EFFECTS OF VARYING ENVIRONMENTAL CONDITIONS ON PERFORMANCE OF COMPLETELY AUTOTROPHIC NITROGEN REMOVAL FROM DEWATERED SLUDGE LIQUORS

by

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Abstract

Centrate, an ammonia rich wastewater, usually contains high concentrations of ammonia (around 1000 mg/L) and accounts for about 20 % of total nitrogen loading to wastewater treatment plants. However, the conventional nitrification/denitrification approach for centrate treatment is costly to operate and releases a large quantity of carbon dioxide, due to the addition of organic compounds for denitrification. A brand new approach for nitrogen management is a novel process called **an**aerobic **am**monium **ox**idation (ANAMMOX). ANAMMOX offers a cost effective solution for centrate treatment. Compared to the conventional biological nitrogen removal process, ANAMMOX can save 63 % oxygen and 100 % exogenous carbon source usage.

In this research, a 400 L pilot-scale CANON SBR was studied at the J.A.M.E.S. WWTP in Abbotsford, B.C. and tested for treating on-site centrate. During 317 days of reactor running period, maximum ammonia removal rate (ARR) of 98.9 % was achieved with an average ARR of 91.0 %. The reactor also achieved a maximum of 0.81 kgN/m^3•d ammonia loading rate (ALR) with a minimum hydraulic retention time (HRT) of 1.46 d. The performance of the CANON SBR was highly influenced by environmental conditions. Better reactor performance was achieved at the highest tested temperature of 32 ºC and maximum air flow rate of 14 L/min. Testing under 26 ºC would reduce ARR by 12% and generate high nitrite concentration peaks. Compared to an airflow rate of 10 L/min, the ARR was increased by 9% and HRT was reduced by 10%, at 14 L/min. Higher initial ammonia concentrations in the reactor could also lead to better reactor performance. A maximum nitrogen removal rate of 0.36 kgN/m^3•d and minimum HRT of 1.79 d was achieved at the highest initial ammonia concentration of 455 mg/L. The feeding rate did not affect the results of nitrogen removal. However, slow feeding with 500 mL/min did not create any nitrite concentration peak during the reaction. Solids concentrations in the reactor could be effectively controlled by sludge settling time. Sludge settling times between 4 to 10 min were recommended, for achieving purposes of sludge enrichment or system optimization.

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Nomenclature

Adenosine Triphosphate	ATP
Ammonia-nitrogen	NH_3-N
Ammonia Loading Rate	ALR
Ammonium Oxidation Bacteria	AOB
Ammonia Removal Rate	ARR
Anaerobic Ammonia Oxidation	ANAMMOX
Biochemical Oxygen Demand	BOD
Complete Autotrophic Nitrogen Removal Over Nitrite	CANON
Chemical Oxygen Demand	COD
Continuously Stirred Tank Reactor	CSTR
Dissolved Oxygen	DO
Electronic Microscopic	EM
Fluorescence On-site Hybridization	FISH
Hydraulic Retention Time	HRT
Initial Ammonia-nitrogen Concentration	NH_3-N_i
Membrane Aerated Biofilm Reactor	MABR
Nitrite-nitrogen	NO_2-N
Nitrate-nitrogen	NO_3-N
Nitrite Oxidation Bacteria	NOB
Nitrogen Removal Rate	NRR
Polymerase Chain Reaction	PCR
Sequencing Batch Reactor	SBR
Sludge Retention Time	SRT
Single Reactor System for High Activity Ammonium Removal Over Nitrite	SHARON
Total Suspended Solids	TSS
Upflow Anaerobic Sludge Blanket	UASB
Upflow Biofilm Reactor	UBF
Volatile Suspended Solids	VSS
Waste Water Treatment Plant	WWTP

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Dedication

To my parents

1. Introduction

1.1 Background

In the natural environment, nitrogen exists in the form of protein, ammonia-nitrogen (NH_4, NH_3), nitrite-nitrogen (NO_2^-), nitrate-nitrogen (NO_3^-), and nitrogen gas (N_2). In fresh water, nitrogen is primarily in organic form like proteinaceous matter and urea. Decomposition of organic nitrogen by microorganisms changes the nitrogen to ammonia. With the presence of oxygen, ammonia can be oxidized to nitrites and nitrates, which can be used by plants and animals for protein generation. However, the death and decomposition of plants and animals returns ammonia back to the environment for a nitrogen balance in the environment.

In secondary wastewater treatment, nitrogen is always present as ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen. Since nitrogen and phosphorus are both fundamental elements for the growth of microorganism, part of nitrogen in wastewater is removed through secondary wastewater treatment processes. The ideal nutrient balance for activated sludge treatment process is BOD : N : P = 100 : 5 : 1. For a typical wastewater with BOD_5 = 150 mg/L and 30% BOD removal in primary treatment process, only 5 ~ 6 mg/L nitrogen could be theoretically removed through a secondary treatment process. However, sewage usually contains about 40 mg/L NH_4-N and 20 mg/L organic nitrogen, which is excessive to microorganisms in the activated sludge of secondary treatment processes. Insufficient removal of nitrogen from wastewater, before discharging, would lead to adverse effects to aquatic systems. Excessive amount of soluble ammonia is toxic to aquatic lives and also leads to eutrophication in water bodies (Robert and Russo, 1981).

Ammonium can be removed from wastewater through physical, chemical and biological processes. Among them, biological nitrogen removal processes are the most cost-effective technologies and have been widely adopted in wastewater treatment facilities globally. One typical biological nitrogen removal process is known as biological nitrification and denitrification process, in which nitrifying bacteria oxidize ammonia to nitrite and nitrate under aerobic conditions, and then denitrifying bacteria convert nitrite/nitrate to nitrogen gas under anoxic conditions (Sliekers, *et al.*, 2002). Denitrification bacteria are known as heterotrophic

microorganisms and require biodegradable organic compounds (COD) as a carbon source for their reproduction. However, wastewaters commonly contain insufficient COD to achieve complete denitrification. Thus, additional organic compounds, such as methanol, are required to add as an exogenous carbon-source during conventional biological denitrification processes. In addition, conventional biological nitrogen removal process is not suitable for some specific wastewaters such as landfill leachate and dewatered sludge liquids with high concentrations of ammonia and low BOD/COD ratio. The wastewater generated during digested sludge dewatering normally contributes to 15–20 % of total nitrogen loading to wastewater treatment plants; proper treatment of dewatered sludge liquids would therefore, reduce nitrogen loading to most wastewater treatment facilities.

To overcome the limitations of conventional biological nitrification and denitrification processes, several novel nitrogen removal processes have been developed, including the ANAMMOX process, SHARON process, CANON process and OLAND process (Hellinga *et al*., 1998; Kuai and Verstraete, 1998; Third *et al*., 2001; Vlaeminck *et al*., 2009). Among them, OLAND and CANON processes use anammox in a single bioreactor. Reviewing different anammox systems described in the literature, it became clear that:

- ANAMMOX based processes are still under investigation in lab-scale devices, and there is limited experience with pilot-scale reactors. In this research, performance of the CANON process was tested in a pilot-scale sequencing batch reactor (SBR). Throughout the thesis, we will use the CANON SBR to describe the one stage ANAMMOX in a single bioreactor.
- The ANAMMOX research is new to Canada and there is very limited research on dealing the local centrate with the ANAMMOX process.
- Most of the experimental knowledge on autotrophic nitrogen removal is restricted to lab scale reactors using synthetic feed.
- There is limited research on studying the effects of environmental conditions on the CANON process

1.2 Objectives of the study

The objectives of this study were:

1) Perform a field study for dealing with on-site dewatered sludge liquor with high ammonia concentration on-site centrate, using a CANON SBR system, and evaluate the performance of the pilot scale CANON SBR.

2) Study the effects of variable environmental conditions including temperature, aeration, initial ammonia concentration, feeding speed and sludge settling time on the performance and microbial kinetics of CANON SBR.

1.3 Literature review

1.3.1 Conventional nitrogen removal process

In order to removal nitrogen from waste water, a variety of physical, chemical and biological methods have been developed, including air stripping, ion exchange, and biological nitrification and denitrification process.

1.3.1.1 Air Stripping

Air stripping is a controlling process based on the principle of ion equilibrium with ammonia and hydrogen ions exist in wastewater:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \quad [1]$$

The equilibrium moves towards the right side when pH is increased above 7.0. The higher the pH level and temperature, the more ammonium is converted to gas form ammonia. According to the calculation of Emerson *et a*l. (1975) in Table 1.1, the fraction of ammonia is about 85% in aqueous ammonium solution of pH 10 at temperature of 25 ºC. Bubbling through wastewater can strip part of the dissolved ammonia gas from wastewater to some degree, which could be done in an air-stripping tower.

However, air stripping is considered as economically unfavorable. Alfred and Culp (1967) reported a cost estimation of ammonia air stripping in a Tahoe water reclamation plant. The cost to achieve 95% ammonia removal was $13.85/Mgal and $25/Mgal for 98% ammonia removal. This cost did not include the additional cost of cleaning and maintaining the stripping tower. Due to the high pH, scales will form rapidly on the packed media within the tower and needs to be washed frequently by acid (Reeves, 1972).

Table 1.1 Fraction of un-ionized ammonia in aqueous solution at different pH values and temperatures

pH	8	10	12	14	16	18	20	22	24	26	28	30	32
7.0	.0016	.0018	.0022	.0025	.0029	.0034	.0039	.0046	.0052	.0060	.0069	.0080	.0093
7.2	.0025	.0029	.0034	.0040	.0046	.0054	.0062	.0072	.0083	.0096	.0110	.126	.150
7.4	.0040	.0046	.0054	.0063	.0073	.0085	.0098	.0114	.0131	.0150	.0173	.0198	.0236
7.6	.0063	.0073	.0086	.0100	.0116	.0134	.0155	.0179	.0206	.0230	.0271	.0198	.0236
7.8	.0099	.0116	.0135	.0157	.0182	.0211	.0244	.0281	.0322	.0370	.0432	.0482	.0572
8.0	.0156	.0182	.0212	.0247	.0286	.0330	.0381	.0438	.0502	.0574	.0654	.0743	.0877
8.2	.0245	.0286	.0332	.0385	.0445	.0514	.0590	.0676	.0772	.0884	.0998	.1129	.1322
8.4	.0383	.0445	.0517	.0597	.0688	.0790	.0904	.1031	.1171	.1326	.1492	.1678	.1948
8.6	.0593	.0688	.0795	.0914	.1048	.1197	.1361	.1541	.1737	.1950	.2178	.2422	.2767
8.8	.0909	.1048	.1204	.1376	.1566	.1773	.1998	.2241	.2500	.2774	.3062	.3362	.3776
9.0	.1368	.1565	.1782	.2018	.2273	.2546	.2846	.3140	.3456	.3783	.4116	.4453	.4902
9.2	.2008	.2273	.2558	.2861	.3180	.3512	.3855	.4204	.4557	.4909	.5258	.5599	.6038
9.4	.2847	.3180	.3526	.3884	.4249	.4618	.4985	.5348	.5702	.6045	.6373	.6685	.7072
9.6	.3868	.4249	.4633	.5016	.5394	.5762	.6117	.6456	.6777	.7073	.7358	.7617	.7929
9.8	.5000	.5394	.5778	.6147	.6499	.6831	.7140	.7248	.7692	.7933	.8153	.8351	.8585
10.0	.6131	.6498	.6844	.7166	.7463	.7735	.7983	.8207	.8408	.8588	.8749	.8892	.9058

Adopted from Emerson *et al*., 1975

1.3.1.2 Ion exchange

Ion exchange is a chemical process that the specified ions in wastewater are displaced by other ions from an insoluble exchange material based on ion exchange capacity of the material. Acid or cationic resins can exchange cation such as Ca^{2+} and NH_4^+, while base or anionic resins can exchange anions like OH^- or NO_3^- (Reeves, 1972). The selectivity order is $Ca^{2+} < K^+ < NH_4^+ <$

Na^+ and SO_4^{2-} < HPO_4^{2-} < NO_3^- < Cl^-. Thus, NH_4^+ in wastewater can be exchanged by Na^+ ion and NO_3^- can be exchanged by Cl^- ion, in theory.

1.3.1.3 Biological nitrification and denitrification

Biological nitrification and denitrification process is the most widely used method for nitrogen removal from wastewater. The process includes two separate steps of bacterial activities: first oxidize ammonia to nitrates (i.e. nitrification) and then convert the nitrates to nitrogen gas (i.e. denitrification).

Nitrification, which converts ammonia to nitrates, is also a two steps biological process. Ammonium and ammonia in raw wastewater are converted to nitrite by bacteria called *Nitrosomnas*. Then, another type of bacteria, *Nitrobacter*, converts the nitrite to nitrate (Johnson and Schroepfer, 1964). The overall reactions of the two steps are shown in Equation 2 and 3.

$$NH_4^+ + 1.5O_2 = 2H^+ + H_2O + NO_2^- \quad [2]$$

$$NO_2^- + 0.5O_2 = NO_3^- \quad [3]$$

Both *Nitrosomnas* and *Nitrobacter*, know as "nitrifiers", are strict "aerobes". This means they need DO to grow and maintain nitrification under aerobic condition at dissolved oxygen (DO) levels of 1.0 mg/L or above (below DO level of 0.5 mg/L, the growth rate is minimal). It can be calculated from the above Equation 2 and 3, that for completely oxidizing 1 g of ammonia to nitrate, nitrifiers need to consume 4.18 g of oxygen. Nitrification is an alkalinity consuming process; it can be calculated that 7.14 g of alkalinity as calcium carbonate ($CaCO_3$) need to be consumed for oxidizing 1 g of ammonia to nitrite.

