done with the UTHSCSA ImageTool™ (2002) program (developed at the University of Texas Health Science Center at San Antonio, Texas and available free from the Internet by anonymous FTP from ddsdx.uthscsa.edu). Quantification was done by relative area quantification against the total biomass concentration stained by DAPI.

5. RESULTS

5.1 Centrate nitrification - Reactor start-up

5.1.1 Centrate characteristics

Centrate quality was extremely variable over the course of this research. Its characteristics depended greatly on the performance of the anaerobic digesters and the centrifuges at the treatment plant from which it was collected. Quality also varied with season and method of collection.

The centrate was collected from a pipe running directly from the centrifuges to the main influent interceptor (City of Winnipeg, 2000). The NEWPCC also runs hot water in the centrate return line to prevent the build up of struvite mineral in the pipes. On a few occasions centrate was collected from that line while the hot water was still running. This resulted in an approximate dilution of 1:10.

Solids recovery immediately after the start-up of a centrifuge is extremely poor. In an attempt to make the solids fed into our laboratory reactors more uniform, the centrate was strained through a course filter (2 layers of paper toweling) before addition to the reactors. Polymer dosing is done at the treatment plant during winter months to aid in solids recovery.

Centrate NH_3-N and VSS concentrations are depicted for a period of 7 months in Figure 5.1. During this time period, the centrate was not being filtered in the lab.

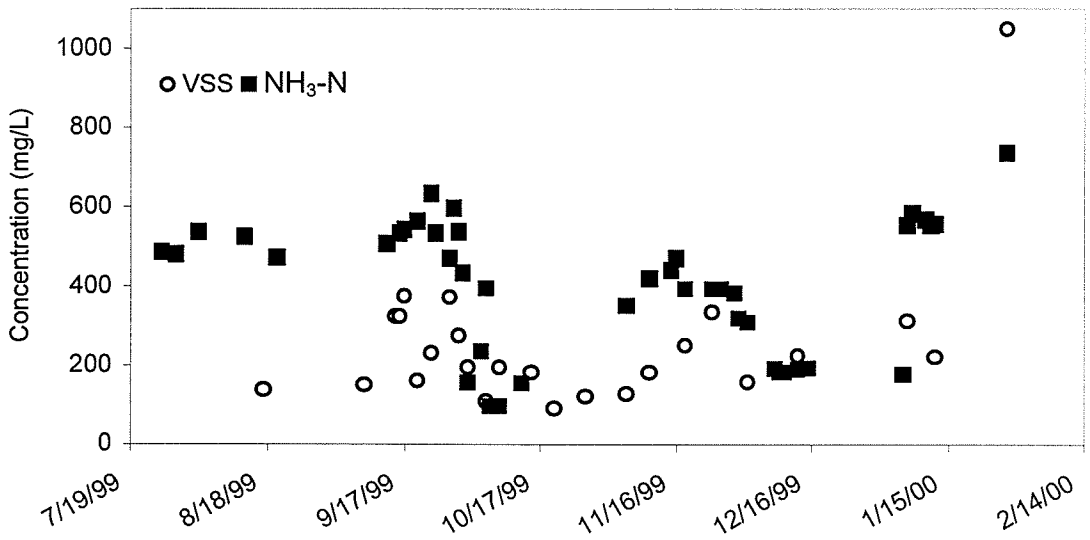


Figure 5.1 Centrate VSS and NH_3-N over a 7 month period before filtering commenced.

During the start-up of this research soluble organic carbon (SOC) of the centrate was monitored. The mean concentration was 118 ± 21.9 mg/L. This was an early indicator that a large concentration of biomass would probably never develop in a reactor fed centrate as the sole substrate. This was expected because the liquor had already undergone anaerobic digestion for 10 to 20 days (City of Winnipeg, 2000). During this time, most of the readily degradable organic compounds would have been converted to the byproducts of anaerobic digestion; namely methane, carbon dioxide and cell mass.

5.1.2. Establishment of nitrifying biomass (Appendix B-1)

Three reactors were operated at 27°C to develop a nitrifying biomass that was acclimated to centrate as a sole substrate. Initially, biomass was not wasted

from these reactors other than that removed with decant liquors. During the first days after seeding the three reactors there was some release of NH_3-N resulting in an effluent NH_3-N concentration greater than that found in the feed (Figure 5.2). The release of NH_3-N was attributed to cell lysis and hydrolysis of organic N due to the addition of excessive inoculum (return activated sludge from the SEWPCC).

After 10 days of operation the effluent NH_3-N concentrations remained below 100 mg/L but the greatest percentage of NH_3-N removal was only 63%. Monitoring of pH and alkalinity indicated that alkalinity was insufficient for complete nitrification. According to the USEPA (1975) a residual alkalinity concentration of 175 mg/L as $CaCO_3$ is required to prevent the inhibition of nitrification rates at pH 7.2. On day 19, alkalinity concentrations in the effluent ranged from 30 to 52 mg/L as $CaCO_3$. On day 21 $NaHCO_3$ was added to the reactors to maintain the pH above 7.2 and by day 26 greater than 90% NH_3–N removal was achieved.

On day 53 a regular wasting schedule of SRT and HRT 5 d was established. When the HRT and SRT were changed, complete NH_3–N removal was maintained at all centrate NH_3-N concentrations (Figure 5.2). NH_3-N removal was generally greater than 99% and always greater than 95% when sufficient alkalinity was supplied. Slight accumulations in effluent NH_3-N on day 66 in R3 was due to malfunctioning of the pump responsible for $NaHCO_3$ addition.

However, full NH_3-N removal was achieved once alkalinity was supplied in sufficient quantities.

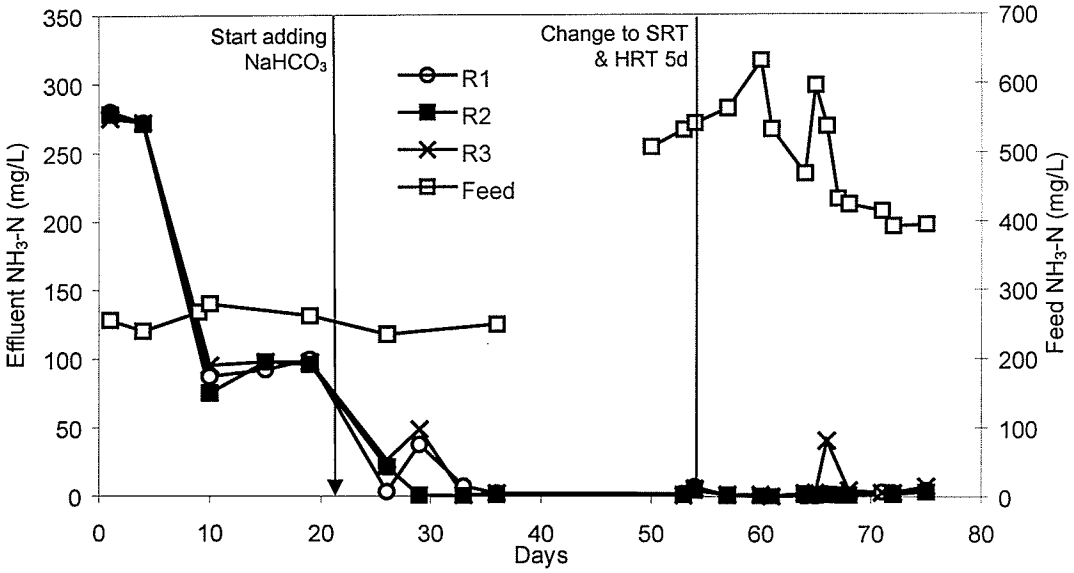


Figure 5.2. Effluent and feed NH_3-N concentrations for 3 parallel reactors treating centrate at 27°C.

After the regular wasting, the VSS in the reactors decreased rapidly, as expected (Figure 5.3). Even though the VSS concentration in the reactors decreased, NH_3-N removal continued to be complete. Over time, the composition of the microbial population likely shifted such that active nitrifiers made up an increased proportion of the total VSS.

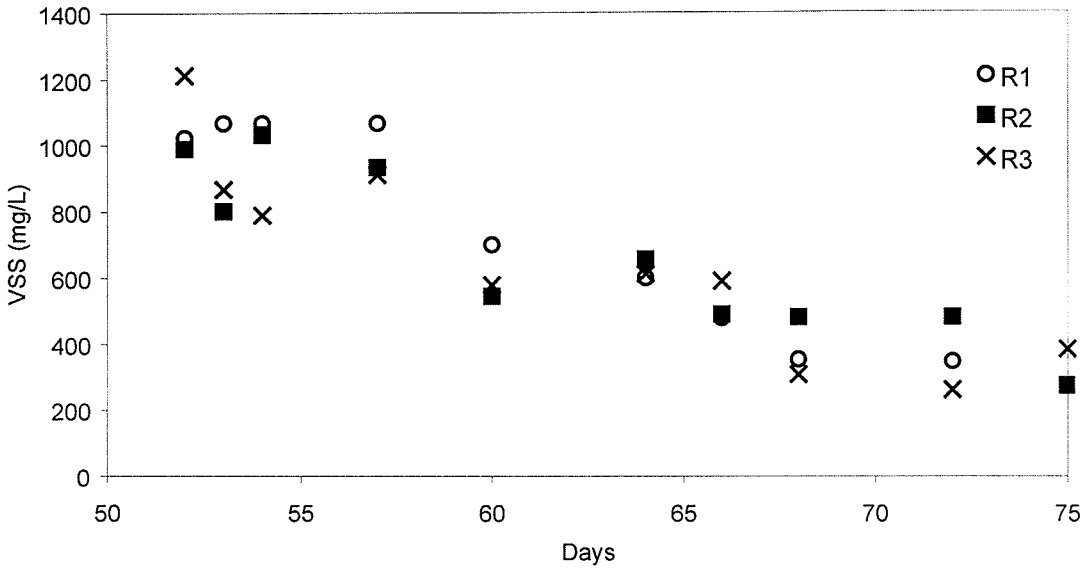


Figure 5.3 Change in VSS concentration after reactors at 27°C changed to SRT and HRT 5 days.

5.1.2.2 Acclimation of biomass to 20, 25 and 30°C (Appendix B-2)

After 75 days of regular operation at 27°C the reactor temperatures were changed to 20, 25 and 30°C. After 7 days of operation at the new temperatures, sampling began (as depicted by day 1 in Figure 5.4). By this time, NH_3-N removal was always greater than 96%. Effluent concentrations ranged from 0.1 to 7.7 mg NH_3-N /L (Figure 5.4). The maximum nitrification rates observed during the start-up of these reactors were 8.4 mg/L*h, 10.6 mg/L*h and 12.7 mg/L*h for NB20, NB25 and NB30, respectively.

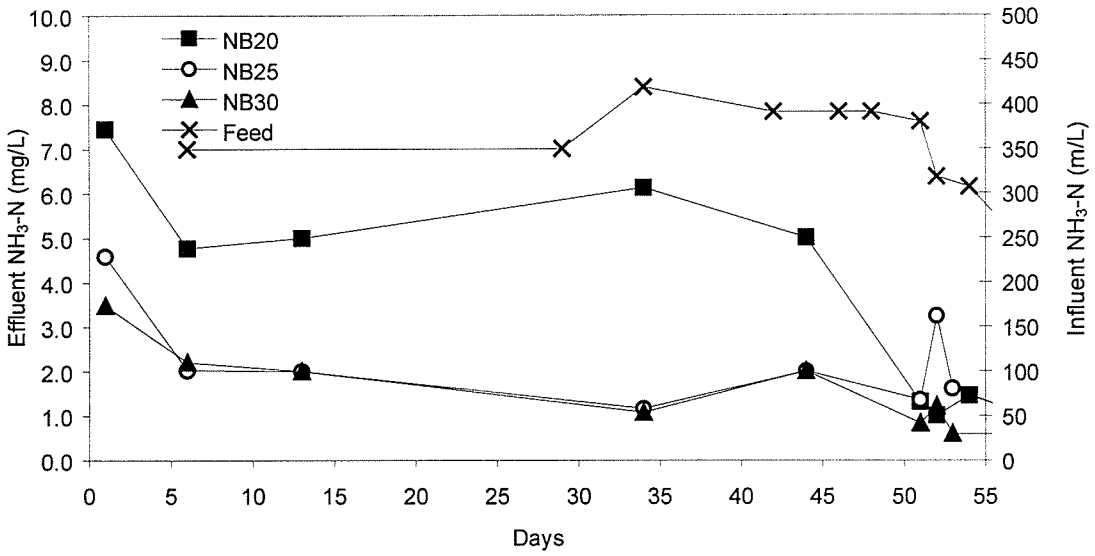


Figure 5.4 Start-up concentrations of NH_3-N in the influent and effluent for NB20, NB25 and NB30.

On day 52 the NO_2-N profile was monitored in the three reactors. There were accumulations of NO_2^--N at all three temperatures with the greatest accumulation in the reactor at 30°C (Figure 5.5). NO_2^--N accumulation was not attributed to low DO concentrations since the concentration was maintained above 4 mg/L at all times. The accumulation of NO_2^--N did not exceed the free nitrous acid toxicity limit of 0.22 to 2.8 mg/L as described by Anthonisen *et al.* (1976) and did not affect nitrification as indicated by excellent NH_3-N removal efficiencies.

The NO_2^--N accumulation was consistent with Mossakowska *et al.* (1997) who found that when nitrifying centrate NO_2^--N always accumulated until all of the NH_3-N was oxidized and that the maximum NO_2^--N accumulation was dependent on the original NH_3-N concentration. However, in Figure 5.5 the accumulation of NO_2^--N could not be attributed to differences in initial NH_3-

N concentration. The accumulation of NO_2^--N can be explained by the difference in temperatures. As the temperature increases, the growth rate of ammonia oxidizing bacteria exceeds that of nitrite oxidizing bacteria (Mulder *et al.*, 2001). An accumulation of NO_2^---N at increased temperatures was expected.

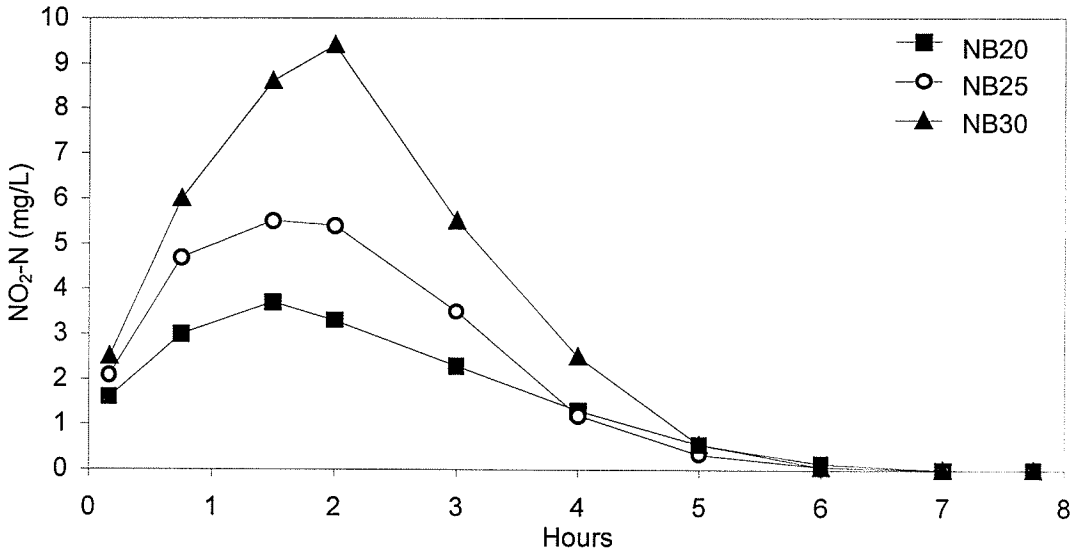


Figure 5.5 NO_2-N accumulation in reactors treating centrate at 20, 25 and 30°C.

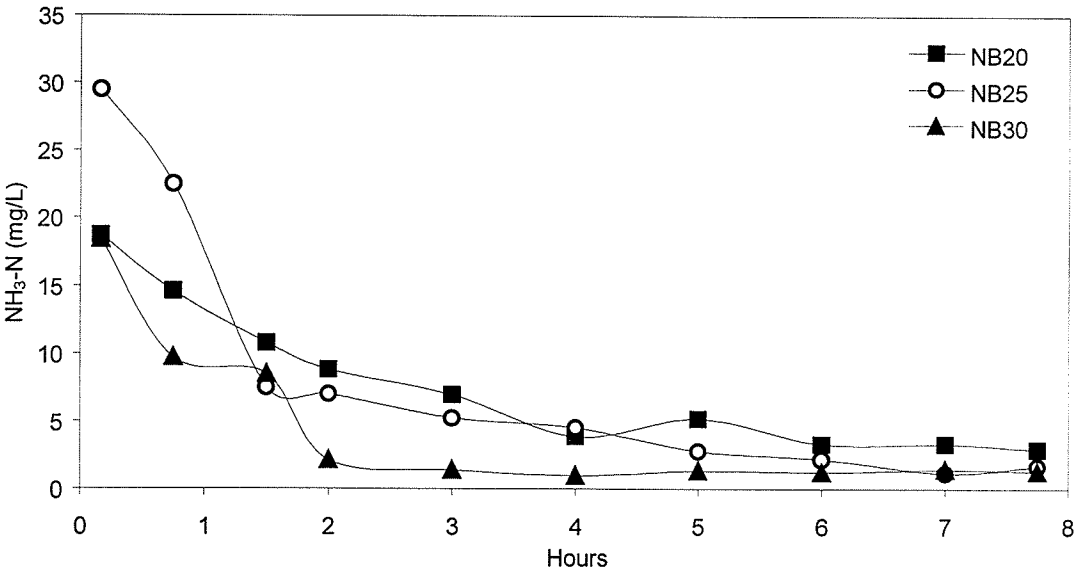


Figure 5.6 An example of NH_3-N reduction in 3 reactors at 20, 25 and 30°C.

At elevated temperatures, there is a greater concentration of free ammonia (FA) which is toxic to NO_2^- oxidizers at low concentrations (0.1 to 1 mg/L) (Anthonisen *et al.*, 1976). At the temperatures and pH used in this study, the toxic range of FA could have been exceeded, causing an accumulation of NO_2^--N. As the FA concentration decreased, NO_2^- oxidizer activity may have recovered such that NO_2^--N was oxidized to NO_3^--N. Within 5 h, the concentration of NO_2^--N had decreased to less than 1 mg/L in all three reactors (Figure 5.5).

The rate of NO_2^--N accumulation exceeded the rate of NO_3^--N production until NH_3-N levels decreased to below a certain concentration (Table 5.1). The concentration of NH_3-N at the point where the NO_2-N accumulation rate becomes less than the consumption rate decreases with increasing temperature (Table 5.1).

Table 5.1 Net NO_2^--N accumulation and consumption in reactors treating centrate at 20, 25 and 30°C.

Temperature (°C)	Net rate of NO_2-N accumulation		Net rate of NO_2-N consumption		NH_3-N at turning point†
	mg/L*h	R^2	mg/L*h	R^2	mg/L
20	1.55	0.93	0.93	1.0	8.8 to 10.8
25	2.48	0.87	1.75	0.97	7.0 to 7.5
30	4.52	0.98	2.96	0.98	1.3 to 2.1

†Turing point: Point at which NO_2^--N consumption becomes greater than the accumulation.

5.1.2.3 Acclimating biomass to 10°C (Appendix B-3)

Because the NB10 reactor was seeded from NB20 it was thought that acclimation of the biomass would occur very quickly. However, after 32 days

consistent nitrification failed to be established with the reactor being operated with an SRT and HRT of 10 days (Figure 5.7). Periodically, feeding and wasting was not done in order for accumulations of NH_3-N to be reduced. This is indicated by feed (centrate) NH_3-N equal to 0 mg/L in Figure 5.7.

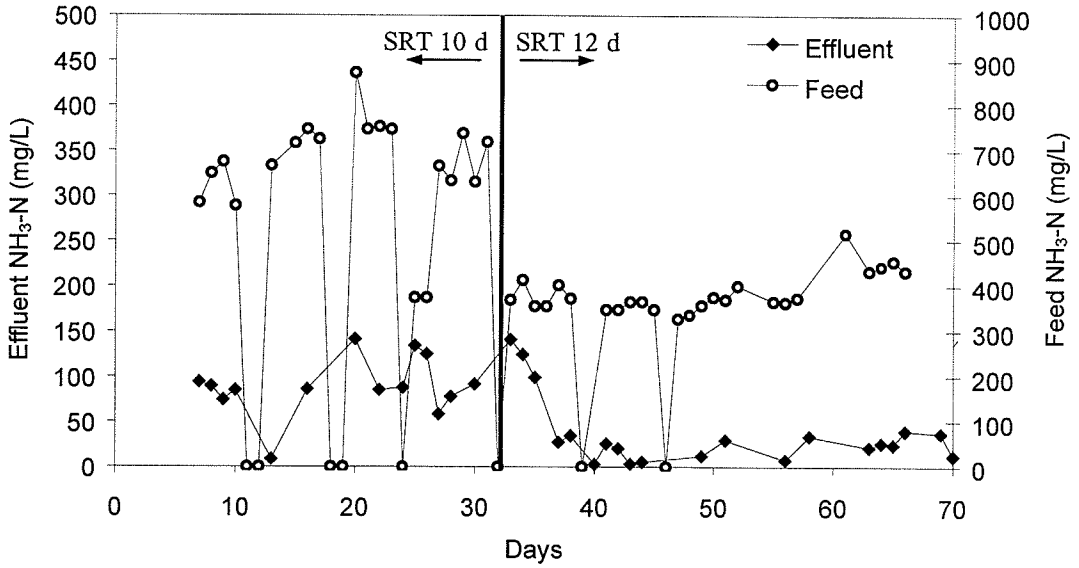


Figure 5.7. Start-up influent and effluent NH_3-N concentrations for NB10.

On day 32 (Figure 5.7) the centrate feed was diluted by 50% with deionized water to decrease the NH_3-N load but not the hydraulic load to the reactor. At this time the SRT and HRT were also increased to 12 days. As a result, nitrification performance improved with effluent NH_3-N concentrations consistently below 50 mg/L. The maximum nitrification rate observed during the time period shown in Figure 5.7 was 5.2 mg/L*h or 41.6 mg/g VSS*h.

5.1.3 Summary and conclusions

- The VSS of centrate was highly dependent on the efficiency of the sludge dewatering centrifuge. This caused a high variability in reactor VSS concentration. Nitrification expressed as mg NH_3-N/g VSS*h therefore was deemed an inaccurate representation of biomass nitrification efficiency. Gravitational settling of centrate solids would decrease the variability in solids concentrations.
- Complete NH_3-N removal from centrate was accomplished only when alkalinity was supplemented. The centrate contained enough alkalinity to achieve approximately 63% NH_3-N removal.
- SRT 5 d was adequate for nitrification of centrate at 20, 25, 27 and 30°C. Partial but unstable nitrification of centrate was possible at 10°C with an SRT of 10 days. Increasing the SRT to 12 days was required for stable nitrification at 10°C.
- The net rate of NO_2^--N production was greater than the net rate of consumption resulting in temporary NO_2^--N accumulation. The rate of production and consumption increased with increasing temperature.
- NO_2^--N accumulation was not a sign of nitrification system failure. NO_2^--N was completely oxidized to NO_3-N as NH_3-N concentrations declined. Temporary NO_2^--N concentration increased with increasing temperature.
- The maximum nitrification rates (mg/L*h) increased with increasing temperature.

5.2 Effect of initial NH_3-N concentration on nitrification rates (Appendix B-4)

5.2.1 Nitrification rate as a function of the initial NH_3-N concentration

When the reactors were compared to each other, the nitrification rate versus initial reactor NH_3-N concentration followed a first-order reaction. From Figure 5.8, the K_N concentration for this biomass was near 15 mg NH_3-N/L.

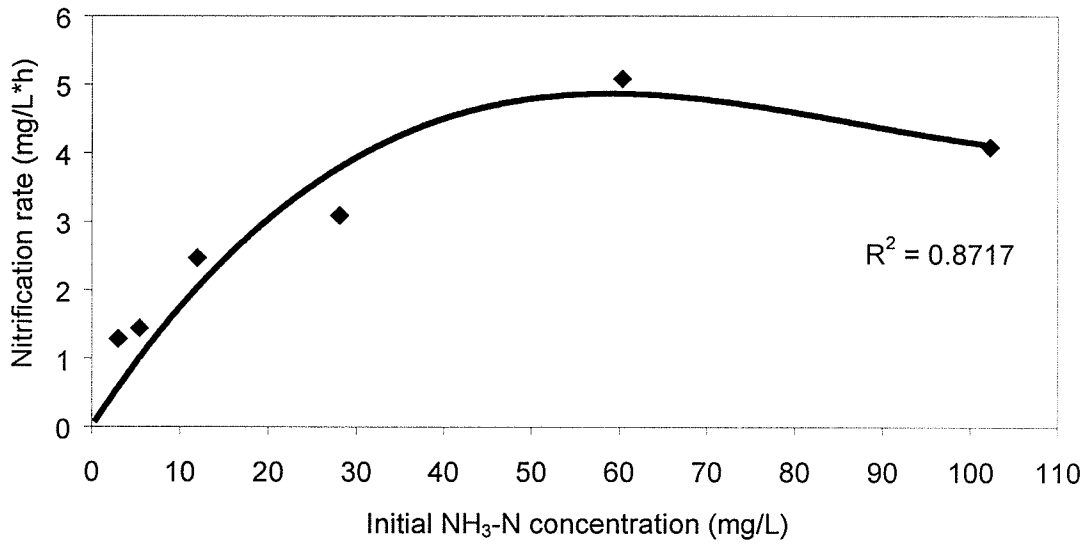


Figure 5.8 Nitrification rate as a function of the initial NH_3-N concentration in the reactor.

In contrast, the nitrification rate in each of the individual reactors followed a zero-order reaction. Once nitrification commenced, the concentration of NH_3-N in the reactor decreased at a linear rate for all initial concentrations of NH_3-N.

One reactor was allowed to reach NH_3-N concentrations of less than 1.0 mg/L (Figure 5.9). When the NH_3-N concentration became very low the nitrification rate decreased by 85%.

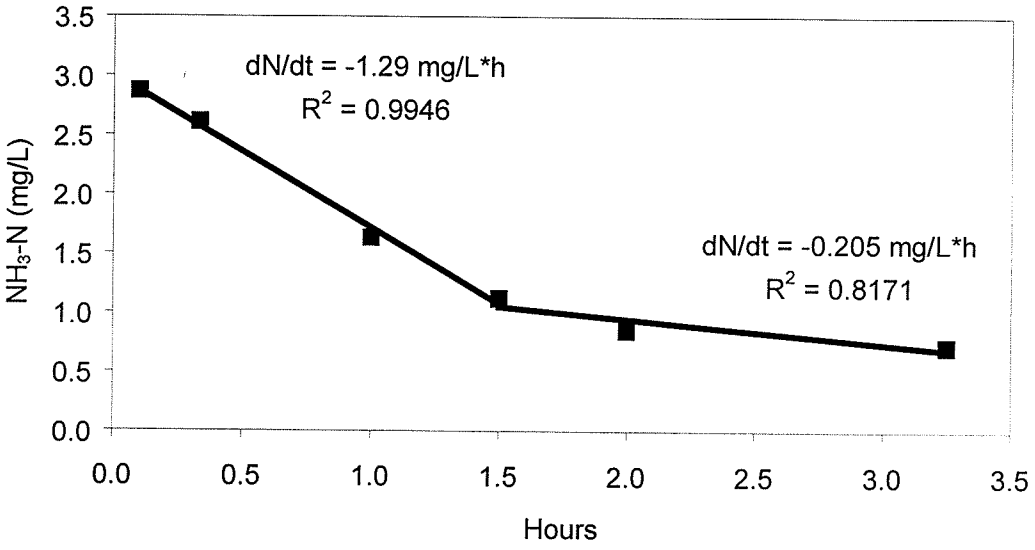


Figure 5.9 Decline in NH_3-N concentration over time.

5.2.2 Discussion

It is generally accepted that the half saturation coefficient (K_N) for ammonia oxidation is a very low concentration. Common values reported range from 0.2 to 3.6 mg/L at 20°C (*e.g.* Metcalf & Eddy 1997; USEPA, 1975; Drtil *et al.*, 1993) and explains why nitrification is usually described as a zero-order reaction. Therefore, in most wastewater treatment systems nitrification rates are very near the maximum. However, there have been several researchers who have found that K_N values for nitrification are much higher. For example, Clarkson *et al.* (1989) found K_N to be 28 mg/L at 23°C while Hanaki *et al.* (1999) found K_N to be 9.4 mg/L at 25°C.

Figures 5.8 and 5.9 suggest that there are two types of reactions occurring. At NH_3-N concentrations greater than approximately 1 mg/L, the nitrification

rate was highly dependent on the initial concentration of NH_3-N in the reactor. The greater the concentration of NH_3-N, the greater the nitrification rate up to an initial concentration of approximately 100 mg/L. The nitrification rate was constant until the NH_3-N concentration became very low (*i.e.*, 1 mg/L). The observed value of 1 mg NH_3-N/L is very near to the most commonly reported values of K_N.

5.2.3 Summary and conclusions

- Nitrification rate is highly dependent on the initial NH_3-N concentration in the reactor (first order reaction). K_N was found to be near 15 mg/L.
- Nitrification rates in each individual reactor were constant for initial NH_3-N concentrations between 1 and 102 mg/L. The nitrification rate decreased by 85% when the concentration of NH_3-N in one reactor was allowed to decrease to less than 1 mg/L.

5.3 Determination of cold shock in a batch test (Appendix C-1)

5.3.1 Laboratory data

NH_3-N removal rates ($\Delta N/\Delta t$) were significantly decreased by sudden cooling, and the magnitude of the decrease was dependent on the change in temperature (ΔT). Figure 5.10 provides an example where the nitrification rates in the warm nitrifying reactors (NB20, NB25 and NB30) were compared with the rates at 10°C. A direct comparison can be made because the initial

concentration of biomass, substrate, pH and aerobic conditions in the warm and cold reactors were similar. The average decrease in nitrification rate was 58 ± 8.2% for NB20, 71 ± 4.7% for NB25 and 82 ± 1.4% for NB30. The differences between the decreases in nitrification rate were found to be statistically significant (t-test, p=0.05) (Appendix C-2). The decrease in nitrification rate with a sudden decrease in temperature is highly dependent on the initial temperature of the biomass.

5.3.2 Comparing observed data with previous studies

Observed decreases in nitrification rates with decrease in temperature were compared with previous studies on nitrifier growth rates (μ). Nitrification rates can be compared with growth rates because they are linearly proportional to each other by Equation 13.

$$\mu_{\max} = \frac{Y \bullet -dN/dt}{X_a} \qquad [13]$$

The theoretical percent decrease in nitrification rate was estimated by taking the ratio of μ_{10C} to μ_T for each temperature. As an example, using Equation 19 from Downing and Hopwood (1964) the growth rate at each temperature was calculated. The theoretical decrease in nitrification rate was then determined by Equation 12.

$$\mu_T = 0.18e^{0.12(T-15)} \qquad [19]$$

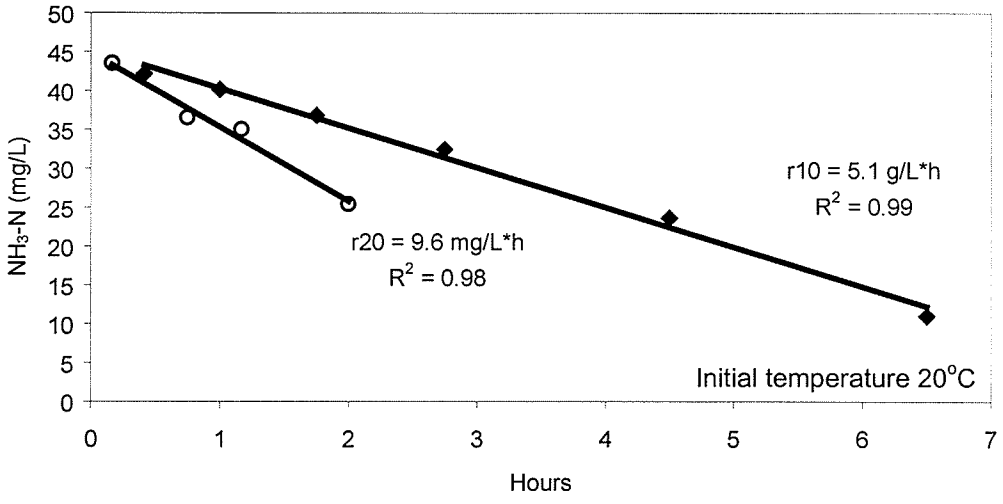
$$\mu_{30C} = 0.18e^{0.12(30-15)} = 1.09d^{-1}$$

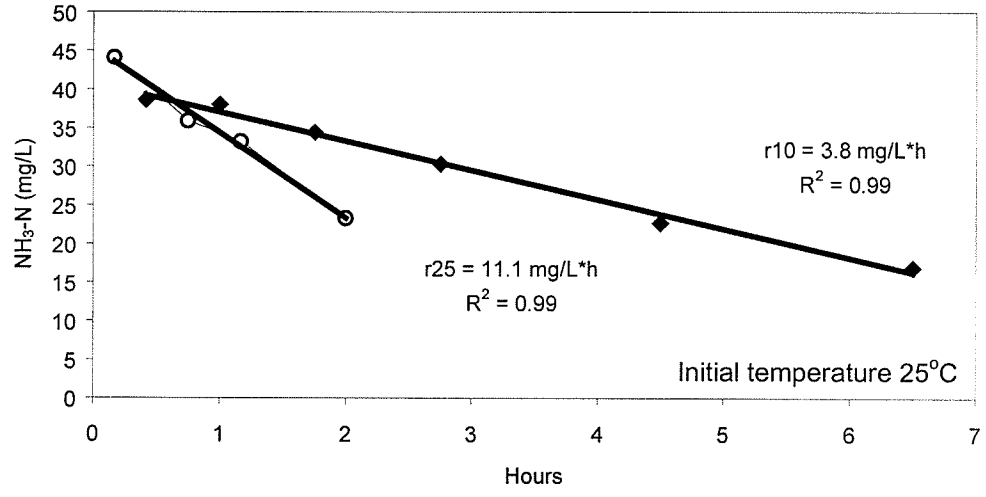
$$\mu_{10C} = 0.18e^{0.12(10-15)} = 0.099d^{-1}$$

$$\textit{Theoretical Decrease in Nitrification Rate} = \frac{\mu_{30C} - \mu_{10C}}{\mu_{30C}} \qquad [12]$$

$$= \frac{(1.09 - 0.099)}{1.09} \times 100\% = 91\%$$

This calculation was repeated with the equations shown in Table 2.7. The observed data was then compared to the theoretical decreases and the results are shown in Figure 5.11. The observed decreases in NH_3-N removal rate after a sudden decrease in temperature were within the range previously seen by other researchers and the rate constant was calculated to be (k_t) be 0.0844 $^oC^{-1}$.





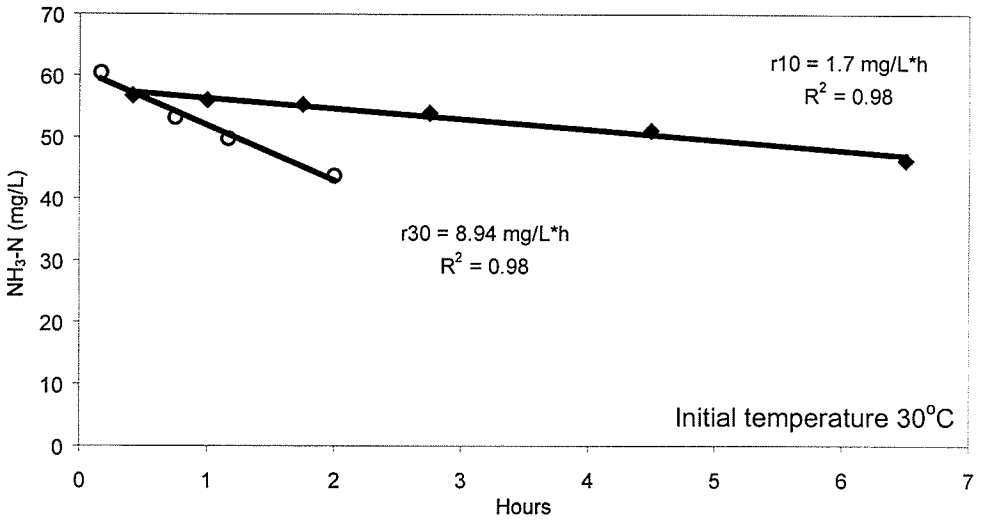


Figure 5.10. Nitrification rates before and after a sudden decrease in temperature to 10°C for NB20, NB25 and NB30.

The temperature correction factors stated by some of the researchers did not indicated whether or not the value was derived from rapid changes in temperature or from biomass acclimated over long term. The similarities amongst the research indicate that nitrification rates immediately after a decrease in temperature behave similarly to biomass that is acclimated to the new, colder temperature.

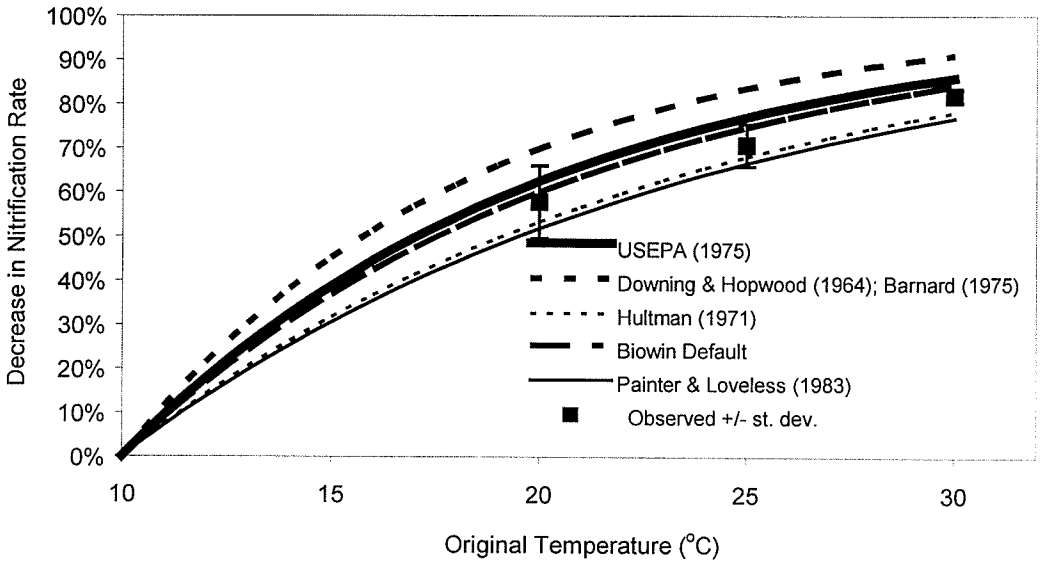


Figure 5.11 Theoretical and observed decreases in nitrification rates after exposure to 10°C

5.3.3 Summary and Conclusions

- Nitrification continued at a slower rate after a sudden decrease in temperature as great as $\Delta T=20^{o}C$.
- The temperature dependence for biomass treating centrate between 10°C and 30°C was observed to be 0.0844 $^{o}C^{-1}$ making the temperature correction factor (Γ_N) equal to 1.088.

- The observed decreases in nitrification rates were within the ranges found by other researchers.

5.4 Seeding nitrifying biomass into a continuous flow system at 10°C (Appendix D)

5.4.1 Characteristics of feed

Synthetic wastewater was used in this research for the purpose of having complete control over influent characteristics. However, despite mixing new synthetic wastewater every few days, keeping the feed refrigerated and cleaning storage containers frequently, variations in feed quality occurred. Degradation of the feed during storage resulted in an increased feed NH_3-N concentration likely due to the hydrolysis of organic nitrogen from the beef extract. The TCOD and NH_3-N concentrations of the feed during this stage of study are shown in Figure 5.12.