In comparison to nitrification, biological denitrification is an anaerobic (anoxic) process that converts the nitrite and nitrate generated by the nitrification process to nitrogen gas (N_2), through several facultative heterotrophic bacteria. These bacteria include species of *Pseudomonas, Achromobacter, Bacillus, and Micrococcus* (Reeves, 1972). The reactions of biological denitrification processes are presented in Equation 4 and 5.

$$2NO_3^- + 10H^+ + 10e^- = N_2 + 2OH^- + 4H_2O \quad [4]$$

$$2NO_2^- + 6H^+ + 6e^- = N_2 + 2OH^- + 2H_2O \quad [5]$$

These heterotrophic bacteria need electron donors to provide electrons for carrying out denitrification reaction. However, many wastewaters do not contain enough organic compounds to act as the electron donors for the denitrification process. Quality electron donors such as methanol, acetate, ethanol, lactate and glucose should be added for complete denitrification (Khin and Annachhatre, 2004). Among them, methanol is relatively cheap and is commonly used in denitrification processes.

Denitrification can occur under anoxic conditions with the DO level less than 0.5 mg/L, ideally less than 0.2 mg/L (Vit Mateju, 1992). Denitrification is an alkalinity production process. From Equation 4, it can be calculated that approximately 3.0 to 3.6 g of alkalinity as $CaCO_3$ is produced per gram of nitrite being converted to nitrogen gas.

1.3.2 Principal of ANAMMOX process

The history of ANAMMOX started in 1977, when E. Broda (1977) published his hypothesis of two kinds of lithotrophs missing in nature based on thermodynamically calculations. Ammonium oxidation reactions (as shown in Table 1.2), that use nitrite as electron acceptor, should be able to occur automatically since their Gibbs Free Energies (G^0) are negative. This hypothesis was not proved until Mulder *et al.* (1995) observed NH_4^+ disappearing with a rate of 0.4 kg•N/m^3•d from a denitrifying fluidized bed reactor treating the effluent of a methanogenic reactor. Both nitrate and ammonium consumption in the reactor increased with denitrogen gas production. They gave the name "ANAMMOX" to this novel biological process.

Table 1.2 Energy produced by ammonium oxidation reactions

Electron acceptor	Chemical reaction	G^0	Possibility
O_2	$2NH_4^+ + 3O_2 = 2NO_2^- + 2H_2O + 4H^+$	-241	Possible
NO_2^-	$NH_4^+ + NO_2^- = N_2 + 2H_2O$	-355	Possible
NO_3^-	$NH_4^+ + 3NO_3^- = 4N_2 + 9H_2O + 2H^+$	-278	Possible
Fe_3^+	$NH_4^+ + 6Fe_3^+ = N_2 + 6Fe_2^+ + 8H^+$	-100	Possible
SO_4^{2-}	$8NH_4 + + 3SO_4 2- = 4N_2 + 3H_2S + 12H_2O + 5H^+$	-22	Possible

Later, van de Graaf *et al.* (1995) identified that ANAMMOX is a process using nitrite as an electron acceptor. The reaction can thus be expressed as:

$$NH_4^+ + NO_2^- = N_2 + 2H_2O \quad [6]$$

Strous *et al*. (1998) estimated the ANAMMOX stoichiometry based on mass balance principles; they found out that the ratio of converted ammonia and nitrite is 1:1.32 anoxically, as presented in Equation 7:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ = 1.02N + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad [7]$$

Parameters of this novel anaerobic ammonia oxidation process are compared with the conventional aerobic nitrification process (Table 1.3). Obviously, the ANAMMOX process does not require additional aeration and organic carbon sources.

Up to 90% of operational cost can be saved in comparison to conventional biological nitrification denitrification and other typical nitrogen removal processes (Jetten *et al*., 2001). The advantages of ANAMMOX make it a promising technology, to replace the traditional nitrification and denitrification technologies for nitrogen removal.

Table 1.3 Parameters of aerobic and anaerobic ammonia oxidation (Jetten *et al*., 2001)

Parameter	Nitrification	ANAMMOX	Unit
Free energy	-275	-357	kJ/mol
Biomass yield	0.08	0.07	mol/mol C
Aerobic rate	200-600	0	nmol/min/mg protein
Anaerobic rate	2	60	nmol/min/mg protein
Growth rate	0.04	0.003	/h
Doubling time	0.73	10.6	Days

1.3.3 Anammox bacteria

1.3.3.1 Characteristics of anammox bacteria

The so-called anammox bacteria that involved in the ANAMMOX process were found to be planctomycete-like. The low 16S RNA sequence of anammox organisms present over 90% similarities to other genera of the *Planctomycetes* such as *gemmate*, *Isosphaera*, or *Planctomyces*, indicating that the anammox should be in a second order within the *Planctomycetes* (Schmid *et al*., 2005).

Three typical types of anammox organisms were later identified by their 16rRNA sequences. *Candidatus "Brocadia* anammoxidans" was identified by Jetten *et al.* (2001). Two other species: *Candidatus "Kuenenia Stuttgartiensis"* and *Candidatus "Scalindua sorokinii*" were also found in wastewater treatment plants and marine sediment (Penton *et al*., 2006). A typical ultrastructure profile of the anammox *bacteria Candidatus "Brocadia anammoxidans"* is showed in Figure 1.1. The anammox bacteria are red coccoid bacteria with a diameter of less than 1 μm (Niftrik et al., 2004). The anammox bacteria are slow growing microorganism, with average doubling time of 11 days. Like other Planctomycetes, these anammox bacteria have a proteinaceous cell wall and are lacking in peptidoglycans. But they are distinct for autotrophic and anoxic. Electronic microscopic (EM) studies indicated that all anammox bacteria have one separate, ribosome-free cytoplasm intracellular compartment, termed as anammoxosome (Lindsay *et al*., 2001). The anammoxosome is bounded by a laddered lipids structure membrane, which is unique in nature and was only found in anammox bacteria (Niftrik *et al.*, 2004). The bacterial nucleoid is located on the outside of the anammoxosome membrane. The anammoxosome can provide a site for catabolism, generating proton motive force for adenosine triphosphate (ATP), as well as protecting the rest of cell from the proton diffusion and toxic intermediates of the ANAMMOX process (Lindsay *et al*., 2001; Niftrik *et al*., 2004).

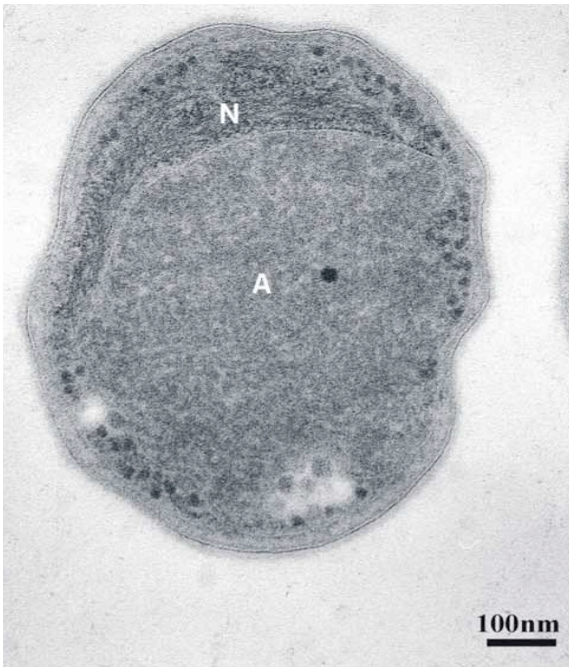

Figure 1.1 Ultrastructure of *Candidatus "Brocadia anammoxidans".* A: anammoxsome; N: bacterial nucleoid (Schmidt *et al*., 2003)

1.3.3.2 Detection of anammox bacteria

The detection methods of anammox bacteria include tracer experiments, PCR amplification and FISH analysis.

Tracer experiment with ^{15}N-labeled ammonium and nitrite is the most basic method to indicate the presence and activity of anammox bacteria. Through anammox reactions, labeled [^{15}N] ammonium reacts with unlabeled [^{14}N] nitrite, generating $^{29}N_2$ ($^{14}N^{15}N$). This method is especially widely used for studying anammox activities and its mechanism principles (van de Graaf *et al*., 1997; Jettan *et al*., 2001)

Polymerase Chain Reaction (PCR) amplification, with general 16S rRNA gene-targeted primers, is an advanced method to detect the presence of anammox organisms if the cell counts are too low for fluorescence on-site hybridization (FISH). PCR amplification can amplify selected 16S rRNA gene fragments from mixed DNA and compare them with gene libraries to rapidly identify anammox organisms (Amann *et al*., 1995). However, anammox organisms are still underrepresented in general 16S rRNA gene libraries and PCR amplification is not quantitatively representative in sample investigation (Amann *et al*., 1995).

Fluorescence on-site hybridization (FISH) is becoming a standard method for the detection of anammox organisms and was described by Schmidt *et al*. (2005). FISH has been intensively used to provide both qualitative and quantitative results for anammox bacteria in water and wastewater samples (Sliekers *et* al., 2002; Pynaert *et* al., 2003; López *et al., 2008*).

1.3.4 Metabolism of the ANAMMOX process

In the ANAMMOX reaction, 1 mol ammonium with 1.32 mol nitrite can be converted to 1.02 mol nitrogen gas and 0.26 mol nitrate. ANAMMOX bacteria have been found to be metabolically flexible. Several possible alternative metabolic pathways have been proposed. The first metabolic pathway of ANAMMOX was proposed by van de Graaf *et al*. (1997) based on ^{15}N studies with the dominant species of *Candidatus Brocadia anammoxidans* (Figure 1.2) and considered hydroxylamine (NH_2OH) as an critical intermediate of nitrite reduction. There are five steps involved in this metabolic pathway. Ammonium is first oxidized by hydroxylamine (NH_2OH) to form hydrazine (N_2H_2), which is step 1. Equivalents formed by N_2H_2 then react with nitrite to form finial product N_2 and NH_2OH (step 2, 3, and 4). In the end, parts of the nitrite would convert to nitrate for biomass growth (step 5).

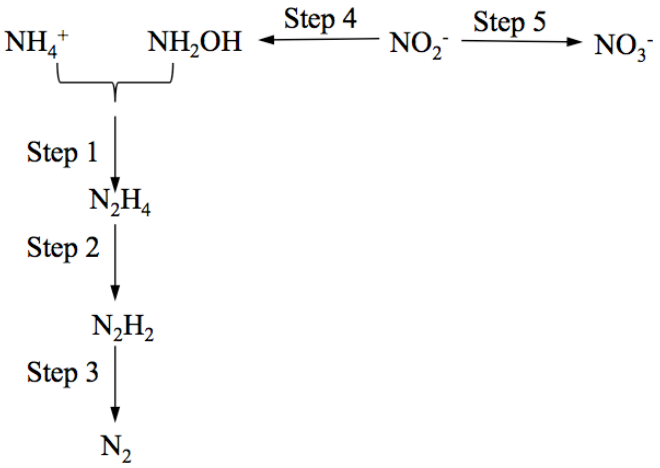

Figure 1.2 Possible metabolic pathway for anaerobic ammonia oxidation (van de Graa*et al*., 1997)

A similar mechanism theory of ANAMMOX in *Candidatus Brocadia Anammoxidans* (Figure 1.3) was postulated by Jettan *et al*. (2001) through the investigation using ^{15}N-labelling experiments. In Figure 1.3, NR is a nitrite-reducing enzyme (NH_2OH is the product); HH is

hydrazine hydrolase that condenses hydrazine out of ammonia and hydroxylamine; HZO is a hydrazine-oxidising enzyme, which may be equivalent to hydroxylamine oxidoreductase. The results showed that nitrite as the electron acceptor was reduced to hydroxylamine and reacted with the electron donor ammonium, generating the ultimate products as denitrogen gas.

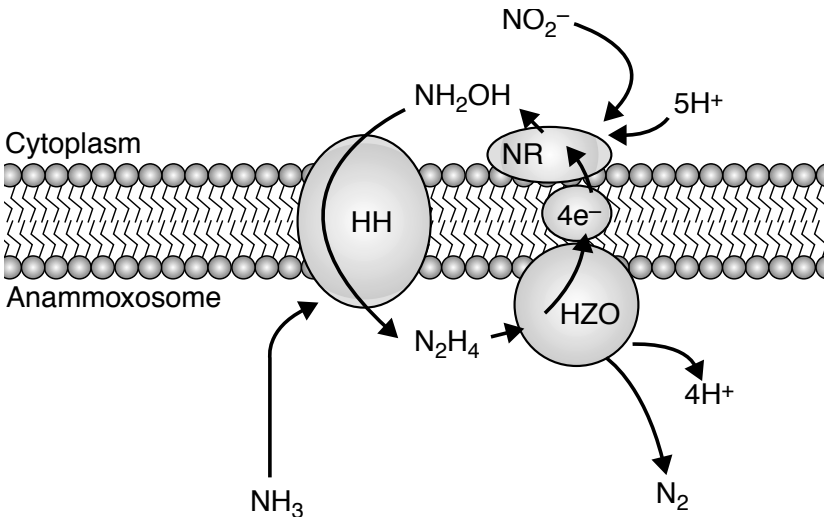

Figure 1.3 Mechanism of anaerobic ammonium oxidation in *Candidatus Brocadia Anammoxidans*. (Jettan *et al*., 2001)

Another slightly different ANAMMOX metabolic mechanism, was proposed by Strous *et al.* (2006), through the study of another species of anammox bacteria, Candidatus Kuenenia stuttgartiensis. Nitric oxide (NO) was postulated to be the intermediate for nitrite reduction instead of hydroxylamine. They found there were two unique enzymes that involved in this ANAMMOX process. Hydrazine hydrolase (hh) is the enzyme that produces hydrazine from nitric oxide and ammonium, while hydrazine dehydrogenase (hd) is the enzyme that can transfer electrons from hydrazine to ferredoxin.