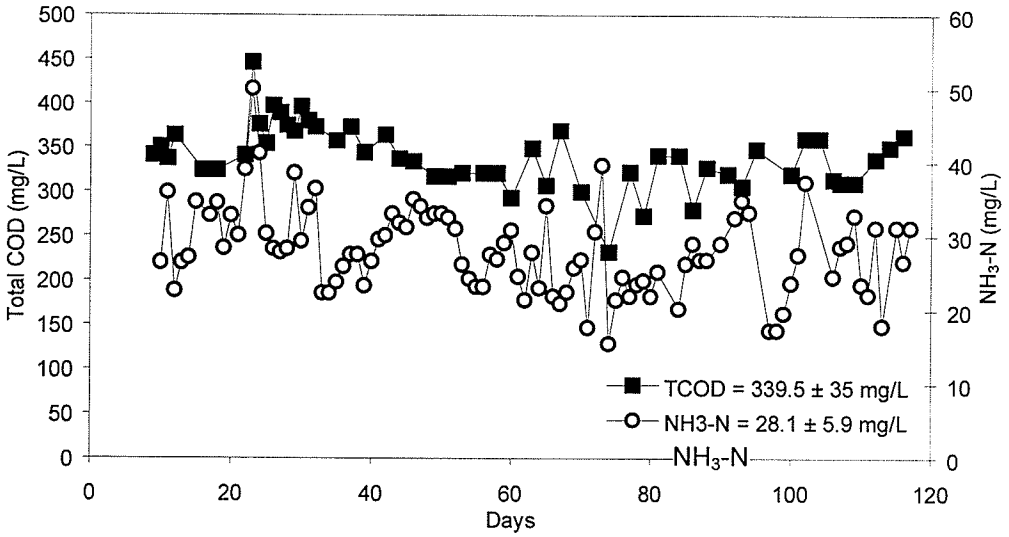


Figure 5.12 Synthetic feed total COD and NH_3-N concentrations during continuous flow study at 10°C

5.4.2 Characteristics of seed (Appendix D-1)

The seed contained high concentrations of SCOD which is likely due to large quantities of slowly degradable or non-degradable COD in the centrate feed. The NH_3-N concentrations from NB20 were highly variable and reached a maximum of over 140 mg/L on Day 53 (equivalent to ~65% NH_3-N removal from centrate) (Figure 5.13). The maximum observed nitrification rate of NB20 was 12.5 mg NH_3-N/L*h or 48.2 mg/g VSS*h.

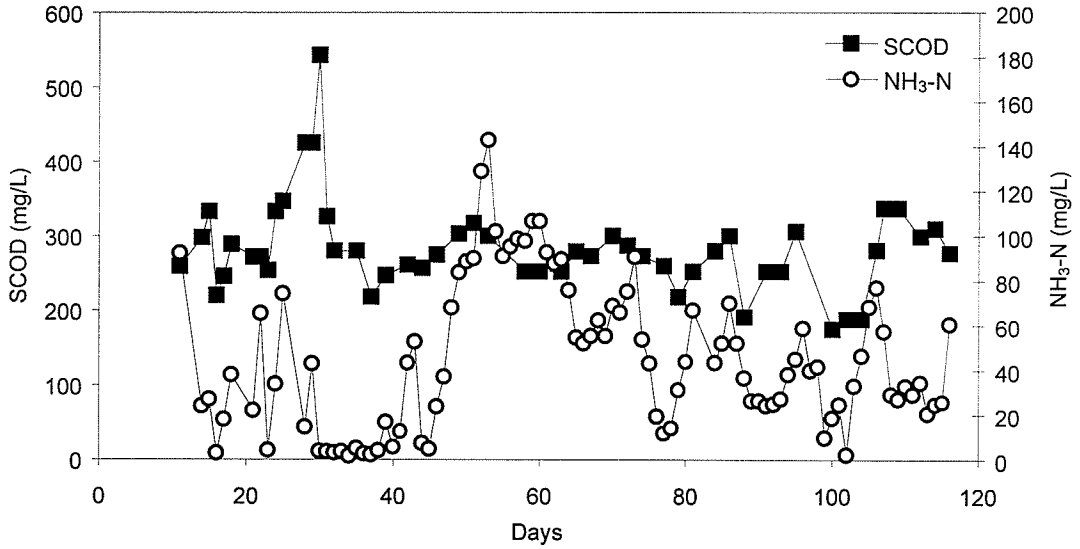


Figure 5.13 NH_3-N and SCOD of NB20 during seeding into a continuous flow reactor at 10°C.

Solids concentration in the seed source (NB20) also declined during seeding. Before seeding commenced, wasting of excess biomass from NB20 was done automatically by a peristaltic pump on a timer. Inadequate mixing before wasting resulted in an accumulation of solids on the sides and bottom of the reactor. This problem was alleviated by manually cleaning, mixing and wasting solids from the reactor. With this more regular wasting regime the

solids concentration began to decline to a final concentration of approximately 200 mg VSS/L (Figure 5.14).

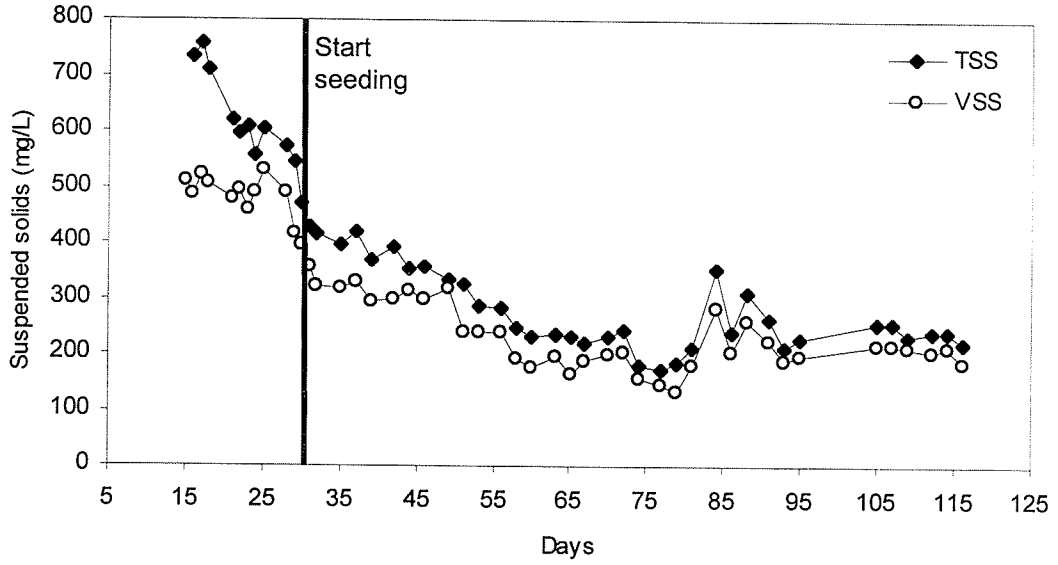


Figure 5.14 Suspended solids concentrations of NB20 during seeding into continuous flow reactors at 10°C.

5.4.3 Results of continuous flow reactors (Appendix D-2 and D-3)

The effluent NH_3-N concentrations for control and seeded continuous flow reactors are shown in Figure 5.15. Starting on day 29 one of the reactors was seeded daily with 100 mL of NB20 which corresponds with a VSS loading rate of approximately 2% ($VSS_{seed}/VSS_{reactor}$). As a result, the NH_3-N concentration in the effluent of the seeded reactor decreased to a level slightly below that of the control reactor. To get a more defined difference between the two reactors, on day 53 the seeding rate was increased to approximately 3.5% ($VSS_{seed}/VSS_{reactor}$) by the daily addition of 200 mL of NB20. No

noticeable difference between the control and seeded reactor effluents was achieved by increasing the seeding rate.

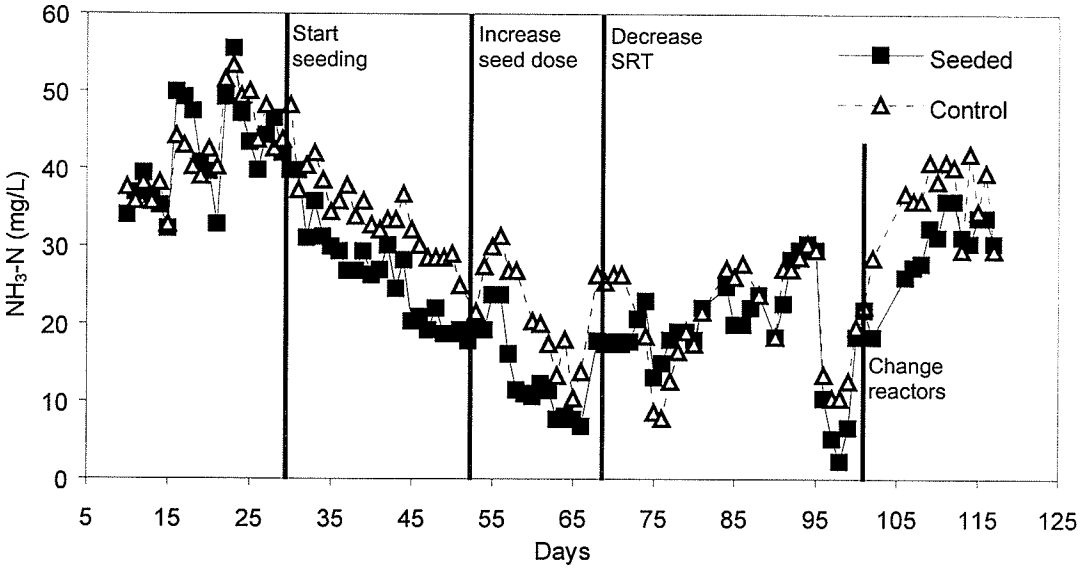


Figure 5.15 Effluent NH_3-N concentrations for 2 continuous flow systems. Dates of major changes in operation are marked by heavy black lines.

Over time, it became quite evident the nitrification was occurring in the control reactor as indicated by the decline in effluent NH_3-N (Figure 5.15). On day 68 the wasting rate for both of the reactors was increased in an attempt to wash-out the nitrifying bacteria from the control reactor. As a result, effluent NH_3-N concentrations increased slightly in both reactors but quickly decreased again around day 95.

Finally, it was deduced that the cause of nitrification in the control reactor was the build-up of attached growth on the walls of the reactors and within the tubing. Despite efforts to change tubing regularly and scrape the sides of the reactors on a daily basis throughout the study, nitrate nitrogen (NO_3^--N) was found in the control reactor in substantial concentrations (Figure 5.16).

Decreasing the SRT did not result in the elimination of NO_3^--N. On Day 101 the entire reactor vessels were replaced with new, clean vessels. As a result, the NH_3-N concentration in both the seeded and control reactors increased but the seeded reactor continued to have a lower effluent NH_3-N concentration than the control (Figure 5.15). NO_3^--N was finally eliminated in the control reactor by changing to new reactors (Figure 5.16).

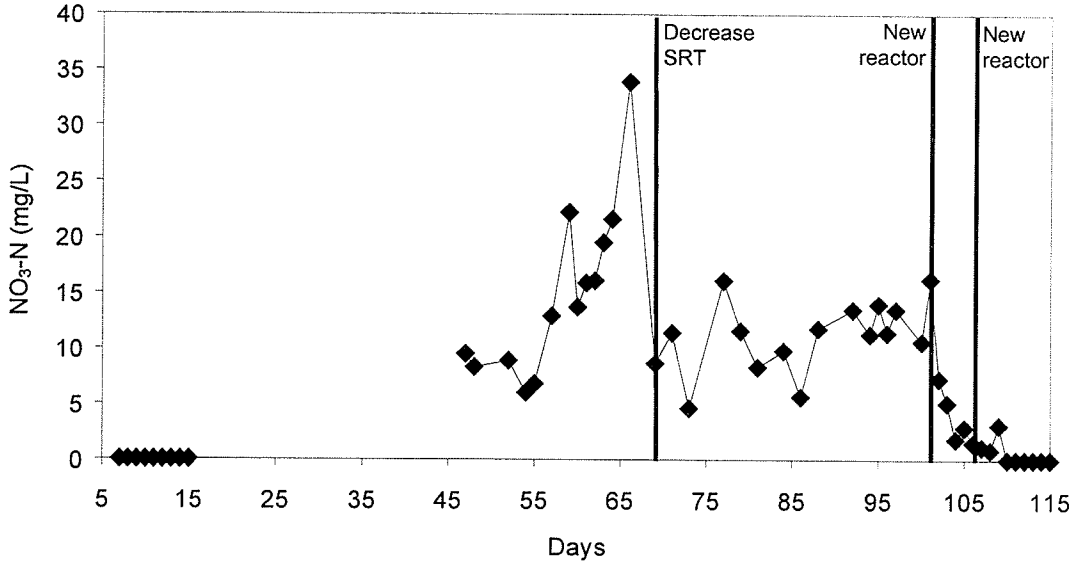


Figure 5.16 Effluent NO_3-N for the control reactor. Dates of major changes in operation are marked by heavy black lines.

To obtain a more defined representation of the differences in effluent quality between the seeded and control reactors, the ratio of Control:Seeded effluent NH_3-N concentrations were determined and plotted in Figure 5.17. It is evident that the control reactor almost always had a higher effluent NH_3-N concentration than the seeded reactor, as indicated by a ratio greater than 1.0. Further statistical analysis demonstrates that the lower effluent NH_3-N

concentration from the seeded reactor was statistically significant (Appendix D-4).

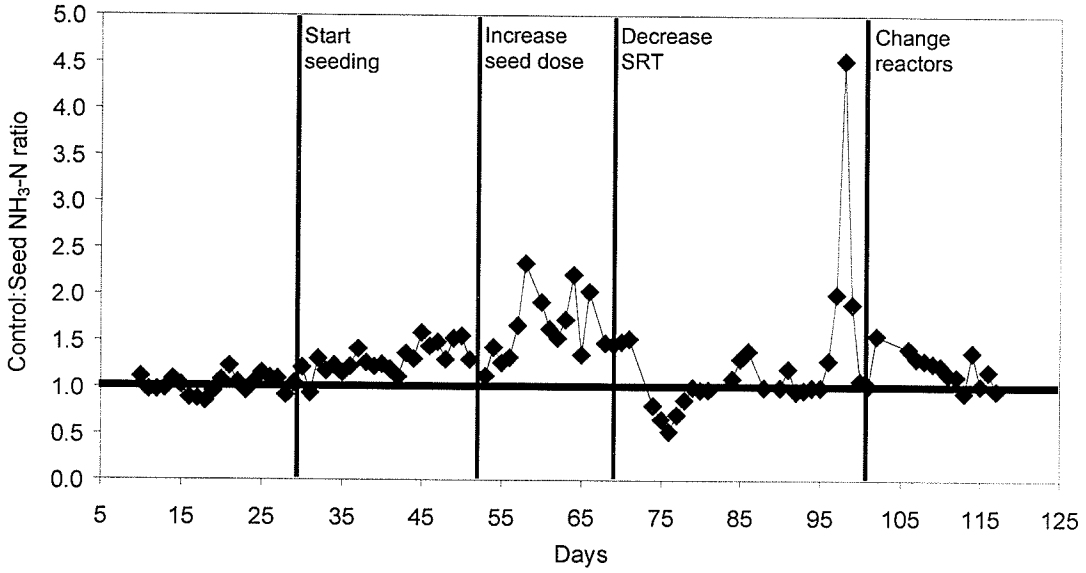


Figure 5.17 Control:Seeded effluent NH_3-N ratio.

Similar effluent SCOD values were observed in both reactors until seeding commenced on Day 29. Effluent SCOD was increased in the seeded reactor over that of the control by the addition of mixed liquor from nitrified centrate which contained high concentrations of SCOD after treatment.

This stage of research was ended after 118 days because of a malfunction in the environmental chamber in which it was housed. During one night the temperature in the chamber dropped to -5°C for approximately 12 hours resulting in complete freezing of reactor and clarifier contents.

5.4.4 Summary and conclusions

- Seeding nitrifiers from a reactor treating centrate at 20°C into the continuous flow system at 10°C sometimes resulted in lower effluent NH_3-N concentrations than a control reactor without the addition of seed.
- Treated centrate caused an increase in effluent SCOD for the reactor into which it was added.
- Attached growth on tubing and reactor walls provided a suitable habitat for the growth of nitrifying bacteria, making SRT control impossible.

5.5 Seeding nitrifying biomass into SBRs at 10°C

5.5.1 Seeding NB20 into SBRs with HRTs 43.6 to 96 h

5.5.1.1 Synthetic feed characteristics

The synthetic wastewater during this phase had 252.8 ± 56.4 mg TCOD/L and 32.1 ± 7.8 mg NH_3-N/L. Synthetic wastewater was used to eliminate variability in influent characteristics.

5.5.1.2 Seed characteristics (NB20) (Appendix E-1)

The concentrations of NH_3-N and NO_3-N in the effluent of NB20 are shown in Figure 5.18. Elevated concentrations of NH_3-N in this reactor from day 10 to 30 were the result of problems with the air supply. Once this problem was corrected several days were required to achieve stable treatment.

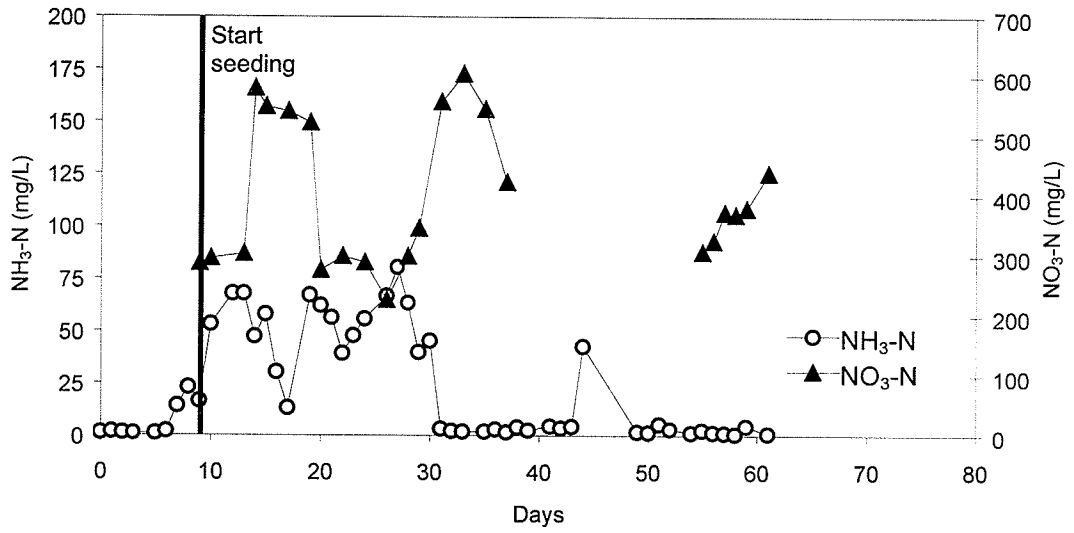


Figure 5.18 NH_3-N and NO_3-N concentrations in NB20.

Other characteristics of the seed are listed in Table 5.2. The mass of VSS added as seed from NB20 was equivalent to 11.3 mg VSS/day or 5.7 mg VSS/L of reactor volume per day. This is a very small mass compared to the total mass of VSS in the seeded reactors.

The estimated concentration of nitrifiers in NB20 (X_a^s) was 95.2 mg/L as determined by Equation 9 which is equivalent to approximately 85% of the VSS in the reactor. This is an unusually high proportion of nitrifiers. The nitrifier fraction usually varies between 4 and 46% for biomass treating wastewater with BOD/NH_3-N ratios from 9 to 0.5, respectively (U.S. EPA, 1975). However, the biodegradable carbon fraction of centrate is very low and the NH_3-N content is high which could both contribute to high proportions of nitrifiers.

Table 5.2 Summary of NB20 characteristics.

Observed Parameter	Units	NB20
θ_x^a	d	5
S^o	mg NH_3-N/L	638 ± 41.0
S	mg NH_3-N/L	2.7 ± 1.3
Mean effluent SCOD	mg/L	325 ± 50.2
X_r	mg VSS/L	113.4 ± 36.5
Maximum dN/dt	mg/L*d	379
Calculations and Assumptions		
b at 20°C	d^{-1}	0.10
X_a^s	mg VSS/L	95.2
U	mg/g nitrifiers*h	166
	mg/g VSS*h	140

5.5.1.3 Results of seeded SBRs (Appendix E-2)

Effluent NH_3-N concentrations became less than 5 mg/L within 26 to 32 days of the start of seeding (Figure 5.19). All four reactors achieved nearly complete NH_3-N removal while seeding continued, but once seeding was stopped, NH_3-N removal dropped off quickly. The rapid increase in effluent NH_3-N with the absence of seeding indicated that the nitrifying bacteria were being rapidly washed out from all of the reactors.

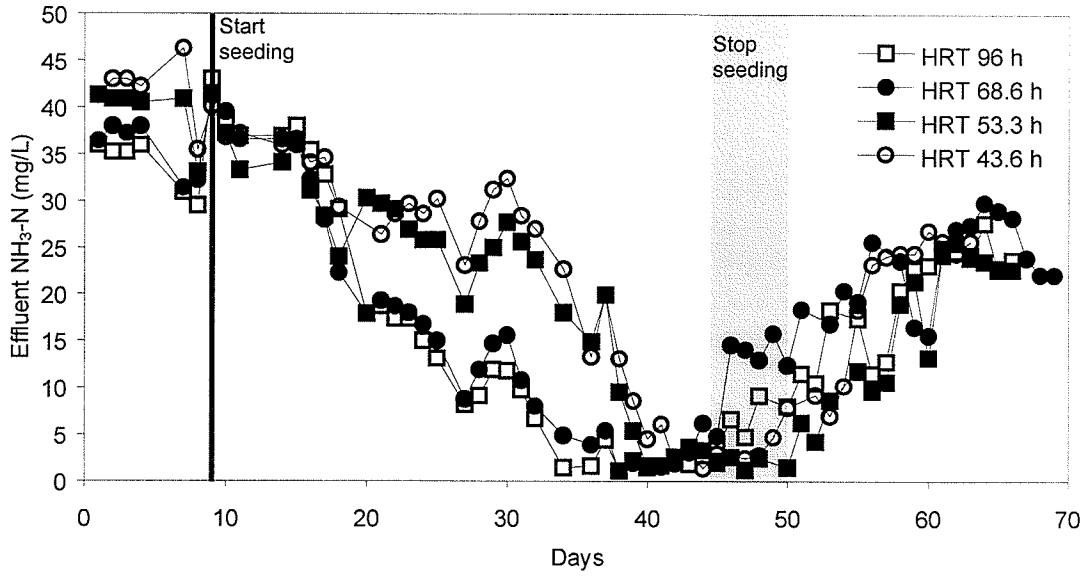


Figure 5.19 Effluent NH_3-N concentrations for cold SBRs at various HRTs.

Figure 5.20 provides an example of changes in the nitrification rate over time for the reactor with HRT-96 h. At the onset of seeding, the removal rate increases until approximately Day 26 after which the removal rate is constant. Even though the removal rate is constant, the effluent NH_3-N continues to decrease; thus is the nature of an SBR system where the volume exchange ratio has an impact on the rate of NH_3-N decrease in the effluent. Then, when seeding is stopped the nitrification rate decreases as the nitrifiers are washed out of the system.

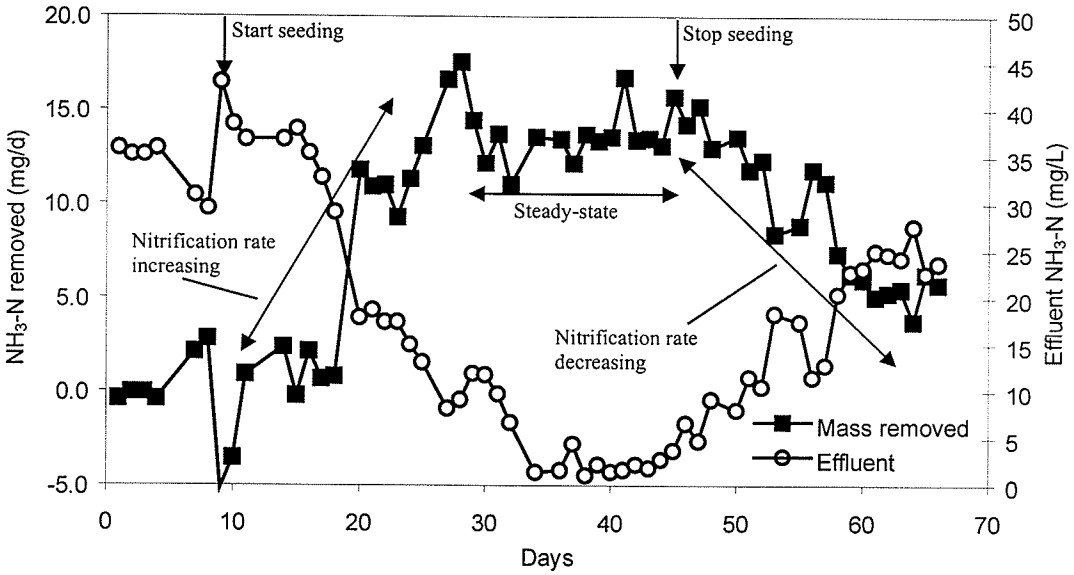


Figure 5.20 NH_3-N removal and effluent NH_3-N for the SBR with HRT-96 h.

Nitrification rates increased and decreased linearly with the start and stop of seeding as shown in Table 5.3. The increase in nitrification rate for all of the reactors was approximately equal. This was expected because the same mass of seed was added to all of the reactors. However, the rate of washout was faster for the reactors with shorter HRTs. The washout rate for the reactor with HRT-43.6 h was 3.4 times greater than the reactor with HRT-96 h.

Table 5.3. Changes in nitrification rates during and after seeding.

Reactor HRT	Increasing nitrification rate with seeding (mg/d/d)	R^2	NH_3-N removal at steady state (mg/d)	Decreasing nitrification rate without seeding (mg/d/d)	R^2
43.6 h	1.12	0.72	34.8±6.51	-1.84	0.86
53.3 h	1.21	0.86	28.0±4.58	-1.23	0.82
68.6 h	0.964	0.74	18.9±2.94	-0.414	0.55
96 h	0.892	0.79	13.9±1.34	-0.542	0.87

At the onset of seeding, effluent NO_3-N concentrations increased sharply due to the nitrified liquor associated with the seed. As expected, the reactors with the longer HRTs had higher concentrations of NO_3-N in the effluent (Figure

5.21). The increases were due to a smaller fraction of liquid being exchanged per day in these reactors than those with shorter HRTs. It is unlikely that NO_3-N concentrations would reach such high values if this process for bioaugmentation was used in full-scale systems. In this study the nitrified centrate made up 9 to 20% of the total flow entering the cold SBRs while in full-scale application the nitrified centrate would contribute only 1 to 2% to the influent flow. The high NO_3-N concentrations in the reactors did not create any problems with settlability or floating biomass due to unintended denitrification.

As a result of seeding, effluent SCOD concentrations rose in the cold SBRs (Figure 5.22). The rise in effluent SCOD followed a similar trend as NO_3-N with higher effluent SCODs in the reactors with shorter HRTs. The increase in effluent SCOD in the seeded reactors was expected from the input of high concentrations of SCOD from NB20.

A summary of the values required for seeded SRT determination are listed in Table 5.4. All of the values reported are from during steady-state conditions; i.e., when minimum effluent NH_3-N concentrations were achieved.

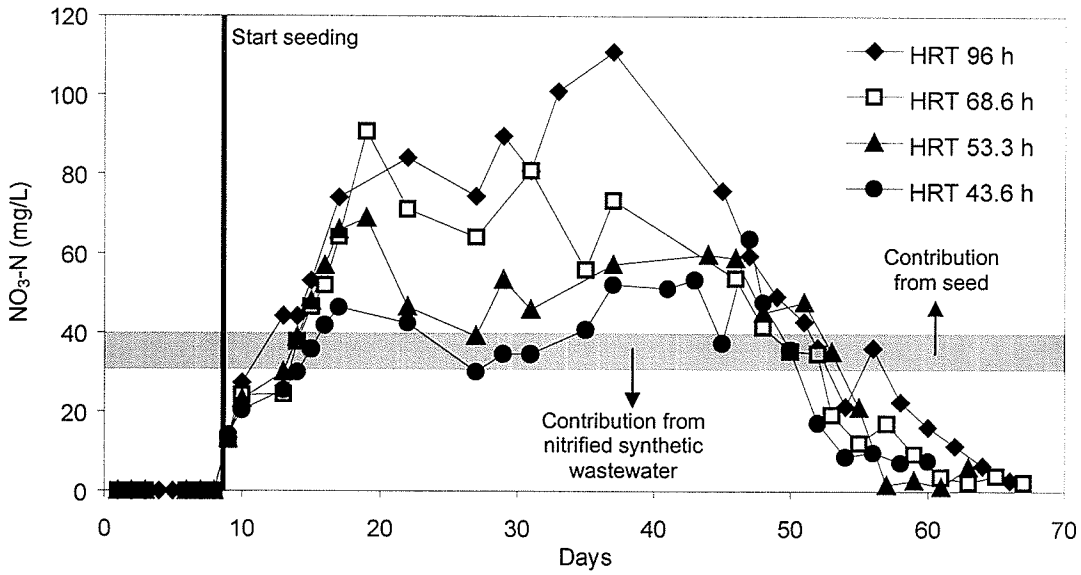


Figure 5.21 NO_3-N concentrations for SBRs with various HRTs.

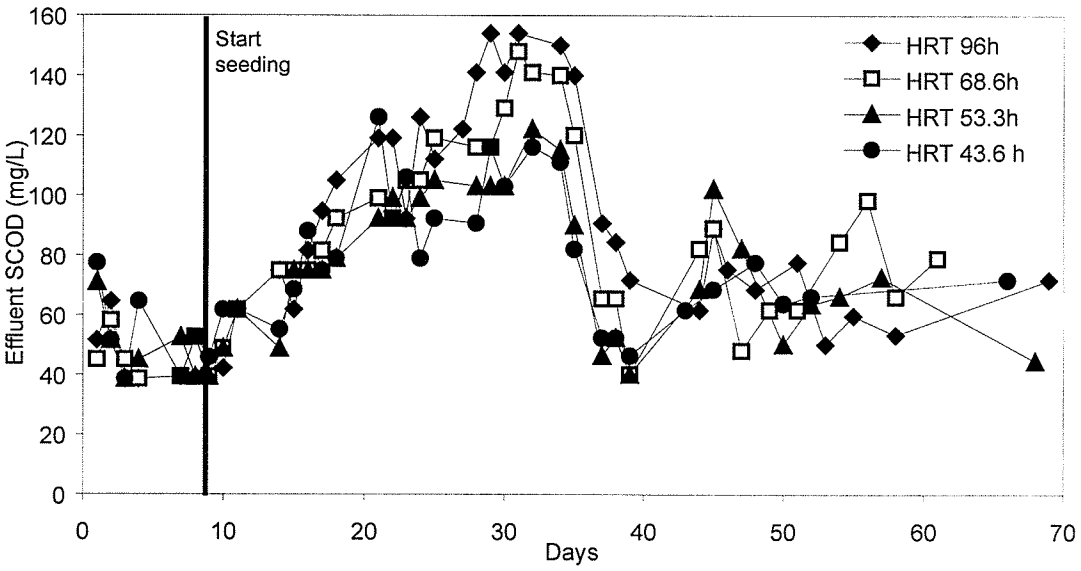


Figure 5.22 Effluent SCOD for SBRs at 10°C with various SRTs.

Table 5.4 Summary of SBRs at 10°C seeded with NB20.

		HRT (h)			
Input parameters	Units	43.6	53.3	68.6	96
θ_x^a	d	3.51	3.63	3.75	4
Q^i	L/d	1.0	0.8	0.6	0.4
Q^s	L/d	0.1	0.1	0.1	0.1
Q^e	L/d	0.6	0.4	0.2	0
Q^w	L/d	0.5	0.5	0.5	0.5
S^o	mg NH_3-N/L	41.9	39.6	35.5	33.8
S	mg NH_3-N/L	1.32	1.2	1.06	1.06
X_r	mg VSS/L	149	140	116	96.2
X_e	mg VSS/L	20	20	20	0
X_a^o	mg VSS/L	8.65	10.6	13.6	19.0
b at 10°C	d^{-1}	0.043	0.043	0.043	0.043
U	mg NH_3-N/g VSS*h	2.85	4.17	3.40	3.01

5.5.1.4 Discussion

Full nitrification was achieved in cold SBRs operating at an apparent SRT too short for nitrification to occur. Before seeding, nitrification was not occurring in the reactors, as indicated by the high effluent NH_3-N concentrations and lack of NO_3-N production (Figures 5.19 and 5.21). With seeding, the concentration of nitrifying biomass in the seeded SBRs was increased such that full nitrification could occur. The mass of nitrifying seed added plus that grown within the seeded SBRs resulted in seeded SRTs longer than the apparent SRT of 4 days.

The ability to achieve full nitrification without decreasing the proportion of biomass wasted daily (to increase the apparent SRT) suggests that the amount of solids wasted daily could be increased while still maintaining full nitrification. This is, in effect, volume savings because the desired effluent quality is achieved without increasing the solids inventory.

Theoretically, with seeding, the nitrification rate should increase until the mass of nitrifiers added as seed is equal to the mass of nitrifiers wasted. The nitrification rates increased at approximately the same rate for all of the seeded reactors, which was expected because the mass of seed added to each reactor was equal. However, the reactors with shorter HRTs experienced a faster decline in nitrification, which was likely due to inadvertent washout of nitrifiers with the decant liquors.

5.5.1.4 Summary and conclusions

- Nitrification was induced by seeding nitrifying bacteria into cold SBRs operating at apparent SRTs that were otherwise too short to sustain nitrification. Effluent NH_3-N concentrations were reduced to less than 5 mg/L within 26 to 32 days as long as seeding was continued.
- Nitrification failed when seeding was stopped. Nitrifying bacteria were washed out of the reactors faster in the SBRs with shorter HRTs. This was indicated by NH_3-N accumulation and NO_3-N decline after seeding was stopped and a more rapid decline in nitrification rate.

5.5.2 Seeding NB10, NB20, NB25 and NB30 into SBRs with HRTs 12 and 24 h and apparent SRT 4 days

5.5.2.1. Synthetic feed characteristics

The synthetic wastewater had average concentrations of TCOD and NH_3-N of 399 ± 19.1 mg/L and 22.8 ± 3.8 mg/L, respectively.

5.5.2.2 Seed characteristics (Appendix F-1)

Inconsistencies in seed quality were an ongoing problem during this stage of the research. There was an inadequate amount of aeration starting on day 30 in NB25 and NB30. This resulted in incomplete NH_3-N removal over days 30 to 50 (Figure 5.23). Full NH_3-N removal was recovered quickly once the aeration problem was corrected. Feeding to NB25 was stopped for 2 days (days 43 and 44) so that the excess NH_3-N in the reactor could be oxidized. After these 2 days, feeding of centrate continued as usual. Seeding into the cold SBRs continued despite elevated effluent NH_3-N concentrations in the seed source and continued for 25 days after recovery. During the period of poor aeration there was a corresponding decrease in NO_3-N concentration in these seed sources which further illustrated the loss of nitrification (Figure 5.24). Elevated levels of NH_3-N were also observed in NB10 until day 25. This was due to insufficient alkalinity for full nitrification, and once alkalinity was provided in adequate quantities, stable nitrification was achieved.

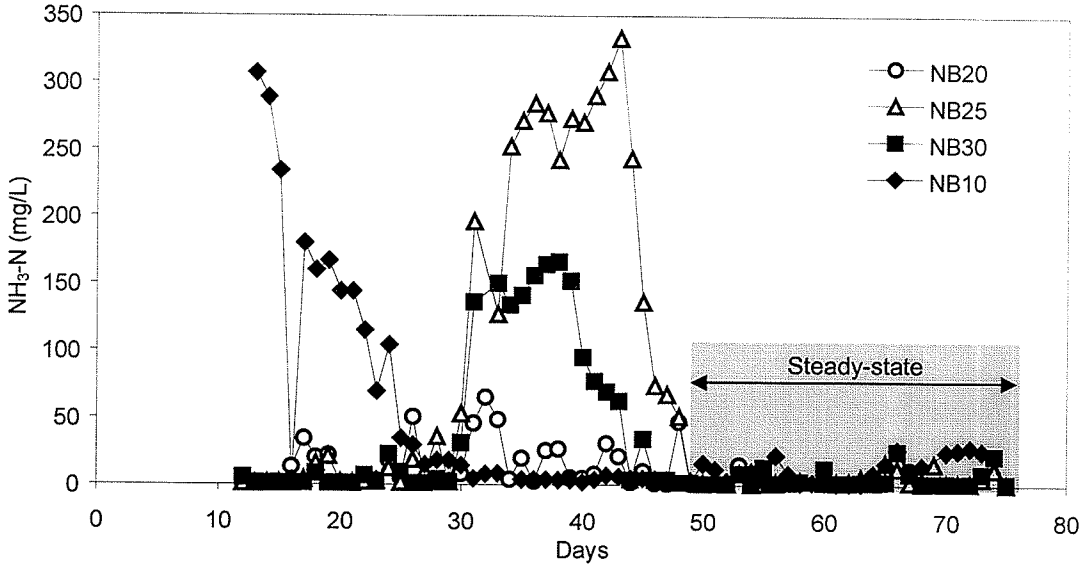


Figure 5.23 NH_3-N concentrations of seed from reactors acclimated to 10, 20, 25 and 30°C

The nitrifying seed liquor contained substantial quantities of SCOD which contributed from 1.8 to 5.4% of the SCOD entering the seeded cold SBRs (Table 5.5). It was unexpected that NB10 would contain much more SCOD when compared to the other seed sources. Possible reasons for this might be increased solubilization of particles with a longer retention time (12 days versus 5 days) or a lack of organisms capable to degrade the SCOD at 10°C.

The steady state conditions between days 49 and 75 were used for the determination of X_a^s and the seeded SRT. A more complete list of seed characteristics during steady state is shown in Table 5.5.

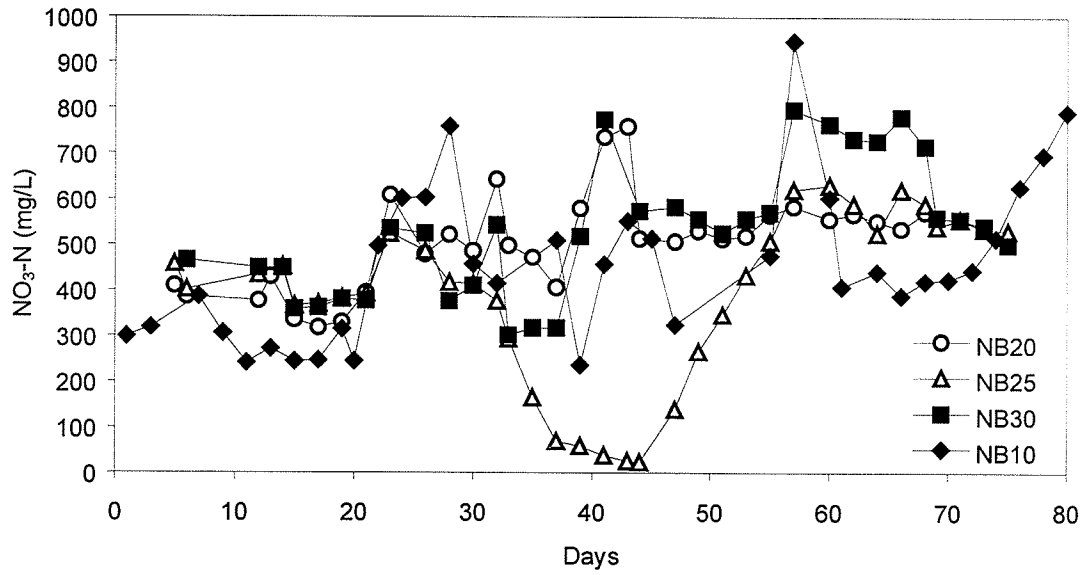


Figure 5.24 NO_3-N concentration of seed from reactors acclimated to 10, 20, 25 and 30°C.

Table 5.5. Summary of nitrifying seed characteristics during steady-state conditions.

		Seed Temperatures			
Observed Parameter	Units	NB10	NB20	NB25	NB30
θ_x^a	d	12	5	5	5
S^o	mg NH_3-N/L	631±47	631±47	631±47	631±47
Mean effluent SCOD	mg/L	351± 80	247± 15	266± 30	237±31
Mean effluent TCOD	mg/L	495	462	480	579
S	mg/L	5.7±4.8	3.5±5.0	4.0±4.8	4.8±6.7
X_r	mg VSS/L	125±32.0	301±45.1	298±46.3	337±55.8
Max. observed dN/dt	mg NH_3-N/L d	125	379	410	430
Calculations and Assumptions					
b	1/d	0.043	0.10	0.15	0.23
X_a^s	mg VSS/L	99.8	100.4	86.0	69.9
U	mg NH_3-N/g VSS*h	41.6	52.5	57.3	53.2
	mg NH_3-N/g nitrifiers*h	52.2	157	199	256

5.5.2.3 Effluent characteristics of seeded SBRs (Appendix F-2 and F-3)

The seeded SBRs were operated with an apparent SRT less than SRT_{min} as demonstrated by the lack of NH_3-N removal and NO_3-N production before the initiation of seeding.

The only reactor that achieved any significant level of NH_3-N removal with HRT-12 h was the SBR seeded with NB10 (Figure 5.25) (Appendix F-4). For NB20, NB25 and NB30 the effluent NH_3-N concentration was slightly greater than that in the influent. During pseudo-steady state in the other three reactors, those with HRT- 24 h had lower effluent NH_3-N concentrations than those with HRT - 12 h (Figures 5.26, 5.27 and 5.28). The exception to this occurred during days 32 to 51 for the 25°C seed (Figure 5.27). There was a significant rise in effluent NH_3-N in the reactors into which NB25 was added during Days 30 to 55 caused by a loss of nitrification in the NB25 seed source reactor (Figures 5.23 and 5.24). The lower concentrations in the 12 h reactor was due to a higher volume exchange ratio in that reactor causing dilution and washout of the extra mass of NH_3-N added from the seed. Poor nitrification efficiency in NB30 also caused a slight rise in effluent NH_3-N in the reactor seeded with 30°C biomass (Figure 5.28).

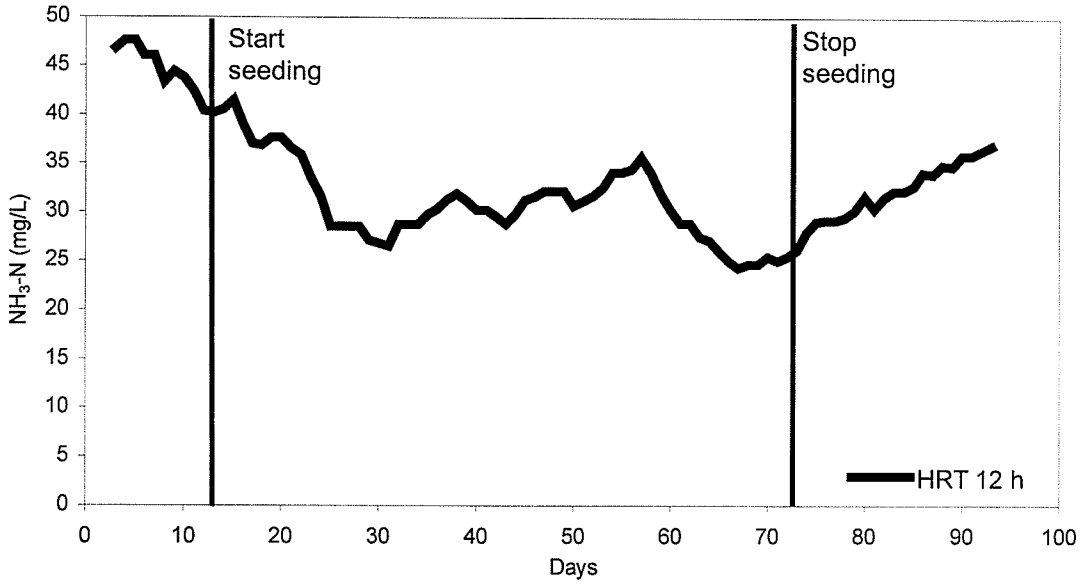


Figure 5.25 Effluent NH_3-N for the reactor seeded with NB10.

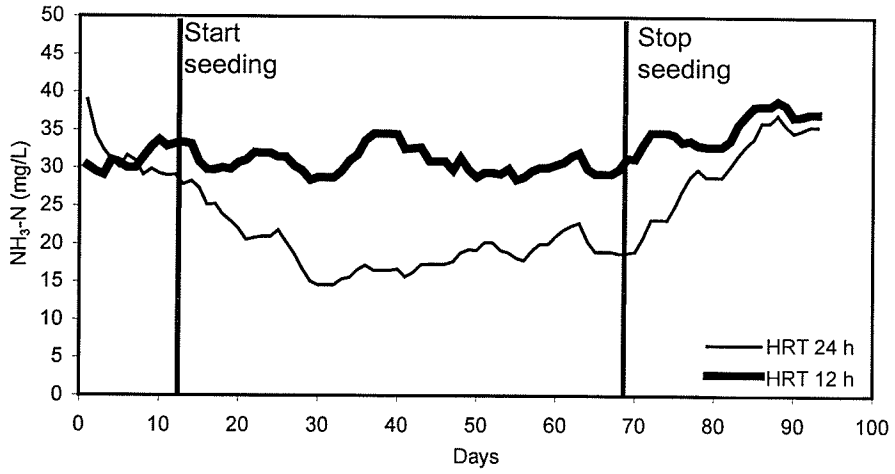


Figure 5.26 Effluent NH_3-N for reactors seeded NB20.

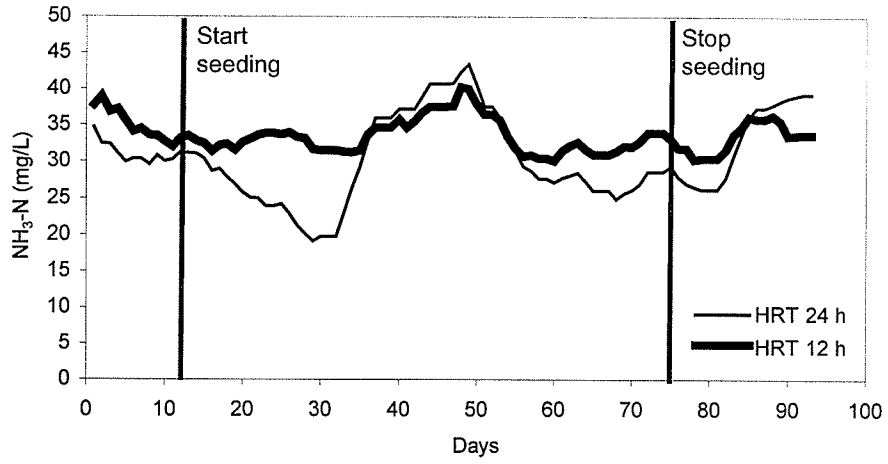


Figure 5.27 Effluent NH_3-N for reactors seeded with NB25.

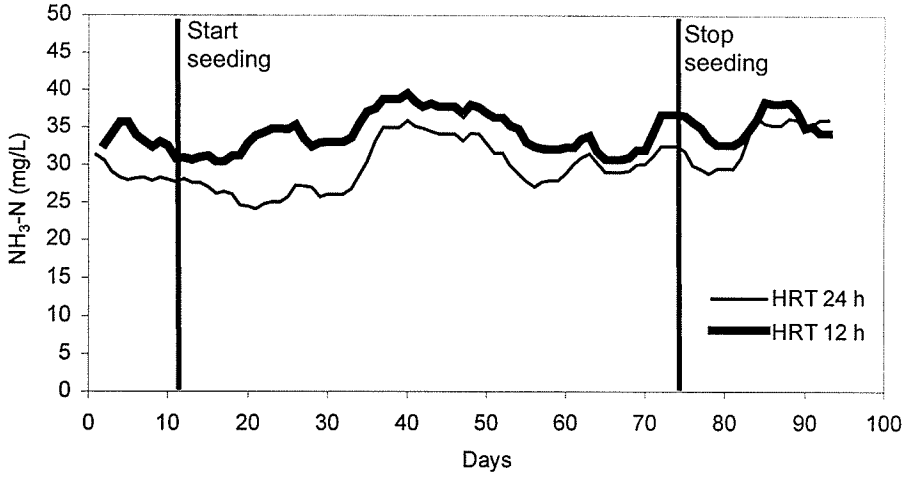


Figure 5.28 Effluent NH_3-N for reactors seeded with NB30.

When the HRT was 24 h, the greatest NH_3-N removal was achieved in the reactor seeded with biomass acclimated to 20°C. Effluent NH_3-N concentrations in this reactor were reduced by approximately 20 mg/L when compared with pre-seeding effluent concentrations. The reactor seeded with 25°C nitrifying biomass achieved lower effluent NH_3-N concentrations than the reactor seeded with 30°C biomass except when nitrification was lost and then recovered over days 30 to 50. Once recovery was complete, the effluent quality resumed as before day 30. The differences between all of the reactors with HRT - 24 h were statistically significant (t-test, $p=0.05$) with the degree of removal of 20°C > 25°C > 30°C (Appendix F-5).

For the reactors with HRT - 24 h, nitrification rates increased at a greater rate as the seed temperature decreased (Table 5.6). The steady-state NH_3-N removal increased as the seed temperature decreased. After seeding was stopped, nitrification failed at a faster rate as the temperature of the seed increased. The reactor seeded with NB10 achieved the highest nitrification rate, removing 54.9 mg NH_3-N/d which is 5 times greater removal than the reactor seeded with NB30.

Table 5.6. Rate of NH_3-N decline during seeding and rate of NH_3-N accumulation in the effluent after seeding has been stopped.

Seed Source	HRT (h)	Increasing nitrification rate with seeding (mg/d/d)	R^2	Steady-state NH_3-N removal (mg/d)	Decreasing nitrification rate without seeding (mg/d/d)	R^2
NB10	12	3.40	0.743	54.9 ± 19.0	-2.17	0.410
NB20	24	4.57	0.340	47.2 ± 17.7	-2.67	0.584
NB25	24	4.28	0.621	37.6 ± 17.2	-3.50	0.703
NB30	24	3.22	0.552	11.1 ± 17.3	-3.70	0.493

The seed sources contributed large quantities of NO_3-N to the reactors into which they were added. As expected, the reactors with HRT - 24 h had higher effluent NO_3-N concentrations than the reactors with HRT - 12 h due to a smaller volume exchange per day in addition to achieving greater NH_3-N removal (Figures 5.29 and 5.30). The concentration of NO_3-N in each of the seed sources was approximately equal during steady-state operation. An average concentration from all of the seed sources was used to approximate the amount of NO_3-N that could be attributed to seed addition. The shaded regions in Figures 5.29 and 5.30 are the theoretical additions of NO_3-N after the third cycle of seeded SBR operation. The theoretical addition was calculated from the concentration of NO_3-N in the seed and the volume exchanged per SBR cycle.

The aeration problems associated with the seed source reactors NB25 and NB30 became evident with the rapid washout of NO_3-N from the seeded SBRs over days 30 to 51 (Figures 5.29 and 5.30). The SBR seeded with NB25 experienced a greater decline in NO_3-N due a more extreme nitrification

failure in the seed source reactor. This reactor also required more time to accumulate NO_3-N once the seed source stabilized.

When seeding was stopped, washout of NO_3-N occurred at a faster rate in the reactors with HRT - 12 h than those with HRT-24 h. In the SBRs with HRT - 12 h, washout occurred within 5 to 7 days for the reactors seeded with NB20, NB25 and NB30 (Figure 5.29). Washout from the reactor seeded with NB10 did not occur as rapidly as the reactors seeded with biomass acclimated to the warmer temperatures and complete washout did not occur after seeding was stopped for 10 days.

When the HRT was 24 h (Figure 5.30) effluent NO_3-N concentrations decreased to less than 1 mg/L within 2 weeks after seeding was stopped in the reactors seeded with NB20 and NB25. The reactor seeded with NB30 biomass had complete washout of NO_3-N within one week. The decline in effluent NO_3-N after seeding was stopped indicated not only the washout of excess NO_3-N added from the seed but the speed at which nitrifying bacteria were being washed out of the system. Partial nitrification in the reactor seeded with NB10 after 10 days of no seeding indicated that NB10 was more resistant to washout when compared to the nitrifying biomass acclimated to warmer temperatures.

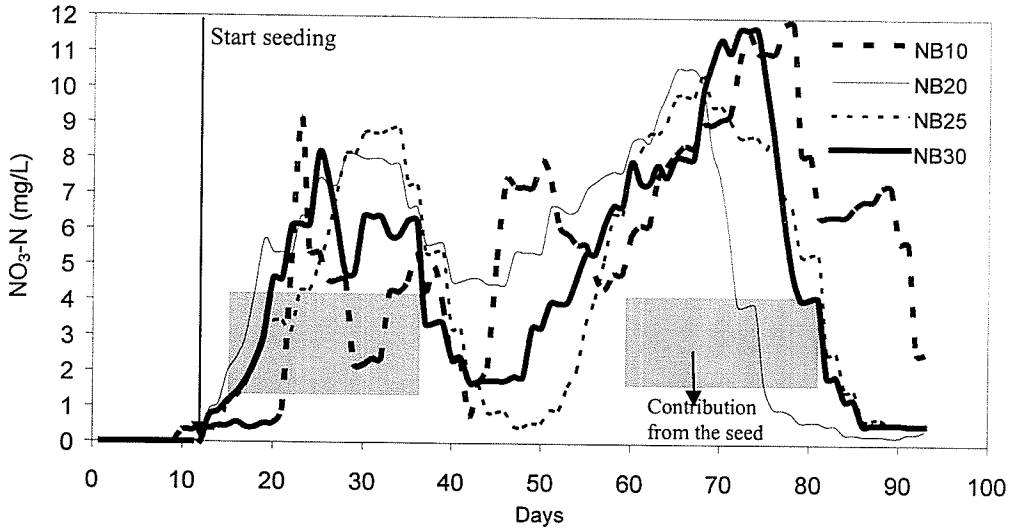


Figure 5.29 Effluent NO_3-N concentrations for SBRs at 10°C with HRT-12 h seeded with NB10, NB20, NB25 and NB30.

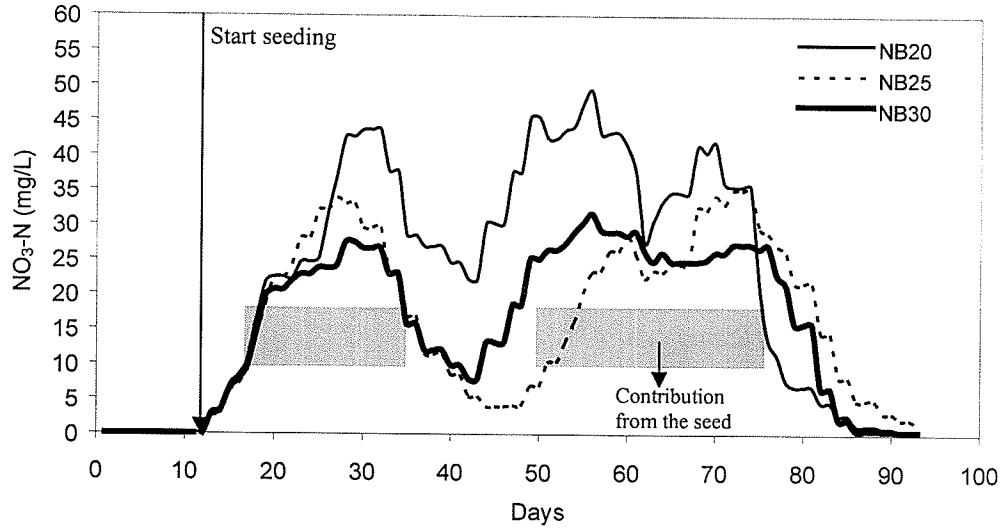


Figure 5.30 Effluent NO_3-N concentrations for SBRs at 10°C seeded with HRT-24 h seeded with NB20, NB25 and NB30.

There was no significant increase in effluent SCOD in the seeded SBRs as a result of seeding even though the seed liquors did contain elevated concentrations of SCOD (Table 5.7). The SCOD associated with the seed is not thought to cause any detrimental effect on the treatment system since the liquor is usually recycled to the front of the plant in untreated form.

Table 5.7 summarizes the steady-state parameters for the seeded SBRs that were used to determine seeded SRT. There was significant loss of solids in the decant liquors (X_e) which decreased the apparent SRT (θ_x^a) from the target of 4 days by 0.2 to 0.8 days. This loss of solids negates the benefit of seeding by decreasing the seeded SRT which will be discussed later. The mass of nitrifiers lost with decant liquors must be subtracted from the mass of seed added.

Table 5.7. Summary of observed and calculated seeded SBR characteristics during steady-state conditions.

		Seed Sources						
		HRT 24h			HRT 12 h			
Input parameters	Units	NB20	NB25	NB30	NB10	NB20	NB25	NB30
θ_x^a	d	3.40	3.42	3.38	3.80	3.30	3.35	3.24
S_o	mg NH_3-N/L	30.8±5.23	30.7±3.66	31.4±4.88	43.1±6.63	32.0±4.25	34.2±4.71	33.9±5.21
S	mg NH_3-N/L	18.7±3.30	26.0±6.85	28.4±4.04	28.3±5.58	31.2±4.02	32.4±3.49	33.7±4.33
X_a^o	mg VSS/L	5.02	4.3	3.5	2.5	2.6	2.15	1.75
X_r	mg VSS/L	412	409	369	638	650	714	585
X_e	mg VSS/L	34.3	36.4	31.0	24.6	28.1	35.9	36.7
Q^s	L/d	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Q^i	L/d	1.9	1.9	1.9	3.9	3.9	3.9	3.9
Q^e	L/d	1.5	1.5	1.5	3.66	3.5	3.5	3.5
Q^w	L/d	0.5	0.5	0.5	0.34	0.5	0.5	0.5
U	mg/g VSS*h	2.39	1.92	0.625	1.79	1.51	0.110	0.395

5.5.2.4 Discussion

In this study, nitrifying activity was always present within the cold SBRs as long as seeding was occurring. The nitrifiers were never completely washed

out of the system even though the system had an apparent SRT less than SRT_{min} for nitrification.

Even though NB10 was treating less than one half of the load entering the other 3 seed source reactors (NB20, NB25 and NB30) the SBR seeded with NB10 achieved the greatest NH_3-N removal. Nitrifying biomass acclimated to the temperature of the reactor into which they are to be seeded contributed the most nitrification potential when compared to nitrifiers acclimated to warmer temperatures.

A malfunctioning of the seed source led to accumulations of NH_3-N in the seeded reactors. This emphasizes the fragility of a seeded system operating at an apparent SRT less than SRT_{min}. The loss in nitrification in the seed source created two problems: a) a decrease in the amount of nitrifying bacteria available to be harvested as seed and b) a rapid rise in the concentration of NH_3-N that is associated with the seed liquor (greater than 300 mg/L on day 44 in the case of NB25). Disruption in the seed source, or lack of seed caused a rapid washout of the nitrifying bacteria and loss of nitrification activity in the reactors seeded with NB25 for approximately one week, while recovery from that incident took more than four apparent SRTs (16 days).

As the temperature difference between the seed and the seeded SBRs increased, the nitrification potential of the seed decreased. The SBR seeded with NB10 was able to achieve 5 times greater NH_3-N removal than the SBR seeded with NB30. The SBR seeded with NB10 was also more resistant to

washout as shown by the slow decrease in nitrification rate after the cessation of seeding. All of these factors suggest that NB10 had the highest growth rate of the four different seeds after addition to SBRs at 10°C.

5.5.2.5 Summary and conclusions

- Partial NH_3-N removal was achieved by seeding nitrifying bacteria acclimated to 20, 25 and 30°C into SBRs at 10°C when the HRT was 24 h. Partial NH_3-N removal was possible with seed acclimated to 10°C when the HRT was 12 h, while very little removal was evident for the seed acclimated to 20, 25 and 30°C. The doses of seed applied were not sufficient for full NH_3-N removal.
- The greater the temperature decrease experienced by the nitrifying seed the greater the decrease in nitrification potential. The order of treatment potential for nitrifying seed grown under the same operating conditions and seeded into reactors at 10°C was: NB10 > NB20 > NB25 > NB30.
- Continual and consistent seeding of nitrifying bacteria was necessary to maintain any degree of NH_3-N removal. Disruptions in the seed supply or cessation of seeding resulted in a rapid accumulation of effluent NH_3-N. Nitrification failure occurred at a faster rate as ΔT between the seed source and seeded reactor increased.

5.5.3 Seeding NB10 and NB20 into SBRs with HRT 8 h and SRT 12 d

5.5.3.1 Synthetic wastewater characteristics

The TCOD and NH_3-N concentrations of the synthetic wastewater at the time of feeding were 258 ± 34.2 mg/L and 23.1 ± 4.09 mg/L, respectively.

5.5.3.2 Seed characteristics (Appendix G-1)

The average concentration of NH_3-N in the centrate fed to NB10 and NB20 was 680 mg/L. NH_3-N removal in these two reactors was always greater than 98% (Figure 5.31). A summary of reactor conditions during seeding is listed in Table 5.8.

Table 5.8 Summary of nitrifying seed characteristics during seeding.

		Seed Temperatures	
Observed Parameter	Units	NB10	NB20
θ_x^a	d	12	5
S^o	mg NH_3-N/L	686±57.6	686±57.6
Mean effluent SCOD	mg/L	316 ± 46.2	203 ± 51.6
Mean effluent TCOD	mg/L	441	348
S	mg/L	3.3 ± 2.38	3.6 ± 1.49
X_r	mg VSS/L	161 ± 30.7	232 ± 42.3
Q^iX_r	mg VSS/d	2.68	3.86
Maximum dN/dt	mg NH_3-N/L*d	125	379
Calculations and Assumptions			
b	1/d	0.043	0.10
X_a^s	mg VSS/L	111	109
U	mg NH_3-N/g VSS*h	32.3	68.1
	mg NH_3-N/g nitrifiers*h	46.9	145

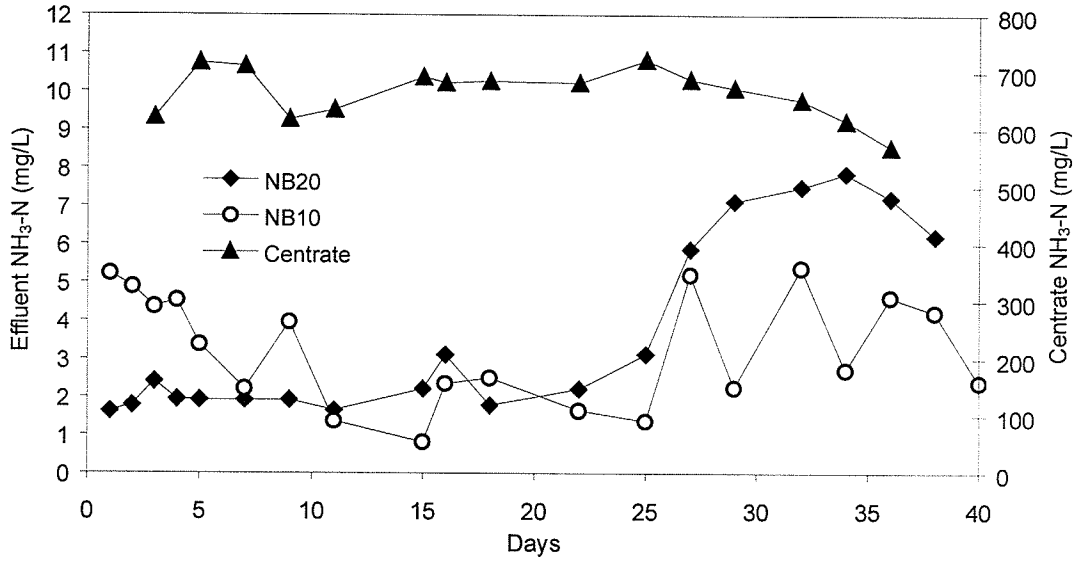


Figure 5.31 Influent and effluent NH_3-N concentrations for NB10 and NB20.

5.5.3.3 Results of seeded SBRs (Appendix G-2)

Both of the seeded SBRs achieved greater than 94% NH_3-N removal within 23 days of seeding (Figure 5.32 and 5.33). The nitrification rates increased at a faster rate in the SBR seeded with NB10. The rate of increase was 21.6 mg NH_3-N/d*d (R^2=0.937) and 16.6 mg NH_3-N/d*d (R^2=0.891) for the reactors seeded with NB10 and NB20, respectively. The maximum removal rates achieved were 188 mg/d for both reactors (complete removal).

Once seeding was stopped, the effluent NH_3-N in the reactor seeded with NB10 continued to decline and nitrification continued for the remainder of the study (Figure 5.32). In the reactor seeded with NB20, the effluent NH_3-N rose rapidly and the rate of NH_3-N removal decreased rapidly at a rate 16.6 mg NH_3-N/d*d (R^2=0.658). Partial nitrification was still achieved in the

reactor seeded with NB20 for 30 days as indicated by the depressed NH_3-N concentrations in the effluent (when compared to pre-seeding concentrations) and the presence of NO_3-N (Figure 5.33).

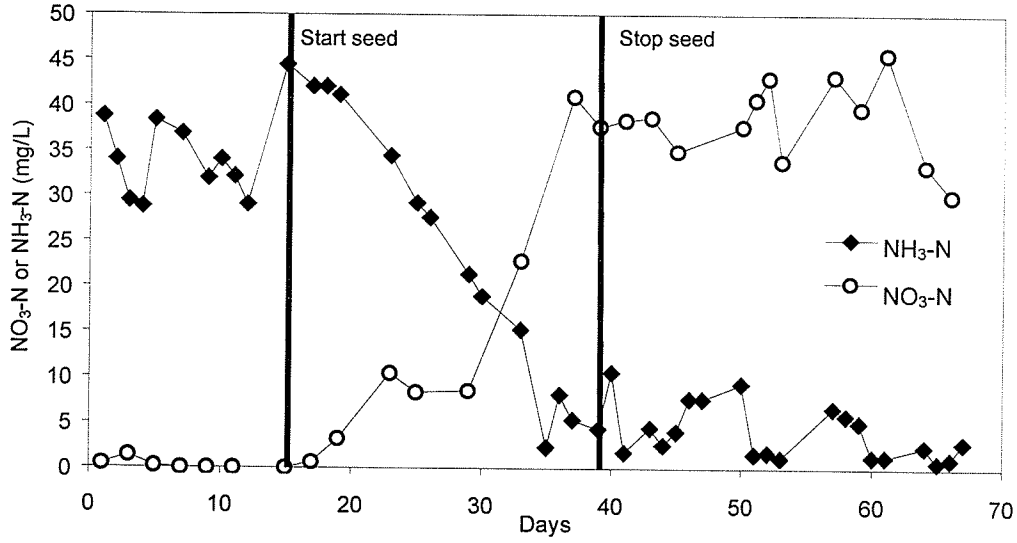


Figure 5.32 Effluent NH_3-N and NO_3-N for SBR seeded with NB10.

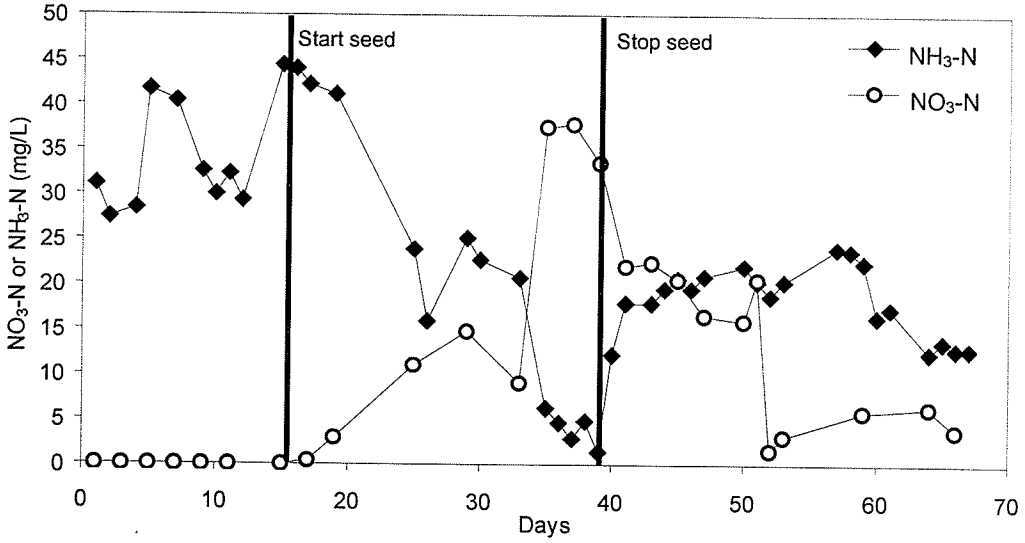


Figure 5.33 Effluent NH_3-N and NO_3-N for SBR seeded with NB20.

A summary of conditions for the determination of seeded SRT are listed in Table 5.9. In order to maintain an apparent SRT near 12 days, the volume of

mixed liquor wasted daily was adjusted to 0.1 L/d (one twentieth of the reactor volume) to compensate for the loss of solids with the effluent.

Table 5.9 Summary of observed and calculated seeded SBR characteristics during steady-state conditions.

		Seed source	
Input parameters	Units	NB10	NB20
θ_x^a	d	11.9	12.4
S_o	mg NH_3-N/L	34.3 ± 4.87	34.7 ± 6.55
S	mg NH_3-N/L	3.21 ± 1.26	3.4 ± 1.61
X_a^o	mg VSS/L	1.85	1.82
X_r	mg VSS/L	2037 ± 261	1966 ± 267
X_e	mg VSS/L	31.7 ± 13.7	25.3 ± 16.6
Q^s	L/d	0.1	0.1
Q^i	L/d	6	6
Q^e	L/d	5.9	5.9
Q^w	L/d	0.1	0.1

5.5.3.4 Discussion

Initially the biomass required to perform nitrification was not residing in either of the cold SBRs. With seeding, the required biomass was introduced and was able to oxidize NH_3 under the operating conditions provided. Once seeding was stopped the reactor seeded with NB20 began to experience nitrification failure within a few days but the reactor that was seeded with NB10 continued to have full nitrification (Figures 5.32 and 5.33). The effluent NH_3-N from the latter reactor actually decreased to a level lower than that achieved when seeding was taking place indicating that seeding was not required to maintain nitrifying bacteria within that system.

Abeysinghe *et al.* (2002) found that maintenance dosing of nitrifying bacteria was necessary in cases of extreme stress due to cold temperature and short

apparent SRT. In this study the stress of short apparent SRT was reduced by operating the reactor near SRT_{min} but maintenance dosing was required to maintain nitrification when NB20 was added to the cold SBR. The NB10 required no maintenance dosing; only the introduction of the right kind of biomass to initiate nitrification.

Previous results showed that nitrifying biomass grown at 20°C experienced a decrease in nitrification rate of 58% when exposed to 10°C. Therefore, it was assumed that the SRT of the seeded reactor would have to be at least 12 days (2.4 times longer than the original SRT of 5 days). It was found in this study that an apparent SRT of 12.4 days was not sufficient to prevent washout of NB20.

The rapid increase in effluent NH_3-N from the reactor seeded with NB20 cannot be completely attributed to washout due to a slow growth rate. The increase and decrease in nitrification rate with the initiation and cessation of seeding were equal. This indicates that nitrifiers were being lost almost as fast as they were being added. Nitrification failure in the reactor seeded with NB20 could be the result of preferential washout of nitrifying bacteria with the effluent stream. Preferential washout would occur if the nitrifying biomass itself was not settling well or not failing to be captured within the sludge floc during settling. If the nitrifiers in the effluent were in a higher proportion than the nitrifiers in the reactor, the mass balance for the apparent SRT calculation would not take this into account.

5.5.3.4 Summary and conclusions

- Full nitrification was achieved in SBRs at 10°C with and apparent SRT of 12 d when nitrifying seed acclimated to 10 and 20°C was added. Greater than 94% NH_3-N removal was achieved within 23 days for both of the seeded SBRs.
- The initial growing conditions of the seed dictated the speed at which nitrification failed after seeding was stopped. Cessation of seeding for 30 days resulted in partial loss of nitrifying activity from the reactor that was seeded with NB20. Partial nitrification was apparent from small quantities of NO_3-N in the effluent. Washout did not occur after 30 days for the reactor that was seeded with NB10.
- The nitrifiers acclimated to 20°C did not have a growth rate sufficient to maintain nitrification even though the apparent SRT of 12 days at 10°C was 2.3 times longer than their original conditions of SRT 5 days.

5.6 Microbial analysis using fluorescence *in situ* hybridization

5.6.1 Results for Seed Source Reactors: NB10 and NB20 (Appendix H-1)

During steady state operating conditions (consistently greater than 98% NH_3-N removal), the seed source reactors were sampled to determine the proportion of ammonia oxidizing bacteria (AOB) in the total biomass. Probe area is expressed as a percentage of the total biomass as measured by DAPI where DAPI is a stain that labels all DNA. Using the probe Nso1225, an

average of 17.9 ± 11.5 % of the biomass in NB10 was labeled while an average of 9.3 ± 6.98 % of the biomass was labeled in NB20 (Figure 5.34). The differences in area labeled for NB10 and NB20 were statistically significant (t-test, Appendix H-1).

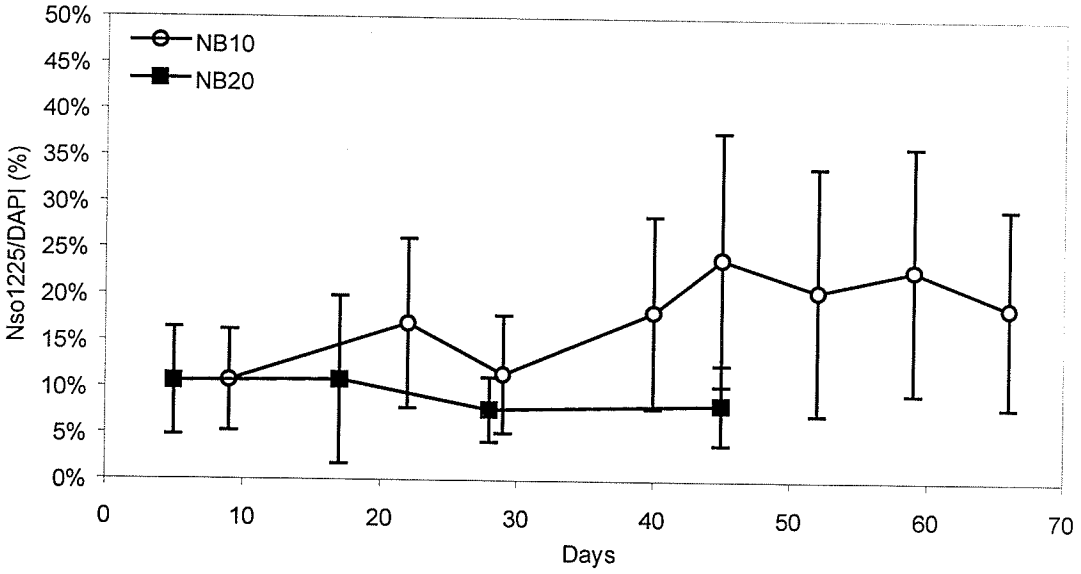


Figure 5.34 Percent Nso1225 of total area stained by DAPI for NB10 and NB20.

5.6.2 FISH analysis of SBRs seeded with NB10 and NB20 with SRT 12 d and HRT 8 h (Appendix H-2)

The Nso1225 signal corresponded well with NH_3-N decreases and NO_3-N increases in the effluent for both reactors (Figures 5.35 and 5.36). The reactor seeded with NB10 had an increase in AOB with the initiation of seeding as shown by the increase in area labeled by the probe Nso1225 (Figure 5.35). When seeding was stopped for this reactor there was a slight decrease in Nso1225 signal but effluent NH_3-N remained low. After seeding was stopped for 30 days, effluent NH_3-N concentrations did not increase and the Nso1225

signal remained high, indicating that AOB washout did not occur. For the SBR seeded with NB20, increases and decreases in Nso1225 signal mirrored the NH_3-N concentrations in the effluent (Figure 5.36).

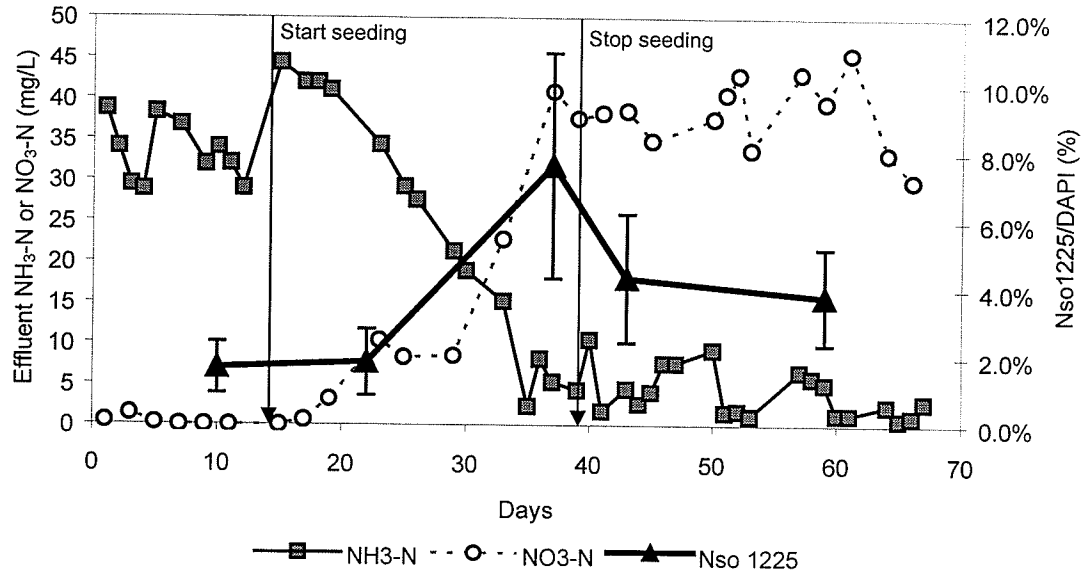


Figure 5.35 Effluent NH_3-N, NO_3-N and mixed liquor AOB proportion for an SBR seeded with NB10. (HRT=8 h and SRT=12 d)

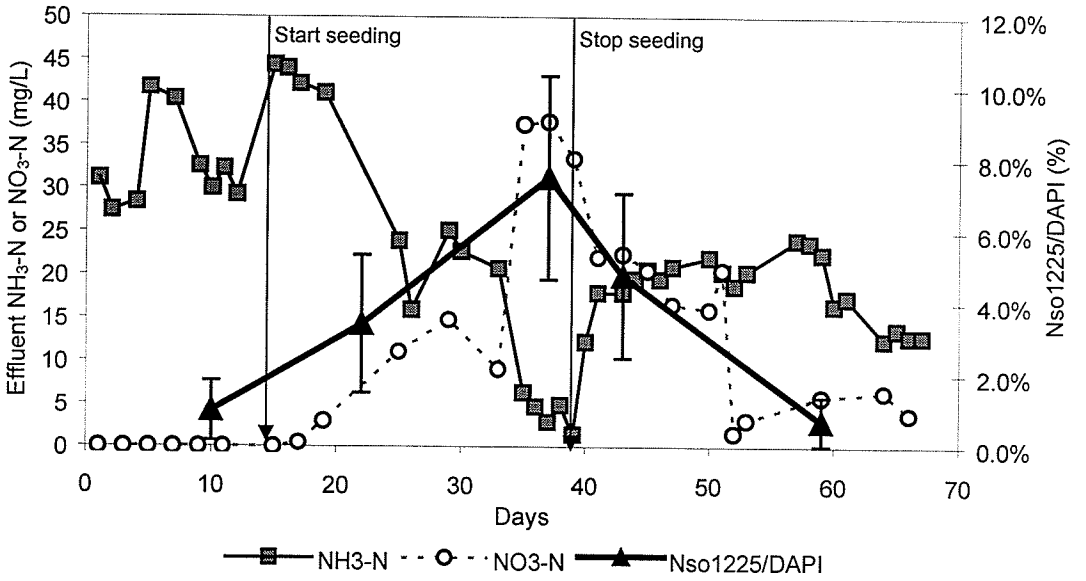


Figure 5.36 Effluent NH_3-N and mixed liquor AOB proportion for an SBR seeded with NB20. (HRT=8 h and SRT=12 d)

The Nso1225 probe signal was compared to the effluent NH_3-N, NO_3-N and NH_3-N removal rates for the SBRs at the time of sampling (Table 5.10). The area of cells stained with DAPI was relatively constant for all sampling periods. The reactor seeded with NB10 contained up to 7.6% AOB by area while the reactor seeded with NB20 contained up to 7.5%.

Table 5.10 Oligonucleotide and staining data for seeded SBRs at 10°C with HRT 8 h and SRT 12 d.