All three hypotheses agreed that hydrazine (N_2H_2) is an important intermediate for the ANAMMOX process, since a transient accumulation of hydrazine was observed. The enzyme which can help the bacteria to oxidize hydrazine to denitrogen gas was identified as a hydroxylamine-oxidoreductase-like protein (HAO) of (Schalk *et al*., 2000). HAO was found to be present only inside anammoxosome of the anammox bacteria cells.

1.3.5 ANAMMOX involved process

The ANAMMOX process has been carried out with ammonium to nitrite ratio of 1:1.32 (Strous *et al*., 1998). For most wastewaters, however, there is not enough nitrite to complete the ANAMMOX process (Zhang *et al*., 2008). NO_2^- should be added additionally unless ANAMMOX is combined with other supplemental process like partial nitrification, that would generate additional nitrite.

1.3.5.1 Partial Nitrification-ANAMMOX

Partial nitrification is a nitrification process that partially oxidizes ammonium to nitrite, but not to nitrate (Equation 8).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad [8]$$

To achieve partial nitrification, conventional subsequent oxidation process of nitrite to nitrate must be prevented. Nitrite oxidation can be prevented in two ways. First, ammonium oxidation is a more temperature dependent process, since activate energies of ammonium oxidation and nitrite oxidation are different, which is 68 kJ mol^{-1} and 44 kJ mol^{-1} respectively (Schmidt, *et* al., 2003). The ammonium oxidation bacteria (AOB) and nitrite oxidation bacteria (NOB) are also quite sensitive to temperature, and their growth rates (or sludge age) change with temperatures (Figure 1.4). At temperatures higher than 15 °C, ammonium oxidizers grow faster than nitrite oxidizers. NOB are outcompeted by AOB at temperatures above 25 °C (Khin and Annachhatre *et al*., 2004). At a temperature of 35 °C, the minimum sludge age of NOB is approximately only half of that of AOB (0.5 and 1 day, respectively). When the hydraulic retention time is controlled higher than the sludge retention time of nitrite oxidizers, but lower than that of ammonia oxidizers, nitrite oxidizers would be selectively washed out and further nitrite oxidation would be prevented. The second way to prevent nitrite oxidation is to use the principle that NOB are unable to grow at low oxygen concentration (i.e. less than 0.4 mg/L or 5% air saturation) with surplus ammonium (Schmidt *et al*., 2003). Nitrites become the stable end product of nitrification under this condition. However, the mechanism of this NOB inhibition by ammonium and low-level dissolved oxygen is not fully understood.

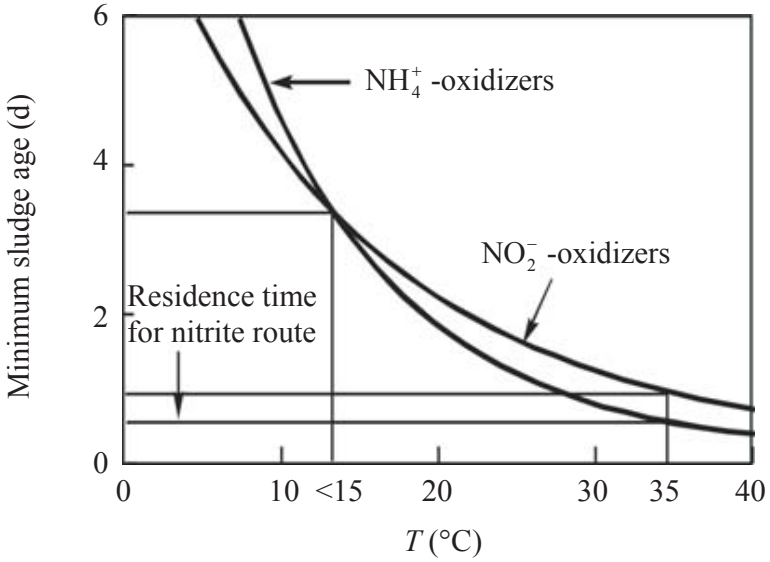

Figure 1.4 Minimum sludge retention time for ammonium and nitrite oxidizers at different temperature (Hellinga *et al*., 1998)

Hellinga et al. (1998) designed a partial nitrification process for treating dewatered sludge liquor with high ammonia concentration (about 1000 mg/L) and named it SHARON (single reactor system for high activity ammonium removal over nitrite). The SHARON process is operated at temperature above 30 °C. High specific growth rates can be enabled under such a high temperature, so that no sludge retention is required for the SHARON process; this means that the sludge age (SRT) equals the hydraulic retention time (HRT). In such a system, the effluent concentration is controlled directly by the bacteria growth rate (1/SRT) instead of influent concentration. Figure 1.5 shows the flow diagram of a SHARON-ANAMMOX process.

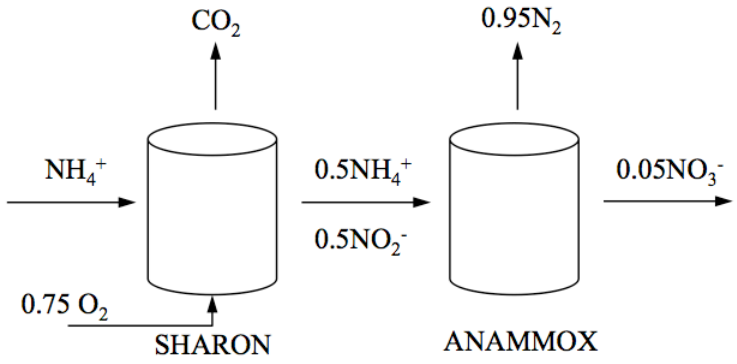

Figure 1.5 Flow diagram of SHARON-ANAMMOX process (Khin and Annachhatre, 2004)

Effluent from a SHARON process is usually used as feed for the ANAMMOX reactor directly. To make an appreciate feed for the ANAMMOX reactor, ammonium and nitrite ratio should be maintained around 1.0 within the effluent of the SHARON process, which means only 50% of the ammonium needs to be converted to nitrite by the SHARON process (Equation 9):

$$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow\ 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O \qquad [9]$$

Van Dongen *et al*. (2001) reported a successful 53% oxidation of ammonium to nitrite in a lab scale continuously stirred tank reactor (CSTR) feed by sludge liquor from the Rotterdam WWTP at 1.2 kgN/m^3•d. The temperature in the reactor was maintained within the range of 30~40 °C and the HRT was 1.0 day.

Fux *et al.* (2002) set up a pilot scale (3.6 m^3) SHARON process with a CSTR reactor and operated at 30 °C without sludge retention. A conversion rate of 58% ammonium to nitrite was achieved over a half of year operation, resulting in nitrite production of 0.35 kgN/m^3•d. They also recommended operating the SHARON ANAMMOX reactor in a weak alkaline condition. The nitrite/ammonium ratio dropped drastically when pH increased higher than 7.2.

Van Kempen et al. (2001) reported a SHARON® system experience constructed at the Utrecht WWTP and at the Rotterdam WWTP. The ammonia concentration in the feed of the SHARON system is quite concentrated, ranging from 0.5 to 1.5 g N/L. Stable nitrogen conversion rates of 90% were achieved in both WWTPs.

1.3.5.2 Completely autotrophic nitrogen removal over nitrite (CANON)

When partial nitrification and ANAMMOX process are integrated into one reactor, the nitrogen removal process is named as completely autotrophic nitrogen removal over nitrite (CANON) process. Both AOB and anammox bacteria grow and cooperate within the CANON process reactor to achieve nitrogen removal. The AOB take oxygen to oxidize ammonium to nitrite (Equation 10), producing an oxygen depletion condition for anammox bacteria to convert nitrite and the ammonium remained in the reactor to nitrogen gas (Equation 11). The overall CANON process reaction is shown in Equation 12.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad [10]$$

$$NH_4^+ + 1.32NO_2^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \quad [11]$$

$$NH_4^+ + 0.85O_2 \rightarrow 0.44N_2 + 0.11NO_3^- + 1.08H^+ + 1.43H_2O \quad [12]$$

As shown in the reaction, the CANON process does not require a carbon source and saves up to 63% oxygen (Khin and Annachhatre, 2004) in comparison to the traditional nitrification-denitrification nitrogen removal process (Equation 2, 3, 4 and 5). With nitrogen removal achieved in a single reactor with a small amount of aeration, the CANON process can greatly reduce the space and energy requirement, outcompeting the conventional nitrification and denitrification process or novel SHARON-ANAMMOX process, from economical points of view.

Oxygen and ammonia concentrations are critical for balancing the competitions between AOB, NOB, and anammox bacteria in CANON reactors. Since NOB can compete for oxygen for nitrite oxidation with AOB and compete for nitrite with anammox bacteria, the activity of NOB should be inhibited. It can be simply achieved by maintaining anoxic conditions in CANON reactors with DO level lower than 0.5 mg/l (Strous *et al.,* 1997; Khin and Annachhatre, 2004). When the DO level is higher than 0.5 mg/l (or 0.5% air saturation), anammox bacteria are reversibly inhibited and will be eventually washed out from the reactor (Strous *et al.,* 1997). The balance of the three groups of bacteria may also be destroyed by ammonium limited feeding to the reactor. For ammonium limited influent, ammonium in the reactor is exhausted rapidly by the AOB. The accumulated nitrite will facilitate the growth of NOB but inhibit anammox bacteria, leading to poor performance of the CANON the process for nitrogen removal. Third *et al*. (2001) reported a limit of effective and stable nitrogen removal (92% total nitrogen removal) in a CANON system as loading rate of 0.1 $kgN/m^3 \cdot d$. Lower than this critical point, a third of the bacteria developed was later identified as *Nitrobacter* and *Nitrospira* species that resulted in nitrogen removal decreasing from 92% to 57%, temporarily.

The CANON process is usually operated with a sequencing batch reactor (SBR) or gas-lift reactor. Sliekers *et al.* (2002) reported a CANON system using a 2 L SBR, which was performed two cycles per day (11.5h filling period, 0.25h settling period, and 0.25h drawing period). The

reactor was inoculated with sludge from an ANAMMOX-SBR-reactor, in which 80% of the biomass consisted of anammox bacteria. The reactor achieved a nitrogen removal rate of 0.16 kgN/m^3•d and maximum anammox activity of 0.15 kg $NH4^+$–N per kg dry waste per day, after 70 days of operation under oxygen-limited conditions. Third *et al*. (2005) investigated an operation strategy of CANON SBR system with intermittent aeration. AOB and anammox bacteria were thus activated alternatively, under this intermittent aeration strategy. The reactor achieved nitrogen conversion rate of 0.08 kgN/m^3•d. Sliekers *et al.* (2003) choose a 1.8 L gas-lift reactor for carrying out the CANON process under an oxygen concentration around 0.5 mg/L. The reactor achieved N-removal rate of 1.5 kgN/m^3•d, which was much higher than previously reported.

A recent study also indicated that membrane-aerated biofilm bioreactor (MABR), equipped with non-woven fabrics, is also feasible for the CANON process treating ammonium-rich wastewater (Gong *et al.*, 2007). The oxygen was forced to penetrate through the non-woven fabrics, generating an oxygen concentration gradient in the biofilm reactor. Partial nitrification that consumed dissolved oxygen occurred in the inner layer of fabrics. ANAMMOX occurred in the outer layer of fabrics that was absent of oxygen. The MABR achieved 0.77 kgN/m^3**.**day of N conversion after 83 days.

1.3.6 Start up of an ANAMMOX reactor

1.3.6.1 Reactor configuration

Since anammox bacteria are slow growth autotrophs with maximum specific growth rate of 0.0027 h^{-1} and doubling time of 11 days (Strous *et al*., 1999), reactors carrying out the ANAMMOX process need to have effective retention of biomass, preventing biomass washed out from the reactors, and also should be suitable for long term enrichment, cultivation and quantification.

A fluidized-bed reactor was first take into consideration since ANAMMOX process was originally discovered within a fluidized-bed reactor (Mulder *et al*., 1995). Anammox microorganisms should be able to grow as biofilms on the sand particles in the reactor (Strous *et* al., 1998). Strous *et al*. (1997) studied the feasibility of fluidized-bed reactor to hold anammox sludge with a 2.5 L (height 70cm, diameter 7cm, liquid volume 2.25L) glass column reactor

inoculated with anammox sludge. After 100 days, the sludge granules changed from brownish to red and anammox bacteria became the dominant bacteria in the reactor. The reactor achieved 82% ammonium removal efficiency and 99% nitrite removal efficiency. However, two out of 15 attempts in total ended by complete washout of the sludge and activity loss. This was mainly due to a lack of complete bulk mixing in a fluidized bed reactor, causing the biofilm structure to change over different reactor runs. If some areas of the reactor were unable to receive substrate, the biomass would starve and thus decrease anammox activity.