	Day	DAPI* (%)	Nso1225† (%)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	dN/dt (mg NH_3-N/L*d)
Seeded with NB10	10	3.62± 1.22	1.68± 0.75	34	0	1.39
	22	3.47± 1.17	1.85± 0.97	34.4	10.4	12.7
	37	5.00± 4.77	7.64± 3.32	5.3	40.9	160.7
	43	2.70± 1.25	4.32± 1.89	4.47	38.6	134
	59	3.06± 0.712	3.78± 1.41	5.04	39.6	132
Mean±St. Dev.		3.62± 0.88				
Correlation Coefficients§				0.507	0.643	0.763
Seeded with NB20	10	4.88± 2.33	1.00± 0.84	30.0	0	3.1
	22	2.82± 0.928	3.42± 1.92	32.5	7.0	10.7
	37	3.36± 1.47	7.51± 2.85	2.88	37.8	132.7
	43	2.71± 1.45	4.77± 2.30	17.9	22.4	75.6
	59	4.85± 2.27	0.71± 0.68	22.4	5.8	55.3
Mean±St. Dev.		3.72± 1.07				
Correlation Coefficients				0.615	0.901	0.627

* The percentage area stained by DAPI was determined by taking the average number of pixels stained and dividing by the total number of pixels in each photograph (total pixels per photograph = 2 150 400).

† The percentage of Nso1225 coverage was determined by taking the area covered divided by the area covered by DAPI to give percent biomass bound by the probe.

§ Correlation coefficients were determined by linear regression of the FISH signal and either NH_3-N, NO_3-N or dN/dt at the time of sampling.

Biesterfeld *et al.* (2002) used FISH to track nitrifying bacteria activity in a nitrifying trickling filter where tracking was defined as a linear correlation or R^2 greater than 0.5. By their definition, our FISH data for Nso1225 signal can be used to predict nitrification rates in seeded SBRs. However, the

nitrification potential of each seed type has to be determined first and then the correlation determined.

5.6.3 FISH analysis of SBRs seeded with NB10 and NB20 with SRT 4 d and HRT 12 h (Appendix H-3)

The SBRs operated with an HRT of 12 hours and SRT of 4 days failed to achieve significant levels of NH_3-N removal (Figures 5.37 and 5.38) despite the daily addition of AOB. It was earlier suspected that the AOB were being wasted from the system inadvertently with the effluent in higher proportions than was present in the reactor contents. Because of this suspicion, effluent samples were collected and examined by FISH analysis.

FISH showed that the effluent from the reactor seeded with NB10 had up to 5 times more AOB in the effluent than in the reactor biomass (Figure 5.37). The effluent from the reactor with NB20 had up to 4 times more AOB in the effluent than in the reactor biomass (Figure 5.38). The proportion of AOBs in the effluent was found to be significantly higher than the proportion in the reactor for each seed source (t-test, Appendix H-3). AOB loss with the decant liquor could not be accounted for in the original total solids balance without further microbial analysis.

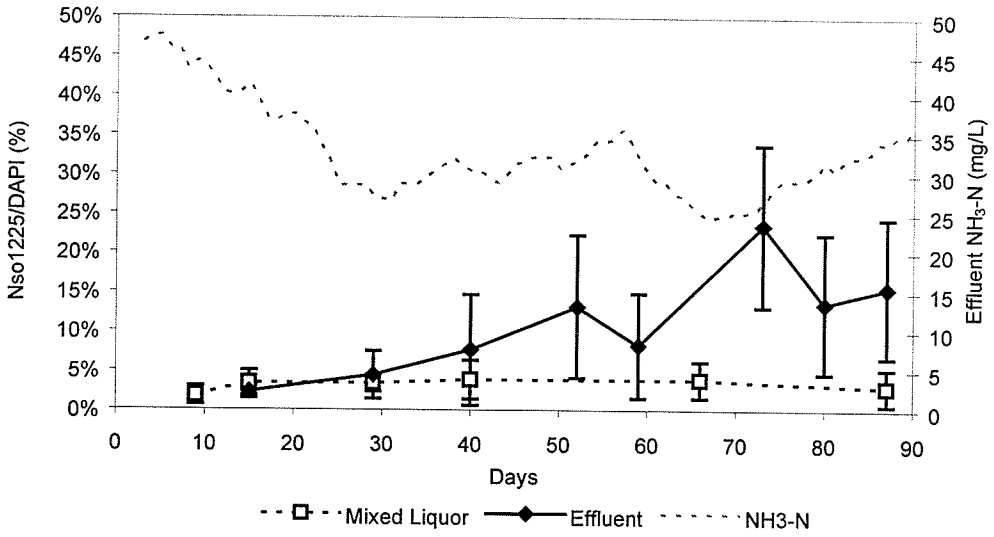


Figure 5.37 Percentage of biomass labeled with Nso1225 in the reactor mixed liquor and effluent solids for SBR seeded with NB10.

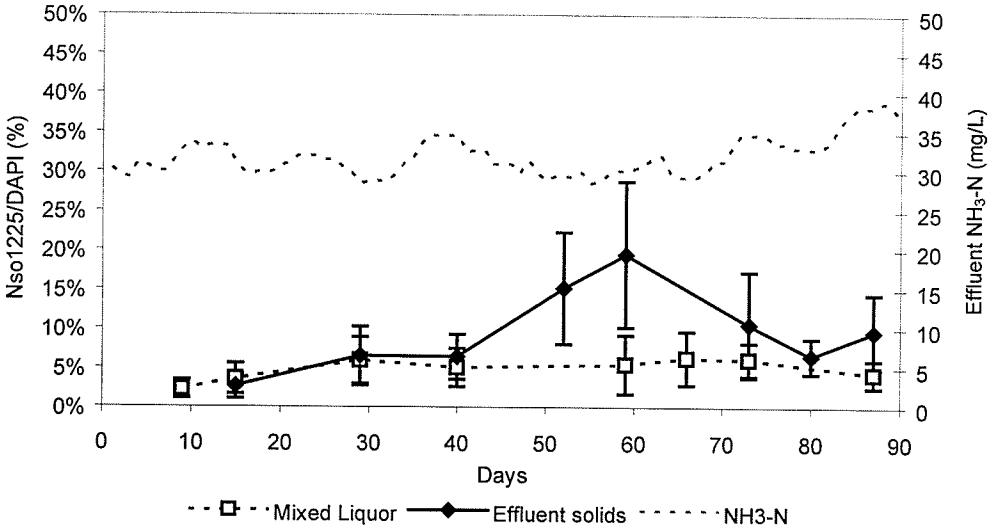


Figure 5.38 Percentage of biomass labeled with Nso1225 in the reactor mixed liquor and effluent solids for SBR seeded with NB20.

5.6.4 Discussion

FISH analysis was found to be an effective method for observing AOBs in reactors treating centrate and in reactors that were seeded with nitrifying biomass. FISH analysis showed that a reactor treating centrate at 10°C could produce a higher proportion of AOBs to biomass than a reactor treating

centrate at 20°C. The degree of difference in AOB proportion between these reactors was not expected since it was calculated earlier that these reactors should have approximately the same concentration of nitrifiers (X_a^s). This observation can further explain the differences in effluent SCOD from the seed source reactors (Table 5.6). The particulate matter in centrate is made up of solids that were not captured during the dewatering of anaerobically digested sludge. These particles are then exposed to aerobic treatment conditions during centrate nitrification; *i.e.*, NB10 was operated with an SRT of 12 d while NB20 was operated with an SRT of 5 d. It was suggested earlier that there could be increased solubilization of solids with a longer retention time. These particles could have contained DNA that was labeled by DAPI stain during FISH analysis. Because NB10 could contain less residual particles due to increased solubilization, a higher proportion of AOB relative to the total area of DAPI may have been labeled. This is further supported by the concentration of solids (X_r) in each of the reactors (Table 5.5 and 5.9). NB10 contained a lower concentration of solids than NB20 but NB10 contained a higher concentration of SCOD (Table 5.5).

Because the concentration of solids in both the seed sources and the seeded SBRs were constant over time, relative area determination was a good choice for comparing AOB population over time. Initially, the reactors operating with an SRT of 12 d and HRT of 8 h did not contain AOB that were suitable for growth in the conditions that were provided. This was verified by a

relatively low Nso1225 signal (<2%) . But full nitrification was achieved in the SBRs when NB10 and NB20 were added as seed. FISH analysis showed that the proportion of AOBs in the reactor increased as a result of seeding and there was a good correlation with effluent NH_3-N, NO_3-N and nitrification rates (Table 5.10).

The probe Nsm156 was also used to determine the presence of *Nitrosomonas spp.* in the SBRs with SRT-12 d and HRT-8 h. Nsm156 signal was always less than 2% of the total area stained. This indicates that *Nitrosomonas* was not the major AOB present but some other AOB of the β subclass of proteobacteria. This might include *Nitrosolobus, Nitrosospira* or *Nitrosovibrio spp.* These findings are in agreement with other researchers who found that *Nitrosomonas* is not the major AOB in wastewater treatment systems (Biesterfeld *et al.*, 2001; Jusetschko *et al.*, 1998). However, it has also been suggested that *Nitrosococcus mobilis* of the γ subclass of proteobacteria is a dominant AOB in some wastewater treatment systems (Juretschko *et al.*, 1998). This species was not examined in our reactors.

For the SBRs with an SRT of 4 d and HRT of 12 h, FISH analysis suggested that that poor seeding results were due to inadvertent AOB wash out with the decant liquors. It showed that the proportion of AOB in the effluent solids was higher than that in the reactor mixed liquor solids. The loss of AOB was likely due to the poor settling properties of the seeded nitrifying biomass and failure of the AOBs to be incorporated into, or captured by, the sludge floc

during settling. In order for seeding to be successful, the AOBs would have to be maintained in the reactor by using a physical barrier such as membrane filtration or by the use of a carrier material like foam blocks. Other methods might include improving settling properties by the addition of WAS, primary sludge or another carbon source.

5.6.5 Summary and conclusions

- In the seed source reactors treating centrate, NB10 had a higher proportion of AOB to total labeled biomass when compared to NB20.
- Nso1225 signal correlated well with effluent NH_3-N and NO_3-N concentrations and with nitrification rates in the seeded SBRs with SRT-12 d and HRT-8 h. Low Nsm156 labeled area showed that *Nitrosomonas* was not the major AOB in the system but likely some other AOB of the β subclass of proteobacteria.
- For reactors operated with an apparent SRT of 4 d and HRT of 12 h, FISH analysis showed that the proportion of AOB in the effluent solids was greater than that in the reactor. Calculating seeded SRT based on a solids balance would not take this into consideration and would thus be overestimated.

5.7 Computer modeling using BioWin™

5.7.1 Feed centrate 8 h/d, 5 d/wk

Traditionally, centrate is recycled to the front of a treatment plant as it is produced. As a baseline for further modeling, a simulation was conducted to determine the effluent quality for treatment plants that recycle centrate for 8 hours per day 5 days per week. Figure 5.39 is an example of effluent NH_3-N levels for a BNR plant that recycles centrate in this manner while Figure 5.40 depicts the effluent for a non-nitrifying treatment plant with the same centrate feed pattern. Firstly, the peak effluent NH_3-N is decreased by 50 to 70% by increasing the SRT from 4.5 d for the non-nitrifying plant to 12 d for the BNR plant. The increased SRT in the BNR plant allows nitrifying bacteria to be maintained within the system and nitrification to occur.

During weekdays, the centrate NH_3-N load corresponds with high NH_3-N loads in the main-stream influent. On the weekends the centrate load is eliminated and improved effluent quality is achieved in both types of treatment plants.

The "wedding cake" flow pattern is also visible in Figures 5.39 and 5.40. In the BNR plant (Figure 5.39) effluent NH_3-N is increasing as the flow increases from day 27 to the peak flow on day 45. This increase is due to nitrifying bacteria being washed from the system with the higher influent flows coupled with a shorter amount of time for nitrification to take place. In the non-nitrifying treatment plant, the effluent NH_3-N is decreasing over the

similar time period due to dilution of the NH_3-N load by increased influent flow. Similar patterns will be seen in many of the simulations to follow.

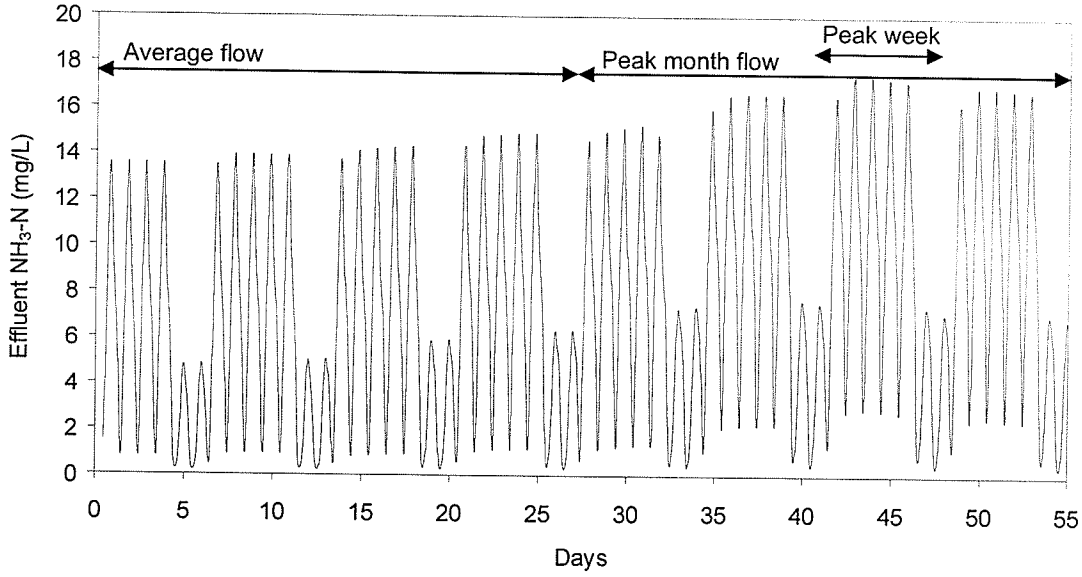


Figure 5.39 Effluent NH_3-N for a BNR plant that is fed centrate 8 hours/day, 5 days/week.

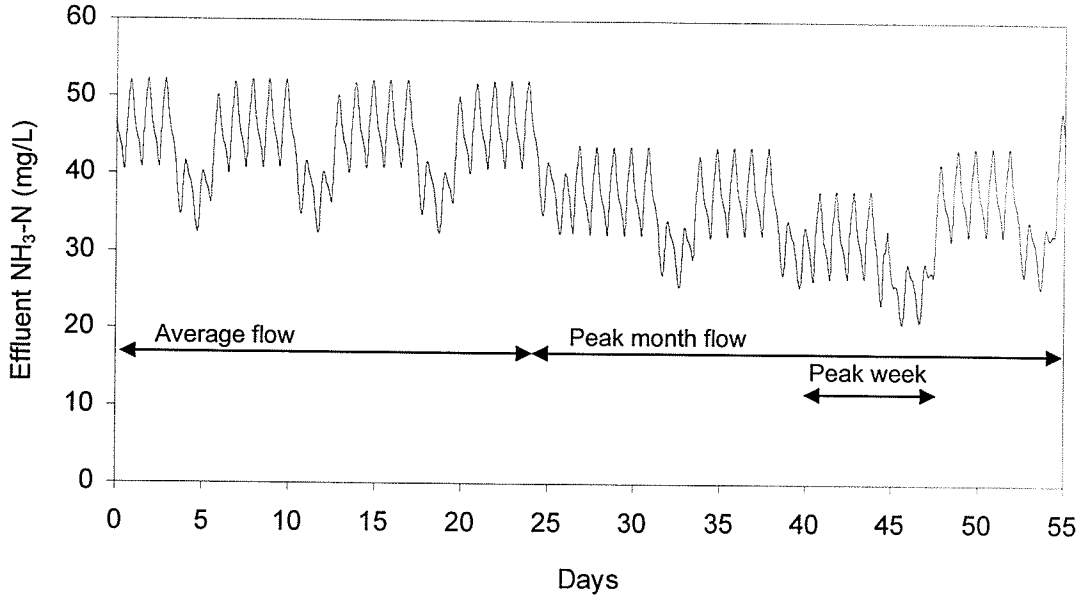


Figure 5.40 Effluent NH_3-N in a non-nitrifying treatment plant fed centrate 8 hours/day, 5 days/week.

5.7.2 Ammonia removal from centrate

Figures 5.41 and 5.42 are the results of modeling the effects of removing NH_3 from the centrate in a side-stream before its return to the main-stream. Similar outcomes would be expected for physical, chemical or biological methods of NH_3 removal. These processes might include ammonia stripping, chemical precipitation or nitrification without biomass recycling. As a result, the peak effluent NH_3-N concentration was decreased by approximately 30% in the BNR plant while it was decreased by 25% in the non-nitrifying plant

In Figures 5.41 and 5.42 "weekday/weekend" effects of centrate feeding are eliminated because the NH_3 load from the centrate is completely removed. The variability in effluent NH_3 is only due to high and low diurnal loads.

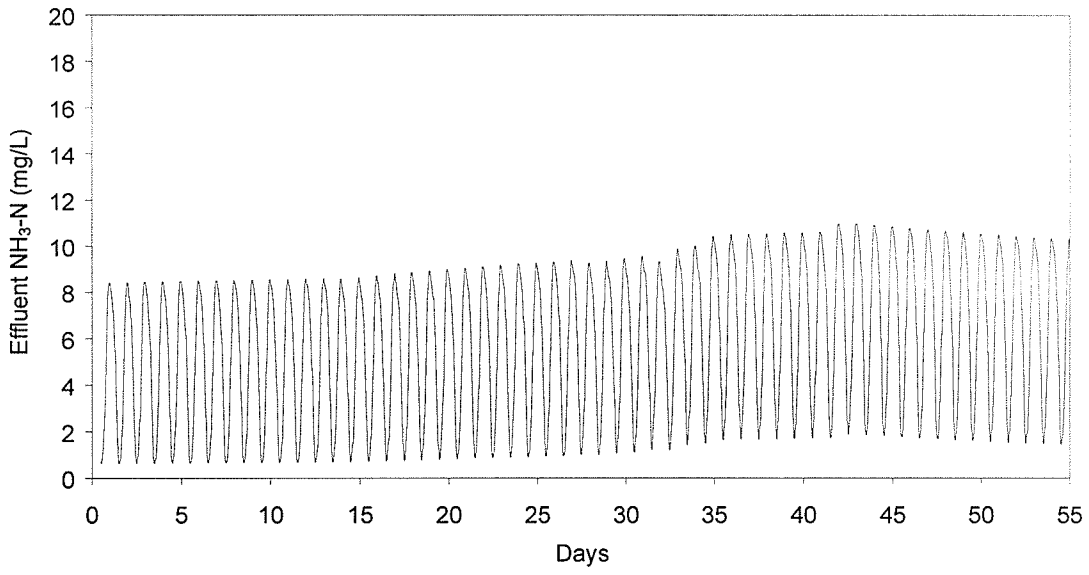


Figure 5.41 Effluent NH_3-N for a BNR plant with NH_3 removal from centrate prior to recycling back to the main-stream.

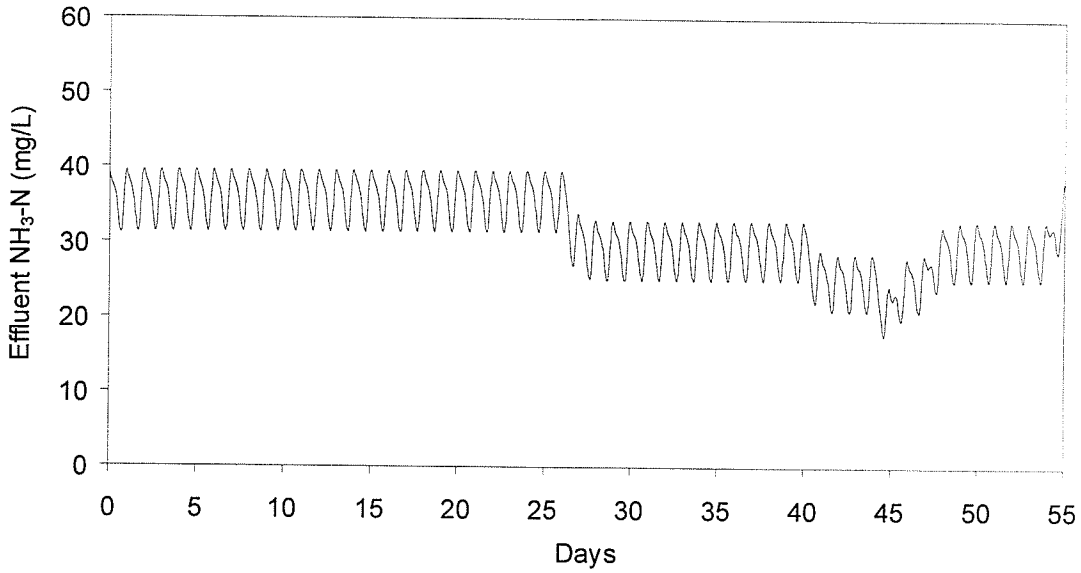


Figure 5.42 Effluent NH_3-N for a non-nitrifying treatment plant with NH_3 removal from centrate prior to recycling back to the main-stream.

5.7.3 Feeding centrate during low ammonia loads

Centrate can be used as an NH_3 substrate supplement to create a more stable supply of substrate for nitrifying bacteria and to produce a more consistent effluent quality; diurnal variations in NH_3 concentration are virtually eliminated. This option decreased the peak effluent NH_3-N concentration by about 45% in the BNR plant (Figure 5.43) when compared with conventional feeding practices (Figure 5.39). By feeding the centrate NH_3 during low influent NH_3 loads, the peak effluent NH_3-N concentrations were decreased by approximately 15% in the non-nitrifying plant (Figure 5.44) when compared with feeding centrate 8h/d, 5d/wk.

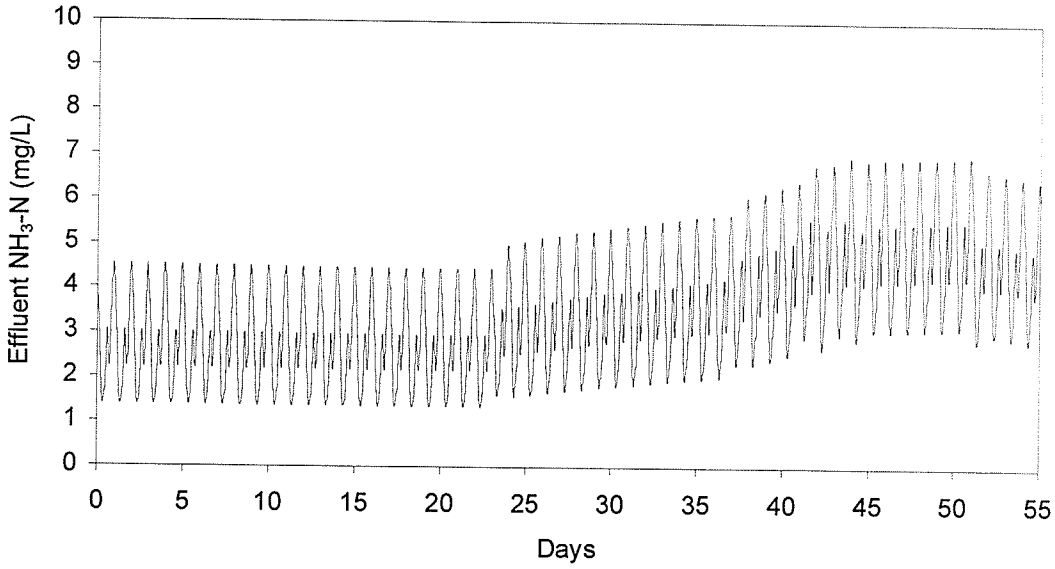


Figure 5.43 Effluent NH_3-N from a BNR plant that is fed centrate only during low NH_3-N loads.

In the BNR plant, the minimum NH_3-N concentration in the effluent was increased by 2 to 3 mg/L when using centrate as a NH_3-N supplement. This is due to the elimination of extremely low loads that would normally cause very low NH_3-N concentrations in the effluent.

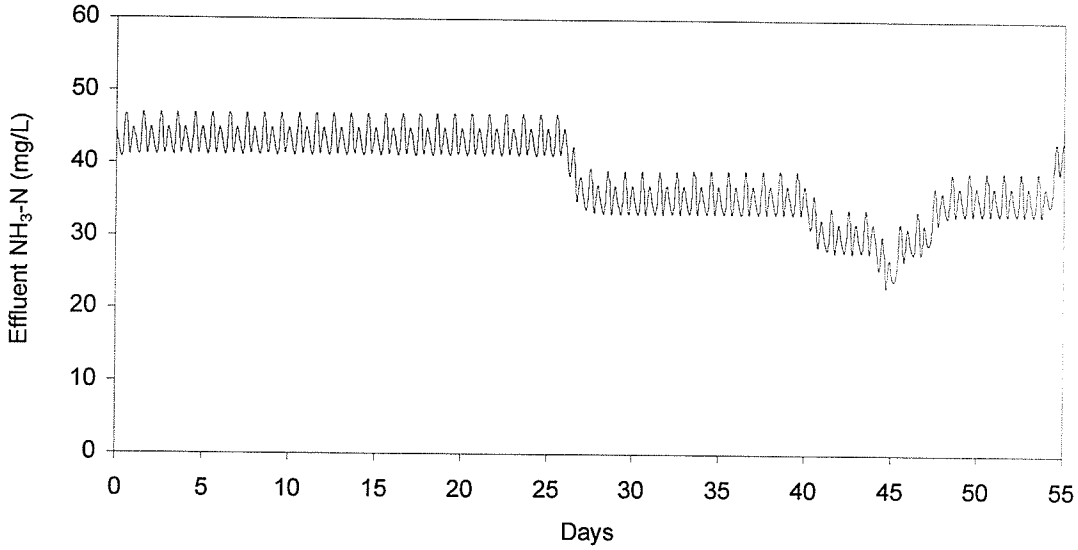


Figure 5.44 Effluent NH_3-N in a non-nitrifying treatment plant fed centrate only during low NH_3-N loads.

5.7.4 Centrate fed continuously, 24 hours per day

Continuous addition of centrate produced the same effluent quality as NH_3 removal from centrate for the BNR plant. Because, in the model, the concentration of nitrifiers is directly proportional to the NH_3 load, the NH_3 oxidation rate per unit volume increases with increased concentrations of nitrifiers. Therefore, a BNR plant currently feeding centrate at a constant rate would not benefit from removing NH_3 from the centrate before recycling it back to the main-stream tanks. In the BNR system, feeding centrate continuously guaranteed a food source for nitrifiers and maintained a greater concentration of nitrifiers than removing the NH_3 from the centrate before recycling. Peak effluent NH_3-N concentrations were decreased by 30% when compared to traditional centrate management (Figure 5.39).

In the non-nitrifying system (Figure 5.45), feeding centrate continuously decreased the peak effluent NH_3-N concentrations by approximately 10% when compared with Figure 5.40. Peak effluent NH_3-N concentrations were less exaggerated than when centrate was recycled to the main-stream only during the day.

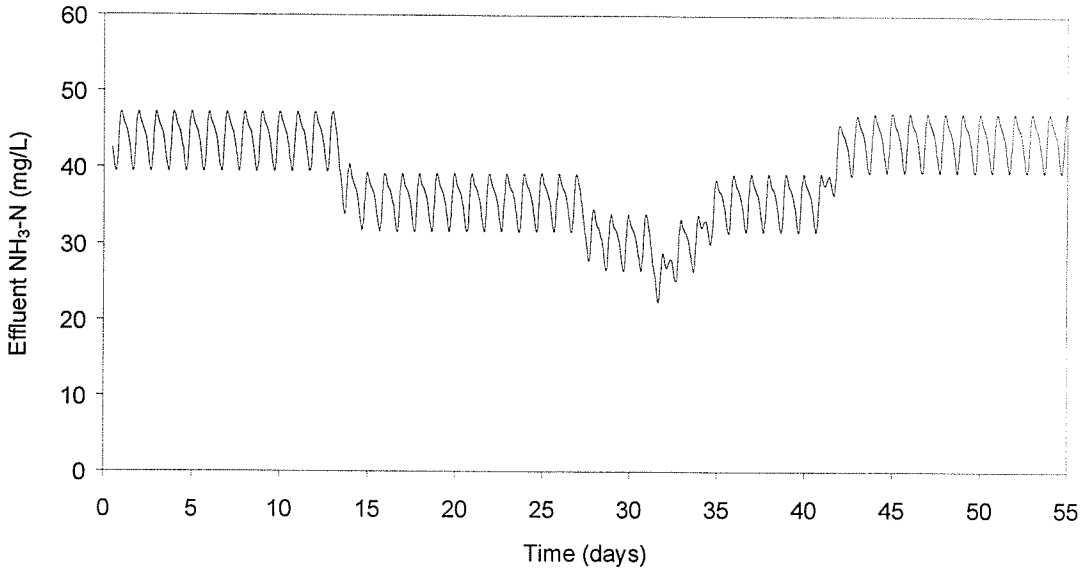


Figure 5.45 Effluent NH_3-N in a non-nitrifying treatment plant fed centrate continuously (equalized centrate flow).

5.7.5 Centrate nitrification for the production of nitrifying seed

5.7.5.1 Determining the amount of nitrifying seed that can be produced

The reactors treating centrate were simulated to estimate the concentration of nitrifiers that could be produced for seed. The operating conditions of the laboratory reactors and the input parameters for centrate characteristics were described previously (Table 4.5). The concentration of nitrifiers in the reactors is independent of the growth rate (μ), thus the growth rate input to the model need only be high enough to achieve the observed level of NH_3-N removal in the laboratory reactors treating centrate. The predicted concentrations of nitrifying bacteria (X_a^s) in each of the seed sources are shown in Figure 5.46.

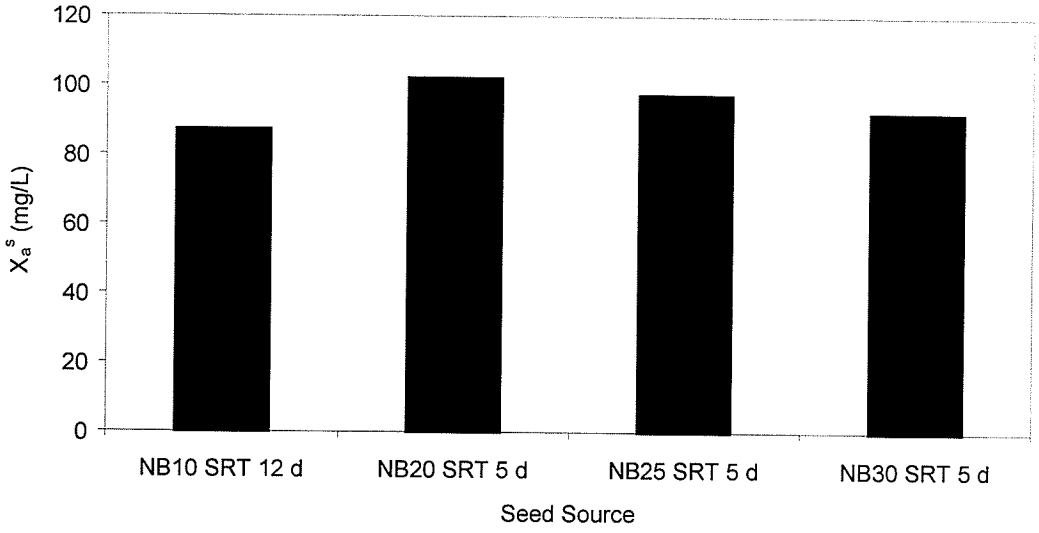


Figure 5.46 Model output for concentration of nitrifiers in the seed source reactors. (Y=0.24 g/g, b=0.1 $e^{0.0844(T-20)}$, S^o=650 mg NH_3-N/L)

The concentration of nitrifiers in each of the systems does not differ substantially. However, the decay rate increases as the temperature increases. Even though NB10 is treating an NH_3-N load 60% smaller than the other seed sources†, the concentration of nitrifiers in the seed sources differs by less than 15 mg/L. This means that NB10 can generate 2.4 times more nitrifiers than NB20 for the same mass of NH_3-N nitrified.

Using the data from Table 5.5, the proportion of nitrifying bacteria to VSS in NB10 and NB20 were calculated to be 69% and 34%, respectively (two times as much). These calculations for X_a^s are consistent with the FISH data that showed NB10 contained a proportion of AOBs two times greater than NB20. However, the absolute values for the proportion of AOBs determined by FISH were much lower than those shown in Figure 5.46.

†NB10 had an SRT and HRT of 12 d while NB20, NB25 and NB30 had an SRT and HRT of 5 d. Therefore, the load to NB10 was 60% smaller than the other seed sources.

5.7.5.2 Using nitrified centrate as a seed source

In a typical wastewater treatment plant, the centrate flow is expected to be 1 to 2% of the total influent flow. The nitrifiers from the side-stream reactor will be diluted approximately 100 times as the stream is added to the main-stream influent line. Based on the amount of nitrifies produced in the seed sources, the concentration of nitrifiers in the influent stream (X_a^o) was calculated to be 1.0 mg/L.

Figures 5.47 and 5.48 show the impact of seeding nitrifiers continuously into a main activated sludge tank with X_a^o equal to 1.0 mg/L and the model's default kinetic parameters. In the BNR plant, the peak effluent NH_3-N concentration is reduced by more than 65% when compared to feeding centrate 8 h/d, 5d/week (Figure 5.39) and by approximately 50% when compared to simply removing NH_3-N from the centrate (Figure 5.41). The benefits of treating centrate from a BNR plant were only realized when the centrate was used to produce a nitrifying biomass. In the non-nitrifying system the effluent NH_3-N is reduced by 60% and 50% when compared to these two methods of recycling, respectively.

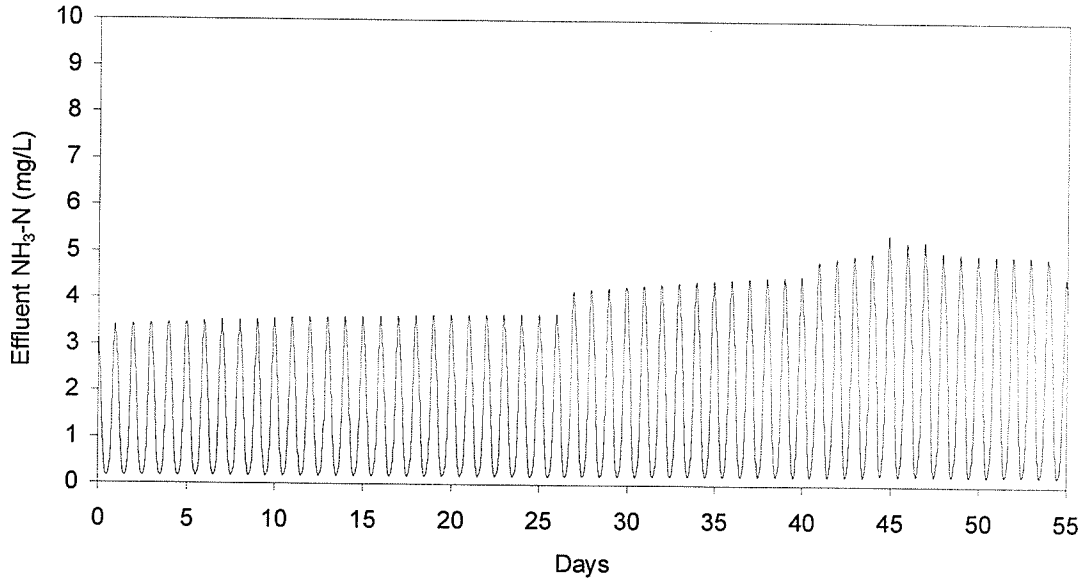


Figure 5.47 Effluent NH_3-N for a BNR plant seeded with 1.0 mg/L nitrifier produced from the nitrification of centrate.

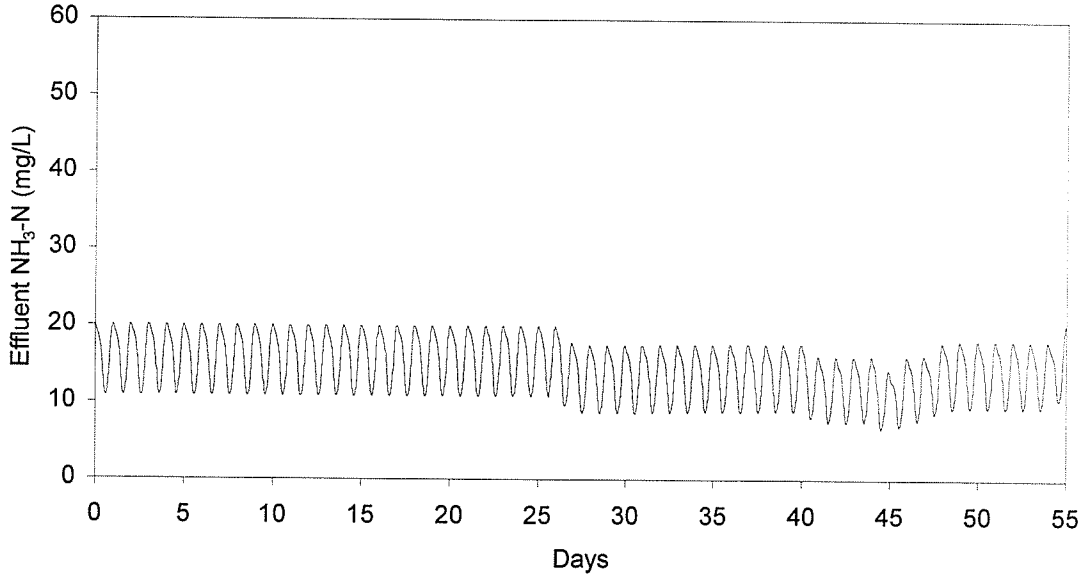


Figure 5.48 Effluent NH_3-N in a non-nitrifying treatment plant seeded with 1.0 mg/L nitrifiers produced from the nitrification of centrate.

In the BNR plant, the indigenous nitrifier population is augmented such that a higher concentration of nitrifiers is present than if the raw centrate was recycled directly (data not shown). This is due to NH_3 being used to produce nitrifier mass (autotrophs) in the side-stream rather than being consumed by

heterotrophic assimilation in the main-stream. Heterotrophs consume organic carbon and divert NH_3 away from nitrifiers for use in building cell mass. This phenomena was observed by de Silva and Rittmann (1999) where nitrifying biomass decreased proportionally to the increase in COD:TKN ratio. Hanaki *et al.* (1990) also found that assimilation by heterotrophs reduced the NH_3 available for nitrification.

In the non-nitrifying treatment plant, recycling the nitrifiers induces nitrification where none existed previously. The continuous addition of nitrifiers maintains some level of nitrification even though the main activated sludge system apparent SRT is too short to otherwise sustain nitrification. This process shows potential for application at the NEWPCC in Winnipeg.

5.7.6 Summary and conclusions

Managing centrate as a separate stream offers flexibility for specialized treatment and can be operated on an as needed basis to meet specific treatment goals. BioWin was used to determine the impact of centrate on two types of wastewater treatment systems: a non-nitrifying, BOD removing plant and a BNR plant. BioWin showed that centrate management can substantially improve effluent quality by decreasing peak NH_3-N concentrations in non-nitrifying plants and stabilizing effluent quality in BNR plants. Full centrate treatment may not be necessary depending on the desired level of treatment.

The greatest improvement in effluent quality for both types of plants occurred when the centrate was used to produce a nitrifying biomass that could be used as seed for the main-stream tanks.

5.8 Integration of model and laboratory data

5.8.1 Implications of inadvertent nitrifier loss with decant liquors

FISH analysis showed that nitrifiers were being lost from the laboratory reactors with the decant liquor. When calculating seeded SRT, a simple solids balance could not account for this loss. Due to the results of FISH analysis, an additional parameter should be incorporated into the seeded SRT determination. The term P, as defined by Equation 20, acknowledges that the proportion of nitrifiers in the effluent may be different from that in the reactor. As a result, Equation 8 then becomes Equation 21.

$$P = \frac{X_a^e / X_e}{X_a / X_r} \qquad [20]$$

$$\theta_x^s = \frac{X_a V}{Q^w X_a + Q^e P X^e - Q^i X_a^o} \qquad [21]$$

The seeded SRTs of the SBRs were then determined by simultaneous calculation of Equations 9, 20 and 21. The seeded SRTs were "hand calculated" to determine the impact of inadvertent wasting of nitrifiers. Figure 5.49 shows the impact of P on the estimation of seeded SRT for SBRs seeded with NB10 and NB20.

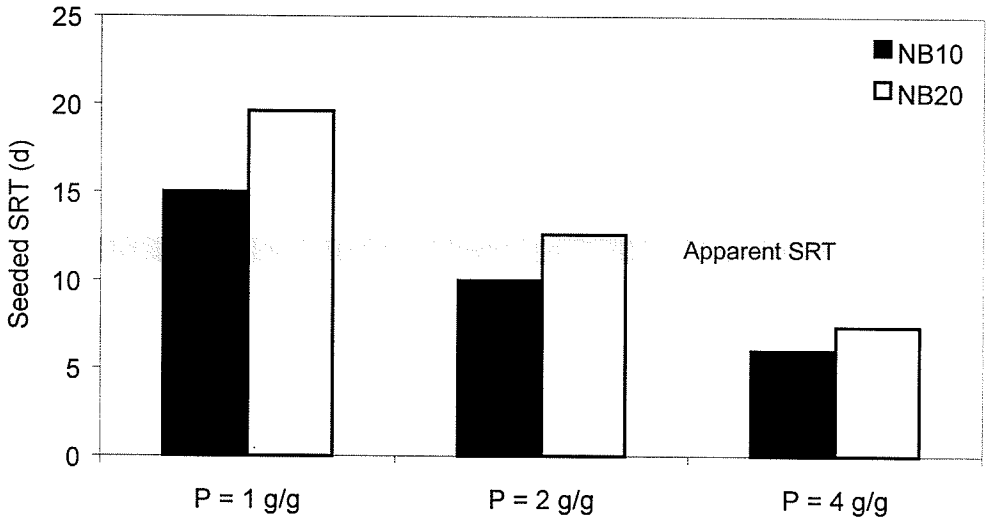


Figure 5.49 The effect of P on the estimated seeded SRT for SBRs seeded with NB10 and NB20 (HRT = 8 h, T = 10°C, S = 3.0 mg NH_3-N/L, Xe = 31.6 mg/L for NB10 and Xe = 25.3 mg/L for NB20) (data from Table 5.10).

The original temperature of seed dictates the seeded SRT required to achieve a desired effluent NH_3-N concentration. NB20 requires a longer seeded SRT than NB10 (Figure 5.49) because NB20 experiences a large decrease in growth rate upon exposure to 10°C.

When the proportion of nitrifiers in the effluent solids is the same as that in the reactor (P = 1 g/g), the seeded SRT will always be greater than the apparent SRT. However, if the proportion of nitrifiers in the effluent solids is increased, the seeded SRT can be shorter than the apparent SRT (Figure 5.49). The mass of nitrifiers lost with the decant liquor could exceed that added as seed. This is a particularly important consideration in systems operating near SRT_{min} for nitrification and short HRTs. In this case, the concentration of nitrifiers in the reactor becomes quite high due to seeding and growth making high proportions of nitrifiers in the effluent possible. Continuously

losing high quantities of nitrifiers with the effluent negates the benefits of seeding and would not be a sustainable nitrification system.

Effluent NH_3-N will continue to decline as long as the mass of nitrifiers added to the system is greater than that removed with the waste stream or the effluent. When the mass added equals the mass removed (steady-state) the final achievable effluent NH_3-N is reached.

5.8.2 Predicting required seed dose to achieve desired level of treatment

BioWin in combination with the observed laboratory data was used to estimate the seeded SRTs of the seeded SBRs and to predict the dose of nitrifying seed that must be added daily. In order to compensate for model limitations a procedure for predicting seeded SRT was developed and is described here.

Simply, there are two different types of seeded systems; those that are able to achieve nitrification without seed and those that are not. The system operating with an SRT long enough for nitrification to take place without seeding will contain two different types of nitrifying biomass once seeding is initiated. The nitrifier population will be made up of those nitrifiers that are indigenous to the system and those that were added as seed. However, BioWin does not have provisions for defining two different types of nitrifying biomass. Only one input parameter for nitrifier growth rate and decay is

possible in the model and the entire nitrifier population must be considered as one entity. Therefore a net growth rate and net decay rate must be determined and adjusted based on laboratory observations. Previously, FISH analysis showed that the proportion of nitrifiers in the decant liquor solids can be up to five times greater than the proportion in the reactor solids. The modeling procedure used here for determination of seeded SRT already takes this into consideration by using net kinetic values.

In order to model the seeded SBRs, an inventory list of the known and unknown parameters for each of the reactors was developed. The following parameters were known based on laboratory observations:

- Centrate characteristics
- Wastewater characteristics
- Apparent SRTs and HRTs of all reactors
- Final achievable effluent NH_3-N after seeding
- Apparent SRTs at which nitrification did not occur (these values are therefore less than SRT_{min})
- Temperature correction factor for nitrification

The procedure used to determine growth rates of the different seed sources was as follows:

To determine the kinetic parameters, a seed reactor that was able to achieve partial nitrification without seeding was selected. Based on the data shown in Figure 5.37, it was known that the SRT_{min} for nitrification at 10°C was near

12.4 days when NB20 was added as seed. This assumption is based on the fact that partial nitrification was occurring in the SBR after seeding was stopped for 25 days.

The apparent SRT of the modeled system was then set at 12.4 d and the maximum growth rate (μ_{max}) in the model was decreased until nitrification failed. The final value was adjusted to 0.279 d^{-1} at 20°C. This value is approximately 45% lower than the model default value of 0.5 d^{-1} at 20°C. Then, by keeping the growth rate set at 0.279 d^{-1}, the apparent SRT was increased until the laboratory-observed level of treatment (3.4 mg NH_3-N/L) was achieved. The apparent SRT was increased to 17.2 d to reach this level of treatment and is therefore the seeded SRT of the laboratory system. The net growth rate of nitrifiers (μ) in the seeded system was then calculated to be 1/17.2 d = 0.0581 d^{-1}.

Using the Arrhenius relationship, μ_{max} of the seed sources at 10°C were then calculated by Equation 22 and are listed in Table 5.11.

$$\mu_{max\ after\ seeding} = 0.279\ e^{0.0844(10-T)} \qquad [22]$$

The model estimated seeded SRTs of the reactors and the corresponding net growth rates are also listed in Table 5.11.

Table 5.11. Summary of seeded SRT determination by BioWin based on laboratory observations.

Seed source	HRT (h)	Apparent SRT (d)	Mean effluent NH_3-N (mg/L)	μ_{max} after seeding† (d^{-1})	Seeded SRT, θ_x^{s*} (d)	Net growth rate with seeding, μ (d^{-1})
NB10	8	11.9	3.21	0.164	>11.9	<0.084
NB10	12	3.80	28.3	0.164	8.52	0.12
NB20	8	12.4	3.40	0.120	17.2	0.058
NB20	12	3.3	31.2	0.120	12.1	0.083
NB20	24	3.40	18.7	0.120	12.5	0.080
NB20	43.6	3.51	1.32	0.120	>26.5	<0.038
NB20	53.3	3.63	1.20	0.120	>28.1	<0.036
NB20	68.6	3.75	1.06	0.120	>29.9	<0.033
NB20	96	4.00	1.06	0.120	>28.0	<0.038
NB25	24	3.42	26.0	0.077	20.8	0.048
NB30	24	3.38	28.4	0.0516	38.6	0.026

*SRT required to achieve observed level of treatment
†Maximum growth rate of seed after addition to 10°C

For the reactors that achieved very low effluent NH_3-N concentration, the model can only determine the lower and upper limits to seeded SRT and net growth rate, respectively. Only a minimum value for seeded SRT and maximum value for net growth rate can be determined because at low NH_3-N concentrations, the biomass growth was limited by the mass of NH_3-N available.

Based on the values listed in Table 5.11, Figures 5.50 and 5.51 were created to determine the seed dose required to achieve a desired level of treatment. The μ_{max} values for each seed type were input to the model. Then seed was added (X_a^o) at various concentrations and the effluent NH_3-N and X_a were determined. Then the apparent SRT required to achieve the same level of treatment without seeding was determined; this is equivalent to the seeded SRT.

The apparent SRT of the reactor modeled in Figure 5.50 is very near the SRT_{min} required to achieve nitrification. When NB10 and NB20 were added,

very small doses are required to initiate nitrification and establish a population of nitrifiers in the reactor. As the temperature of the seed increased, greater doses were required to reach a given level of treatment. Similarly, the seeded SRT required to achieve a given level of treatment is greater as the seed temperature increases.

The concentration of nitrifiers in each of the reactors becomes similar as the effluent NH_3-N approaches zero (Figure 5.50). This is due to the fact that a limited mass of nitrifiers can be supported on a given mass of NH_3-N. The amount of nitrifiers in the reactor is a function of yield and not the growth rate of the seed.

In Figures 5.51 and 5.52 the apparent SRT is much below the SRT_{min} for nitrification at 10°C. A much larger seed dose is required for all levels of treatment when compared to the doses in Figure 5.50. Because the SRT is so short, the seed is washed out a rapid rate thus requiring very large inputs of seed to establish a population of nitrifiers. Even with very large doses of seed, the concentrations of nitrifiers in Figure 5.51 and 5.52 are less than that in Figure 5.50.

Figures 5.50, 5.51 and 5.52 can be created for any type of treatment system once the growth rates and temperature correction factor is determined for the nitrifying seed source. They can be used to determine the required dose of seed to achieve a certain level of NH_3-N removal or to estimate the effluent NH_3-N based on a known seeding rate.

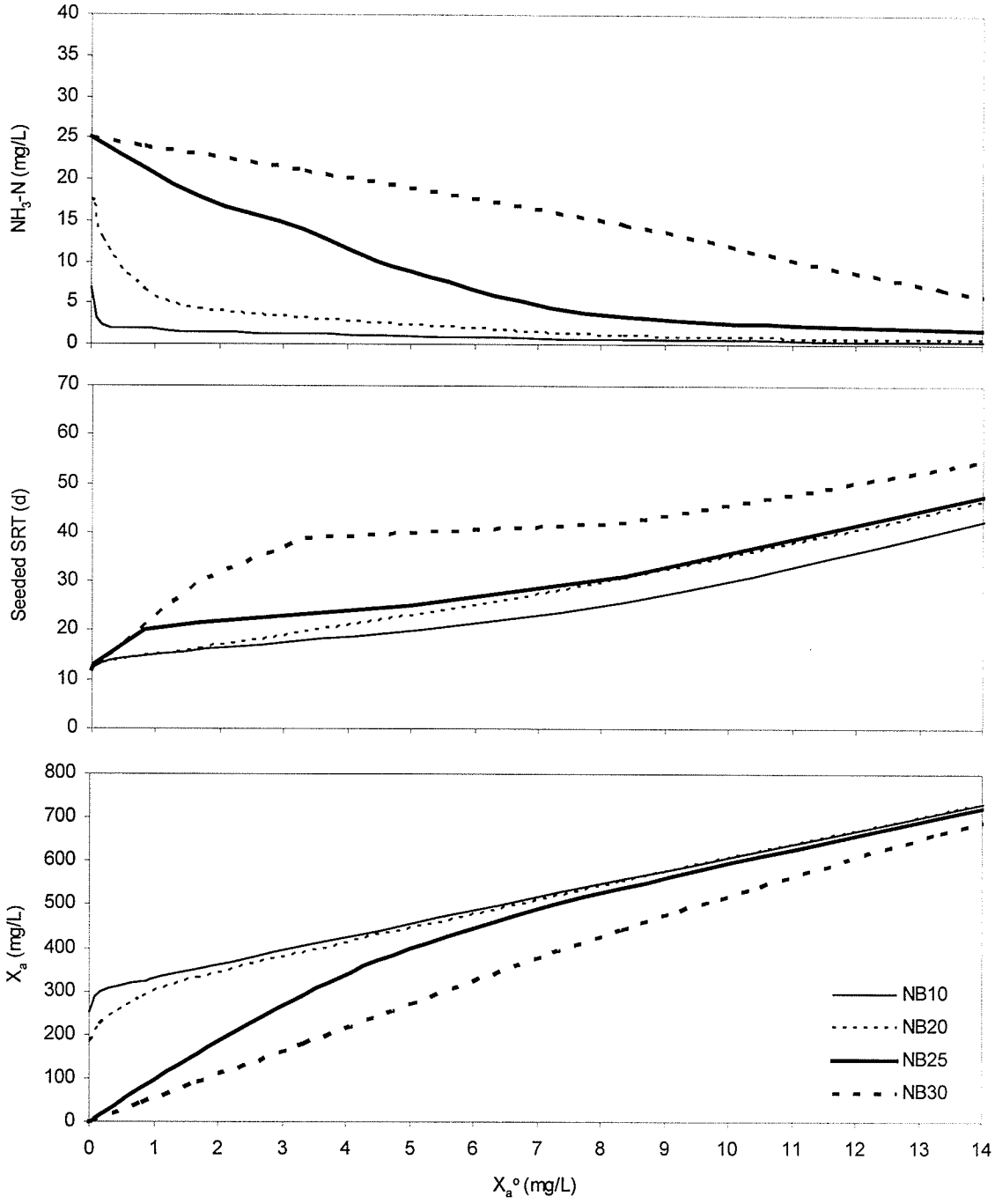


Figure 5.50 Seed dose required to achieve a given level of NH_3-N in the effluent and the corresponding seeded SRT and X_a. (HRT = 8 h, apparent SRT = 12 d, T = 10°C, μ values are listed in Table 5.13).

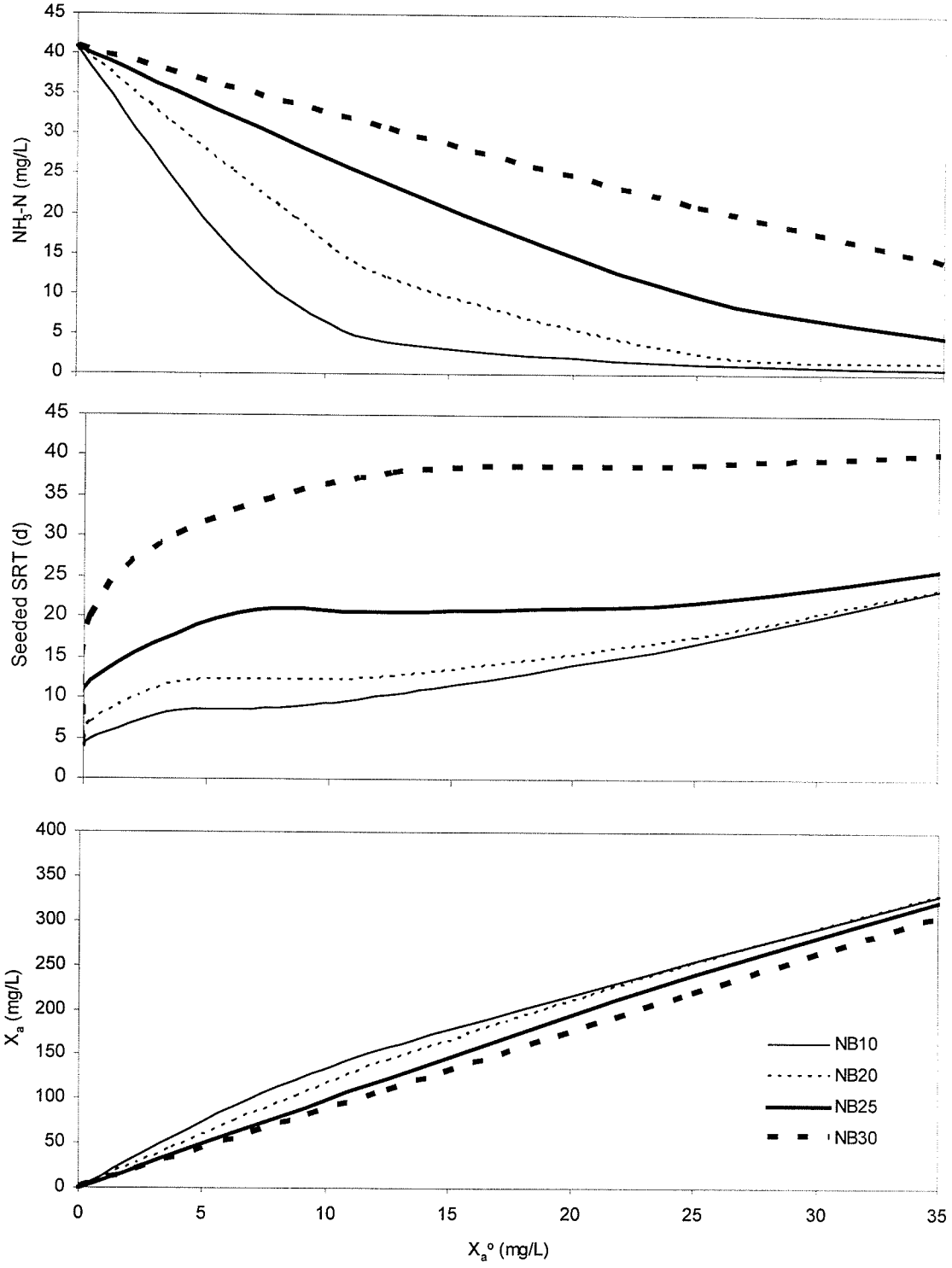


Figure 5.51 Seed dose required to achieve a given level of NH_3-N in the effluent and the corresponding seeded SRT and X_a. (HRT = 12 h, apparent SRT = 4 d, T = 10°C, μ values are listed in Table 5.13).

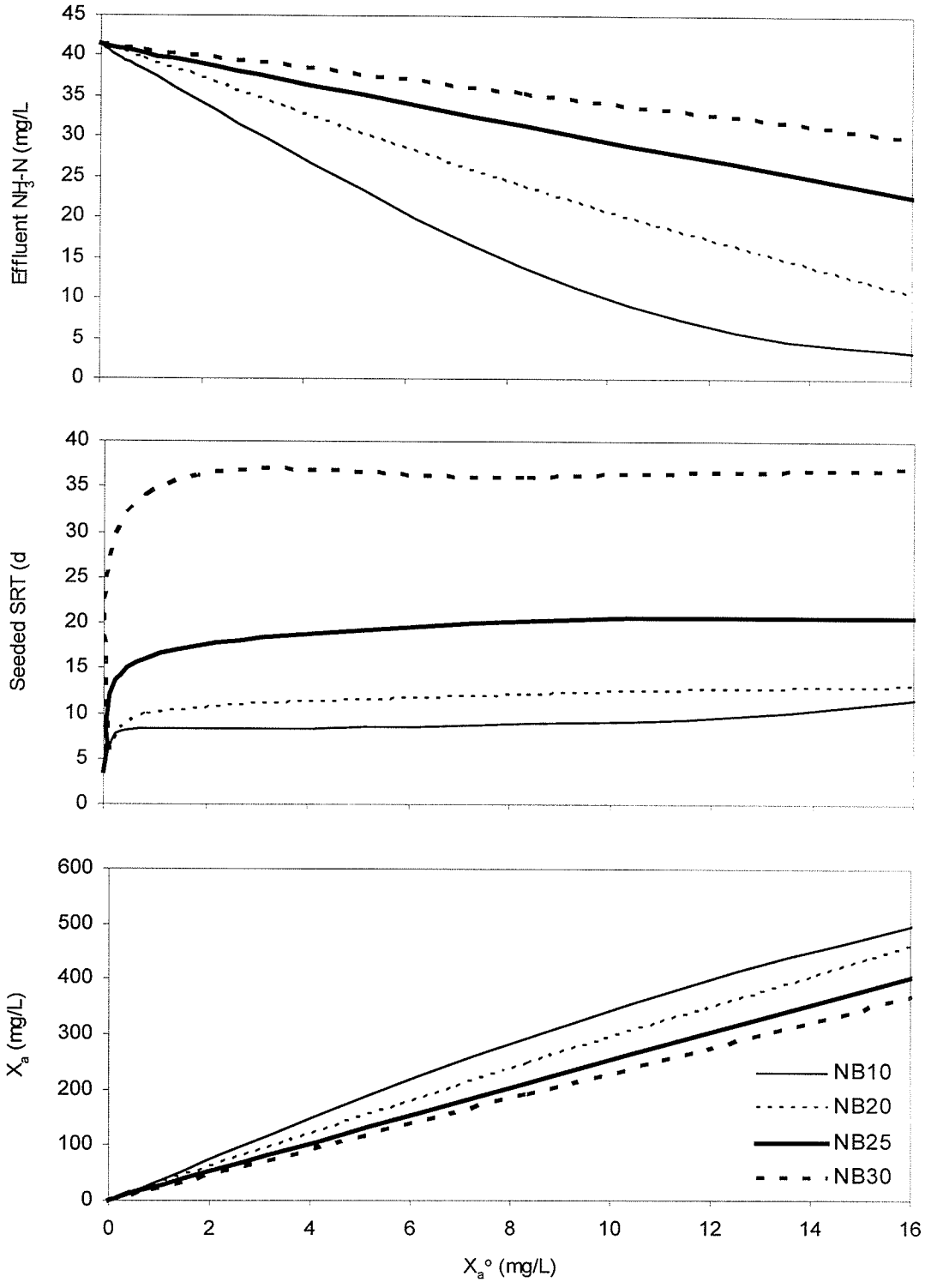


Figure 5.52 Seed dose required to achieve a given level of NH_3-N in the effluent and the corresponding seeded SRT and X_a (HRT 4 h, apparent SRT 3.5 d, T=10°C). The operating conditions are similar to those at NEWPCC.

5.8.3 Summary and conclusions

When seeding, it is desirable to increase the seeded SRT as much as possible because the seeded SRT dictates the final effluent NH_3-N concentration. It was shown that a disproportionate loss of nitrifiers with the effluent decreases the seeded SRT and thus negates the benefits of seeding. If the mass of nitrifiers lost with the effluent is high enough, the seeded SRT can become shorter than the apparent SRT.

Using the BioWin wastewater treatment simulation model in conjunction with laboratory observations, the dose of seed that was required to achieve a given level of treatment was estimated. As the apparent SRT decreased, the dose of seed required increased because the seed was being washed from the system more quickly when the apparent SRT was short. If the apparent SRT of the seeded system was near SRT_{min} for nitrification, very small doses of seed were required to initiate nitrification.

It was shown that the greater the difference in temperature between the seed and the seeded reactor, the greater the seed dose that was required. Much greater doses of NB30 were required than NB10 to achieve the same level of NH_3-N in the effluent.

5.9 Volume savings as a result of seeding

5.9.1 Determination of volume savings

To upgrade a wastewater treatment plant to include nitrification requires an increase in SRT. Increasing the SRT usually means an increase in the solids inventory within the plant and an increase in the required volume by 2 to 3 times. Any method that can decrease the solids inventory while still maintaining nitrification is desirable.

It has been shown that an SRT of at least 12 days is required to accomplish nitrification in the cold SBRs without seeding. To determine the volume savings that can be achieved with seeding we must determine how much the apparent SRT can be reduced when seeding is provided.

Using BioWin, nitrifiers at various concentrations were seeded into an SBR at 10°C. The apparent SRT of the SBR was then reduced until the final effluent NH_3-N was 2.0 mg/L. The growth rate was set at 0.38 d^{-1} which was the observed growth rate of nitrifiers at 10°C. The decrease in apparent SRT is a good approximation of the volume savings because the solids inventory increases linearly with increasing SRT (Figure 2.2).

Figure 5.53 shows that as the seed dose increases, the volume savings increases. Additionally, the greater the temperature difference between the seed and the seeded SBR, the less volume that can be saved.

The inset of Figure 5.53 depicts the volume savings that can be expected from seed generated "in-house" from the nitrification of centrate. Centrate has a

limited supply of NH_3-N thus the mass of nitrifiers that can be produced is limited. The volume savings was determined to be less than 20% for nitrifiers produced from centrate at 10°C. Kos (1998) suggested that the volume savings could be 40% by seeding at a rate of X_a^o = 1.3 mg/L. However, Kos used a greater growth rate of 0.114 d^{-1} at 10°C while we observed a growth rate of 0.083 d^{-1}. In addition, Kos did not account for washout of nitrifying bacteria with decant liquors.

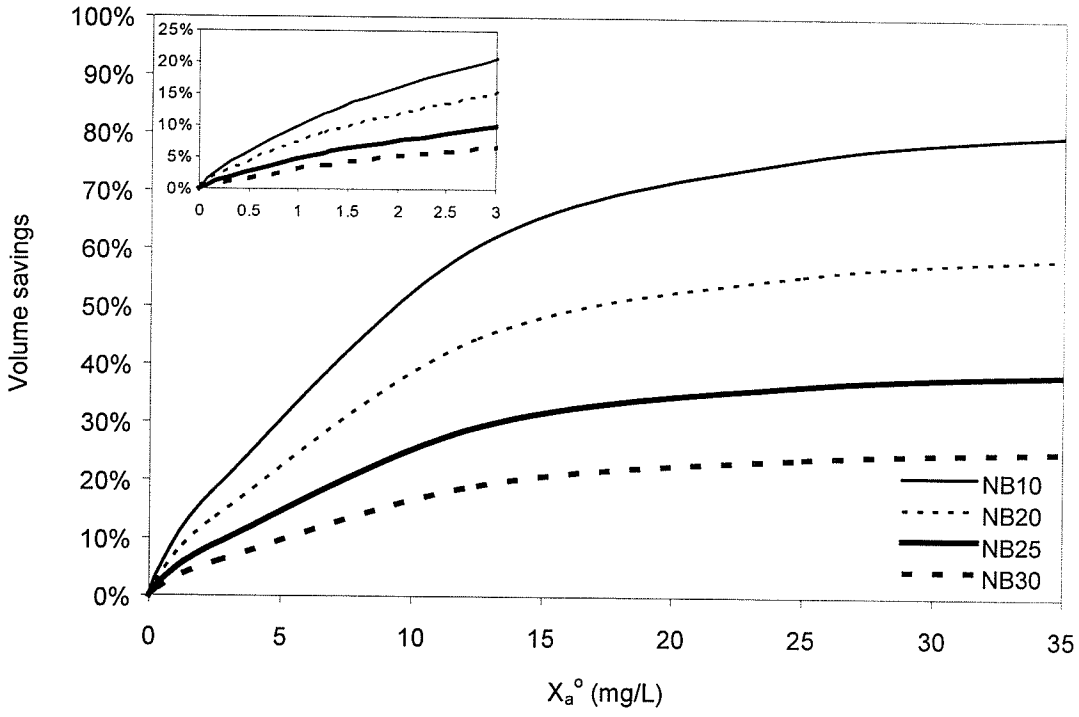


Figure 5.53 Volume savings that result from seeding nitrifiers acclimated to different temperatures (T=10°C, SRT_{min} = 12 d).

5.9.2 Summary and conclusions

The volume required for nitrification can be decreased by seeding. The degree to which the volume can be decreased was dependent on the seed

dose and the temperature at which the seed was grown. Seed that was acclimated to the temperature into which it was seeded provided the greatest benefit for a given seed dose. The greatest volume savings from seed produced from the nitrification of centrate is expected to be less than 20%.

6 RESEARCH OVERVIEW

6.1 Summary

This study was originally initiated to determine treatment options for centrate from the North End Water Pollution Control Centre in Winnipeg. Centrate was identified as a problem because it contributes up to 25% of the nitrogen load entering the plant. Future plans to upgrade the plant have considered treating centrate in a side-stream reactor with nitrification.

This research has shown that the NH_3-N can be completely removed from centrate using nitrification over a range of 10 to 30°C as long as a sufficient SRT is maintained and alkalinity is supplied from an external source. An SRT of 12 days was required at 10°C while 5 days was sufficient for the reactors with temperatures greater than 20°C. Free ammonia toxicity was not a problem as demonstrated by complete NH_3-N removal and absence of NO_2^--N accumulation.

The nitrifying biomass produced from the treatment of centrate was found to continue nitrification when cooled to 10°C. Previous studies on the effect of temperature on nitrification did not study the changes in growth rate over longer term cold exposure in a diverse mixed culture. It was suspected that that other environmental stresses would result in a greater decrease in growth rate than could be expected based on temperature change alone. These other stresses might include substrate competition by heterotrophic NH_3-N assimilation as a result of carbon-rich wastewater (Hanaki *et al.* 1990)

or predation of the nitrifying bacteria by higher organisms (Lee and Welander 1994; Martinage and Paul 2000). Another important concern is that poor settling properties of nitrifying bacteria could limit the benefits of seeding. However, there was a lack of microbial evidence to support this latter claim.

The nitrifying biomass produced from centrate treatment was seeded into SBR at 10°C that were operated with various HRTs and SRTs. In some cases full NH_3-N removal was achieved while only partial removal was possible in others. Using the laboratory data in conjunction with the BioWin wastewater treatment simulation model and microbial analysis, seeded SRTs of the nitrifiers in the SBRs were determined. Additionally, the required seed doses to achieve a desired level of treatment were determined.

Microbial analysis using FISH showed that *Nitrosomonas spp.* were not the dominant ammonia oxidizers in the seeded SBRs. FISH also showed that nitrifiers were in fact settling poorly and being inadvertently lost with the effluent. BioWin was used to demonstrate that seeded SRTs were substantially reduced by this loss of nitrifiers and that lower effluent NH_3-N could be achieved if washout was eliminated.

Using the model, we were able to demonstrate that the initial growing condition of the seed dictates the treatment potential in the seeded system. Much larger doses of seed acclimated to 30°C was required than seed

acclimated to 10°C to achieve the same level of treatment. Seed acclimated to warmer temperatures also required much longer seeded SRTs.

It was shown that short-SRT nitrification is possible with the addition of nitrifying bacteria from an external source. The ability to achieve full nitrification without increase the apparent SRT suggests that the amount of solids wasted daily could be increased while still maintaining full nitrification. This is, in effect, volume savings because the solids inventory of the system did not need to be increased to support a nitrifying bacterial population. The volume savings based on seeding rate for a range of seed temperatures was determined. The expected volume savings when seed is generated from centrate is expected to be less than 20% in a reactor at 10°C. The volume savings decreased as the seed temperature increased.

6.2 Engineering significance

Upgrading a treatment plant to include nitrification is expensive because tanks must be enlarged to accommodate an increased solids inventory. One method proposed for the NEWPCC upgrade includes centrate nitrification in a RAS re-aeration tank. However, with this method the SRT of the nitrifiers is the same as the rest of the solids in the process. It has been shown that approximately 25% of the NH_3-N load entering a treatment plant can be eliminated by centrate nitrification in a small dedicated side-stream tank. Side-stream treatment also provides the additional benefit of producing a

concentrated nitrifying biomass that can be added to the main-stream tanks as seed. With this method the SRT of the nitrifiers is longer than the SRT of the other solids in the reactor. This means volume savings.

The greatest benefit of seeding is realized by acclimating the nitrifying seed to the temperature of the reactor into which they are to be seeded. The maximum possible volume savings from the nitrification of centrate was determined to be 20% when the seed was acclimated to 10°C. However, there was a trade-off; the reactor treating centrate at 10°C required an SRT of 12 days while the reactors at temperatures greater than 20°C required less than 5 days. The reactors used to treat centrate in the lab were also operated with the SRT equal to HRT. To minimize the size of the side-stream reactor treating centrate, it must be operated with an SRT longer than the HRT. Due to poor total biomass production the biomass is not conducive to floc formation and has poor settling properties. The size of the side-stream tank can only be minimized by improving the settling or capture of nitrifiers; possibly by membrane filtration or some other physical separation process.

6.3 Recommendations

Centrate nitrification can occur over a wide range of temperatures. However, the greatest benefit from seeding can only occur if the side-stream reactor is at the same temperature as the main-stream process into which the nitrifiers are to be seeded.

The maximum volume savings can only occur by minimizing the size of the side-stream tank while producing the most possible nitrifiers. The size of the side-stream tank can be minimized by making the HRT shorter than the SRT. Because the nitrifiers have poor settling characteristics, a solids separation process should be applied. This might include improving settling properties by increasing the biomass concentration in the side-stream reactor by RAS, primary sludge or carbon addition, or by physical separation with membrane filtration.

Nitrification at ambient temperatures is recommended over the SHARON® process because the SHARON® process requires high temperatures. The high temperature is required to maximize the growth rate of ammonia oxidizers such that the SRT can be reduced to washout nitrite oxidizers. The temperature makes the biomass unsuitable for seeding into the main-stream.

The BABE process configuration is ideal for centrate treatment. However, the temperature should be decreased to the ambient temperature of the main-stream for the greatest seeding benefit.

While removal of NH_3-N from centrate results in a 25% decrease in NH_3-N load entering a WWTP, modeling showed that it did not result in volume savings in a nitrifying plant. The SRT_{min} required for nitrification was independent of the NH_3-N load; therefore the plant must be expanded to the same volume whether or not the NH_3-N load from centrate is present.

6.4 Future research

- FISH analysis showed that the seed ammonia oxidizing bacteria did not settle well. Solids separation to capture nitrifiers is desirable to minimize the size of the side-stream centrate treatment tank and maximize the benefits of seeding. Future research should examine methods for increasing the capture of nitrifiers either by improving settlability or by filtration.
- It was found that nitrification rate was dependent on the initial concentration of NH_3-N in the reactor between 1 and 100 mg/L. However, when nitrification proceeded there was a noticeable decrease in nitrification rate when the concentration was allowed to decrease to less than 1.0 mg/L. These results are contrary to the generally accepted idea that nitrification rate is not dependent on the substrate concentration at concentrations much greater than 1.0 mg/L. Further research into the mechanisms behind this behaviour is required.
- This research has shown that centrate treatment for the production of nitrifying bacteria for seeding is feasible. Pilot- or full-scale application of centrate treatment is the next step.

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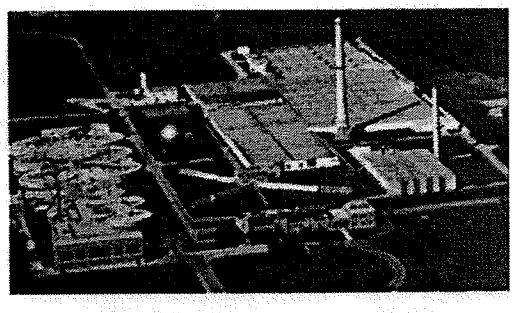
APPENDIX A

Based on public hearings that took place in Winnipeg in 2003, a report titled "Better Treatment: taking action to improve water quality" was published. The document outlines the issues discussed concerning the operation of the City of Winnipeg's wastewater collection and treatment systems. Although the report states that Environment Canada believes that centrate treatment alone is inadequate for addressing the ammonia toxicity problem, ammonia removal from centrate would, in fact, achieve the removal guidelines later recommended in the document. The recommendations for nutrient removal were as follows:

> "The City of Winnipeg should be directed to plan for the removal of nitrogen and phosphorus from its municipal wastewater, and to take immediate steps in support of the nutrient reduction targets established for Lake Winnipeg, The City's nutrient removal plan should be a key element of a licence review hearing to be scheduled within two years.
>
> The City of Winnipeg should develop a plan to remove nutrients from its municipal wastewaters rather than deferring this until completion of Manitoba's nutrient management strategy. Priority should be placed on phosphorus. Other municipal jurisdictions in the Red and Assiniboine river[s] basin have already implemented phosphorus removal, with effluent limits of 1 to 2 mg/L total phosphorus, and are also moving towards nitrogen removal. The City should also take immediate steps to reduce nutrients by accelerating the implementation of technological solutions at one of more of its water pollution control centres and controlling other point and area sources. Targets of 10 per cent for phosphorus and 13 per cent for nitrogen should be achievable within a two-year period."
>
> Source: Manitoba Clean Environment Commission. 2003. Report on Public Hearing, List of Recommendations. pp. 56-57.

Winnipeg, MB



Earth Tech's Winnipeg office has been awarded an engineering assignment for upgrades to the City of Winnipeg's North End Water Pollution Control Centre (NEWPCC). These upgrades are the first steps in implementing the City's long term plan for improving its wastewater system, which was presented to Manitoba Conservation and the Clean Environment Commission's public hearings held in January and April of this year. The first component of the assignment involves the installation of a disinfection system. The preferred technology will be to utilize ultraviolet (UV) light however testing is currently underway to verify its performance acceptability. The second component of the assignment involves the treatment of the centrate stream generated in the biosolids dewatering process. Conceptual planning completed recently by the Winnipeg office determined that implementation of centrate treatment would provide a significant reduction in the risk to the aquatic wildlife in the Red River. Consequently, centrate treatment was selected as the first step of an ammonia control program. Full nutrient control to reduce nitrogen and phosphorous loads to Lake Winnipeg is also being considered.

Source: Anonymous. 2003. News from the field. Western Canada WATER. Summer. 55(2): 7

APPENDIX B-1

Reactor start-up. Centrate nitrification at 27°C in 3 different reactors.

		R1						R2						R3						
		27°C						27°C						27°C						
Days	Centrate NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NH_3-N (mg/L)	
1	256						280	4760	3299	3223	3040		278						275	
3		4363	2400	3043	1799									4377	1943	3040	1477			
4	241						272.3						271.2						272.3	
9	268	3120	1887	2050	1509			3593	2700	2440	2111			3890	2856	2640	2256			
10	280						87.4						75.2						95.3	
15							92.1						97.9						97.9	
16		2667	2062	1747	1561			4133	3092	2863	2292			3183	2767	2123	2000			
18						17.94						20.63						15.77		
19	263						99.6						96.1						97.6	Start adding
23		2180	1555	1380	1122			3023	2411	1950	1756			1640	833	957	678			alkalinity
26	236						3.5						21.6						26.6	
29							37.7						0.7						48.7	
30		4210	3022	2327	1700			4703	3689	2630	2200			5600	4411	3183	2633			
33							7.6						0.5						1	
36	250						2.4						1.2						2.4	
45		4073	967	1883	467			5060	2344	2430	1233			5603	1689	2703	956			
50	508					7.57						6.13						4.27		
52		4910	1800	2363	1022			4750	1611	2563	989			5133	2011	2617	1211			
53	533	5660	1866	2490	1067		2.2	4840	1388	2250	800		1.4	5200	1522	2290	867		1.1	Start regular
54	543	5913	1711	3187	1067		7.2	5627	1578	2957	1033		4.7	5165	1311	2240	789		6	wasting
57	564	5947	1067	3027	1067	13.61	0.7	5503	1300	2460	933	9.90	1	5896	1178	3143	911	10.19	1.4	
60	633	5530	1067	2317	700		0.3	4920	956	2157	544		0.4	5013	1033	2220	578		1.5	
61	534					9.06	0.1					9.26	0.1					5.84	0.3	
64	470	6120	767	4023	600		0.8	4703	911	2737	655		2.1	5030	778	2373	611		2.7	
65	597						0.4						1.3						3.3	
66	538	5207	733	2210	478	11.19	1.9	4817	767	2067	489	10.00	1.8	4537	1022	1970	589	9.83	40.7	
67	433						1.2													
68	425	4863	467	2100	353		1.5	4170	627	2090	480		1.6	4417	487	1800	307		4.8	
71	415						3.5						1.3						3.1	
72	393	4270	733	1560	347		2.9	3940	707	1493	480		1.8	4070	440	1613	260		3	
75	395	3757	487	1600	273		4.6	3410	377	1623	273		3.5	3777	610	1593	383		7.5	

APPENDIX B-2

Data for 3 reactors treating centrate at 20°C, 25°C and 30°C from start-up to beginning of cold shock experiments (October 1999 to July 2000)

		NB20						NB25						NB30					
Date	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NH_3-N (mg/L)	TS (mg/L)	TSS (mg/L)	VS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)
8-Oct-99	94	7.5	3777	610	1593	383		4.6	2750	487	1600	273		3.5	3410	377	1623	273	
13-Oct-99	153	4.8					3.4	2.0					14.0	2.2					10.6
15-Oct-99			3773	497	1983	335			3040	433	1253	350			3320	373	1330	330	
18-Oct-99							4.8						5.8						6.6
20-Oct-99		5.0	2815	340	1390	220	4.1	2.0	2767	327	1353	210	3.9	2.0	3170	480	1640	337	6.6
27-Oct-99			2217	183	1177	187			2327	247	1250	207			2628	240	1473	255	
5-Nov-99	350		2637	243	1237	190			2603	243	1066	173			2870	213	1033	140	
10-Nov-99	419	6.1	2820	190	913	225		1.2	2850	290	913	223		1.1	3107	197	860	177	
18-Nov-99	391		3523	413	1707	270			3610	282	1823	297			4000	455	1983	335	
22-Nov-99		5.0					8.4	2.0					10.6	2.0					12.7
24-Nov-99	391		3707	393	1420	305			3693	373	1190	357			4257	417	1373	340	
26-Nov-99	391																		
29-Nov-99	380	1.3						1.4						0.8					

APPENDIX B-3

Data for start-up and operation for the reactor treating centrate at 10°C before its use as seed.