Sequencing batch reactor (SBR) seems to be more feasible and also widely used for carrying out the ANAMMOX process (Strous, *et al*., 1998; Arrojo, *et al*., 2006; Chamchoi and Nitisoravut, 2007; López, *et al*., 2008; Jin, *et al*., 2008). This is mainly because of the efficient biomass retention of SBR. In comparison with stoichiometry predicted results, Strous *et al*. (1998) reported a 90% biomass retention efficiency of their SBR for anammox sludge accumulation. This means that only 10% of biomass would be washed out from the reactor. A large amount of anammox sludge can thus be enriched in the reactor after a period of time.

In addition, SBR is an easy control process and is feasible to be optimized by changing its operational strategies. A typical SBR cycle includes three stages: mixed filling with substrate, settling and drawing, which can be controlled by PLC controllers (Figure 1.6).

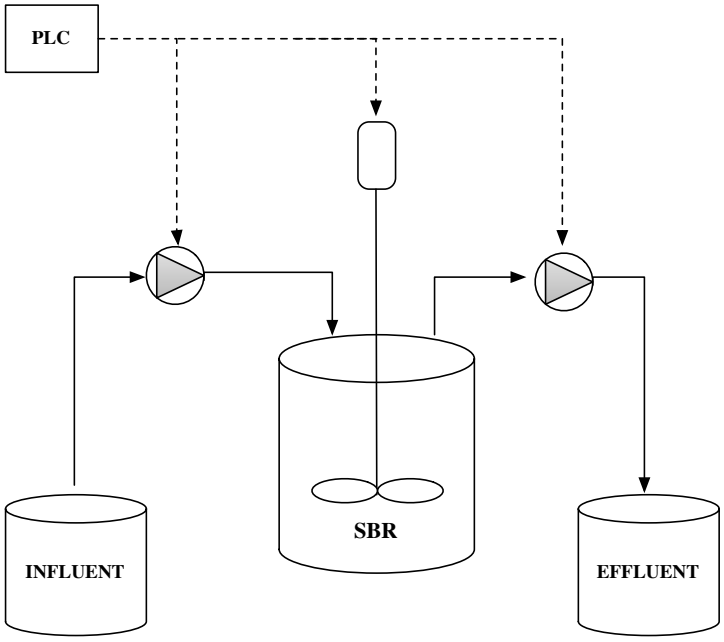

Figure 1.6 A typical sequencing batch reactor set up

Depena-Mora *et al.* (2004) slightly modified their SBR cycle by adding a 30 minutes mixing stage with substrate supply between mixed fill stage and settling stage. The modification helped overcome the floatation problems in SBR and significantly improved the settling property of anammox sludge in the SBR reactor; the sludge volume index (SVI, volume of 1g suspension sludge after 30 min settling) decreased from 108 to 60 cm^3/g. This is due to nitrogen gas bubbles can be better released during this mixing period without substrate supplying and because further nitrogen gas production was prevented by consuming remained nitrite in the reactor within the 30 min mixing stage. When carrying out a lab-scale ANAMMOX process, the anaerobic environment of SBR can be maintained by flushing with argon gas (Jin et al., 2008). While conducting the CANON or OLAND process, which requires an anoxic environment with DO level around 0.5 mg/L, the SBR can be modified with a pH-controlled aeration system where aeration is activated when pH is high in the reactor and off when pH drops, due to nitrification in the reactor (Kuai and Verstraete; Wett, 2006).

One limitation to the nitrogen conversion rates of the reactors is the efficiency of oxygen transfer through gas and liquid mass phase (Sliekers *et al*., 2002). Gas-lift reactor (Figure 1.7) was supposed to increase the N-conversion rate of Anammox related process due to its relatively high gas-liquid mass transfer of oxygen (Sliekers *et al*., 2003). Sliekers *et al*. (2003) proved that a gas-lift reactor was suitable for carrying out both ANAMMOX and CANON process; N-removal rates of 8.9 $kgN/m^3 \bullet d$ and 1.5 $kgN/m^3 \bullet d$ were achieved, respectively. The removal rate was almost 20 times higher than which was achieved in previous SBR laboratory reactors of 0.07 $kgN/m^3 \bullet d$ (Sliekers *et al*., 2002). However, a decrease of sludge settling ability was observed when the applied nitrogen load rate (NLR) exceeded the specific anammox activity rate of the biomass. This means that the NLR of gas-lift reactor may be limited by the specific anammox activity in the reactor.

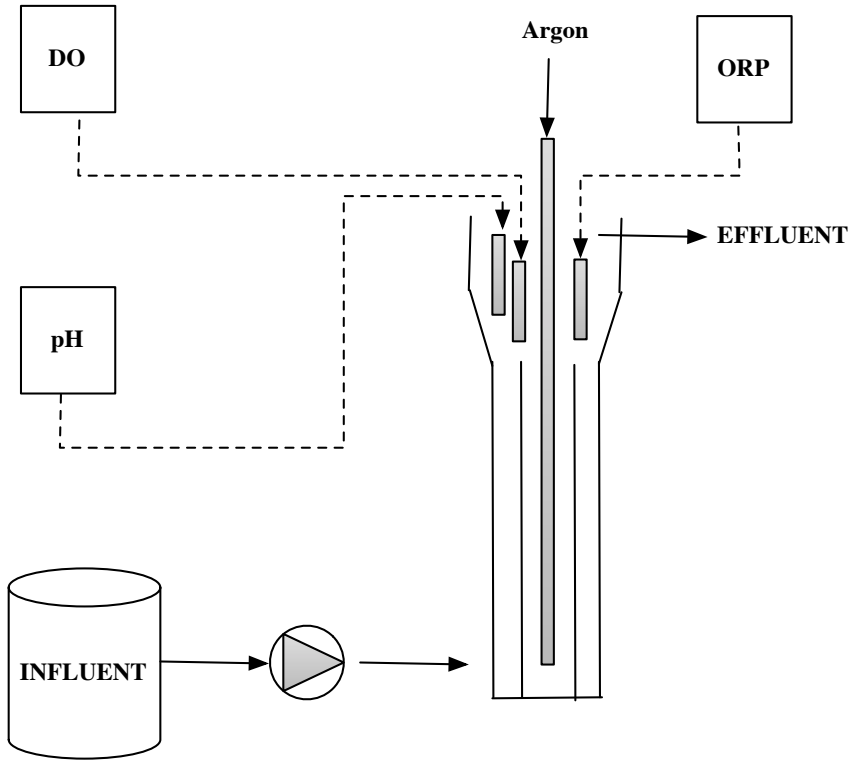

Figure 1.7 Gas lift reactor set up (Depena-Mora *et al*., 2004)

According to Jin *et al*. (2008), an anaerobic sludge blanket (UASB) reactor is a more stable reactor configuration to tolerate substrate concentration shocks than both SBR and upflow biofilm reactor (UBF), when carrying out the ANAMMOX process. Since granular sludge is easily achievable, UASB reactor might be more suitable to carry out high-loaded ANAMMOX process than other reactors. Super high nitrogen removal rates (NRR) of 74.3–76.7 kgN/m^3•d were achieved by Tang *et al.* (2011), which were almost three times higher than other previous reported NRR values.

Recent studies indicated that biofilm reactors (Figure 1.8), such as upflow biofilm reactor (UBF) and Membrane-aerated biofilm reactor (MABR), are also feasible to carry out the ANAMMOX process; the nonwoven fiber material used within the reactor was proved to be effective to attach biomass in anammox cultivation. Compare with SBR, UBF might be a better reactor in terms of reducing start-up time. Jin *et al*. (2008) reported that it took 57 days to start up an ANAMMOX SBR reactor, while UBF reactor needed only 31 days. However, a biofilm reactor is not feasible in tolerating high nitrogen loading because of the limitation of surface area. Kim *et al.* (2009) reported that the nitrogen-loading rate of biofilm ANAMMOX reactor cannot be higher than 1000 mg TN/L.

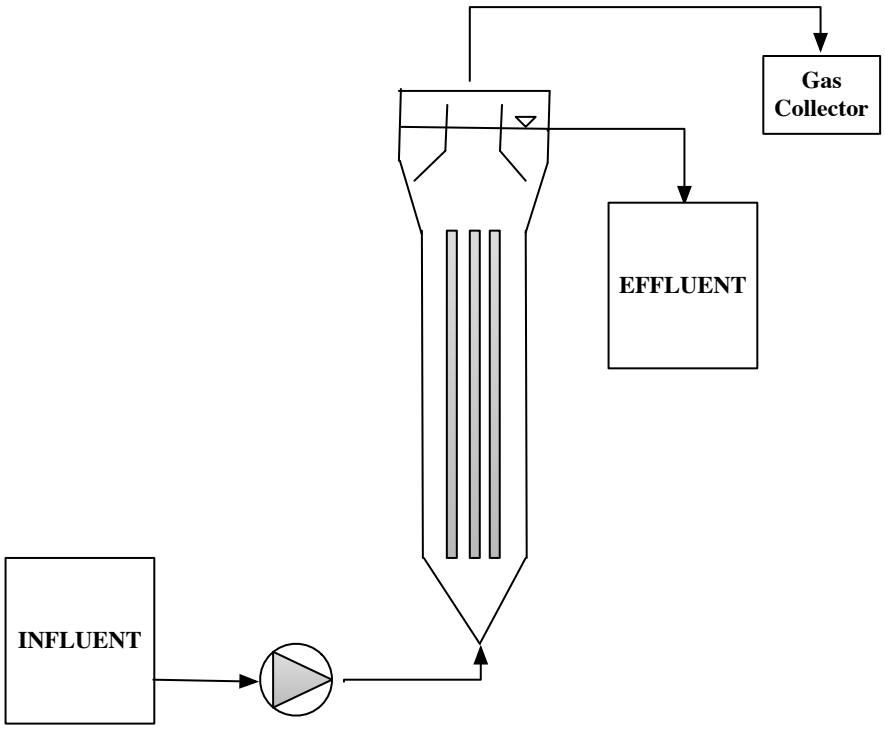

Figure 1.8 Schematic diagram of a biofilm ANAMMOX reactor (Kim *et al.*, 2009)

1.3.6.2 Reactor inoculation and start up

Anammox bacteria are widely found in the environment. Based on PCR analysis, anammox bacteria were identified in different water and wastewater treatment units including membrane bioreactors, denitrifying tanks, nitrifying RBCs, oxidation ditches, anaerobic digesters and aeration tanks (Zhang *et al*., 2008). Variety types of conventional sludge have been used as inocula for carrying out the ANAMMOX process in certain reactor configurations (Table 1.4). However, even in small-scale lab reactors, starting an ANAMMOX reactor with conventional sludge as inocula may take a period over 100 days. For example, start-up experience from a full scale UASB ANAMMOX reactor in Rotterdam, NL, with nitrifying sludge as inocula, indicated that the start-up times for full-scale reactors might be longer than 3 years (van de Star *et al*., 2007). In order to achieve a fast reactor start-up, the addition of mature anammox sludge from exotic sources might be necessary. Ni *et al* (2011) reported a successful start-up of a 50 L pilot-scale UASB granular reactor within 2 weeks, by using sludge containing mature anammox bacteria.

Table 1.4 Start-up ANAMMOX reactors with different seeding sludge

Sludge	Start-up time (d)	Reactor used	References
Upflow blanket anaerobic sludge	150	SBR	Chamchoi and Nitisoravut, 2007
Activate sludge	150	SBR	Chamchoi and Nitisoravut, 2007
Anaerobic digestion sludge	150	SBR	Chamchoi and Nitisoravut, 2007
Nitrifying sludge	105	UASB	Zheng *et al*., 2004
Denitrification sludge	100	UASB	Zheng *et al*., 2004

1.3.7 Operational concerns of ANAMMOX reactor

In order to control and optimize the ANAMMOX process, parameters that might affect the performance of the ANAMMOX reactor had been widely investigated and are summarized in Table 1.5.

Table 1.5 Summary of studies that investigated inhibitions on ANAMMOX process

Factors	References	Amount	Effects
Ammonium	Strous *et al*., 1999	1g N L^{-1}	No effect
	Bettazzi *et al*., 2010	90 mg NH_4^+- N L^{-1}	No effect
	Dapen-Mora *et al*., 2007	55mM	50% inhibition
	Ni and Meng *et al*., 2011	988.3 mg NH_4^+- N L^{-1}	50% inhibition
Nitrate	Strous *et al*., 1999	1g N L^{-1}	No effect
	Bettazzi *et al*., 2010	57 mg NO_3 - N L^{-1}	No effect
Nitrite	Strous *et al*., 1999	100 mg NO_2 - N L^{-1}	Complete inhibition
	Bettazzi *et al*., 2010	60 mg NO_2 - N L^{-1}	Activity decrease
	Egli *et al*., 2001	185 mg NO_2 - N L^{-1}	Complete inhibition
	Dapen-Mora *et al*., 2007	25 mM	50% inhibition
Methanol	Isaka *et al*., 2008	5 mM	70% inhibition
DO level	Strous *et al*., 1997	>0% O_2	Complete inhibition
	Jung *et al*., 2007	>0.2 ppm	Activity decrease

Anammox bacteria are strictly anaerobic. The anammox activity is complete inhibited by the presence of oxygen (Strous *et al*., 1997). However, the inhibition of oxygen is reversible, making it possible to combine partial nitrification and the ANAMMOX process within one reactor. Ni and Meng (2011) observed a 50% inhibition of anammox activity with ammonia concentrations

as high as 988.3 mg NH_4^+-N/L. The inhibition can be fully recovered by decreasing the substrate concentration. Jung *et al*. (2007) suggested a DO level below 0.2 ppm when anammox bacteria were cultivated in the continuous culture reactor.