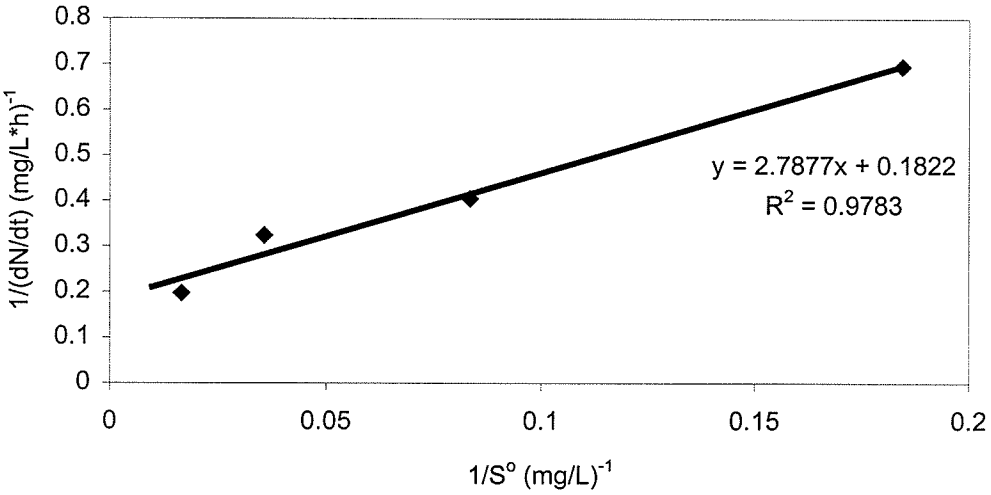
Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	TSS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NO_3-N (mg/L)	
7	585	93.6			0.909	368	SRT 10 d
8	650	89.1	133	90			
9	675	73.9			0.519	408	
10	577	84.6					
11	-						
12	-						
13	667	8.3			0.882	430	
14			133	127			
15	717				-		
16	748	85.9	148	127		458	
17	726				-		
18	-						
19	-						
20	873	141.4				440	
21	748						
22	753	85			-	426	
23	748						
24	-	87.8			-	369	
25	374	134					
26	374	125				372	
27	666	58.4					
28	634	78			-	403	
29	739						
30	631	91.5				324	
31	720						
32	-						Increase SRT to 12 d
33	370	141	139	126		362	Dilute centrate by 50%
34	413	124				475	
35	355	98.7	138	109	4.86		
36	355		183	144			
37	402	27.3	226	204	3.78	533	
38	372	34.6	175	150		446	
39	-						
40		2.57				430	
41	348	25.4					
42	348	20.2	168	153		239	
43	365	2.98					
44	365	5.39	263	188	3.7	359	

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	TSS (mg/L)	VSS (mg/L)	dN/dt (mg/L*h)	Effluent NO_3-N (mg/L)
45	348					
46	-					
47	328					363
48	336					
49	357	11.6	135	125	5.2	366
50	375					
51	370	29.6	158	133		
52	400					
53						
54						
55	365					
56	363	7.2	90	68.3		322
57	373					
58		33.9	133	90		
59						
60						
61	516					
62						
63	433	21.1				322
64	443	25.8	133	127	0.94	
65	455	24.4				301
66	433	39.5	148	127	0.22	294
67	-					
68	-					
69		37				290
70	270	11.8			0.77	
71	304					
72	315	5.28			1.43	
73						
74						
75	-					
76						
77						
78						
79						
80	261	8.47				
81	522					
82	522					

APPENDIX B-4

Data for the determination of the effect of initial NH_3-N concentration on nitrification rate for NB20.

Time (hours)	Centrate dilution					
	1to100	1to50	1to20	1to10	1to5	1to3
	mg NH_3-N/L	mg NH_3-N/L	mg NH_3-N/L	mg NH_3-N/L	mg NH_3-N/L	mg NH_3-N/L
0.10	2.87	5.39	11.6	28.0	60.3	102
0.33	2.61	4.84	10.9	26.8	56.1	101
1.00	1.63	3.91	9.8	25.3	50.0	95.6
1.50	1.12	3.33	8.42		49.3	
2.00	0.85	2.44	7.21	21.3	46.3	93.0
3.25	0.72	0.98	3.72	18.3	43.3	89.2
dN/dt (mg NH_3-N/L*h)	1.29	1.44	2.47	3.09	5.08	4.08
R^2	0.995	0.987	0.993	0.991	0.887	0.947
1/(dN/dt)	0.778	0.696	0.405	0.324	0.197	0.245
S^o (mg NH_3-N/L)	3.00	5.42	12.0	28.1	60.3	102
$1/S^o$ $(mg/L)^{-1}$	0.3333	0.1844	0.0835	0.0356	0.0166	0.0098
VSS (mg/L)	71.4	71.8	73.0	75.1	79.1	84.6
U (mg NH_3-N/g VSS*h)	18.0	20.0	33.8	41.1	64.2	48.3



K_N = 2.7877 / 0.1822 = 15.3 mg NH_3-N/L

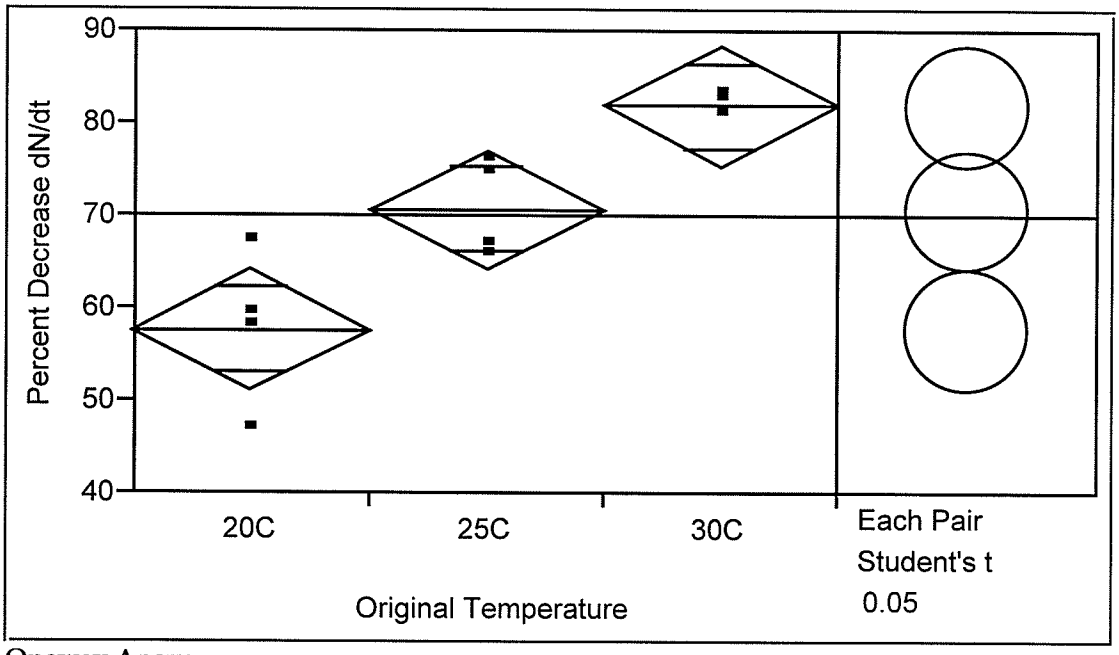
APPENDIX C-1

Data for the determination of the effect of sudden decrease in temperature on nitrification rates for NB20, NB25 and NB30.

	NB20								NB25								NB30							
	Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 1		Rep. 2		Rep. 3		Rep. 4		Rep. 1		Rep. 2		Rep. 3		Rep. 4	
Time	20°C	10°C	20°C	10°C	20°C	10°C	20°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C	30°C	10°C	30°C	10°C	30°C	10°C	30°C	10°C
(hours)	NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)		NH_3-N (mg/L)	
0.17			43.5								44.1								60.4					
0.25	39.3	35.5			37.1	40	22.5	21.5		31.8			29.8	35.8	22.6	19.3	29.8	33.6			38.3	44.3	37	50
0.42				42.1								38.6								56.7				
0.75		30.9	36.5		31	37.8				29.1	35.9		23.3	34.4				30.3	53.1		33.3	43.5		
0.83	33.2								24.1								27.9							
1.00				40.1			15.8	18.8				38			22.3	11.6				56			31	48.6
1.17			35								33.2								49.7					
1.50	24.6	27.2			20.3	35.1	11		9.41	23.3			15.4	33.7		7.8	26	29.3			28.7	40.9		
1.75				36.8								34.4								55.3			24.3	
2.00	13.7		25.4		13.7		7.07	16.7	4.52		23.3		10.3		20.2	5.7	22.6		43.7		22.8		20.7	
2.25						31.1								33.1								43.5		
2.50		21.9								19.4								29.3						
2.75				32.4								30.3								53.9				
3.00								12.9							18									45.8
3.50						23.3								27.4								39.2		
4.00								10.5							15.6			28.9						43.8
4.50				23.6		17.9						22.7		25						51.1		38.4		
6.00						8.2								21.6				27.4				35.2		
6.50				11								16.9								46.3				
7.00						2.14								16.4				27.4				32.8		
dN/dt (mg/L*h)	14.3	5.8	9.6	5.1	13.5	5.7	8.9	2.9	17.1	5.7	11.1	3.8	11	2.8	7.9	1.9	3.9	0.68	8.9	1.7	8.4	1.6	9.6	1.6
Decrease	59.3%		46.8%		57.9%		67.0%		66.7%		65.8%		74.5%		75.9%		82.6%		80.9%		81.0%		83.3%	
Mean Decrease	57.7%								70.7%								81.9%							
St.Dev.	8.2%								4.7%								1.4%							

APPENDIX C-2

Comparison of decreases in dN/dt after a sudden decrease in temperature - cold shock test.



Oneway Anova

Summary of Fit

Rsquare	0.799838
Adj Rsquare	0.755358
Root Mean Square Error	5.711854
Mean of Response	70.14167
Observations (or Sum Wgts)	12

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Original Temperature	2	1173.3217	586.661	17.9818	0.0007
Error	9	293.6275	32.625		
C. Total	**11**	**1466.9492**			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
20C	4	57.7500	2.8559	51.289	64.211
25C	4	70.7250	2.8559	64.264	77.186
30C	4	81.9500	2.8559	75.489	88.411

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	30C	25C	20C
30C	0.0000	11.2250	24.2000
25C	-11.2250	0.0000	12.9750
20C	-24.2000	-12.9750	0.0000

Alpha= 0.05

Comparisons for each pair using Student's t

t
2.26216

Abs(Dif)-LSD	30C	25C	20C
30C	-9.1366	2.0884	15.0634
25C	2.0884	-9.1366	3.8384
20C	15.0634	3.8384	-9.1366

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. However, the reverse does not hold true. Upon further analysis using the Student's t Test, the difference between all of the seed sources was shown to be statistically significant.

APPENDIX D-1

Seed source characteristics (NB20) for seeding into continuous flow reactors

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent pH	dN/dt (mg/L*h)	
5							4.2	
6								
7								
8								
9								
10								
11	855	92.5			259		12.5	
12							5.3	
13							7.2	
14	425	24			298			
15	358	27		510	333			
16	458	3	732	485	220			
17	489	18	757	521	246			
18	688	38	710	507	289			
19	545						8.5	
20	514							
21	484	22.1	620	480	272			
22	474	65.43	598	495	272			
23	661	4.3	607	460	254			
24	730	33.8	555	492	333			
25	703	74.2	605	528	346			
26	730							
27	710							
28	703	14.7	573	490	425			
29	710	43	546	414	425	7.1		Start seeding
30	288	3.85	471	395	543	7.4		
31	212	3.49	429	356	326	8		
32	366	3.13	416	320	280	8.1		

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent pH
33	406	3.7				
34	434	1.67				
35	417	5.17	398	316	280	7.4
36	384	2.69				8.1
37	389	2.34	419	329	218	7.9
38	406	4.09				7.7
39	384	16.8	369	294	247	7.1
40	322	5.62				
41	378	12.7				
42	339	43.2	391	298	261	7.4
43	356	52.8				7.4
44	356	7.4	353	315	257	7.4
45	317	4.9				
46	350	23.8	356	299	275	
47	409	37.1				
48	257	68				
49	409	83.8	332	316	303	7.4
50	393	88.6				7.4
51	383	90	325	240	317	
52	403	129				
53	424	143	287	241	300	
54	342	102.2				
55	258	91				
56	433	95.3	281	240		
57	354	98.7				
58	325	97.9	248	191	252	
59	478	106.7				
60	433	106.7	231	178	252	

APPENDIX D-1 cont'd

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)
61	280	92.7			
62	270	87.8			
63	181	89.6	237	197	252
64	241	75.8			
65	251	54.6	230	165	279
66	186	52			
67	366	55.3	220	190	273
68	284	62.3			
69	315	55.4			
70	255	68.9	230	200	300
71	294	65.8			
72	299	75.2	244	203	287
73	142	90.8			
74	301	53.7	181	156	273
75	146	43.1			
76	146	19.4			
77	275	11.8	174	144	260
78	301	14.1			
79	245	31.1	185	132	218
80	317	43.8			
81		66.9	212	179	252
82					
83					
84	308	43.4	351	282	280
85	331	52.1			
86	243	70	239	203	300
87	178	52.1			
88	187	36.5	310	260	191
89	200	26.3			
90	200	26.3			

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)
91	325	24.2	264	223	252
92	315	24.9			
93	308	27.1	210	190	252
94	315	38.1			
95	357	44.9	227	197	306
96	315	58.8			
97	343	39.8			
98	301	41.5			
99	249	9.8			
100	324	18.7			175
101	348	24.6			
102	324	2.34			188
103	268	33			
104	300	46.4			188
105	244	68.3	255	214	
106	260	76.9			281
107		57.3	256	216	337
108	400	29.1			
109	389	27	231	211	337
110	383	32.8			
111	350	29.1			
112	333	34.4	240	205	299
113	328	20.6			
114	271	24.6	240	210	310
115	353	25.8			
116	714	60.6	220	186	277
117	718				

APPENDIX D-2

Data for influent and effluent for the continuous flow reactor that was not seeded (control reactor).

Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Influent TCOD (mg/L)	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)
1		341.4		89					
2									
3									
4									
5									
6									
7				97.7	35.8	0	8.1	861	760
8				67.2	35.8	0		538	502
9		333	341	106.4	36.4	0		543	482
10	26.4	341	350	80.3	37.6	0		561	507
11	35.9	315	337	89.0	35.8	0		613	520
12	22.6	354	363	80.3	38.2	0			
13	26.4	315.3		89.0	35.7	0			
14	27.1	267		80.3	38.2	0		729	662
15	34.6			80.3	32.8	0			
16			324	80.3	44.2			790	678
17	32.8		324	89.0	43				
18	34.5		324	80.3	40.2			1143	980
19	28.4				39				
20	32.8				42.5				
21	30.1			62.9	40.2			1035	908
22	39		341		51.5				
23	50		446	80.3	53.3			1017	906
24	41.2		376	71.6	49.4				
25	30.3		354	67.3	50			1020	968
26	28.3		397	110	43.7				
27	27.8		389	67.3	48.2				
28	28.3		375	60.1	42.6			999	919

Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Influent TCOD (mg/L)	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)
29	38.5		368	88.8	43.7				
30	29.3		396	187	48.2		8.1	804	706
31	33.8		380	118	37.2		8.2		
32	36.4		373	94.4	40.4		8.1	1010	831
33	22.3				42		8.1		
34	22.3				38.5		8		
35	23.8		357	94.9	34.4		8	937	809
36	25.9				35.8		8.1		
37	27.5		373	48.0	37.8		8.2	885	777
38	27.5				33.8		8.2		
39	23.3		344	95.6	35.8		8.1	1049	899
40	26.6				32.7				
41	29.5				32.1				
42	30.1		364	68.0	33.4		8.1	927	766
43	33				33.4		7.9		
44	31.8		337	68.0	36.7			669	631
45	31.2				32.1				
46	35		334	77.4	30.1			688	581
47	34				28.5	9.5			
48	32.5				28.5	8.3			
49	33		317	77.4	28.5			637	591
50	33				29				
51	32.5		317	77.4	24.9			634	572
52	31				18.3	8.9			
53	26.2		321	99.2	21.5			675	620
54	24.3				27.4	6			
55	23.2				29.9	6.8			
56	23.2		321	78.4	31.3			567	527

APPENDIX D-2 cont'd

Days	Influent NH_3-N (mg/L)	Influent TCOD (mg/L)	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	
57	27.5			26.8	12.9			
58	26.9	321	99.2	26.8		488	428	
59	29.1			18.3	22.2			
60	30.8	293	71.5	20.3	13.7	397	335	
61	24.6			20.0	15.9			
62	21.4			17.4	16.1			
63	27.8	349	71.5	13.3	19.5	651	591	
64	23			17.9	21.6			
65	34.1	307	113	10.4		686	607	
66	21.9			13.8	33.9			
67	20.9	369	80.5			609	564	
68	22.5			26.3				Increase
69	25.7			25.3	8.7			wasting rate
70	26.8	300	53.0	26.3		503	480	
71	17.7			26.3	11.4			
72	30.6		66.7			756	690	
73	39.6				4.6			
74	15.5	232	66.7	18.4		653	607	
75	21.4			8.6				
76	24.5			7.8				
77	21.9	322	80.5	12.6	16.1	727	633	
78	23.5			16.4				
79	24	273	53.0	19.0	11.6	634	543	
80	21.9			17.4				
81	25.2	341	88.5	21.5	8.3	646	571	
82								
83								
84	20.2	341	61.3	27.1	9.8	611	530	
85	26.3			26.0				
86	29	280	61.3	27.7	5.7	538	469	
87	26.8							

Days	Influent NH_3-N (mg/L)	Influent TCOD (mg/L)	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	
88	26.8	327	74.9	23.7	11.8	559	458	
89								
90	29			18.3				
91		320	74.9	27.1		489	422	
92	32.5			27.0	13.5			
93	34.8	306	68.1	28.6		615	539	
94	33.2			30.3	11.3			
95		348	61.3	29.5	14	483	439	
96				13.4	11.4			
97	17.3			10.4	13.5			
98	17.3			10.4				
99	19.6			12.6				
100	23.7	320	69.4	19.6	10.6			
101	27.5			22.3	16.2			Change reactor
102	37.3	360	56.2	28.5	7.3			
103					5.1			
104		360	69.4		1.8			
105					2.9			
106	24.6	314	43.0	36.8	1.5			
107	28.6	310	55.0	35.9	1.1	645	564	Change reactor
108	29.1			35.9	0.87			
109	32.8	310	74.6	40.9	3.1	555	482	
110	23.5			38.4	0			
111	22.1			40.9	0			
112	31.2	337	75.4	40.2	0	579	530	
113	17.9			29.5	0			
114		350	80.6	42.0	0	467	426	
115	31.2			34.5	0			Change reactor
116	26.6	363	70.0	39.5	0	545	518	
117	31.2			29.5	0			

APPENDIX D-3

Data of effluent characteristics for the continuous flow reactor that was seeded with NB20.
Influent characteristics are as listed for the control reactor.

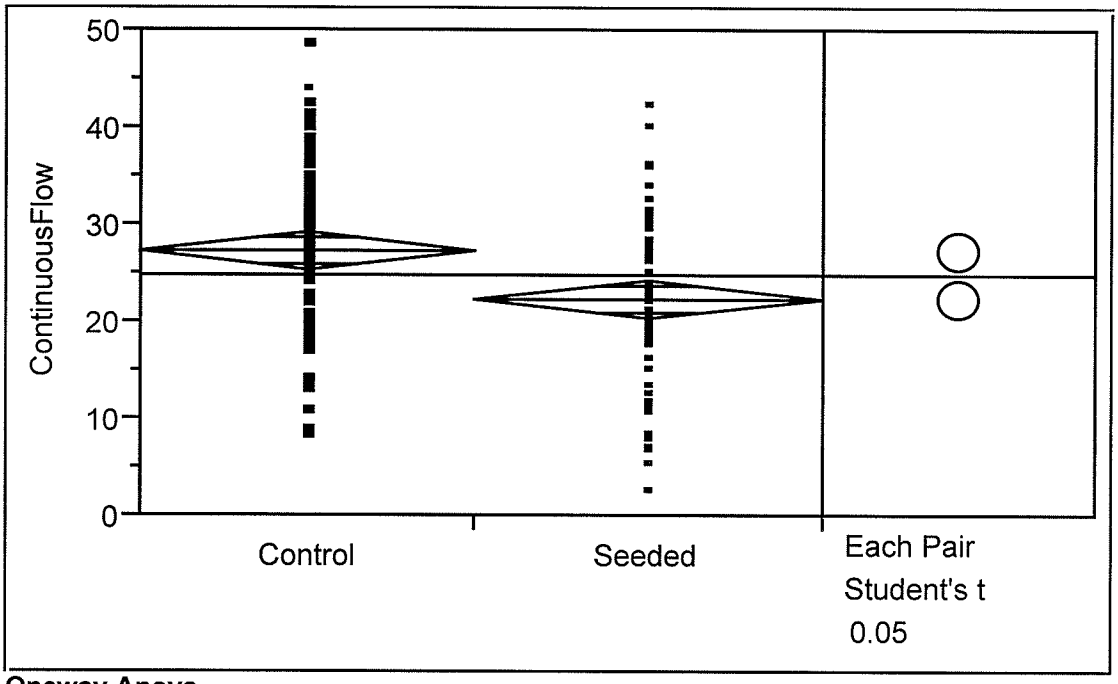
Days	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)	Days	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)		Days	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)	
1	133						29	60.1	42		8.1			Start seeding	57		16.1					
2							30	78.9	39.8		8.2	775	676		58	196	11.5			916	797	
3							31	125	39.8		8.2				59		11					
4							32	110	31		8	736	603		60	99.2	10.6			621	523	
5							33		35.8		8.1				61		12.3					
6							34		31.2		8				62		11.4					
7	71.6	31	0	8.1	591	507	35	125	29.9		8	688	588		63	130	7.7			879	771	
8	97.7	31.6	0		592	558	36		29.3		8				64		8.1		7.4			
9	71.6	34	0		514	466	37	63.5	26.8		8	703	631		65	120	7.7		7.3	667	589	
10	89	34	0		664	582	38		26.8		8				66				6.8			
11	106	36.9	0		683	570	39	109	29.3		8	546	453		67	94.3				669	602	
12	89	39.4	0				40		26.3						68		17.8					Increase
13	71.6	36.4	0				41		26.9						69		17.3					wasting rate
14	80.3	35.2	0		726	648	42	95.6	30.1		8	715	606		70	115	17.7			1093	986	
15	80.3	32.2	0				43		24.5		7.8				71		17.3					
16	80.3	49.9			829	722	44	95.6	28.2			729	677		72	66.7	17.7		7.7	848	716	
17	80.3	49.2					45		20.3						73		20.6		7.7			
18	71.6	47.4			858	690	46	120	20.9			779	688		74	80.5	22.9			500	422	
19		40.8					47		19.2						75		13.1					
20		39.6					48		22						76		14.9					
21	62.9	32.8			653	559	49	91.5	18.7			823	751		77	84.3	17.9			485	375	
22	62.9	49.2					50		18.7						78		19					
23	80.3	55.5			599	530	51	106	19.2			865	735		79	66.7	19			665	577	
24	80.3	47.1					52		17.8						80		17.9		7.7			
25	88.8	43.4			758	717	53	99.2	19.2			558	513	Double seed	81	102	22.1			703	617	
26	53	39.8					54		19.2					dose	82							
27	53	44.3					55		23.7						83							
28	67.3	46.5			913	879	56	113	23.7			705	627		84	81.7	24.8			761	673	

APPENDIX D-3 cont'd

Days	Effluent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	pH	MLSS (mg/L)	MLVSS (mg/L)	
85		19.9					
86	81.7	19.9			688	638	
87		22.1					
88	74.9	23.7			682	553	
89							
90		18.3					
91	88.5	22.6			642	555	
92		28.3					
93	88.5	29.5			696	614	
94		30.3					
95	88.5	29.5			559	498	
96		10.4					
97		5.2					
98		2.3					
99		6.65					
100	122	18.3					
101		21.8					Change reactor
102	69.4	18.3					
103							
104	82.6						
105							
106	76.0	26					
107	74.6	27.3			921	829	Change reactor
108		27.8					
109	87.7	32.4			824	707	
110		31.2					
111		35.9					
112	70.0	35.9			809	744	
113		31.2					
114	91.8	30.4			793	724	
115		33.7					Change reactor
116	64.5	33.7			843	712	
117		30.4					

APPENDIX D-4

Compare effluent NH_3-N for continuous flow reactors - Control (unseeded) vs. Seeded reactor.



Oneway Anova

Summary of Fit

Rsquare	0.07489
Adj Rsquare	0.069072
Root Mean Square Error	9.03429
Mean of Response	24.78106
Observations (or Sum Wgts)	161

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	5.10976	3.588	159	0.0004
Std Error	1.42425			
Lower 95%	2.29687			
Upper 95%	7.92265			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Reactor 2	1	1050.550	1050.55	12.8715	0.0004
Error	159	12977.325	81.62		
C. Total	160	14027.875			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Control	79	27.3835	1.0164	25.376	29.391
Seeded	82	22.2738	0.9977	20.303	24.244

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Control	Seeded
Control	0.00000	5.10976
Seeded	-5.10976	0.00000

Alpha=0.05

Comparisons for each pair using Student's t

t

1.97500

Abs(Dif)-LSD	Control	Seeded
Control	-2.83898	2.29687
Seeded	2.29687	-2.78656

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that the seeded reactor had a lower effluent NH_3-N concentration than the control reactor and the difference between the effluents was statistically significant.

APPENDIX E-1

Influent and effluent data for NB20 during seeding of SBRs at 10°C with an apparent SRT of 4 d and HRT of 43.3 to 96 h.

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)
1		1.42		156	141	
2		1.78		91	83	
3		1.42		155	130	
4		1.07		135	115	
5						
6		1.07				
7	711	2.13		130	96.9	
8	651	14.2		163	130	
9	703	23.1		130	99.2	231
10	708	16.6	686	130	100	232
11	659	53.3	697	129	109	298
12						
13	629	67.8				
14	639	67.8	656	152	122	311
15	665	47.3		136	121	324
16		58	648	126	103	324
17	640	30.3		103	88.4	318
18	639	13.2	600	91.1	83.5	333
19						
20	648	67	637			
21		62.2		100	83.6	346
22	603	56.5		89.2	70.7	360
23	676	39.25	621	93.9	83.7	387
24	624	47.8		116	83.1	373
25	587	55.8	567	105	75.2	387
26						
27	595	66.8	551			
28	639	80.5		67.9	60.4	
29		63.4	566	128	92.7	
30	665	39.9		108	90.5	
31		45.3		123	87.7	

Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)
32	611	6	589	95	85	
33	627	2.33				
34		2.04	604			
35						
36		2.04	545			
37		3.06		276	241	
38		1.62	623	258	182	
39	650	4.1		216	109	
40		2.54				
41						
42	708	4.62				
43		3.58				
44	656	4.36				
45		4.28		138	98.4	
46						
47						
48	586					
49						
50		1.77		138	128	
51	612	1.51				
52	602	5.41				
53		3.06		185	163	
54						
55	587	1.32				
56		2.63	543	234	167	
57		1.46		197	154	
58		1.18	531	166	142	
59	601	0.81		140	125	
60	590	4.48	579	158	125	
61						
62		0.9	555			

APPENDIX E-2

Data for SBRs at 10°C with an apparent SRT of 4 days and various HRTs, seeded with NB20.

HRT 43.6 h

	Influent NH_3-N	Influent SCOD	Effluent NH_3-N	Effluent NO_3-N	MLSS	MLVSS	Effluent SCOD	Effluent TCOD	Waste TCOD
	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
1	29.7	385	41.3	0	174	151	77.5		
2	31.6	266	43	0	161	146	51.5		
3	32.7	246	43	0	181	151	38.6		
4	38.1	233	42.2		191	166	64.5		
5									
6	31.3			0					
7	21.0	317	46.3	0	130	96.9	39.2		
8	25.6	291	35.5	0	171	138	52.5		
9	30.6	291	40.1	14.2	188	155	45.8	Start seeding	
10	31.8	286	36.8	20.4	193	158	61.8		
11	38.2	274	37.2		291	249	61.8		
12									
13	38.0			25.5					
14	40.7	223	35.9	30	182	159	55.2		
15	38.8	227	35.9	35.9	199	184	68.4		
16	41.6	229	34.1	41.9	186	168	88		
17	39.1	211	34.6	46.4	152	132	74.9		
18	39.6	186	29.3		125	108	78.9		
19									
20	46.0								
21	29.6	230	26.4		165	130	126		
22	33.0	273	28.6	42.4	146	120	92.3		
23	33.4	256	29.7		130	116	106		
24	36.8	252	28.6		178	145	78.9		
25	39.9	239	30.2		166	131	92.3		
26									
27	37.5	180	23.1	30.2					
28	43.1	244	27.8		95.2	85.2	90.7		
29	42.9	152	31.2	34.7	141	116	116		
30	38.8	215	32.4		138	92.5	103		
31	43.1	175	28.4	34.7	111	85.8			
32	39.6		27				116		
33	24.4								
34	20.8		22.7				111		
35				40.9			82		

Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Waste TCOD (mg/L)
36	22.1	140	13.3						
37	31.1	140	19.9	52.2	165	143	52.3		
38	31.2		13.1		177	116	52.3		
39	35.0		8.59				46.4		
40	32.6		4.52						
41	27.8	341	6.08	51.2	238	181			
42	39.9		1.93						
43	33.6	285	3.19	53.5	213	181	61.6		
44	24.0		1.32						
45	35.1	279	2.93	37.5	246	194	68.4	75.2	
46									
47	38.5		2.43	63.8				Stop seeding	
48			2.75	47.7	253	222	77.5	91.3	
49		313	4.7						
50			7.91	35.8	230	201	63.7	119	
51		307							
52			9.22	17.4	237	207	66	124	278
53			7.02						
54	40.9		10.2	8.8					
55	35.3		18.3		252	216			
56	42.5	229	23.2	9.86					
57			24.1						
58	23.0	329	24.4	7.27					
59	16.1		24.4						
60	18.6		26.8	7.72	262	219			
61	23.9	310	25.7						
62	21.2		24.4		195	166			
63	22.7		25.7						
64	21.9				169	146			
65	20.9	224							
66	19.8				191	165	71.7	71.7	276
67	19.8								
68	20.9								
69	17.2								
70	23.3								

APPEENDIX E-2 cont'd

	HRT 53.3 h								
Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Waste TCOD (mg/L)
1	29.7	385	41.3	0	183	163	71		
2	31.6	266	40.9	0	186	158	51.5		
3	32.7	246	40.9	0	173	153	38.6		
4	38.1	233	40.5		133	113	45		
5									
6	31.3			0					
7	21.0	317	40.9	0	123	93.2	52.5		
8	25.6	291	33.1	0	161	128	39.2		
9	30.5	291	41.3	13.1	168	155	39.2	Start seeding	
10	31.4	284	37.2	23.2	174	149	48.7		
11	38.5	273	33.3		220	195	61.8		
12									
13	38.6			30					
14	41.3	218	34.1	38.9	177	152	48.7		
15	39.0	229	36.3	47.9	176	166	74.9		
16	42.0	231	31.1	56.9	175	162	74.9		
17	38.9	214	28.4	65.9	145	128	74.9		
18	39.0	189	24		121	104	78.9		
19				68.9					
20	46.5		30.3						
21	30.3	225	29.7		133	103	92.3		
22	33.5	275	29.1	46.5	133	110	99		
23	33.5	259	26.9		127	109	92.3		
24	37.0	255	25.8		155	122	99		
25	40.2	242	25.8		144	114	105		
26									
27	38.2		18.9	39.2					
28	44.0	176	23.3		113	90.3	103		
29	43.3	238	25	53.4	138	111	103		
30	38.8	148	27.7		133	90	103		
31	43.2	210	25.6	45.9	122	106			
32	38.8	171	23.7				122		
33	24.4								
34	20.4		18				115		
35							90		
36	21.6		14.9						
37	30.5	137	19.9	57.2	124	101	46.4		
38	30.6	137	9.51		166	107	52.3		
39	34.3		5.37				40.1		
40	31.9		1.62						
41	34.1		1.62						
42	34.9		2.61						
43	38.0		3.63						
44	27.2	278	3.31	59.7			68.4		
45	39.0		1.93		226	192	102		
46	32.8	273	2.56	58.9					
47	23.5		1.2		229	189	82.1	75.2	313
48	34.5		2.43	45.1					
49		270							
50	37.7		1.46		210	197	50	119	
51		307	6.27	47.7				Stop seeding	
52			4.23		190	173	63.4	84.4	
53			8.69	35.2					
54	40.9				181	155	66	98.1	265
55	35.3		11.8	21.1					
56	42.5	229	9.62						
57			10.6	1.67	201	179	72.4		
58	23.0	329	19						
59	16.1		21.4	2.75					
60	18.6		13.2						
61	23.9	310	24.2	1.12					
62	21.2		25.5		199	175			
63	22.7		23.9	6.09					
64	21.9		23.5		166	133			
65	20.9		22.8						
66	19.8	224	22.6		153	143			
67	19.8								
68	20.9				144	126	44.5	58.1	262
69	17.2								
70	23.3								

APPENDIX E-2 cont'd

	HRT 68.6 h								
Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Waste TCOD (mg/L)
1	29.7	385	36.4	0	154	131	45		
2	31.6	266	38	0	158	133	58		
3	32.7	246	37.2	0	165	133	45		
4	38.1	233	38		180	153	38.6		
5									
6	31.3			0					
7	21.0	317	31.4	0	100	75.3	39.2		
8	25.6	291	32.2	0	123	95.7	52.5		
9	30.2	291	41.3	12.9	143	108	39.2	Start seeding	
10	30.9	282	39.5	24.2	158	138	48.7		
11	39.1	271	36.6		169	143	61.8		
12									
13	39.7			24.4					
14	42.3	210	36.6	37.7	150	128	74.9		
15	39.3	232	36.6	46.5	158	145	74.9		
16	42.6	234	32.3	52	131	113	74.9		
17	38.6	218	28	64.1	106	93.2	81.5		
18	38.1	194	22.3		95	82.5	92.3		
19				90.7					
20	47.2		17.9						
21	31.4	217	19.3		96.2	70.9	99		
22	34.3	277	18.7	71.1	91.4	73.6	92.3		
23	33.7	262	18		87.7	87.7	105		
24	37.4	260	16.8		121	98.2	105		
25	40.8	247	15		110	80.2	119		
26									
27	39.2		8.8	64.1					
28	45.3	216	11.9		75	60	116		
29	44.0	276	14.7		101	85.4	116		
30	38.9	190	15.6		118	82.9	129		
31	43.2	249	10.8	80.9	111	93.4	148		
32	37.5	211	8				141		
33	20.9								
34	19.7		4.9				140		
35				55.9			120		
36	20.9		3.9						
37	29.5	178	5.4	73.4	124	101	65.4		
38	29.5		1.06				65.4		
39	33.2		1.94				40.1		
40	30.9		1.51						
41	32.9		1.51						
42	33.8		1.89						
43	36.8		3.05						
44	29.6	315	6.24				82.1		
45		292	4.8		118	90.1	88.9		
46	24.3		14.6	53.8					
47	30.3	368	14.1		594	229	48	Stop seeding	
48	43.7		13.0	41.6					
49	36.8	313	15.8		440	210	61.6	61.6	286
50	26.3		12.4	35.5					
51	38.2	307	18.4		427	201	61.6		
52				34.9				77.5	
53	42.3		16.9	19.4					
54	40.9	270	20.4		223	164	84.4	84.4	
55	35.3		19.2	12.2					
56	42.5	229	25.6		189	140	98.2	91.6	220
57				17.2					
58	23.0	329	23.6		166	124	66		
59	16.1		16.5	9.51					
60	18.6		15.6						
61	23.9	310	24.9	3.69	180	134	78.8		
62	21.2		27.0						
63	22.7		27.4	2.22	177	146			
64	21.9		29.8						
65	20.9		29.0	3.9					
66	19.8	224	28.2						
67	19.8		23.9	2.22					
68	20.9		22.1		154	116			
69	17.2		22.1						
70	23.3				142	129.0		58.1	262

APPENDIX E-2 cont'd

	HRT 96 h								
Days	Influent NH_3-N (mg/L)	Influent SCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Waste TCOD (mg/L)
1	29.7	385	35.9	0	155	130	51.5		
2	31.6	266	35.2	0	158	148	64.5		
3	32.7	246	35.2	0	140	118	38.6		
4	38.1	233	35.9		146	124	38.6		
5									
6	31.3			0					
7	21.0	317	30.9	0	90.2	77.7	39.2		
8	25.6	291	29.5	0	130	103	39.2		
9	29.7	291	43	13.3	117	93.8	45.8	Start seeding	
10	30.0	279	38.5	27.3	129	108	42.1		
11	40.0	269	36.9		137	118	61.8		
12									
13	41.6			44.3					
14	44.0	196	36.9	44.2	116	109	74.9		
15	39.9	237	38	53.1	120	115	61.8		
16	43.6	240	35.4		115	92.7	81.5		
17	38.1	225	32.8	74.1	104	88.4	94.6		
18	36.4	202	29.1		92.7	82.7	105		
19									
20	48.5		17.9						
21	33.5	202	18.7		97.5	80	119		
22	35.8	282	17.4	84.1	100	80.1	119		
23	34.1	269	17.4		96	93.5	92.3		
24	38.1	269	15		100	85.4	126		
25	41.8	255	13.1		107	86.4	112		
26									
27	41.0		8.2	74.4			122		
28	47.6	223	9.1		70.5	55.6	141		
29	45.3	279	11.9	89.6	103	82.7	154		
30	38.9	199	11.8		104	70.7	141		
31	43.4	254	9.75	80.9	87.5	75	154		
32	35.2	219	6.7		100	95			
33	20.0			101					
34	18.6		1.4				150		
35							140		
36	19.7		1.6						
37	27.7	188	4.4	111	109	91.4	90.7		
38	27.7	188	1.06		116	74.5	84.4		
39	31.3	188	2.2				71.7		
40	29.0		1.45						
41	38.4		1.64						
42	31.9		2.2						
43	34.6		1.82						
44	27.9	315	2.77				61.6		
45		294	3.7	75.9			88.9	Stop seeding	
46		365	6.6		135	113	75.2		
47			4.7	59.5					
48	43.7		9.1		120	103	68.4	119	225
49	36.8	313		49.2					
50	26.3		8.0						
51	38.2	307	11.5	42.9	167	130	77.5	91.3	
52			10.5	36.3					
53	42.3		18.3		166	131	50	91.3	
54	40.9	270		21.5					
55	35.3		17.4		158	121	59.6	78.8	207
56	42.5	229	11.5	36.3					
57			12.8						
58	23.0	329	20.4	22.6	154	123	53.2		
59	16.1		22.7						
60	18.6		23.1	16.3	174	132			
61	23.9	310	25.0						
62	21.2		24.6	11.5					
63	22.7		24.2						
64	21.9		27.6	6.4					
65	20.9		22.6		116	89.7			
66	19.8	224	23.7	2.75					
67	19.8				102	91.3			
68	20.9								
69	17.2				104	95.5	71.7	98.9	181
70	23.3								

APPENDIX F-1

Centrate and seed characteristics for NB10, NB20, NB25 and NB30 that were added to SBRs at 10°C with an apparent SRT of 4 d and HRTs of 12 h and 24 h

Days	Centrate			NB10					
	NH_3-N (mg/L)	SCOD (mg/L)	TCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	469	259	680	251	365	472	299	139	126
2									
3				251	321	434	319	138	109
4	503			350	409			183	144
5		215	597	253	428	604		226	204
6	486			160					
7				305			387		
8	193			320	379			255	190
9				347			306		
10	250			375	388			253	182
11				345			241		
12	212	116	380	345	372			230	165
13				307			272		
14				289				194	141
15	193			234	364		244		
16									
17	221			180			246	232	182
18				160					
19		94.4	300	167	385	506	315	131	121
20	334			144			245		
21				144					
22	309			115	391		497	155	120
23				69.5					
24	323			104	385	415	601	139	114
25				34.4					
26	505			29.4	494	560	603		
27	480			14.7					
28				17.9			759		
29	524			17.9	463			201	145
30				14.2			457		
31	522	227	463	4.6	403			192	142
32	698			7.8			415		
33	729	215	427	8.3	409	554		170	120
34	505								
35	516			3.7					
36	644			3.7					
37	722			3.7			509		
38	710			3.5					

Days	Centrate			NB10					
	NH_3-N (mg/L)	SCOD (mg/L)	TCOD (mg/L)	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
39	698			3.5			236		
40	747	197	385	1.9	373	457		128	106
41	710			3.8			457		
42	412			6.9					
43	398			6.1	428		551	128	95
44	646			4.8					
45	384			4.8	436		513	162	121
46	347			4.4					
47	379	96.2	155	3.8	371	421	324	130	92.5
48									
49	616			2.5					
50	305			16.1	340			108	97.5
51	432			11.3					
52				2.7	358			115	97.5
53									
54	813		341	9.9	363	453		103	87.5
55				2.7			476		
56	805			22.0					
57				8.0	332		946	123	103
58	813			1.5					
59				1.8	287			133	113
60	781			1.8			603		
61	747	172	282	1.1	204	297	408	123	100
62	765			1.1					
63	711			1.1					
64	711			8.3	201		440	130	104
65	707			15.7					
66	689			26.5	197		389	155	110
67	689			9.7					
68	718	172	255	14.2	197	289	420	118	100
69									
70	662			24.7			424		
71				25.9	231			122	100
72	683			27.9			444		
73				25.3					
74	704			9.7			514		
75	715			29.1					

APPENDIX F-1 cont'd

Days	NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1						
2						
3						
4						
5	0.93			409	241	219
6	0.93			386		
7	0.75				230	190
8	0.6					
9	3.26	273			230	196
10						
11						
12	1.37			377	320	270
13	1.37			430		
14					335	275
15	1.23			336		
16	13.1				359	323
17	34.2			318		
18	20.2					
19	21.8	250	388	330	323	253
20	1.37					
21	1.08			395	324	269
22	3.75					
23	2.79	235		608	328	308
24	18.1					
25	5.13					
26	50.1			479	389	332
27	2.41					
28	1.65			521	345	249
29	2.22					
30	7.79			486	356	304
31	46					
32	64.9			643		
33	48.4			498	378	308
34	3.78					

Days	NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
35	19.7			471	349	303
36	2.61					
37	25.3	240	473	406	306	245
38	26.6					
39	5.45			579		
40	4.76				340	310
41	7.77			736		
42	30.9				374	280
43	21.1			759		
44	1.6		451	513	365	295
45	9.79					
46	1.33					
47	0.78			507	403	326
48	47					
49	1.06			530	347	316
50	0.79					
51	0.79	239	536	513	335	309
52	0.79					
53	14.8			519		
54	0.65				367	335
55	0.65			565		
56	1.02				307	275
57	1.62			583		
58	2.03	249			351	378
59						
60	1.62			556		
61	1.41				342	286
62	1.62			567		
63	1.62				378	276
64	1.41			551		
65	2.03	249	656		435	363
66	16.4			535		
67	11.1					
68	1.76			572	402	342

APPENDIX F-1 cont'd

Days	NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1						
2						
3						
4						
5	1.4			458	410	345
6	1.4			402		
7	1.06				324	261
8	0.6					
9	1.06	273			343	278
10						
11						
12	1.06			434	369	313
13	0.84					
14	1.12			452	385	310
15	1.37			366		
16	1.37				393	337
17	3.82			370		
18	16					
19	21	323		383	422	312
20	1.65					
21	1.35			393	407	347
22	2.03					
23	2.41	242		524	315	285
24	12.1					
25	1.47					
26	18.6			486	500	425
27	9.48					
28	35.6			417	359	276
29	1.65					
30	52.7			414	401	335
31	196					
32				376		
33	127			293	406	345
34	252					
35	271			165	344	308
36	284					
37	277	250	431	68.7	311	268
38	242					

Days	NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
39	273			57.8		
40	270				266	198
41	290			37.6		
42	308				242	199
43	333			25.1		
44	243	253		23	309	219
45	136					
46	73.8					
47	67.25			138	309	263
48	50.75					
49	2.4			265	347	286
50	1.02					
51	1.28	253	529	346	331	295
52	1.02					
53	8.45			432		
54	0.63				335	284
55	0.89			507		
56	1.41				335	262
57	1.68			620		
58	2.54	233			386	310
59						
60	1.89			630		
61	1.68				339	297
62	1.68			588		
63	5.23				439	321
64	1.68			525		
65	16.4	266	664		395	342
66	12.1			620		
67	1.68					
68	1.37			588	398	326
69	15.8			540		
70	1.26				387	319
71	1.26			555		
72	1.26	260	571		349	296
73	5			535		
74	11.6					
75	0.62			530	330	255

APPENDIX F-1 cont'd

Days	NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1						
2						
3						
4						
5	0.93				478	389
6	0.93			465		
7	0.84				491	382
8	0.56					
9	1.12	273			446	364
10						
11						
12	5.24			449	466	383
13	1.27					
14	1.12			449	497	401
15	1.35			359		
16	1.35				505	425
17	1.35			362		
18	8.27					
19	1.08	259		381	503	402
20	1.08					
21	0.81			378	471	386
22	6.55					
23	1.84	193		535	416	376
24	22.6					
25	9.48					
26	0.91			524	524	426
27	1.1					
28	3.95			376	384	284
29	2.03					
30	31.1			410	374	327
31	136					
32				542		
33	150			301	658	465
34	134					
35	141			317	398	344
36	156					
37	164	235	487	317	376	304
38	166					

Days	NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
39	152			517		
40	94.9				457	400
41	77.1			774		
42	69.3				406	324
43	62.5					
44	3.77		699	573	462	366
45	34.2					
46	3.92					
47	3.92			582	399	312
48	1.55					
49	1.55			555	418	330
50	0.63					
51	0.5	224	551	524	415	336
52	0.63					
53	8.28			556		
54	0.5				360	300
55	13.2			570		
56	1.02				283	237
57	2.4			796		
58	2.4	241			329	257
59						
60	12.2			764		
61	1.18				328	292
62	1.18			732		
63	1.79				383	268
64	1.59			727		
65	2.4	249	599		388	311
66	24.8			780		
67	8.34					
68	0.97			716	393	307
69	1.26			560		
70	1.26				401	300
71	0.62			555		
72	0.94	255	534		328	297
73	8.38			540		
74	21.7					
75	1.26			500	355	280

APPENDIX F-2

Data for SBRs at 10°C seeded nitrifiers acclimated to different temperatures. The apparent SRT was 4 d and the HRT was 12 h.