Concentration of nitrite in the ANAMMOX reactor is considered as a critical factor that may cause severe inhibition to the activity of anammox bacteria even at a low concentration level. Although nitrite is an electronic acceptor in the ANAMMOX process, nitrite accumulation should be avoided when carrying out the ANAMMOX process in any reactor. Strous *et al*. (1999) detected an activity loss of anammox bacteria in a SBR at concentration higher than 100 mg/L NO_2-N, while Egli *et al* (2001) reported a complete activity loss at concentration at 185 mg/L NO_2-N within a RBC system. Adding intermediate compounds (hydroxylamine and hydrazine) could help to recover the anammox bacteria activity after complete or partial inhibition by nitrite toxin, however, permanent recovery is hard to achieve (Bettazzi *et al*., 2010). The concentration of ammonia, as substrate of ANAMMOX reaction, could also inhibit anammox bacteria. However, compared with nitrite, the toxicity concentration of ammonia is relatively high.

Nitrates would be accumulated within the reactor as simultaneous products of ANAMMOX reaction. The concentration of nitrate compounds would not inhibit to anammox bacteria (Bettazzi *et al*., 2010). However, the production of nitrate should be limited in a SHRAON or CANON process, to protect AOB from partial nitrification and limit nitrite oxidation.

Organic compounds, especially methanol, may contaminate the ANAMMOX reactor and inhibit anammox bacteria, since nitrate would be produced in the ANAMMOX process and might be removed by the heterotrophic denitrification process. Methanol would be added as hydrogen donor and recycled to ANAMMOX reactor with effluent from heterotrophic denitrification process to dilute high ammonium concentration or to avoid nitrite accumulation in the ANAMMOX reactor. Batch experiments done by Isaka *et al*. (2008) demonstrated that the anammox activity decreased about 70% when 5 mM methanol was added. The inhibition effect was found irreversible, suggesting that it is important to avoid methanol contamination in an ANAMMOX reactor.

1.3.8 Applications of ANAMMOX

Since the ANAMMOX process uses nitrite instead of organic carbon as an electronic acceptor, it is more suitable to be applied to treat wastewater with low carbon to nitrogen ratio. Recent research indicated that the ANAMMOX process could be successfully applied to treat sludge digester effluent (centrate), landfill leachate, and coke-oven wastewater (Table 1.6).

Table 1.6 Summary of the applications of ANAMMOX process

Wastewater	Process	NRR[a]	Scale	Reference
Centrate	Partial nitrification-ANAMMOX	0.71	10 L	van Dongen *et al*., 2001
	Partial nitrification-ANAMMOX	9.50	70 m^3	van der Star *et al*., 2007
	Partial nitrification-ANAMMOX	1.10	1 L	Gali *et al*., 2007
Landfill leachate	Partial nitrification-ANAMMOX	14	12 L	Liang and Liu, 2008
Coke-oven WW	ANAMMOX	0.062	1 L	Toh and Ashbolt, 2002

[a] Nitrogen removal rate, unit: kg N/(m^3•d)

Centrate from a municipal plant is a typical wastewater with a very low carbon to nitrogen ratio. In addition, the pH value (7.0 ~ 8.5) and temperature (35 ºC ~ 40 ºC) of centrate are commonly good for the growth of anammox bacteria. Thus, it is very suitable to use the ANAMMOX process to treat centrate. Van Dongen *et al*. (2001) first established a 10 L lab-scale Partial nitrification-ANAMMOX process treating centrate from Rotterdam WWTP, Netherlands. The SHARON process used a continued stirred tank reactor (CSTR), with hydraulic retention time (HRT) of 1 d. The ANAMMOX process applied a granular SBR. Over 80% of NH_4^+ could be converted to N_2 in this case. Based on the lab results, a full-scale SHARON-ANAMMOX process was built in Rotterdam WWTP (van der Star *et al*., 2007). Stable NRR of 9.5 kg N/(m^3•d) was finally reached after 3.5 years of start-up operation.

Landfill leachate is also characterized by low biodegradable organic contents and high concentrations of ammonia-nitrogen. Liang and Liu (2008) already indicated that leachate could be successfully treated with an ANAMMOX-soil infiltration system. Landfill leachate obtained from a municipal solid waste (MSW) sanitation landfill in Beijing, China with 1400~2800 mg/L NH_4^+-N and 1100-2600 mg/L COD_{Cr} was treated with a partial nitrification-ANAMMOX system. Both partial nitrification and ANAMMOX were carried out in a fixed bio-film reactor.

Results showed that stable removal efficiencies of 97% NH_4^+-N and 89% COD_{Cr} could be achieved by this system, indicating the process is feasible for leachate treatment.

Coke-oven wastewater, which is different from centrate and landfill leachate, contains not only high concentration of ammonia-N (330 ~ 650 mg/L) and organic matters (2000 ~ 2500 mg/L COD), but also toxic compounds like phenol (300 ~ 800 mg/L), cyanides (10 ~ 90 mg/L) and thiocyanates (300 ~ 500 mg/L) (Toh *et al*., 2002). Toh and Ashbolt (2002) reported a successful application of ANAMMOX process treating synthetic coke-ovens wastewater. Phenol was added stepwise to the influent from 50 to 550 mg/L to avoid instant toxic effect. After 15 months of enrichment, the NRR reached 0.062 kg N/(m^3•d) in this system.

2. Materials and Methods

2.1 Description of the sludge treatment facility

The research project was carried out at the J.A.M.E.S. wastewater treatment plant (WWTP). The J.A.M.E.S. WWTP (Figure 2.1), located on the bank of the Frasier River in the city of Abbotsford, BC, is the third largest secondary treatment plant in British Columbia. Serving over 240,000 residents the plant treats an average of 81,700 m^3/d (18 MGD). The plant consists of both primary and secondary treatment, producing a high quality effluent with an average biochemical oxygen demand (BOD) of 45 mg/L and total suspended solids (TSS) of 45 mg/L.

Figure 2.1 Picture of J.A.M.E.S. WWTP

The influent is discharged into the plant pre-treatment area by two screw pumps. Large particles in the influent like rags, sticks and plastics are screened out from the influent. Water would then proceed through a separator system (Figure 2.2). Next, the particles are allowed to settle down in primary sedimentation tanks (Figure 2.3 and Figure 2.4) with a residence time of 2 to 7 hours. Lighter particles float to the surface and are collected using skimmers (Figure 2.5), while heavier particles sink to the bottom of the tanks where they are raked to a sump, and pumped to the anaerobic digesters for treatment. The particles collected by the screens and grit tanks are dewatered and transported off site to the regional landfill.

Figure 2.2 Primary grit separation tank of J.A.M.E.S. WWTP

Figure 2.3 Primary sedimentation tank of J.A.M.E.S. WWTP

Figure 2.4 Decant of primary sedimentation tank of J.A.M.E.S. WWTP

Figure 2.5 Skimming removal tanks of J.A.M.E.S. WWTP

The wastewater is then pumped into two biological trickling filters (Figure 2.6), where the water is distributed over plastic media plates (Figure 2.7) (covered by growing biomass) after being mixed with return sludge from the clarifier and effluent from the solids contact tank. On the surface of the filter, bacterial colonies would form, producing bio-films that adsorb particles and

consume dissolved organics in the wastewater, resulting in a cloudy effluent that flows to solids contact tanks.

Figure 2.6 Top view of the Trickling Biological Filter

Figure 2.7 Close up of water distributor and trickling biofilter media

In the solids contact tanks (Figure 2.8), the biological trickling filter effluent is combined with sludge from the secondary clarifiers. This results in a TSS of about 2000 mg/L. With aeration, further removal of nitrogen and dissolved organic compounds is achieved. After residing for 1-2

hours, the water is pumped to the secondary clarifiers (Figure 2.9 and Figure 2.10) where the sludge and treated wastewater is separated. The clarifier effluent has an average BOD of 45 mg/L and a TSS of 45 mg/L. As clarified secondary effluent enters the final chlorine contact tank, a chlorine solution is injected as disinfectant. Before the effluent flow is discharged into the Fraser River, a sulphur dioxide solution is injected to neutralize any residual chlorine.

Figure 2.8 Solids contact tank

Figure 2.9 Full view of secondary clarifier

Figure 2.10 Close view of secondary clarifier

Sludge from the secondary clarifiers is pumped back to the primary sedimentation tank, where it is mixed with the sludge from the raw effluent. This sludge, now consisting of the skimming and suspended solids from the primary sedimentation tanks, and the sludge from the secondary clarifiers, is pumped into a thermophilic (high temperature) pasteurization process tank to achieve full pasteurization. Solids from the pasteurization process are then pumped to three anaerobic digesters (Figure 2.11) for solids stabilization. Organic contents and odours are greatly reduced in stabilized solids. Methane gas, produced as a by-product in this process, is combusted in the Treatment Centre's boilers for digester heating and building areas.

Figure 2.11 Roof of anaerobic digester

Once the sludge is digested, the stabilized biosolids are pumped to a dry, soil-like consistency centrifuge (Figure 2.12) for dewatering, producing a dry cake of solids (Figure 2.13) and are stored on-site. The high strength dewatered sludge liquor (centrate) from the centrifuges, contains relatively high concentration of ammonia (850 mg/L to 1200 mg/L) and is pumped into nitrifying tanks (Figure 2.14). Treated effluent from the nitrifications tanks is returns back to the head of the plant and mixed with incoming sewage.

Figure 2.12 Centrifuge

Figure 2.13 Stabilized bio-solids after anaerobic treatment and centrifuging

Figure 2.14 Nitrification tanks

2.2 Influent media

The pilot reactor used in this project was fed by on-site centrate collected from the dewatered sludge liquor of centrifuge process in J.A.M.E.S. WWTP. Main characteristics of the centrate as feed to ANAMMOX reactor is summarized in Table 2.1

Table 2.1 Main characteristics of centrate feeding to the ANAMMOX reactor

Parameter	Abbreviate	Unit	Minimum	Maximum	Average
Ammonia-nitrogen	NH_4-N	mg/L	713.1	1177.0	910.5
Nitrite-nitrogen	NO_2-N	mg/L	0.04	4.5	0.6
Nitrate-nitrogen	NO_3-N	mg/L	0	4.0	0.3
Phosphate	PO_4-P	mg/L	NA	NA	122.5
pH	pH	NA	7.6	8.7	8.2
Total suspended solids	TSS	mg/L	73	500	280
Volatile suspended solids	VSS	mg/L	33	435	218.1
Chemical oxygen demand	COD	mg/L	NA	NA	429
Biochemical oxygen demand	BOD_5	mg/L	9	33	18
Alkalinity	ALK	mg/L	2460	3870	3019

The centrate was mainly ammonium concentrated liquid, with an average ammonia-nitrogen concentration of about 910 mg/L. There was almost no nitrite in the centrate (Avg. = 0.6 mg/L), which means partial nitrification process is necessary to partially convert ammonium to nitrite for carrying the ANAMMOX process. Biochemical oxygen demand (BOD_5) of the centrate was relatively low (Avg. = 18 mg/L), thus conventional biological nitrification and denitrification would not work under such a low C/N ratio. The Chemical Oxygen Demand (COD) was as high as 429 mg/L, which was mainly due to the addition of polymer in the sludge dewatering process. Total suspended solids (TSS) in centrate (73 mg/L ~ 500 mg/L) were also highly depended on the dosage of polymer added in the sludge dewatering process. The dosage of polymer used at J.A.M.E.S. WWTP may range from 0.65 L/s to 0.85 L/s. Increasing the dosage of polymer would reduce TSS in the centrate directly. The alkalinity of the centrate is around 3000 mg/L and the ratio of alkalinity and ammonium-nitrogen was around 3.0, indicating alkalinity was enough for carrying out the CANON process. Ortho-P levels averaged about 122 mg/L.

2.3 Experimental set-up

2.3.1 Pilot-scale SBR

A 400 L, pilot-scale, sequencing batch reactor (SBR) was designed for the slow growth of the anammox bacteria (Figure. 2.15). SBR has been proved to be an effective reactor for carrying out ANAMMOX reaction, due to its ability of biomass retention (Kuai and Verstraete *et al*., 1998; Strous, *et al*., 1998; Arrojo, *et al*., 2006; Chamchoi and Nitisoravut, 2007; López, *et al*., 2008; Jin, *et al*., 2008). Two, 500-Watt Electronic control heaters (HMA-500, JSK Merchandising Inc.) were used to control the temperature of the reactor. Isolate capsules were also used to wrap the reactor for maintaining reactor temperature. A mechanical stirrer was installed within the SBR to achieve a complete mix condition in the reactor. Mixing is important to help mass transfer in the reactor and sludge granulation (Arrojo *et al*., 2006). The formation of well-settling, anammox granular biomass would not only improve anammox sludge retention and also increase its tolerance to shock loading (van der Star *et al*., 2007; Fernandez *et al*., 2008). However, granular anammox sludge may break up when exposed to high shear force conditions, at high stirring speeds. Thus, a stirring speed of 15 rpm was chosen to provide sufficient mixing to the reaction without breaking the granular sludge.

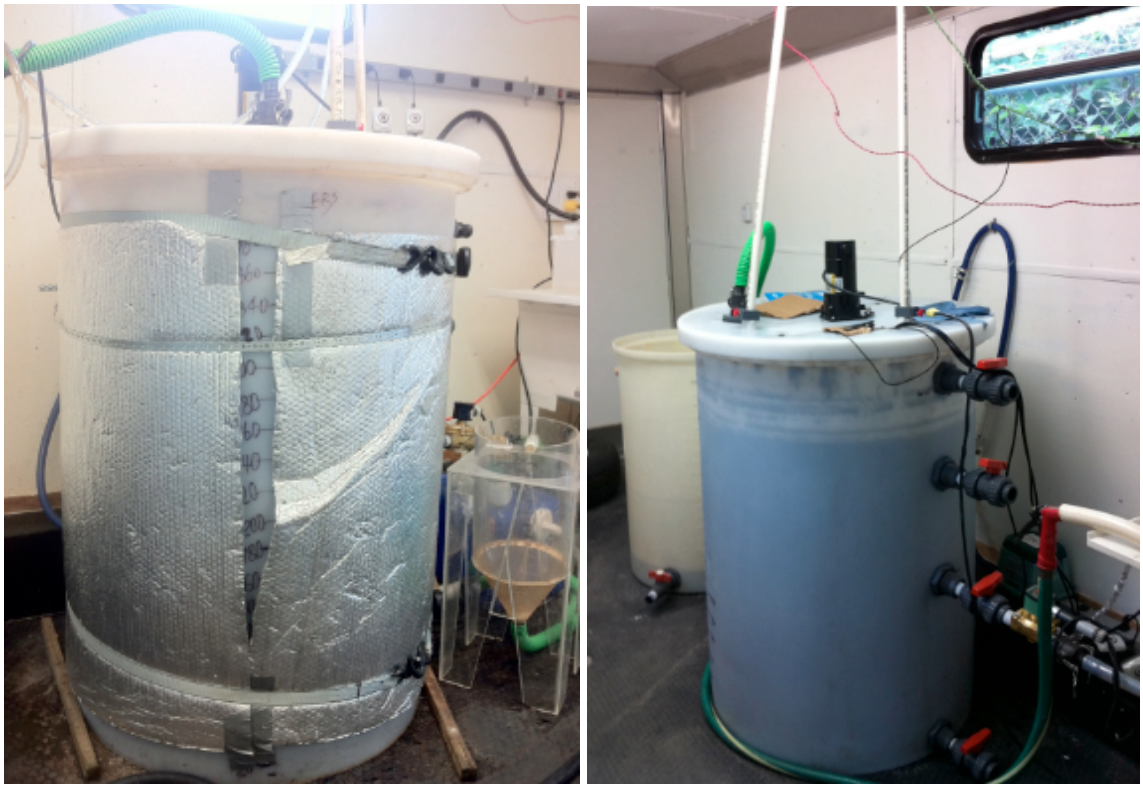

Figure 2.15 Sequencing batch reactor (SBR) for the CANON process

2.3.2 CANON SBR system

A CANON SBR system was designed for treating the on-site centrate at the J.A.M.E.S. WWTP. The schematic diagram of this CANON system was presented in Figure 2.16. Centrate was collected from the sampling spot of sludge dewatering effluent and pumped to a 550 Gal (or 2082 L) centrate storage tank. Centrate was then pumped periodically into the CANON reactor through a submersible feeding pump (No.49, Zoeller Pump Co.) for treatment. A mechanical impeller with a mixing speed controller was used to ensure a complete mixing condition in the reactor. An air compressor (HP-40-0110, Hiblow Technology Co. LTD.) was used to maintain a certain DO level in the reactor. The aeration condition in the reactor is adjustable through an air flow meter (0 ~ 40 STD L/min) located after the air compressor. A time controller fixed on the aeration control panel was able to adjust the aeration time. A decant valve on the reactor was used to discharge centrate, after being treated. The volume of feed and decant was controlled by level switchers that connected to the feed and decant control panel.

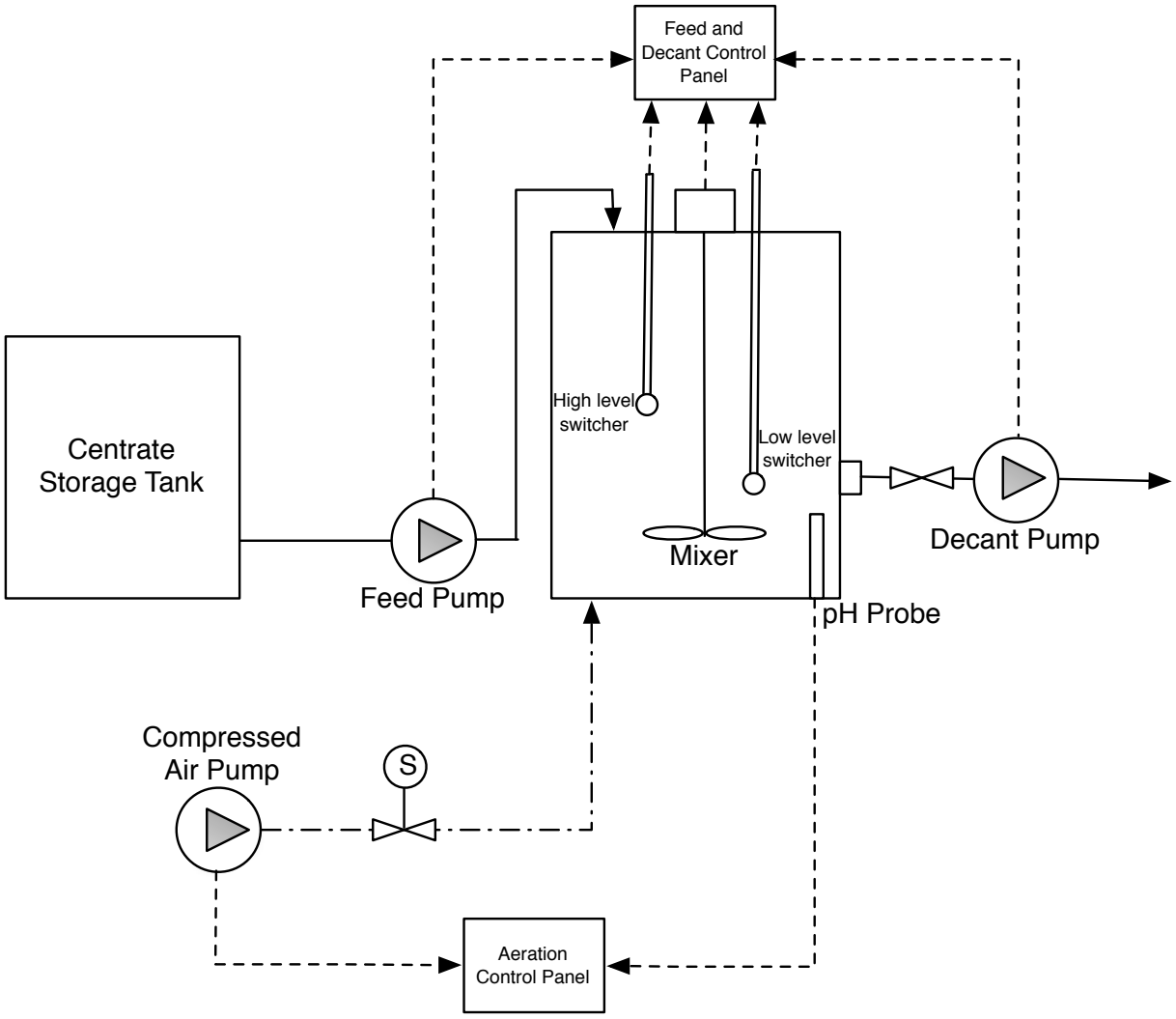

Figure 2.16 Schematic diagram of CANON-SBR system

The CANON SBR reactor worked continuously with fresh substrate feed. Each cycle of the nitrogen removal process consisted of five stages: feeding stage, anaerobic protection stage, anoxic reaction stage, settling stage, and decanting stage. In the feeding stage, a certain volume of centrate in the storage tank was pumped into the reactor at a certain feeding speed. The volume of feeding centrate can be easily adjusted through changing the level of the feeding controller within the reactor. After feeding substrate, the reactor went through an anaerobic protection stage (typically 1 to 2 h) that only stirrer works in this stage. The purpose of this stage is to protect anammox bacteria from instant nitrite toxic at the beginning of the cycle and also avoid nitrite accumulation in the reactor. In the following anoxic reaction stage, the air compressor supplied oxygen to the reactor, maintaining anoxic condition (DO level less than 0.5 mg/L) for the CANON SBR. When the reaction was finished, 4 to 10 min sludge settling time was applied to the settling stage to ensure anammox bacteria would settle and remain in the

reactor. After sludge settling, the supernatant was decanted from the effluent valve on the lower part of the reactor. Decant volume could be easily be adjusted by changing the level of the decanting controller within the reactor.

2.3.3 Control of the reactor

The SBR is designed to be automatically controlled by the system control panels (Figure 2.17). The control is based on real-time SBR control, as described by Yu *et al.* (2000), Kim *et al.* (2004) and Wu *et al.* (2007). The automatically control system (ACS) consisted of four different controllers: a pH controller, a feed controller, a decant controller, an aeration and mixing controller (Figure 2.18).

Figure 2.17 Control panel for the CANON-SBR reactor

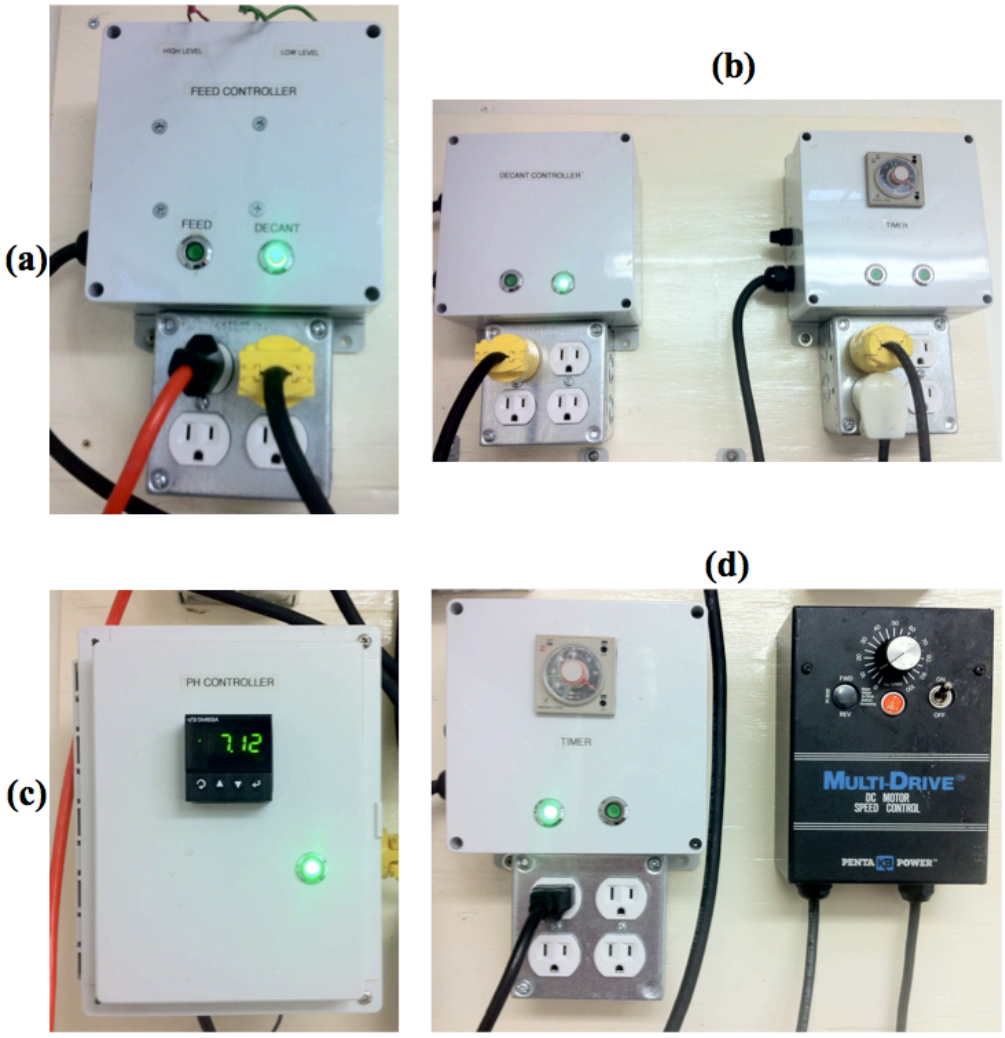

Figure 2.18 Four main parts of the control panel: a) feed controller; b) decant controller; c) pH controller; d) aeration and mixing controller

The core part of the system is the pH and air controller, since pH and aeration was used for real-time control of the SBR cycle. The pH would drop in parallel with nitrogen removal, which is the basis of the control in the CANON SBR. An industry pH probe (from Cole-Parmer Instrument Co.) (for continuous pH measurements) was used to detect pH variation in the CANON SBR and was calibrated once every three months for quality control. Detected signals were transported to the pH controller and the pH value was shown instantly on the LED panel. A pH level of 6.0 was set as the set point of the pH controller. When pH in the reactor was higher than the set point, the air compressor would start to work and initiate the CANON reaction (pH controlled intermittent aeration). The intermittent aeration can be controlled by setting the on and off timer of the aeration controller (Figure 2.18d). When pH dropped below the set point, the aerator and mixer would be turned off automatically for sludge settling. Sludge settling time was controlled by the decant controller (as shown in Figure 2.18b). The settling time was adjustable,

allowing anammox bacteria to settle within a period of time (Typically 4 ~ 10 min). After settling, a solenoid decant valve would be turned on automatically and start decanting. When the liquid level within the reactor reached the low level switcher, the decanting valve turned off automatically and the feed pump started to work. The feeding pump will be turned off automatically, until the liquid level reached the high level switcher. When the pH level increased above the set point due to the feeding of centrate, the pH controller and aeration controller started to work again.