Days	Influent NH_3-N (mg/L)	Influent COD (mg/L)	Seed source NB10								Days	Influent NH_3-N (mg/L)	Influent COD (mg/L)	Seed source NB10							
			Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)				Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
1	18.2		44.8	26.6	89.4	0.0	715	653			32			32.9							
2	20.3										33	20.1	403		58.1	82.3	8.6	613	537	31	26
3	19.1		48.7	58	76.8	0.0	780	545	21.6	17.7	34	21.1									
4	22.5		49.4	32.9			890	758	44.3	36.2	35	20.8	403	33.6			5.5				
5	17.3			32.9	64.3	0.0	820	765	46	42	36	20.8		32.9							
6	20.9		41.4								37	20.8	389	31.5			0.8				
7	21.1					0.0					38	19.7		28.9							
8			33.9	21.8			839	739	22	20	39	15.1		29.3			0.7				
9	22.2	431	48.7								40	20.8	380	25.8	94.4	155		484	445	36.3	31.3
10	19.6		45.5	9.7		1.0	817	700	28	22	41	19.9									
11	22.6										42	22	373								
12	23.5	409	32.7	54.7	54.7	0.2	541	449	24	20	43	25.6			48.1		3.6	620	541	34	30
13	21.1								Start seeding		44	22	380	35.5							
14	23.4	402	42.0			0.2	717	616	36	26	45	23.7		34.7	37.6		11.3	528	453	26	22
15	24.4		38.6	27.8							46	21.6		30.6							
16	23.6	402	37.3			0.9					47	22.5	423	28.0	17.3	30.1	6.8			9.3	8
17	19.4		34.5	39.9			605	545	32	24	48	19.1									
18	22.6		36.1								49	21.8	395								
19	24.2	409		15.7	52		665	600			50	23		24.9	22.3		5.9	619	539	15.3	14.7
20	24.6										51	22	380	38.0			5.5				
21	25	445									52	18.4		37.0	27.4			626	545	25	21
22	31.1		35.7	9.7		9.1	625	555	22	20	53	18.8		35.0			5.3				
23	27	382	28.1								54	25.4	380	36.0	27.4	54.7		892	811	23	21
24	28.3		26.7	27.8		1.6	590	530	32	30	55	18.8									
25	23.1		23.9								56	23.2	388	36.0			2.2				
26	27.3	403		9.7	33.9	3.0	580	545			57	20.6		32.0	37.6			1050	945	17	15
27	27.3										58	23	388	28.6			6.8				
28	25.2	410									59			24.5	22.3			901	799	18.3	16.7
29	20.1		29.8	21.8	137	2.0	525	440	92	80	60	21.8		24.5			8.5				
30	25.9	417	27.0								61	25.2	461	28.0		38	7.1	918	777	13	7
31	21.3		25.6	33.9		2.1	636	567	42	30	62	21.2									

APPENDIX F-2 cont'd

Days	Influent NH_3-N (mg/L)	Influent COD (mg/L)	Seed source NB10							
			Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
63	15.8		30.2	37.6		8.9	1050	905	31	21
64	24.8	412	22.7							
65	13.4		19.8	48.1		9.1	885	765	21	17
66	23.9		21.0							
67	30.1	412	29.9	37.6	46.2		870	780	25	23
68	22.8									
69	25.5	399	29.2			9.5				
70	25.5		27.8	32.5			890	795	19	17
71	30.6	392	25.1			13.7			Stop seeding	
72	24.3		24.4				940	810	21	19
73	27.4		31.3			10.0				
74	24.7	392	36.9	46.2	69.7		765	675	19	18
75	24.9		29.8							
76	22.3	384								
77	18.7									
78	23.1	377	28.6			6.4				
79	22.5			42.8			1025	915	26	20
80	34		26.5			6.5				
81	25.3	392	41.6	27.4	59		911	845	28.6	24.3
82	29.1									
83	24.2	377								
84	35.3		34.3	58.8		7.3	1019	802	38	23
85	24.2	392	33.9							
86	24		33.5	20.3			1053	976	11.5	
87	14.6		31.0							
88	22.7	399	41.1	27.4	91.3		1117	989	52	50
89	16.7		41.5			4.1				
90	23.7	399								
91			37.3	17.3		1.0	1278	1046	50	39
92		399								

APPENDIX F-2 cont'd

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	30.3			0	607	529
2	28.8					
3	28.2			0		
4	37					
5	29.1			0	1024	886
6	26.7					
7				0		
8	38.5					
9	37.4	109	130	0	919	759
10						
11	32.8			0		
12	30.9	87.3			1057	743
13	27.3			6.42	Start seeding	
14	32.1	87.3			981	819
15	24.4			5.42		
16	31	130	130			
17						
18	34.7			5.27		
19	29.7	137			744	593
20	32.4					
21	34.1	109			809	673
22	30.2					
23	30.8	117	152	7.4	841	744
24						
25						
26	29.9	124		7.11	657	568
27	26.6					
28	31.1	172		9.79	568	458
29	23.8					
30	32.5	145	249	7.04	787	680
31						

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32	34.1	166		6.64	696	593
33	33.7					
34	34.7	152		6.21	658	606
35	33.4					
36	37.1	182	228	3.92	622	528
37						
38						
39	33.7	139		3.5	699	631
40	23.7					
41	35.4	139		6.47	595	525
42	33.6					
43	28.2	125	189	3.45	604	532
44						
45						
46	28.2	139		6.16	718	625
47						
48	29.1	161		6.51	714	615
49	29.8					
50	30.7	154	196	7.26	673	568
51						
52	28.6			5.99		
53	31.4	125			728	661
54	21.3			8.87		
55	31.2	111			663	600
56	34.8			8.14		
57	33.4	139			749	623
58						
59						
60	33.4	78.7		8.87	939	828
61	25.4					
62	33.4	70.5		10.9	921	788

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	24.1					
64	30.3	108	115	11.9	882	816
65						
66						
67	36	152		7.53	896	786
68	33			3.04	Stop seeding	
69	33				785	681
70						
71	33			1.16		
72	38.7		272		788	697
73						
74						
75	34.4	92.9		0.88	865	748
76	28.3					
77	33.7	100		0.88	741	678
78	30.1					
79	38	115	138	0.13	671	624
80						
81						
82	38.4	175		0.58	763	654
83	38.8					
84	40.2	153		0.17	650	606
85	35.5					
86		153	168	0	646	548
87						
88	41.7					
89	35.5	168		0.47	759	642
90	31.3					
91	40.6	138		0.47	744	636
92						
93		138	182	0.27	719	635

APPENDIX F-2 cont'd

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	37.7			0	724	624
2	40.2					
3	32.7			0		
4	38.3					
5	29.1			0	918	817
6	27					
7	36.8			0	950	853
8	31.5					
9	39.4	159	166	0	852	722
10	27.1					
11	33.6			0		
12	36.8	130			905	774
13	29.4			2.37	Start seeding	
14	32.1	102			1047	811
15	29			3.97		
16	32.4	130	173			
17						
18	34.7			2.83		
19	31.9	130			1005	735
20	36.1			3.4		
21	34.3	116			954	778
22	32.5					
23	33.7	95.6	145	6.56	937	793
24						
25						
26	33.1	124		7.52	833	700
27	33.4					
28	33.4	159		10.3	876	708
29	24.7					
30	33.1	131	172	8.34	1028	873
31						

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32						
33	32.2	124		7.86	1018	847
34	32.9					
35	34.8	138		5.61	833	749
36	35.4					
37	38	124	214	2.59	897	737
38						
39						
40	38	132		1.45	671	580
41	27.1					
42	39.4	125		0.96	705	597
43	41.2					
44	42	132	182	0.23	630	531
45						
46						
47	38.6	125		0.23	693	605
48						
49	38.3	147		1.27	599	507
50	32.8					
51	36.5	125	175	1.32	740	638
52						
53	32.5			3.63		
54	27.1	139			847	715
55	24.1			7.32		
56	31.4	139			709	628
57	33.9			8.38		
58	33.9	123			772	680
59						
60						
61	34.2	160		9.11	911	793
62	27.9					

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	33.6	70.5			889	759
64	29.1					
65	30.4	86.8	175	10.5	949	846
66						
67						
68	36.3	119		10.3	870	753
69	31.2			7.8		
70	33.2	108			850	711
71	32.5			7.89		
72	36.8	100	138		774	707
73						
74						
75	31.6	115		9.34	805	691
76	25.1				Stop seeding	
77	32.8	100		3.98	779	665
78	25.4					
79	37.3	92.9	138	2.66	741	644
80						
81						
82	37.3	163		1.09	816	711
83	35.6					
84	37.6	108		0.78	750	589
85	33					
86		175	182	0.27	718	613
87						
88	38					
89	33.7	138		0.68	860	738
90	25.3					
91	38	130		0.58	787	670
92						
93		130	182	0.47	836	732

APPENDIX F-2 cont'd

Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1				0	750	650
2	32.7					
3	35.4			0		
4	38.9					
5				0	743	657
6	28.5					
7	30.1			0	682	607
8	28.5					
9	36.5	145	188	0	637	579
10						
11	30.3			0		
12	31.3	94.4			724	638
13	27.1			5.22	Start seeding	
14	32.1	102			832	705
15	29.7			1.69		
16	31.7	102	130		748	662
17						
18	35.2			2.11		
19	31.5	137			650	538
20	37.5			10		
21	37.6	123			689	597
22	32.2					
23	34.6	103	166	6.29	631	567
24						
25						
26	34.6	92		6.02	658	554
27	28.2					
28	32.5			1.98	688	575
29	34.6					
30	35.2	124	214	11	679	594
31						

Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32						
33	37.4	145		4.46	767	657
34	37.7					
35	40.7	138		3.29	729	641
36	36.7					
37	41.4		235	2.35	581	480
38						
39						
40	41.4	132		1.57	786	664
41	32.2					
42	37	125		1.26	763	631
43	38.9					
44	39.2	161	161	2.42	679	581
45						
46						
47	37.1	132		1.96	645	536
48						
49	35.7	132		5.36	587	515
50	35.7					
51	36.8	125	178	4.49	660	592
52						
53	30.9			4.2		
54	34.8	139			565	500
55	24.3			7.37		
56	32.2	139			536	483
57	34.2			8.5		
58	36.2	115			596	493
59						
60						
61	34.9	86.8		6.17	779	696
62	29.7					

Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	34.2	86.8		8.87	747	613
64	23.9					
65	31	100		9.11	794	685
66						
67						
68	36.3	119		11.6	663	576
69	34.8			13.5		
70	34.1	108			578	490
71	34.8			9.92		
72	43.7	108	153		599	531
73						
74						
75	35.4	123		7.22	721	621
76	30.6				Stop seeding	
77	30.3	108		2.66	755	655
78	27.8					
79	39.2	108	138	2.38	628	521
80						
81						
82	38.3			0.68	677	547
83	38					
84	37.6	115		0.68	675	589
85	39					
86		108	175	0.37	575	500
87						
88	39					
89	32.7	163		0.68	708	634
90	26.5					
91	39	153		0.58	642	554
92						
93		138	196	0.37	601	512

APPENDIX F-3

Data for SBRs at 10°C seeded with nitrifying biomass acclimated to different temperatures. The apparent SRT was 4 d and the HRT was 24 h. Influent and seed characteristics as listed in Appendix E-1 and E-2, respectively.

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	38.9			0	243	215
2	29.7					
3	28.7			0		
4	26.7					
5	26.7			0	449	411
6	39.3					
7	26.8			0	380	346
8	25.7					
9	34.9	102	159	0	394	303
10	24.7					
11	24.9			0		
12	27.3	145			383	301
13	30.6			18.7	Start seeding	
14	29.9	94.4			500	384
15	18.9			23		
16	19.9	102	230		658	465
17						
18	16.8			23.5		
19	21.6	130			485	357
20	24.6			20.9		
21	21.6	137			432	390
22	20.4					
23	20.9	124	138	29.4	423	412
24						
25						
26	14.1	138		44.4	390	375
27	16.7					
28	11.7	172		53.9	500	424
29	12.1					
30	18.5	145	193	32.5	502	421
31						

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32						
33	17.8	152		26.4	452	406
34	17.8					
35	17	124		26.6	474	427
36	15					
37	15.2	125		27.6	548	442
38						
39						
40	18.4	139		19.4	418	402
41	12.7					
42	20	147		19.3	484	421
43	19.9					
44	15.7	178	224	51.8	519	475
45						
46						
47	20	161		41.8	503	445
48						
49	21.6	161		42.8	468	400
50	19.5					
51	19.8	125	182	42.8	478	418
52						
53	15			47.2		
54	18.6	154			438	395
55	14.8			57.6		
56	19.3	154			442	416
57	28			24.8		
58	24.4	154			433	368
59						
60						
61	23.1	78.7		30.4	554	478
62	17.5					

Days	Seed source NB20					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	21	123		36.1	476	405
64	15.8					
65	18	92.9	144	37.4	518	456
66						
67						
68	21.7	130		50.3	482	405
69	18			37.7	Stop seeding	
70	21.9	103			477	404
71	24.3			18.6		
72	30.1	92.9	108		436	386
73						
74						
75	30.9	100		8.38	460	395
76	27.9					
77	31.6	108		6.83	432	365
78	28.7					
79	25.4	92.9	108	5.22	423	381
80						
81						
82	37.9	123		1.53	425	350
83	35.1					
84	38.3	115		1.43	429	370
85	33.1					
86		123	108	0.79	421	351
87						
88	40.8					
89	30.8	138		0.47	442	371
90	30.8					
91	39.9	138		0.26	450	393
92						
93		153	168	0.26	365	310

APPENDIX F-3 cont'd

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	34.7			0	450	368
2	30.3					
3	32.1			0		
4	27.3					
5	25.3			0	413	352
6	32.7					
7	30.3			0	408	354
8	29.2					
9	38.7	87.3	116	0	411	318
10	26.8					
11	29.2			0		
12	31.7	87.3			457	352
13	32.3			17.1	Start seeding	
14	29.9	94.4			555	416
15	24			18.6		
16	26.9	94.4	130		601	481
17						
18	22.7			20.1		
19	25.4	130			477	375
20	25.7			28.3		
21	25.6	159			608	487
22	23.7					
23	20.4	117	166	35.9	590	537
24						
25						
26	18.8	138		31.8	528	492
27	18					
28	19.6	152		31.3	441	377
29	18.5					
30	23.7	145	207	25.6	474	393
31						

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32						
33	34.6	166		12.8	495	439
34	34.6					
35	35	124		12.3	494	467
36	36.1					
37	39.6	118	193	9.18	487	387
38						
39						
40	40.8	125		4.04	444	398
41	34.6					
42	35	139		3.06	407	352
43	44.7					
44	48.7	111	182	4.76	495	413
45						
46						
47	41.1	125		4.52	441	360
48						
49	39.2	139		11.4	460	385
50	33.2					
51	36.7	111	125	15	439	384
52						
53	30.3			21.7		
54	27.3	139			442	366
55	22.3			32.8		
56	26.4	125			388	347
57	29.3			24.1		
58	30.3	125			395	344
59						
60						
61	30.6	78.7		20.6	535	465
62	24.1					

Days	Seed source NB25					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	28	86.8		25.7	470	413
64	23.8					
65	23.5	92.9	103	26.8	482	431
66						
67						
68	25.1	123		44.9	450	373
69	27.9			31		
70	30.1	92.9			458	399
71	27.6			29		
72	32.4	92.9	130		438	402
73						
74						
75	28.7	92.9		28.6	500	418
76	20.9				Stop seeding	
77	25.1	115		23.4	472	384
78	25.4					
79	30.6		123	12.8	406	361
80						
81						
82	36.7	108		7.17	431	335
83	35.9					
84	39.1	108		4.54	417	387
85	37.9					
86		123	108	2.92	366	314
87						
88	39.5					
89	39.5	123		1.64	459	388
90	37.9					
91	40.3	138		1.85	468	415
92						
93		138	153	0.58	434	379

APPENDIX F-3 cont'd

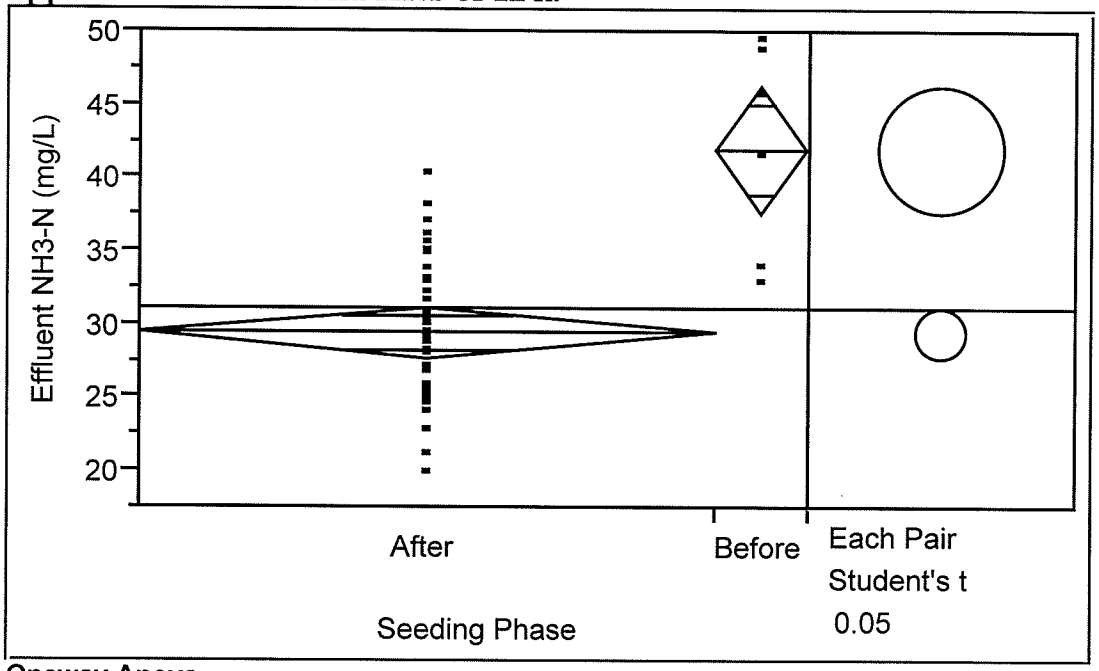
Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	31.2			0	417	367
2	29.7					
3	26.1			0		
4	26.1					
5	26.7			0	439	389
6	29.1					
7	29.1			0	414	368
8	28.3					
9	32.5	87.3	109	0	389	311
10	24.4					
11	23.9			0		
12	29.4	87.3			462	352
13	25.4			20.5	Start seeding	
14	29.1				500	395
15	24.2			18		
16	26.5	130	145		521	421
17						
18	21.6			20.9		
19	20.9	145			446	342
20	24.4			23		
21	27.2	116			460	395
22	28					
23	28	103	138	24.6	446	414
24						
25						
26	28.6	152		23.6	440	393
27	24					
28	26.3	124		34.1	448	361
29	21.5					
30	29.8	138	207	22	512	438
31						

Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
32						
33	32.7	152		12.6	440	381
34	32.7					
35	35.4	152		12.9	449	394
36	35.4					
37	38.8	154	214	10.5	436	335
38						
39						
40	37.1	178		6.18	379	358
41	28.9					
42	34.1	154		7	402	352
43	33.3					
44	37.4	154	210	26.18	444	368
45						
46						
47	32.1	154		22.4	405	245
48						
49	33.6	161		26.6	394	347
50	28.4					
51	32.1	125	161	30.2	420	362
52						
53	23.1			30		
54	26.6	139			400	350
55	23.1			34.7		
56	29.2	147			355	330
57	32.2			22.2		
58	33.4	139			367	305
59						
60						
61	32.7	119		30.2	484	431
62	27.3					

Days	Seed source NB30					
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
63	31.7	94.9		21.8	425	365
64	26.6					
65	27	119	108	22.2	427	361
66						
67						
68	33.2	103		30.2	474	392
69	31.9			25.7		
70	31.9	103			451	390
71	31.9			25.6		
72	33.7	92.9	168		416	376
73						
74						
75	29.7	92.9		28.4	462	387
76	22.6				Stop seeding	
77	29.3	108		14.2	376	299
78						
79	36.6	77.9	123	4.74	384	342
80						
81						
82	35.5			0.79	379	318
83	34.8					
84	38.4	108		0.79	335	304
85	32.7					
86		138	130	0.26	311	275
87						
88	39.9					
89	34.8	138		0.58	404	343
90	29.6					
91	39.9	123		0.58	378	347
92						
93		138	168	0.15	373	321

APPENDIX F-4

Compare effluent NH_3-N before and after seeding NB10 for an SBR with an apparent SRT of 4 d and HRT of 12 h.



Oneway Anova

Summary of Fit

Rsquare	0.421175
Adj Rsquare	0.407058
Root Mean Square Error	5.200362
Mean of Response	31.17674
Observations (or Sum Wgts)	43

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	-12.5009	-5.462	41	<.0001
Std Error	2.2887			
Lower 95%	-17.1231			
Upper 95%	-7.8788			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Seeding Phase	1	806.8023	806.802	29.8332	<.0001
Error	41	1108.7944	27.044		
C. Total	42	1915.5967			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
After	37	29.4324	0.8549	27.706	31.159
Before	6	41.9333	2.1230	37.646	46.221

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Before	After
Before	0.0000	12.5009
After	-12.5009	0.0000

Alpha=0.05

Comparisons for each pair using Student's t

t
2.01954

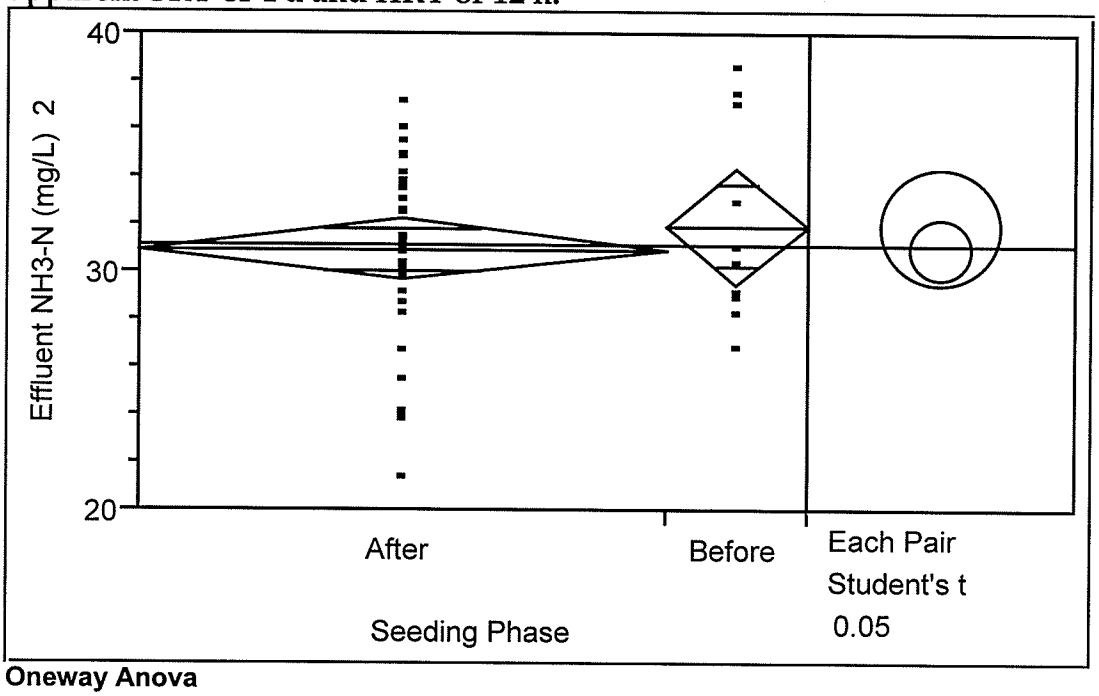
Abs(Dif)-LSD	Before	After
Before	-6.06353	7.87875
After	7.87875	-2.44175

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that the effluent NH_3-N after seeding was lower than before seeding.

APPENDIX F-4 cont'd

Compare effluent NH_3-N before and after seeding NB20 for an SBR with an apparent SRT of 4 d and HRT of 12 h.



Oneway Anova

Summary of Fit

Rsquare	0.011033
Adj Rsquare	-0.01047
Root Mean Square Error	3.861528
Mean of Response	31.19167
Observations (or Sum Wgts)	48

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	-0.98316	-0.716	46	0.4774
Std Error	1.37242			
Lower 95%	-3.74570			
Upper 95%	1.77939			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Seeding Phase	1	7.65225	7.6522	0.5132	0.4774
Error	46	685.92442	14.9114		
C. Total	47	693.57667			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
After	38	30.9868	0.6264	29.726	32.248
Before	10	31.9700	1.2211	29.512	34.428

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Before	After
Before	0.000000	0.983158
After	-0.98316	0.000000

Alpha=0.05

Comparisons for each pair using Student's t

t
2.01290

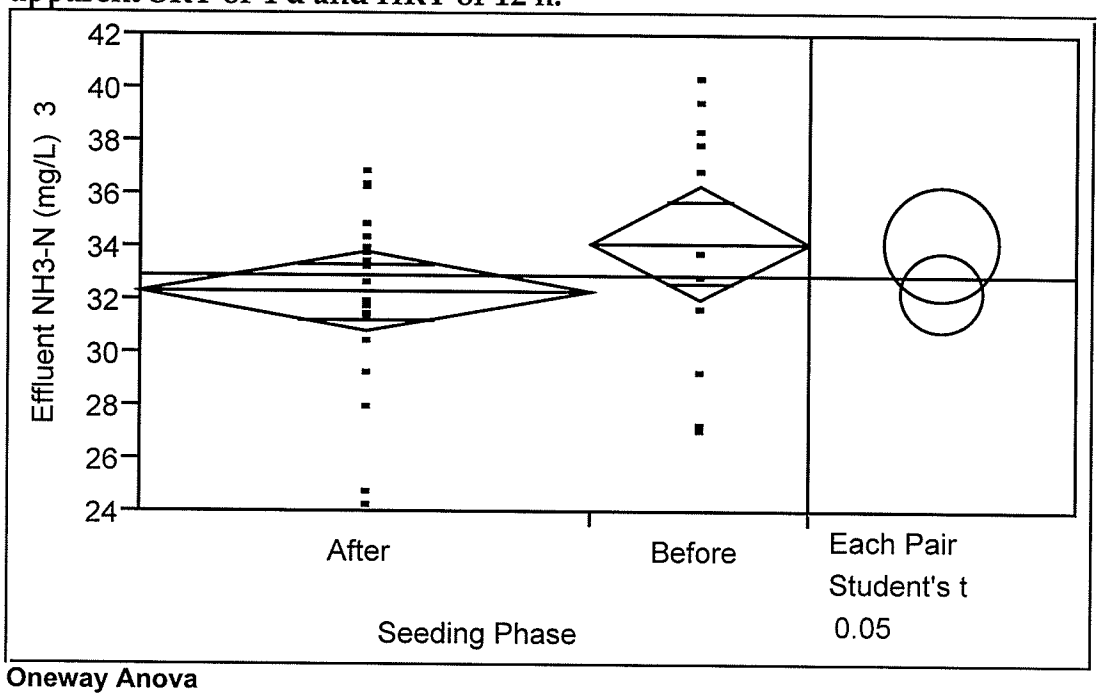
Abs(Dif)-LSD	Before	After
Before	-3.47613	-1.77939
After	-1.77939	-1.78321

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. The effluent NH_3-N after seeding was not lower than before seeding.

APPENDIX F-4 cont'd

Compare effluent NH_3-N before and after seeding NB25 for an SBR with an apparent SRT of 4 d and HRT of 12 h.



Oneway Anova

Summary of Fit

Rsquare	0.055263
Adj Rsquare	0.028271
Root Mean Square Error	3.707794
Mean of Response	32.92027
Observations (or Sum Wgts)	37

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	-1.86317	-1.431	35	0.1613
Std Error	1.30213			
Lower 95%	-4.50664			
Upper 95%	0.78031			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Seeding Phase	1	28.14641	28.1464	2.0473	0.1613
Error	35	481.17089	13.7477		
C. Total	36	509.31730			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
After	25	32.3160	0.7416	30.811	33.821
Before	12	34.1792	1.0703	32.006	36.352

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Before	After
Before	0.00000	1.86317
After	-1.86317	0.00000

Alpha=0.05

Comparisons for each pair using Student's t

t

2.03011

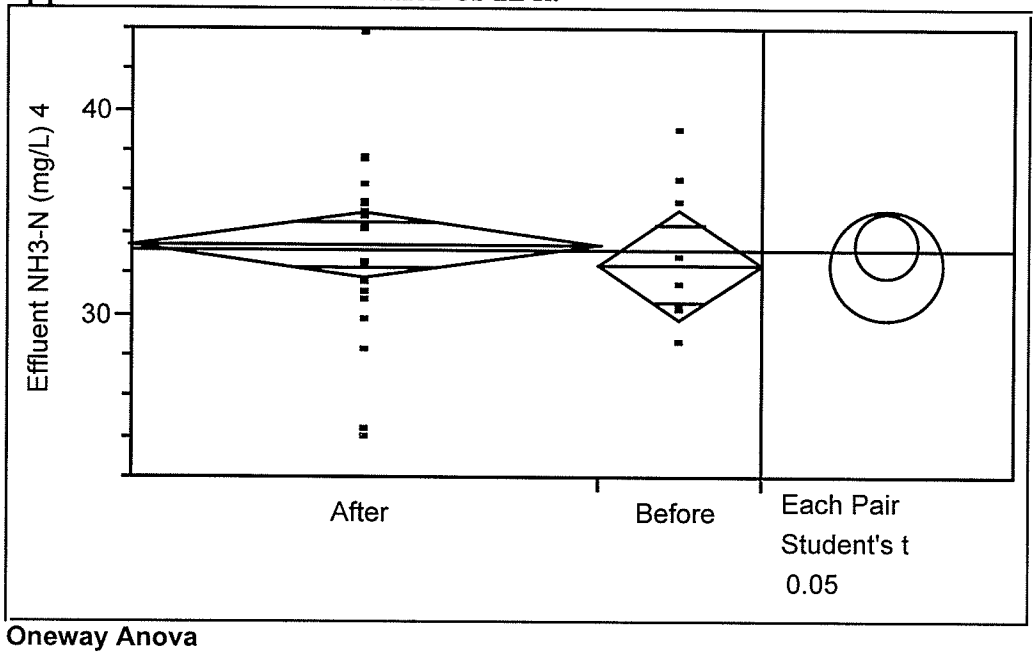
Abs(Dif)-LSD	Before	After
Before	-3.07298	-0.78031
After	-0.78031	-2.12902

Positive values show pairs of means that are significantly differe

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. The effluent NH_3-N after seeding was not lower than before seeding.

APPENDIX F-4 cont'd

Compare effluent NH_3-N before and after seeding NB30 for an SBR with an apparent SRT of 4 d and HRT of 12 h.



Oneway Anova

Summary of Fit

Rsquare	0.011431
Adj Rsquare	-0.01853
Root Mean Square Error	3.971161
Mean of Response	33.17143
Observations (or Sum Wgts)	35

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	0.94872	0.618	33	0.5410
Std Error	1.53583			
Lower 95%	-2.17595			
Upper 95%	4.07339			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Phase 4	1	6.01758	6.0176	0.3816	0.5410
Error	33	520.41385	15.7701		
C. Total	34	526.43143			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
After	26	33.4154	0.7788	31.831	35.000
Before	9	32.4667	1.3237	29.774	35.160

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	After	Before
After	0.000000	0.948718
Before	-0.94872	0.000000

Alpha=0.05

Comparisons for each pair using Student's t

t

2.03452

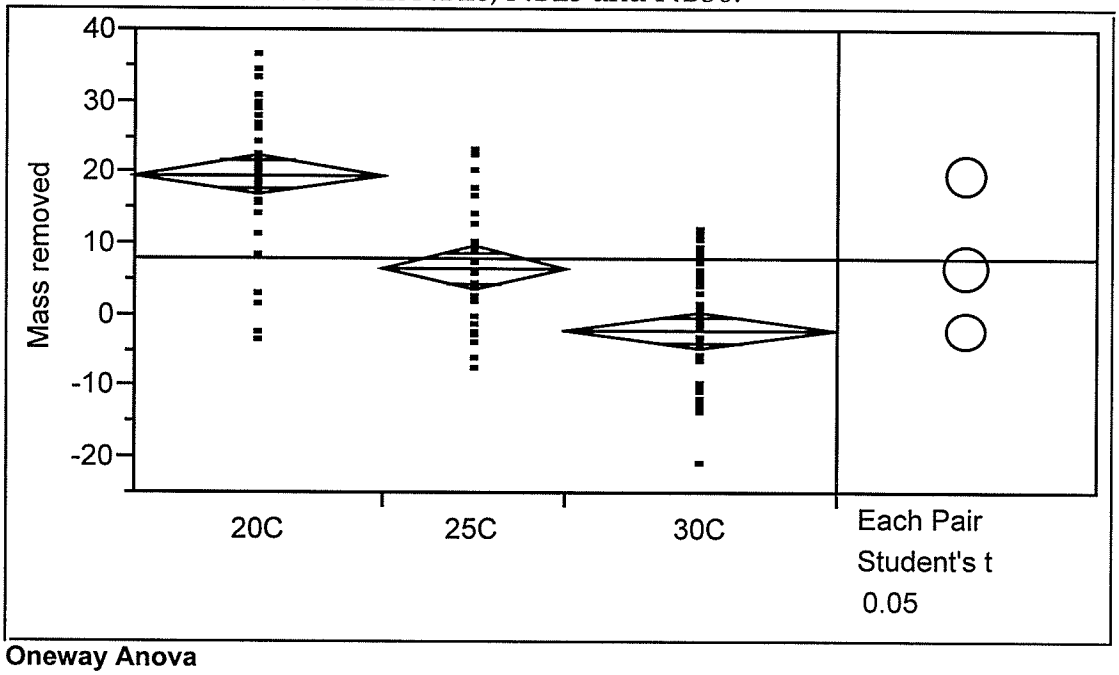
Abs(Dif)-LSD	After	Before
After	-2.24082	-2.17595
Before	-2.17595	-3.80866

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. The effluent NH_3-N after seeding was not lower than before seeding.

APPENDIX F-5

Comparison of NH_3-N removal from SBRs with an apparent SRT of 4 d and an HRT of 24 h seeded with NB20, NB25 and NB30.



Oneway Anova

Summary of Fit

Rsquare	0.547503
Adj Rsquare	0.539701
Root Mean Square Error	8.667426
Mean of Response	7.905686
Observations (or Sum Wgts)	119

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Seed Temperature 2	2	10544.078	5272.04	70.1776	<.0001
Error	116	8714.416	75.12		
C. Total	118	19258.495			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
20C	42	19.7762	1.3374	17.127	22.425
25C	31	6.6210	1.5567	3.538	9.704
30C	46	-2.0668	1.2779	-4.598	0.464

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	20C	25C	30C
20C	0.0000	13.1552	21.8430
25C	-13.1552	0.0000	8.6878
30C	-21.8430	-8.6878	0.0000

Alpha=0.05

Comparisons for each pair using Student's t

t
1.98063

Abs(Dif)-LSD	20C	25C	30C
20C	-3.7461	9.0903	18.1792
25C	9.0903	-4.3604	4.6987
30C	18.1792	4.6987	-3.5796

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that when the HRT was 24 hours:

a. NB20 removed more NH_3-N than NB25 and NB30.
b. NB25 removed more NH_3-N than NB30.