2.4 Process operations

The CANON SBR reactor was running continuously for 317 days. Within this period of time, the running of the reactor was conducted under three experimental periods: (1) start-up stage; (2) sludge enrichment and (3) system optimization.

2.4.1 Inoculation and start up

The start-up of the reactor took 115 days. The start up period of the reactor can be separated into two different stages. During the first stage (from day 1 to day 24), the CANON SBR was inoculated by anaerobic sludge from the digester of the Lulu Island wastewater treatment plant (WWTP). 30 L anaerobic sludge was added into the reactor and was mixed with 300 L water and 30 L centrate. Feeding and decanting of the system was operated manually, since the reactor was extremely unstable at the beginning. The airflow rate was maintained at 5 L/min, with an intermittent period of 1 hour on and 5 hours off. DO in the reactor was measured by a DO meter (HQ30d, from HACH Company), calibrated once every two months. The concentrations of nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and ammonia-nitrogen (NH_3-N) in CANON SBR were measured daily with quick test kits (from API co.). No sample was collected from the reactor for analysis because the nitrogen removal was done by biological nitrification and denitrification, instead of the ANAMMOX process, during this period. Since the DO level was below 0.5 mg/L and temperature was above 30 ºC in the reactor, nitrite oxidation process could be inhibited (Schmidt *et al*., 2003), leading to the outgrowth of AOB over NOB. Partial nitrification could be achieved to provide appropriate amount of nitrites as electron acceptors for the ANAMMOX process.

During the second stage of start up period (from day 25 to day 115), 10 L of mature anammox flocculent sludge (from the Environmental Engineering lab at the of University of British Columbia) was added into the reactor. The aeration flow rate was still maintained at 5 L/min but the intermittent duration was reduced to 1 h on/ 9 h off, to avoid nitrite inhibitory effects to anammox bacteria. The aeration intermittent period was then slowly increased from 1 h on/ 9 h off to 15 min on/ 15 min off, to increase the reaction speed. Starting from day 76, samples of influent, decant effluent and mixed liquor in the reactor were collected, respectively, for the analysis of NH_3/NH_4^+-N, NO_2-N, and NO_3-N.

2.4.2 Sludge enrichment

In the second period for sludge enrichment (from day 115 to day 148), the system was adjusted for automatic running and continuous aeration. The airflow rate was increased step-wisely from 5 L/min to 10 L/min, for improving the growth rate of AOB. Once the airflow rate was increased, the nitrite concentration in the reactor was checked with quick test kits. The aeration could be further improved if only nitrite concentration in the CANON SBR was maintained below the safety limit (considered as 20 mg/L).

2.4.3 System optimization

During the last period (from day 148 to day 317), the CANON system was optimized under variable environmental conditions. Since the reactor was controlled by an automatically controlled system, all parameters of the process could easily be adjusted and tested for system optimization. Operational parameters include: feeding rate, initial ammonia concentration temperature, airflow rate and sludge settling time. Tests under variable environmental conditions are summarized in Table 2.2. The optimization tests were conducted at the J.A.M.E.S. WWTP with on-site centrate collected directly from on-site sludge dewatering process for the purpose of pilot testing. Since centrate quality may vary from plant to plant, the optimization results of this research project may only be suitable, accurate and appropriate for dealing with the centrate at the J.A.M.E.S. WWTP. Whether the optimization results are universally compatible for treating centrate from other WWTPs may need further investigation.

Table 2.2 Summary of tested conditions for system optimization

Tests No.	Test time	Feeding volume (L)	Feeding time (min)	Reactor volume (L)	Temp (ºC)	Airflow rate (L/min)	Settling time (min)	pH set point
1	148d~150d	70	1	350	28	8	10	6
2	151d~153d	70	1	350	30	8	10	6
3	154d~158d	70	1	350	26	8	10	6
4	158d~165d	70	1	350	32	8	10	6
5	166d~229d	70	1	350	32	10	10	6
6	230d~240d	70	1	350	32	10	8	6
7	241d~244d	90	1	350	32	10	8	6
8	245d~253d	90	1	350	32	12	8	6
9	254d~260d	120	1	350	32	12	8	6
10	261d~271d	120	1	350	32	14	8	6
11	272d~281d	190	1	380	32	12	8	6
12	282d~290d	190	1	380	32	12	4	6
13	291d~297d	190	380	380	32	12	6	6
14	298d~317d	190	380	380	32	12	4	6

2.5 Sample analysis

For evaluating the performance of the CANON SBR, samples from influent centrate, reactor mixed liquor and decanting effluent were collected four times per week. In order to describe the nitrogen removal within cycles, mixed liquor samples from the reactor were also taken once per hour from the beginning to the end of representative cycles.

Before being analyzed, samples were preserved in the laboratory refrigerator at 4 ºC. Samples were analyzed for ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), phosphate (PO_4-P), Biochemical Oxygen Demand (BOD5), Chemical Oxygen Demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, and pH. All analyses were conducted at the Environmental Engineering Laboratory of the Department of Civil Engineering, University of British Columbia. Methods for each analysis were summarized in Table 2.3. NH_3-N, NO_2-N, NO_3-N and PO_4-P were analyzed by flow injection analysis of spectrophotometry (Quickchem 8000, Lachat). COD was determined colorimetrically by using a Hach DR/2000 direct spectrophotometer at 600 nm. To ensure the accuracy of analysis of NH_3-N, NO_2-N, NO_3-N and PO_4-P, samples were centrifuged under 25000 rpm for 10 min before

being analyzed. Only supernatant was used for analysis, since particles in the samples would significantly influence on the accuracy of the results. In addition, blanks prepared by distilled water were used to calibrate the results.

Table 2.3 Sample preparation and analysis methods

Parameter	Method [a]
Chemical oxygen demand (COD)	Standard Methods 5220
Biochemical oxygen demand (BOD_5)	Standard Methods 5210
Ammonia-nitrogen(NH_3-N)	Standard Methods 4500 – NH_3
Nitrite-nitrogen (NO_2-N)	Standard Methods 4500 – NO_2
Nitrate-nitrogen(NO_3-N)	Standard Methods 4500 – NO_3
Phosphate(PO_4-P)	Standard Methods4500 – P
Total suspended solids (TSS)	Standard Methods 2540D
Volatile suspended solids (VSS)	Standard Methods 2550
Alkalinity (as $CaCO_3$)	Standard Methods 2320
Hardness (as Ca_2^+)	Standard Methods 2340

[a] Adapted from Standard Methods for the Examination of Water and Wastewater (APHA,1989)

Base on the analytical results of influent ammonia concentration ($NH_{3\ Inf}$) and effluent ammonia concentration ($NH_{3\ Enf}$), ammonia removal rate (ARR) could be calculated through Equation 13:

$$ARR = \frac{NH_{3\ Inf} - NH_{3\ Eff}}{NH_{3\ Inf}} \quad [13]$$

Hydraulic retention time (HRT) of the reactor could be determined by measuring cycle length time (T), liquid volume of the reactor (V_R) and feeding volume (V_f), as presented in Equation 14:

$$HRT = \frac{V_R}{V_f/T} \quad [14]$$

Ammonia loading rate (ALR) could be calculated with Equation 15:

$$ALR = \frac{NH_{3\ Inf}}{T}, \ kgN/m^3 \cdot d \quad [15]$$

3. Performance of the CANON SBR

In order to evaluate the performance of the CANON SBR system for nitrogen removal from centrate, concentrations of influent and effluent ammonia-nitrogen (NH_3-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) were measured from day 76 to day 317. Figure 3.1 shows the variation of influent and effluent NH_3-N concentration and ammonia-nitrogen removal rate (ARR). The concentrations of influent and effluent NO_2-N and NO_3-N are shown in Figure 3.2. TSS and VSS, as presented in Figure 3.3, are important to evaluate sludge enrichment and the sludge holding ability of the reactor. Since the CANON process is an alkalinity consuming process, the differences between influent and effluent pH value, as well as alkalinity difference between influent and effluent, is presented in Figure 3.4. The influent alkalinity varied from 2460 mg/L to 3870 mg/L, while alkalinity in the effluent changed from 15 mg/L to 134 mg/L, indicating most of alkalinity was consumed by the CANON process. The consumption of alkalinity would lead to pH decreasing. pH of feeding centrate varied from 7.56 to 8.76, with an average of 8.19. Effluent pH could be as low as 5.53 to 6.41, with an average value of 6.04 after the reaction. Figure 3.5 shows the cycles length of CANON cycles and HRT of the reactor, which are critical parameters for the evaluation of system stability and future design of a full-scale reactor.

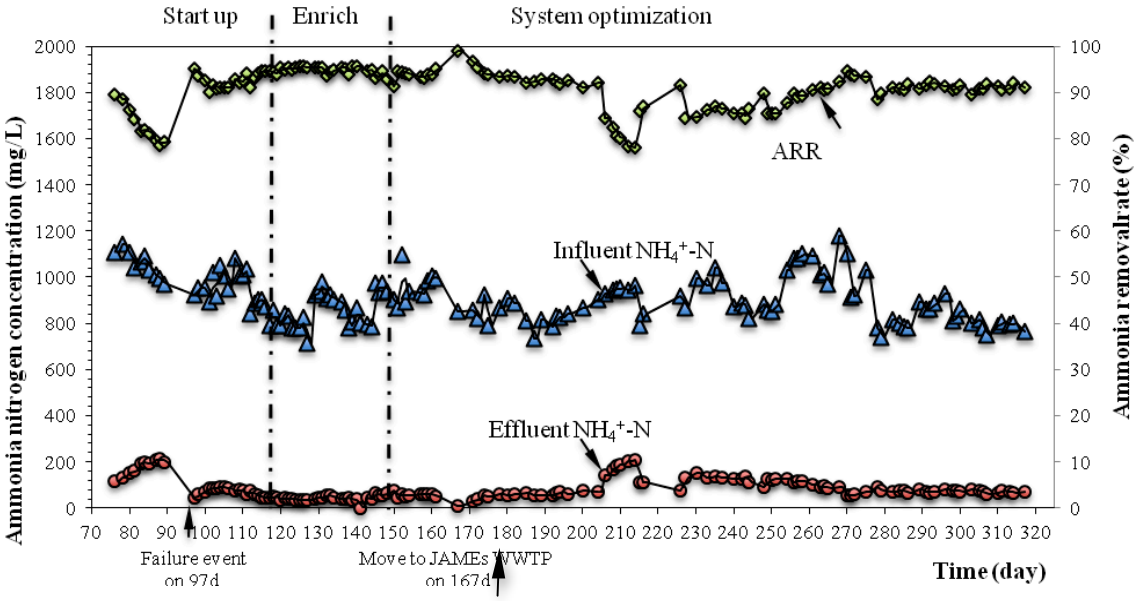

Figure 3.1 Variation of influent and effluent ammonia-nitrogen concentration and ammonia-nitrogen removal rate (ARR) during reactor running period

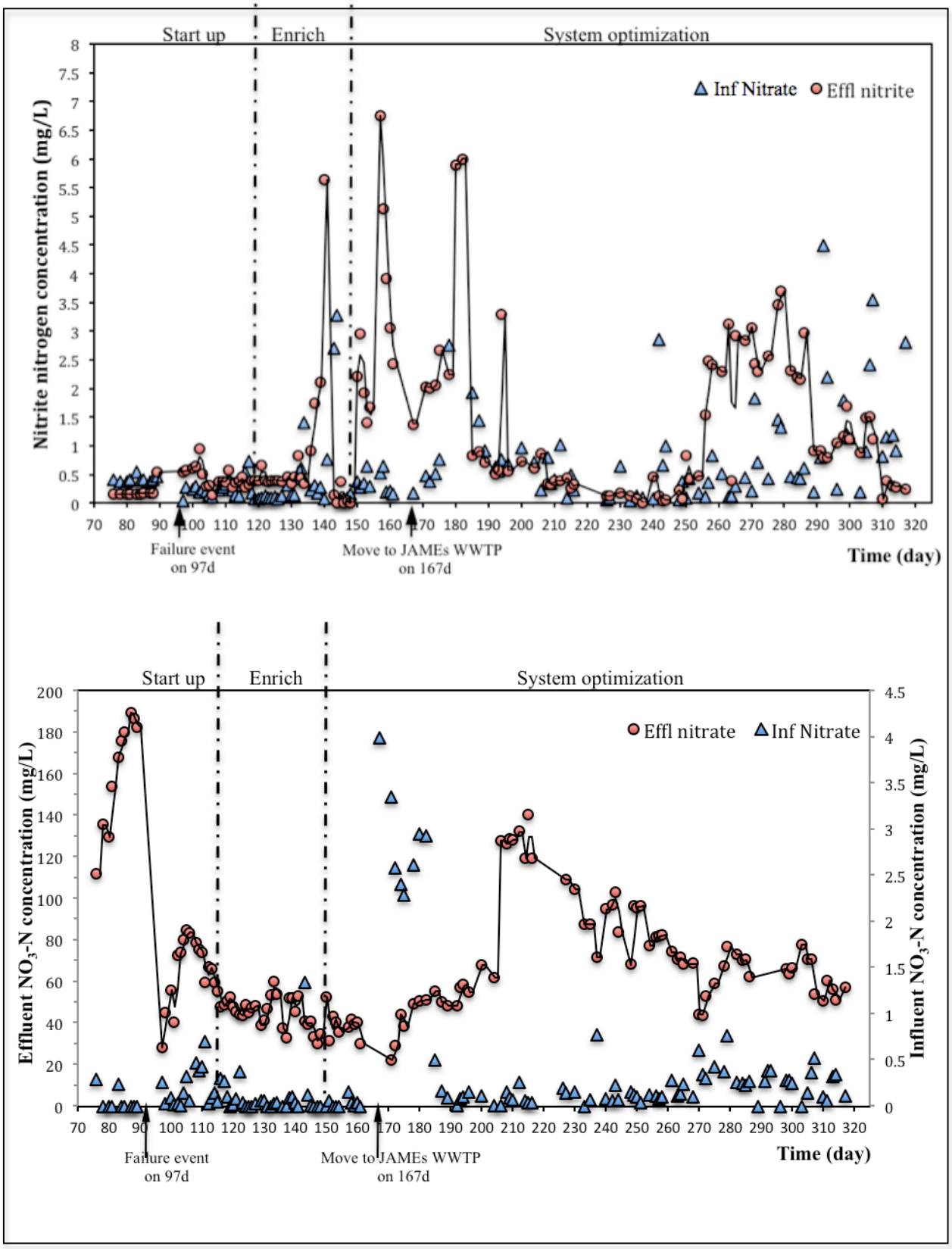

Figure 3.2 Concentration variations of the influent and effluent nitrite-nitrogen and nitrate-nitrogen during reactor running period