APPENDIX G-1

Seed characteristics for NB10 and NB20 that were added to SBRs at 10°C with SRTs of 12 d and HRTs of 8 h.

	NB10						
Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1		5.22			489		
2		4.88					
3	621	4.36					
4		4.53			497		
5	716	3.37					
6							
7	710	2.21			520		
8							
9	617	3.96			503		
10							
11	634	1.36			496		
12						174	151
13							
14							
15	691	0.81			537	185	138
16	681	2.35				Start seeding	
17			83.7			184	149
18	684	2.5			525		
19			149	341		160	147
20							
21							
22	681	1.64			508	145	135
23							
24							
25	721	1.36			496		
26							
27	687	5.2			502		
28							
29	672	2.24			488		
30							
31							
32	651	5.37			458		
33							
34	615	2.7			448		

	NB10						
Days	Centrate NH_3-N (mg/L)	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
35							
36	569	4.61			437		
37							
38		4.21			406	187	173
39						Stop seeding	
40	743	2.37				203	168
41							
42							
43	718	5.01					
44							
45	650	3.56					
46	755	2.86					
47	681	3.03					
48							
49							
50	808	2.51					
51							
52	674	1.66					
53	598	17.6					
54		21.4				268	230
55							
56							
57		1.52					
58							
59	788	4.06					
60							
61							
62							
63							
64	610	1.95					
65							
66	665	3.4					
67							

APPENDIX G-1 cont'd

Days	NB20 Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
1	1.62			557		
2	1.78					
3	2.4					
4	1.93			608		
5	1.92					
6						
7	1.92			673		
8						
9	1.92			694		
10						
11	1.64					
12				673	247	212
13						
14						
15	2.21			701	275	215
16	3.1			713	Start seeding	
17		239			235	200
18	1.78					
19		166	438		220	194
20						
21						
22	2.21			701	211	193
23						
24						
25	3.12			627		
26						
27	5.86					
28						
29	7.12			560		
30						
31						
32	7.5			673		
33						

Days	NB20 Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)
34	7.85			559		
35	7.2			517		
36						
37	6.21			502	318	286
38				496		
39					215	265
40					Stop seeding	
41						
42	7.85					
43						
44	8.23					
45	46.1					
46	57.1					
47						
48						
49						
50						
51	4.1					
52	54.7					
53	109				332	294
54						
55						
56	111					
57						
58	91.6					
59						
60						
61						
62						
63	13.6					
64						
65	50.4					
66						

APPENDIX G-2

Data for SBRs at 10°C with an SRT of 12 d and HRT of 8 h, seeded with NB10 and NB20.

Days	Influent NH_3-N (mg/L)	Influent COD (mg/L)	Seed source NB10							
			Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
1	19.7	302	38.7	34.1		0.41	2506	2168	100	92
2	18.8		34							
3	15	255	29.4	46.2		1.34	2505	2124	20.8	19.2
4	15.9		28.8							
5	20.4	255	38.3	46.2	54.7	0.22	2613	2273	52	50
6										
7	25.2	202	36.8			0	2925	2562	37	32
8										
9	23.8		31.9		26.3	0				
10			34				2871	2529	36	34
11	17.4		32.1	38		0				
12			29	34.1	38		2780	2301	33	31
13										
14										
15	22.8		44.4			0	2814	2307	26	15
16									Start seeding	
17	28.2		42			0.65	2386	2064	20	18
18			42							
19	26.3	221	41.1	18.8	26.3	3.2	2343	2057	18.5	15.9
20										
21										
22	30.6						2662	2331		
23			34.4			10.4				
24										
25	32.5		29.2			8.32				
26			27.6							
27	24.8									
28										
29	29.2		21.3			8.48				
30			18.9							
31										
32	24									
33			15.2			22.8				
34	26.3									

Days	Influent NH_3-N (mg/L)	Influent COD (mg/L)	Seed source NB10							
			Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
35			2.3							
36	25.6		8.1							
37			5.3			40.9				
38							1935	1695	27	24
39			4.3			37.6			Stop seeding	
40	23.9	202	10.5	42.1	54.7				22	20
41			1.76			38.3				
42										
43	24.5		4.47	22.5		38.6	2229	1959	14.7	13
44	30.1		2.57							
45	21.4		4.04			34.9	2329	2024	39	34
46	22.5		7.61							
47	25.4		7.58	16.3	21.7		2884	2512	36	34
48										
49										
50	25.4		9.27			37.6	2562	2223	36	34
51	25.1		1.56			40.6				
52	21.7		1.76			43	2813	2440	64	62
53	19.9		1.16			33.8				
54							2494	2213		
55										
56										
57	26.6		6.58			43.2	2953	2552	23	20
58			5.79							
59	18.3		5.04			39.6	3188	2841	33	31
60	19.6		1.28							
61	20		1.28			45.6	2206	2006	27	24
62										
63										
64	25.1		2.39			33.3	2412	2171	30	25
65	22.3		0.66							
66	25.3		1.03	16.3	16.3	30	2529	2276	29	27
67	18.1		2.8							

APPENDIX G-2 cont'd

Days	Seed source NB20							
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
1	31.1	30.1		0	1895	1682	54	45.7
2	27.4							
3				0	2454	2177		
4	28.4							
5	41.7	50.4	54.7	0	2540	2253	34	30
6								
7	40.4	54.7		0	2238	1981	28	26
8								
9	32.6	22.5		0				
10	30				2487	2218	37	32
11	32.3			0				
12	29.3	30.1	34.1		2480	2053	30	28
13								
14								
15	44.4			0	2364	1986	30	21
16	44						Start seeding	
17	42.2			0.41	2514	2143	20	15
18								
19	41.1	26.3	67.9	2.96	2280	2003	30	23
20								
21								
22					2464	2141		
23								
24								
25	23.9			11				
26	15.9							
27								
28								
29	25.1			14.7				
30	22.7							
31								
32								
33	20.7			9.02				
34								

Days	Seed source NB20							
	Effluent NH_3-N (mg/L)	Effluent SCOD (mg/L)	Effluent TCOD (mg/L)	Effluent NO_3-N (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)	Effluent VSS (mg/L)
35	6.27			37.5				
36	4.63							
37	2.88			37.8				
38	4.83	30.1			1805	1578	19	18
39	1.4			33.5			Stop seeding	
40	12.2	50.4	63.4				18	17
41	17.9			22				
42								
43	17.9	15.2		22.4	2529	2218	12.7	12
44	19.5							
45	20.6			20.5	2218	1947	18.7	16
46	19.5							
47	20.9	21.7	18.9	16.5	2706	2394	16	14
48								
49								
50	22			15.9	3222	2811	28	25
51	20.3			20.5				
52	18.7			1.55	2541	2406	47	73
53	20.3			3.06				
54								
55								
56								
57	24				2418	2171	17.3	12.7
58	23.7							
59	22.4			5.78	2635	2435	22	21
60	16.4							
61	17.3				2605	2400	27	24
62								
63								
64	12.4			6.31	2529	2288	37	32
65	13.6							
66	12.8			3.73	2465	2247	21	21
67	12.8							

APPENDIX H-1

Relative area quantification of Nso1225 versus DAPI for NB10.
Total pixels per photo=2150400

Days	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
9	124314	7269	5.8%
	129616	20119	15.5%
	136425	8516	6.2%
	192609	10660	5.5%
	276677	50884	18.4%
	232991	15623	6.7%
	400769	29301	7.3%
	239059	20300	8.5%
	77837	15020	19.3%
	173529	24064	13.9%
Mean	198383	20176	10.7%
St.Dev.	93796	12831	5.5%
22	45781	10464	22.9%
	109211	11858	10.9%
	136342	4890	3.6%
	39681	9150	23.1%
	37948	8619	22.7%
	84908	5980	7.0%
	126124	40846	32.4%
	93365	20526	22.0%
	85071	7631	9.0%
	171024	27138	15.9%
Mean	92946	14710	16.9%
St.Dev.	44169	11491	9.1%
29	68155	9185	13.5%
	36461	1431	3.9%
	86819	7190	8.3%
	57916	4835	8.3%
	110272	14206	12.9%
	63608	11744	18.5%
	65948	3739	5.7%
	24575	5117	20.8%
	41378	7824	18.9%
	62460	2486	4.0%
Mean	61759	6776	11.5%
St.Dev.	24713	4078	6.4%
40	33261	2142	6.4%
	22127	4121	18.6%
	23112	8651	37.4%
	20485	5548	27.1%
	29465	6464	21.9%
	27350	3163	11.6%
	49852	5904	11.8%
	25847	2571	9.9%
	310965	89860	28.9%
	42457	3511	8.3%
Mean	58492	13194	18.2%
St.Dev.	89199	27012	10.4%

Days	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
45	48109	10068	20.9%
	134302	31810	23.7%
	66604	9510	14.3%
	74313	40858	55.0%
	90822	6469	7.1%
	32571	9920	30.5%
	136715	29815	21.8%
	210489	27553	13.1%
	60027	10681	17.8%
	116630	41378	35.5%
Mean	97058	21806	24.0%
St.Dev.	53353	13875	13.7%
52	117270	24034	20.5%
	34559	1563	4.5%
	85378	3801	4.5%
	55315	5515	10.0%
	80716	25380	31.4%
	106382	28415	26.7%
	184664	22437	12.2%
	61808	17059	27.6%
	62878	7906	12.6%
	63734	35284	55.4%
Mean	85270	17139	20.5%
St.Dev.	42628	11742	15.6%
59	118326	31937	27.0%
	62056	15811	25.5%
	159846	49014	30.7%
	42291	5076	12.0%
	26545	4150	15.6%
	11620	1706	14.7%
	171163	94654	55.3%
	37984	8760	23.1%
	88300	11698	13.2%
	79974	8843	11.1%
Mean	79811	23165	22.8%
St.Dev.	55041	29066	13.4%
66	27705	2867	10.3%
	55584	3862	6.9%
	99139	26074	26.3%
	35160	3967	11.3%
	56302	16632	29.5%
	52834	9331	17.7%
	22907	3738	16.3%
	27523	3527	12.8%
	70899	10320	14.6%
	47800	20014	41.9%
Mean	49585	10033	18.8%
St.Dev.	23310	8228	10.7%

APPENDIX H-1 cont'd

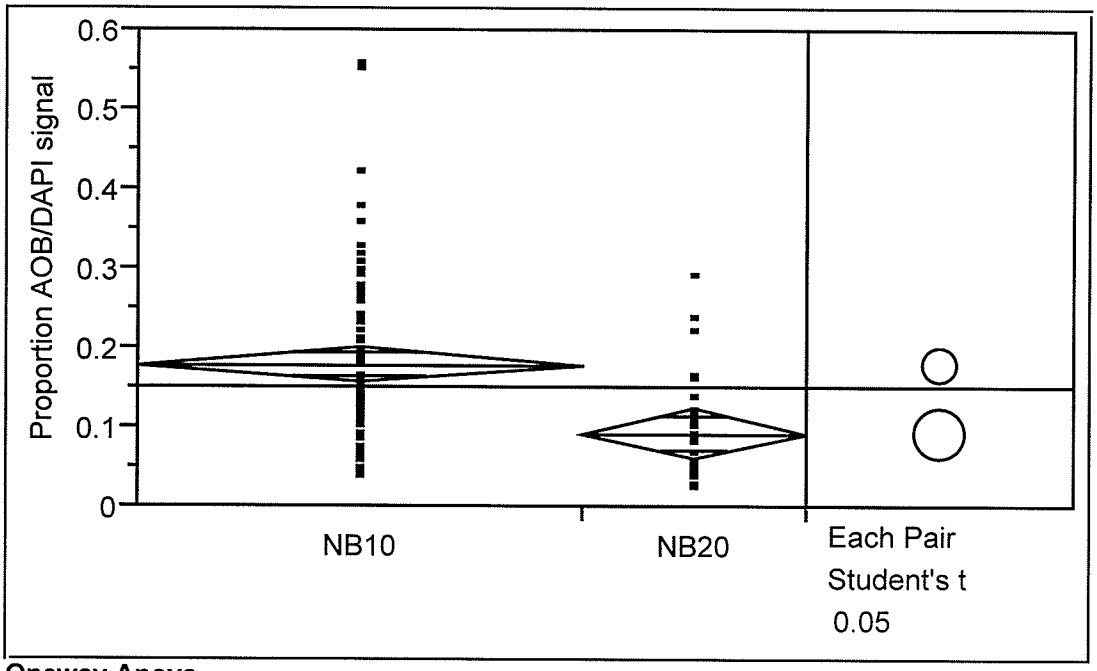
Relative area quantification of Nso1225 versus DAPI for NB20

Total pixels per photo=2150400

Days	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
1	51862	6134	11.8%
	21867	1686	7.7%
	66331	8882	13.4%
	30007	2434	8.1%
	78242	3032	3.9%
	80702	10869	13.5%
	56685	2558	4.5%
	26520	6199	23.4%
	61736	4004	6.5%
	32337	4366	13.5%
Mean	50629	5016	10.6%
St.Dev.	21714	3001	5.8%
17	57413	1412	2.5%
	21574	4705	21.8%
	39361	11406	29.0%
	22357	1906	8.5%
	42393	6653	15.7%
	12831	459	3.6%
	37180	958	2.6%
	84622	8999	10.6%
	40805	4558	11.2%
Mean	38025	4406	11.7%
St.Dev.	21064	3650	9.1%
28	58617	2514	4.3%
	20536	2223	10.8%
	42158	2088	5.0%
	24596	2111	8.6%
	25789	2609	10.1%
	12831	1526	11.9%
	51425	1085	2.1%
	57846	2287	4.0%
Mean	35289	2450	7.1%
St.Dev.	16657	962	3.7%
45	6995	949	13.6%
	45712	2121	4.6%
	64389	5688	8.8%
	37435	3640	9.7%
	86976	13996	16.1%
	37929	3962	10.4%
	43319	2294	5.3%
	34704	1880	5.4%
Mean	40940	3794	9.3%
St.Dev.	22439	3864	4.1%

APPENDIX H-1 cont'd

Compare relative area quantification of AOBs by FISH analysis of NB10 and NB20.



Oneway Anova

Summary of Fit

Rsquare	0.142536
Adj Rsquare	0.135269
Root Mean Square Error	0.100303
Mean of Response	0.150574
Observations (or Sum Wgts)	120

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	0.086025	4.429	118	<.0001
Std Error	0.019424			
Lower 95%	0.047561			
Upper 95%	0.124488			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Reactor 3	1	0.1973394	0.197339	19.6151	<.0001
Error	118	1.1871513	0.010061		
C. Total	119	1.3844907			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
NB10	80	0.179248	0.01121	0.15704	0.20146
NB20	40	0.093224	0.01586	0.06182	0.12463

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	NB10	NB20
NB10	0.000000	0.086025
NB20	-0.08602	0.000000

Alpha=0.05

Comparisons for each pair using Student's t

t

1.98027

Abs(Dif)-LSD	NB10	NB20
NB10	-0.03141	0.047561
NB20	0.047561	-0.04441

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that NB10 had a higher proportion of AOBs than NB20.

APPENDIX H-2

FISH analysis of seeded SBRs at 10°C with an apparent SRT of 12 d and an HRT of 8 h.

Days	Seeded with NB10					
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nsm156 (Pixels)	Nsm156/DAPI (%)
10	58512	202	0.35%			
	69543	1357	1.95%			
	86521	1998	2.31%			
	90548	1504	1.66%			
	52147	488	0.94%			
	76891	1948	2.53%			
	32547	921	2.83%			
	122156	1597	1.31%			
	105879	1587	1.50%			
	84215	1222	1.45%			
Mean	77896	1282	1.68%			
St.Dev	26210	590	0.75%			
22	57167	165	0.29%	138427	1885	1.36%
	71500	1466	2.05%	166529	2897	1.74%
	87200	2678	3.07%	157651	173	0.11%
	92862	1605	1.73%	72508	1603	2.21%
	60140	799	1.33%	56048	1049	1.87%
	77491	2077	2.68%	142688	2300	1.61%
	37832	1312	3.47%	153928	1506	0.98%
	120156	1618	1.35%	87119	1408	1.62%
	113244	1257	1.11%	197686	3513	1.78%
	85929	1236	1.44%	145637	1445	0.99%
Mean	80352	1421	1.85%	131822	1778	1.43%
St.Dev	25257	677	0.97%	45104	943	0.60%
37	38298	2547	6.65%	50927	646	1.27%
	80402	5897	7.33%	55437	5	0.01%
	63037	7851	12.45%	66685	232	0.35%
	90118	4859	5.39%	74877	1455	1.94%
	386724	18164	4.70%	65047	1258	1.93%
	108211	4345	4.02%	76447	171	0.22%
	35843	4389	12.25%	46599	1051	2.26%
	134121	7232	5.39%	54357	824	1.52%
	64241	3956	6.16%	56237	1406	2.50%
	73310	8858	12.08%	56663	189	0.33%
Mean	107431	6810	7.64%	60328	724	1.23%
St.Dev	102552	4438	3.32%	10004	554	0.93%

Days	Seeded with NB10					
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nsm156 (Pixels)	Nsm156/DAPI (%)
43	82464	4336	5.26%	90536	1469	1.62%
	77207	2381	3.08%	40089	294	0.73%
	58872	1309	2.22%	82981	1214	1.46%
	23501	1394	5.93%	157858	765	0.48%
	18904	1393	7.37%	119660	1419	1.19%
	96022	1570	1.64%	127985	2816	2.20%
	54626	2217	4.06%	92087	1200	1.30%
	50320	1926	3.83%	93239	1283	1.38%
	33873	2219	6.55%	94209	1192	1.27%
	84448	2791	3.30%	41849	518	1.24%
Mean	58024	2154	4.32%	94049	1217	1.29%
St.Dev.	26842	912	1.89%	35986	684	0.47%
59	68240	3709	5.44%	78326	639	0.82%
	51363	2173	4.23%	35750	943	2.64%
	62122	1692	2.72%	46379	699	1.51%
	60521	1416	2.34%	63114	1124	1.78%
	78202	1628	2.08%	76549	3420	4.47%
	86448	5305	6.14%	72989	700	0.96%
	76607	2658	3.47%	62173	128	0.21%
	69842	1720	2.46%	44234	333	0.75%
	71359	2819	3.95%	87300	2953	3.38%
	32518	1625	5.00%	51791	1086	2.10%
Mean	65722	2475	3.78%	61861	1203	1.86%
St.Dev.	15303	1226	1.41%	16974	1096	1.32%

APPENDIX H-2 cont'd

Days	Seeded with NB20					
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nsm156 (Pixels)	Nsm156/DAPI (%)
10	86200	1523	1.77%			
	55676	1273	2.29%			
	62097	1904	3.07%			
	45605	1383	3.03%			
	60728	2842	4.68%			
	47613	3428	7.20%			
	62824	1727	2.75%			
	33023	377	1.14%			
	50956	3003	5.89%			
	101185	2376	2.35%			
Mean	60591	1984	3.42%			
St.Dev	19947	928	1.92%			
22	143006	9354	6.54%	22107	319	1.44%
	102210	11946	11.69%	62531	703	1.12%
	39983	2549	6.38%	30824	659	2.14%
	61236	3498	5.71%	31731	405	1.28%
	59496	3597	6.05%	39984	300	0.75%
	33581	1567	4.67%	57600	832	1.44%
	62020	7067	11.39%	273332	650	0.24%
	83154	8956	10.77%	50867	283	0.56%
	66994	5437	8.12%	111472	1557	1.40%
	71485	2697	3.77%	24044	280	1.16%
Mean	72317	5667	7.51%	70449	599	1.15%
St.Dev	31581	3499	2.85%	75970	394	0.53%
37	87264	7964	9.13%	206631	1285	0.62%
	104481	2557	2.45%	478452	5836	1.22%
	113000	7156	6.33%	190566	4291	2.25%
	51343	1346	2.62%	113798	201	0.18%
	27601	1591	5.76%	51427	561	1.09%
	47142	3049	6.47%	149371	2131	1.43%
	42613	795	1.87%	91667	1373	1.50%
	51764	1834	3.54%	56777	889	1.57%
	44116	1626	3.69%	45910	590	1.29%
	12619	738	5.85%	61589	974	1.58%
Mean	58194	2866	4.77%	144619	1813	1.27%
St.Dev	32754	2579	2.30%	130901	1829	0.57%

Days	Seeded with NB20					
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nsm156 (Pixels)	Nsm156/DAPI (%)
43	102954	998	0.97%	81379	632	0.78%
	153617	3090	2.01%	52876	697	1.32%
	49431	144	0.29%	68232	349	0.51%
	37450	155	0.41%	48165	252	0.52%
	91388	948	1.04%	69178	293	0.42%
	63795	1007	1.58%	83852	265	0.32%
	110252	79	0.07%	97359	233	0.24%
	195749	100	0.05%	133865	2007	1.50%
	140151	870	0.62%	185063	877	0.47%
	97592	15	0.02%	49873	159	0.32%
Mean	104238	741	0.71%	86984	576	0.64%
St.Dev.	48819	930	0.68%	43053	556	0.43%
59	115239	1994	1.73%	103055	117	0.11%
	103698	2091	2.02%	50403	718	1.42%
	50365	1123	2.23%	39493	163	0.41%
	40556	165	0.41%	72633	1274	1.75%
	95244	944	0.99%	54203	893	1.65%
	60999	1007	1.65%	73466	732	1.00%
	125665	178	0.14%	35919	280	0.78%
	205789	220	0.11%	51348	451	0.88%
	155498	870	0.56%	46702	431	0.92%
	96235	115	0.12%	80190	1276	1.59%
Mean	104929	871	1.00%	60741	634	1.05%
St.Dev.	49987	731	0.84%	21015	421	0.55%

APPENDIX H-3

FISH analysis of reactor MLVSS and effluent solids for SBRs at 10°C seeded with NB10 and NB20.

The apparent SRT of the seeded reactors was 4 d and the HRT was 12 hours.

Reactor MLVSS

Days	Seeded with NB10			Seeded with NB20		
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
9	16978	328	1.93%	112084	951	0.85%
	32078	361	1.13%	148786	5182	3.48%
	49776	1312	2.64%	73050	876	1.20%
	74603	1056	1.42%	65044	2004	3.08%
	39521	353	0.89%	217634	6692	3.07%
	73873	87	0.12%	161674	5776	3.57%
	77971	587	0.75%	140029	1047	0.75%
	120246	4622	3.84%	265498	7797	2.94%
	84046	2098	2.50%	80020	648	0.81%
	145843	4321	2.96%	128473	3045	2.37%
Mean	71494	1513	1.82%	139229	3402	2.21%
St.Dev	39636	1670	1.16%	64056	2719	1.18%
15	190742	10464	5.49%	230555	3898	1.69%
	58264	2916	5.00%	209214	5011	2.40%
	43767	1607	3.67%	67900	1532	2.26%
	123019	1732	1.41%	84915	2403	2.83%
	171244	2332	1.36%	147770	10039	6.79%
	141288	7553	5.35%	117562	3235	2.75%
	120933	2164	1.79%	46493	1315	2.83%
	123583	3624	2.93%	125810	9176	7.29%
	129193	5696	4.41%	31354	1267	4.04%
	54367	1469	2.70%	152812	4123	2.70%
Mean	115640	3956	3.41%	121439	4200	3.56%
St.Dev	49364	3019	1.61%	66043	3124	1.93%
29	27332	1184	4.33%	226164	26167	11.57%
	25880	1222	4.72%	94525	3251	3.44%
	31787	772	2.43%	90633	3676	4.06%
	30406	1253	4.12%	213630	17697	8.28%
	47322	1079	2.28%	279342	24458	8.76%
	57223	1810	3.16%	69870	3189	4.56%
	24402	1075	4.41%	186552	5316	2.85%
	36504	1241	3.40%	142849	10228	7.16%
	78489	2940	3.75%	56855	3247	5.71%
	38957	595	1.53%	141262	3839	2.72%
Mean	39830	1317	3.41%	150168	10107	5.91%
St.Dev	17021	654	1.06%	74342	9232	2.95%
40	66067	2181	3.30%	51824	4309	8.31%
	39113	1539	3.93%	50949	1544	3.03%
	28501	329	1.15%	89750	3458	3.85%
	130080	3290	2.53%	132253	7595	5.74%
	120231	9184	7.64%	68110	1420	2.08%
	38647	613	1.59%	84697	7058	8.33%
	39173	1934	4.94%	92264	3422	3.71%
	48113	690	1.43%	49213	4017	8.16%
	22390	882	3.94%	71895	2644	3.68%
	25112	2069	8.24%	235902	7561	3.21%
Mean	55743	2271	3.87%	92686	4303	5.01%
St.Dev	38709	2592	2.47%	56260	2340	2.43%

Reactor MLVSS

Days	Seeded with NB10			Seeded with NB20		
	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
59				208062	5601	2.69%
				68616	3353	4.89%
				78130	3238	4.14%
				46658	1881	4.03%
				40135	1020	2.54%
				37334	4150	11.12%
				63445	8180	12.89%
				48446	1462	3.02%
				23799	553	2.32%
				93109	6128	6.58%
Mean				70773	3557	5.42%
St.Dev.				52497	2479	3.72%
66	65066	2167	3.33%	64868	1788	2.76%
	39811	546	1.37%	115027	2634	2.29%
	26511	729	2.75%	42438	3185	7.51%
	100080	2197	2.20%	67242	3247	4.83%
	90230	6174	6.84%	94473	4703	4.98%
	34589	715	2.07%	95862	5343	5.57%
	40070	1334	3.33%	40600	5847	14.40%
	50224	1790	3.56%	55882	3355	6.00%
	21597	1890	8.75%	88098	7255	8.24%
	26345	1226	4.65%	128302	7490	5.84%
Mean	49452	1877	3.89%	79279	4485	6.24%
St.Dev.	27302	1627	2.30%	29848	1959	3.40%
73				32326	1646	5.09%
				23037	1499	6.51%
				25294	2385	9.43%
				13525	451	3.33%
				50641	4342	8.57%
				33451	1077	3.22%
				56164	3024	5.38%
				31662	1496	4.72%
				85015	6688	7.87%
				104880	7024	6.70%
Mean				45600	2963	6.08%
St.Dev.				29198	2321	2.11%
87	23742	52	0.22%	157794	5507	3.49%
	48479	130	0.27%	146314	7919	5.41%
	66540	2478	3.72%	34665	437	1.26%
	110902	8331	7.51%	82321	2594	3.15%
	45170	1510	3.34%	114475	3345	2.92%
	115516	5201	4.50%	42874	3158	7.37%
	54381	359	0.66%	288357	15477	5.37%
	61650	1364	2.21%	114033	4207	3.69%
	107331	4986	4.65%	125577	5019	4.00%
	36044	816	2.26%	183527	10702	5.83%
Mean	66976	2523	2.94%	128994	5837	4.25%
St.Dev.	32906	2764	2.30%	73363	4440	1.75%

APPENDIX H-3 cont'd

Effluent solids

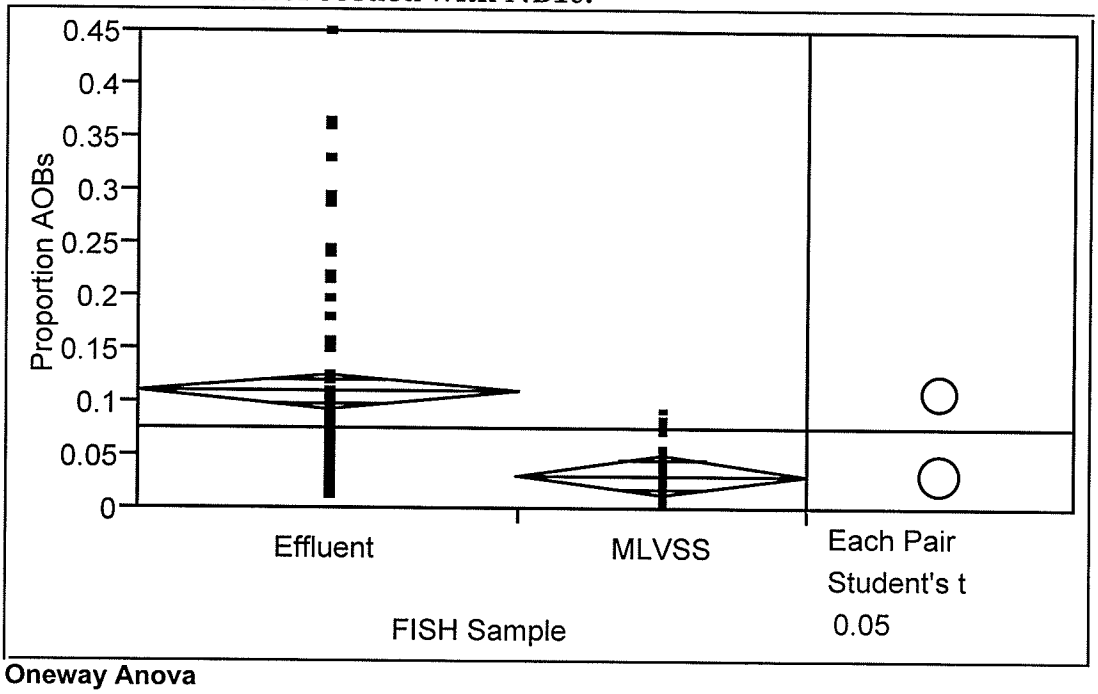
	Seeded with NB10			Seeded with NB20		
Days	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
15	53003	436	0.82%	41394	424	1.02%
	60343	1742	2.89%	124933	1314	1.05%
	52966	1154	2.18%	76309	2895	3.79%
	54279	1389	2.56%	107295	2547	2.37%
	59759	2042	3.42%	67168	1383	2.06%
	79317	1343	1.69%	121698	1958	1.61%
	81354	885	1.09%	74932	1376	1.84%
	63808	1613	2.53%	69966	1487	2.13%
	48413	1316	2.72%	22092	1284	5.81%
	54994	1815	3.30%	112676	5015	4.45%
Mean	60824	1374	2.32%	81846	1968	2.61%
St.Dev	11193	472	0.88%	34456	1277	1.57%
29	35812	457	1.28%	291248	45459	15.61%
	22763	532	2.34%	77702	5348	6.88%
	58547	1191	2.03%	56662	2151	3.80%
	76879	1872	2.43%	32072	935	2.92%
	40582	2398	5.91%	255318	23269	9.11%
	45144	1646	3.65%	50562	2637	5.22%
	76106	8831	11.60%	51811	3371	6.51%
	31502	1676	5.32%	79449	2510	3.16%
	121787	4706	3.86%	78094	4172	5.34%
	62759	3764	6.00%	72206	4435	6.14%
Mean	57188	2707	4.44%	104512	9429	6.47%
St.Dev	29201	2532	3.02%	90631	14200	3.72%
40	26786	1051	3.92%	60502	3990	6.59%
	18064	141	0.78%	55351	2578	4.66%
	62184	1579	2.54%	51620	1162	2.25%
	75007	7214	9.62%	63702	5119	8.04%
	57684	8684	15.05%	53622	1734	3.23%
	531253	124885	23.51%	56025	6000	10.71%
	30268	671	2.22%	55298	5550	10.04%
	35087	1925	5.49%	81478	6680	8.20%
	34455	3211	9.32%	113756	6700	5.89%
	71075	2987	4.20%	53892	2297	4.26%
Mean	94186	15235	7.66%	64525	4181	6.39%
St.Dev	154837	38629	7.04%	19345	2106	2.85%
52	72247	3323	4.60%	121200	18134	14.96%
	53381	6184	11.58%	153206	14747	9.63%
	54529	3567	6.54%	263949	33824	12.81%
	68736	14475	21.06%	186266	39279	21.09%
	26568	3104	11.68%	78607	21557	27.42%
	104233	12532	12.02%	59238	10085	17.02%
	59560	6080	10.21%	78595	14488	18.43%
	145464	14859	10.21%	71604	4993	6.97%
	85645	6821	7.96%	78137	15318	19.60%
	98276	35106	35.72%	109099	3853	3.53%
Mean	76864	10605	13.16%	119990	17628	15.15%
St.Dev	33262	9720	9.06%	64593	11434	7.13%

Effluent solids

	Seeded with NB10			Seeded with NB20		
Days	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)	DAPI (Pixels)	Nso1225 (Pixels)	Nso1225/DAPI (%)
60	26786	1051	3.92%	157190	12086	7.69%
	18064	1141	6.32%	51075	9467	18.54%
	62184	1579	2.54%	46270	5281	11.41%
	75007	7214	9.62%	87410	28132	32.18%
	57684	8684	15.05%	116735	16216	13.89%
	531253	124885	23.51%	122796	21876	17.81%
	30268	671	2.22%	101404	21564	21.27%
	35087	1925	5.49%	96260	22239	23.10%
	34455	3211	9.32%	119690	44053	36.81%
	71075	2987	4.20%	27866	3273	11.75%
Mean	94186	15335	8.22%	92670	18419	19.44%
St.Dev.	154837	38587	6.65%	40248	12088	9.29%
73	177522	51089	28.78%	63273	2863	4.52%
	486251	217329	44.69%	153911	12357	8.03%
	191022	20506	10.73%	81359	4384	5.39%
	86045	10420	12.11%	201402	35713	17.73%
	88860	25142	28.29%	59982	9702	16.17%
	151964	36822	24.23%	240650	15635	6.50%
	56242	11873	21.11%	134736	10677	7.92%
	57339	7009	12.22%	95838	5102	5.32%
	116784	27497	23.55%	87417	20231	23.14%
	130155	37747	29.00%			
Mean	154218	44543	23.47%	124285	12963	10.53%
St.Dev.	125624	62262	10.31%	63584	10193	6.72%
80	72247	5323	7.37%	317398	31015	9.77%
	53381	5224	9.79%	44040	3219	7.31%
	54529	4658	8.54%	33644	2577	7.66%
	68736	13334	19.40%	34071	2494	7.32%
	26568	4106	15.45%	74870	4710	6.29%
	104233	10522	10.09%	80684	6358	7.88%
	59560	7054	11.84%	95750	4882	5.10%
	145464	15120	10.39%	44926	3429	7.63%
	85645	5481	6.40%	48777	1003	2.06%
	98276	35590	36.21%	47111	1931	4.10%
Mean	76864	10641	13.55%	82127	6162	6.51%
St.Dev.	33262	9577	8.85%	85261	8872	2.21%
87	72766	14185	19.49%	32944	1480	4.49%
	25061	5431	21.67%	8970	1043	11.63%
	29992	4421	14.74%	40787	4167	10.22%
	71027	8390	11.81%	74938	8271	11.04%
	61587	20105	32.64%	47742	4133	8.66%
	109898	5357	4.87%	100107	20258	20.24%
	102052	5334	5.23%	56060	2863	5.11%
	47747	2940	6.16%	49185	6138	12.48%
	47663	8364	17.55%	52453	4215	8.04%
	40121	8538	21.28%	37183	1551	4.17%
Mean	60791	8307	15.55%	50037	5412	9.61%
St.Dev.	28592	5202	8.85%	24505	5673	4.80%

APPENDIX H-3 cont'd

Compare relative area quantification of AOBs in the reactor MLVSS and effluent for a reactor seeded with NB10.



Oneway Anova

Summary of Fit

Rsquare	0.218461
Adj Rsquare	0.212798
Root Mean Square Error	0.073768
Mean of Response	0.076931
Observations (or Sum Wgts)	140

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	0.078247	6.211	138	<.0001
Std Error	0.012598			
Lower 95%	0.053336			
Upper 95%	0.103157			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
FISH Sample	1	0.20991546	0.209915	38.5747	<.0001
Error	138	0.75096691	0.005442		
C. Total	139	0.96088237			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Effluent	80	0.110465	0.00825	0.09416	0.12677
MLVSS	60	0.032218	0.00952	0.01339	0.05105

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Effluent	MLVSS
Effluent	0.000000	0.078247
MLVSS	-0.07825	0.000000

Alpha=0.05

Comparisons for each pair using Student's t

t

1.97730

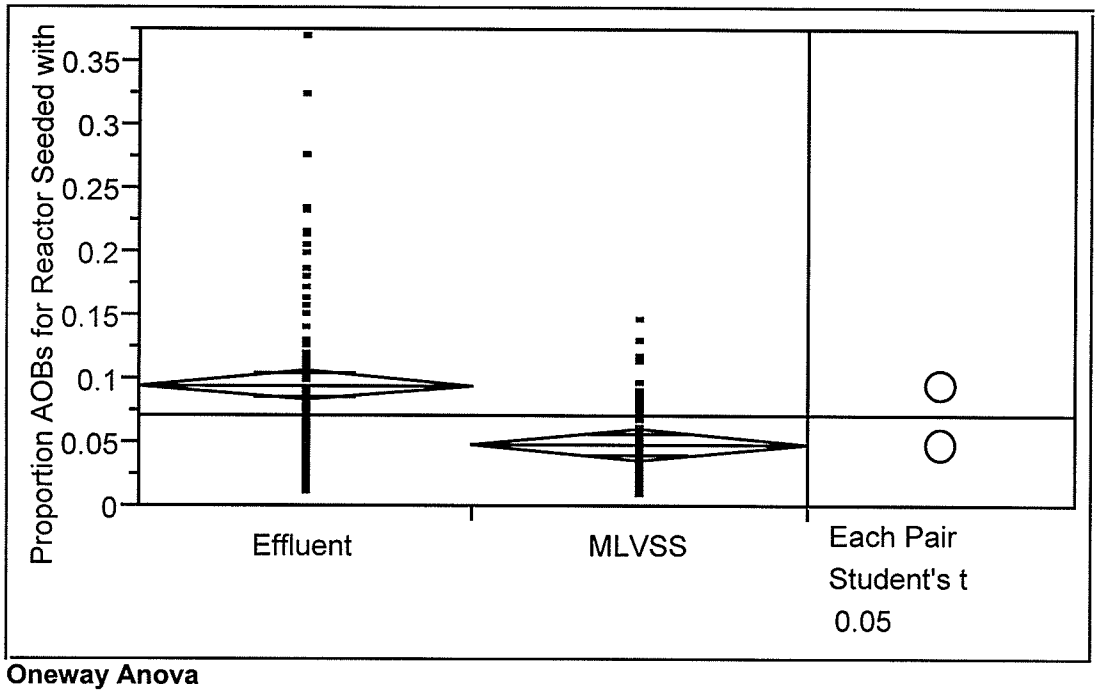
Abs(Dif)-LSD	Effluent	MLVSS
Effluent	-0.02306	0.053336
MLVSS	0.053336	-0.02663

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that the effluent from the reactor seeded with NB10 contained a significantly larger proportion of AOBs than the reactor mixed liquor.

APPENDIX H-3 cont'd

Compare relative area quantification of AOBs in the reactor MLVSS and effluent for a reactor seeded with NB20.



Oneway Anova

Summary of Fit

Rsquare	0.158291
Adj Rsquare	0.15293
Root Mean Square Error	0.055002
Mean of Response	0.071912
Observations (or Sum Wgts)	159

t-Test

	Difference	t-Test	DF	Prob > |t|
Estimate	0.047404	5.434	157	<.0001
Std Error	0.008724			
Lower 95%	0.030172			
Upper 95%	0.064636			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Seed Source 2	1	0.08932027	0.089320	29.5252	<.0001
Error	157	0.47495940	0.003025		
C. Total	158	0.56427967			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Effluent	79	0.095763	0.00619	0.08354	0.10799
MLVSS	80	0.048359	0.00615	0.03621	0.06051

Std Error uses a pooled estimate of error variance

Means Comparisons

Dif=Mean[i]-Mean[j]	Effluent	MLVSS
Effluent	0.000000	0.047404
MLVSS	-0.0474	0.000000

Alpha=0.05

Comparisons for each pair using Student's t

t

1.97519

Abs(Dif)-LSD	Effluent	MLVSS
Effluent	-0.01729	0.030172
MLVSS	0.030172	-0.01718

Positive values show pairs of means that are significantly different.

Summary: The top and bottom of the diamonds form the 95% confidence intervals for the means. The probability is 0.95 that this confidence interval contains the true group mean. If the confidence intervals do not overlap, the groups are significantly different. It can be said that the effluent from the reactor seeded with NB20 contained a significantly larger proportion of AOBs than the reactor mixed liquor.