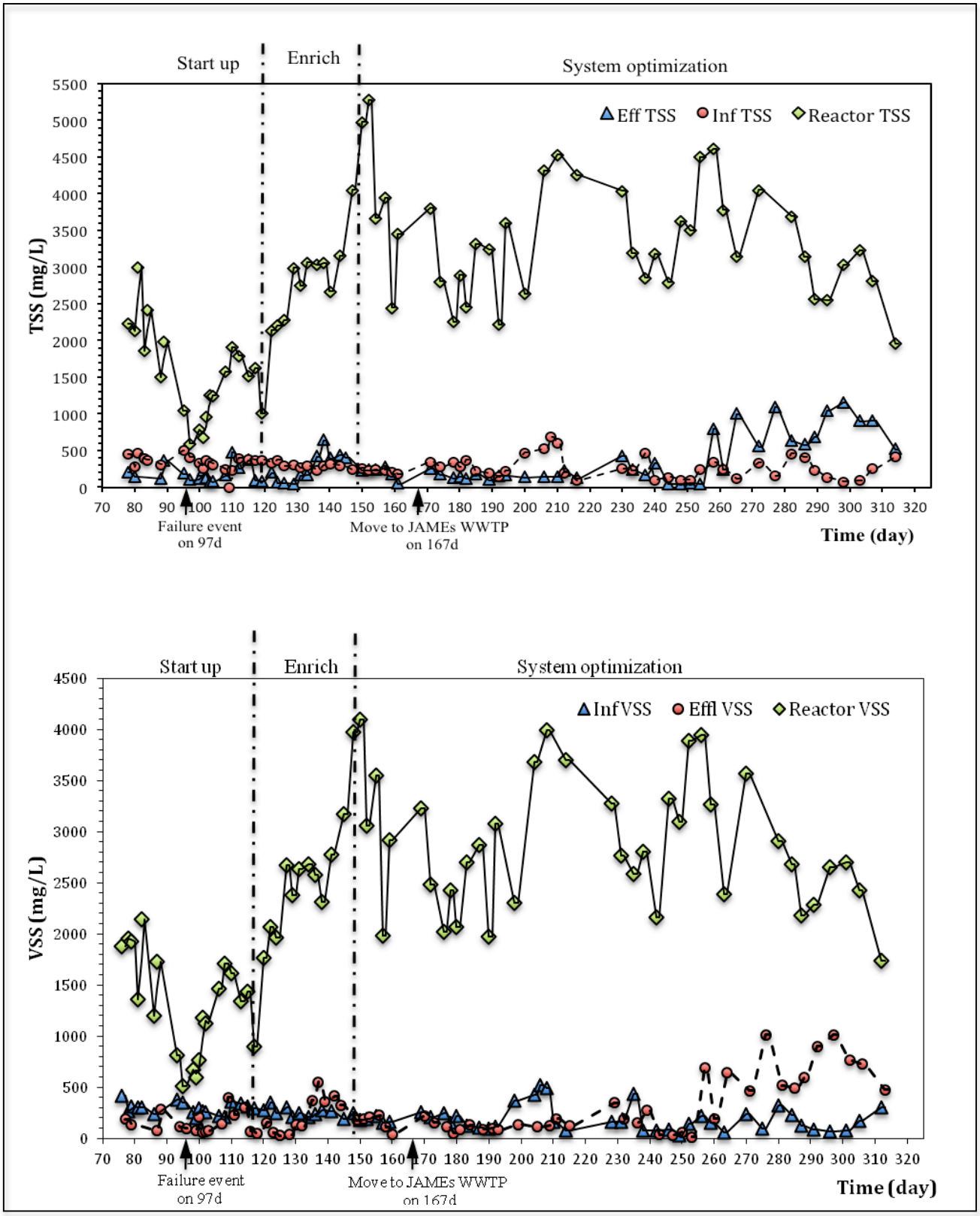

Figure 3.3 Variation of the TSS and VSS of influent, effluent and reactor during reactor running period

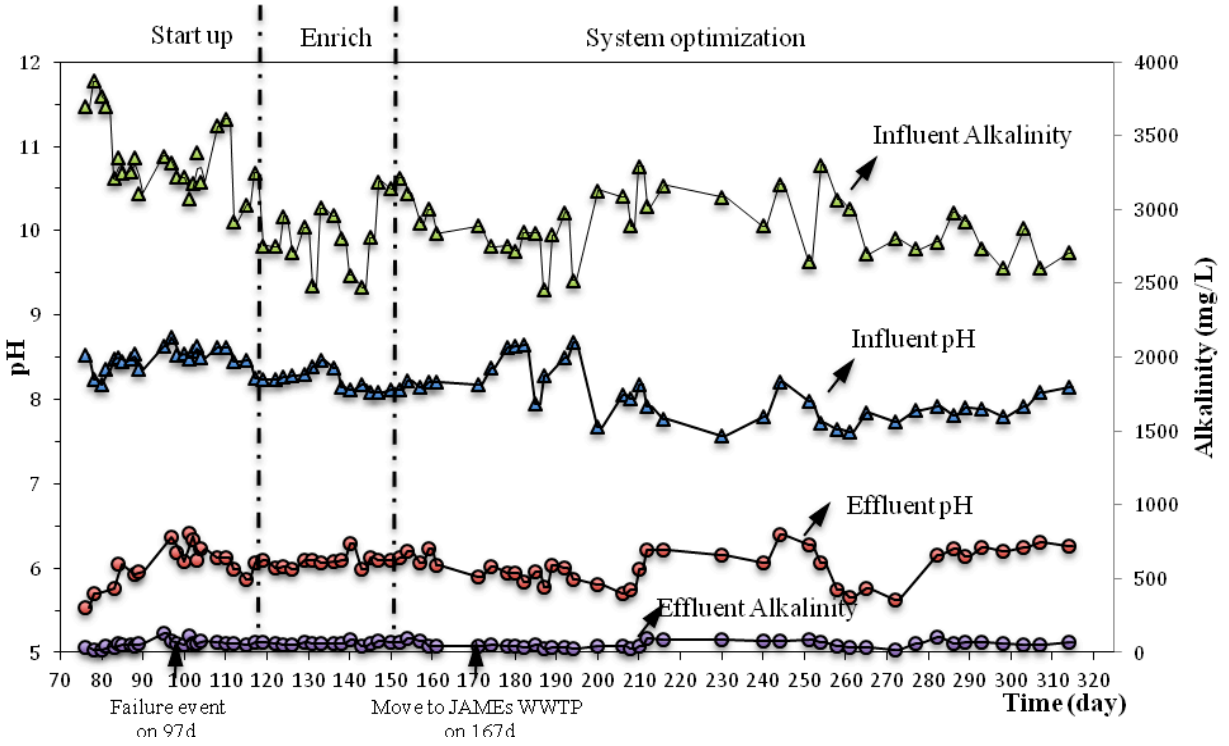

Figure 3.4 Variation of the influent and effluent pH and alkalinity during reactor running period

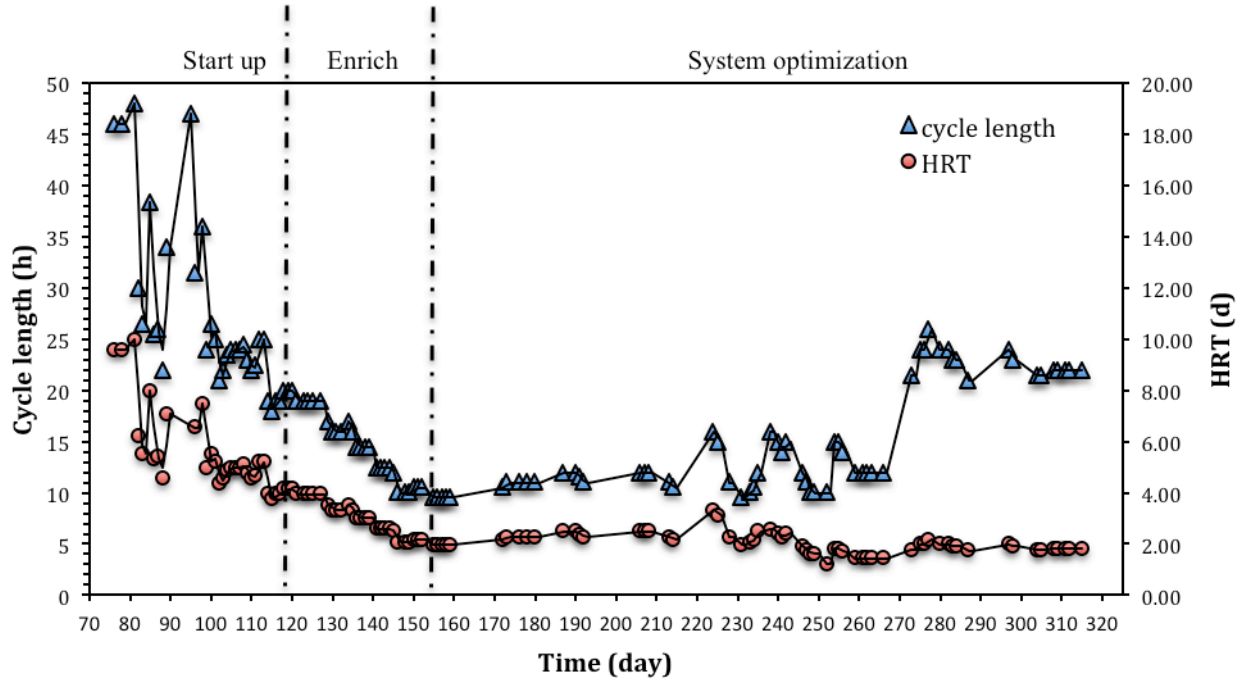

Figure 3.5 Variation of the cycle length and hydraulic retention time (HRT) during reactor running period

3.1 Start-up period

Since no samples were collected before day 76, the performance of the early stage SBR start-up could only be evaluated by the results of quick test kits. During the first stage of start-up period (from day 1 to day 24), when anammox sludge was not yet inoculated, nitrite concentration varied from 20 mg/L to 100 mg/L even at low aeration (5 L/min, 1 h on/ 5 h off). At the same time, nitrate concentration ranged from 50 mg/L to 200 mg/L. At the end of this stage, nitrite concentration decreased and remained at around 10 mg/L, which would not inhibit the growth of anammox bacteria (Bettazzi *et al*., 2010).

During the second stage of start-up period from (day 25 to day 115), mature anammox sludge was inoculated to the reactor. Daily test of nitrite and nitrate concentration through the quick test kits showed that the nitrite concentration dropped constantly from 10 mg/L, on day 25, to less than 2 mg/L on day 75, while the nitrate concentration drop from 200mg/L to 50 ~ 100 mg/L. The reduction of nitrite concentration could be explained by the utilization of ANAMMOX process, indicating the success of the anammox bacteria inoculation. The decreasing nitrate concentration could be caused by partial nitrification, since ammonia oxidizing bacteria could outcompete the nitrite oxidizing bacteria, under the condition of DO below 0.5 mg/L and temperature above 25ºC.

From day 76, samples were collected for analysis. The results showed that the concentrations of NO_2-N in the feeding centrate were both below 0.5 mg/L, which could be ignored in the CANON process. The nitrite concentrations in the decant effluent were also kept below 0.5 mg/L. Thus, the inhibition effects of nitrite to anammox bacteria could be ignored too. However, the ARR of the reactor was found to decrease from 89.5%, on day 76, to 79.4%, on day 88 (Figure 3.1), while the effluent nitrate concentrations increased from about 114 mg/L to 182 mg/L (Figure 3.2). The unexpected decreasing of ARR could be explained by the decreasing of TSS and VSS in the reactor. The TSS dropped from 2230 mg/L, on day 76, to 1500 mg/L, on day 88, while VSS dropped from 1875 mg/L to 1200 mg/L (Figure 3.3), indicating less anammox bacteria available in the reactor. The drastic increase of NO_3-N also indicated that the anammox bacteria were out competed by NOB.

An incident occurred on day 97 that almost caused failure during the start-up of the reactor. The electronic controlled decanting valve broke and failed to close when feeding started, causing most of sludge in the reactor to be washed out. TSS in the reactor dropped drastically from around 2000 mg/L to 593 mg/L (Figure 3.3). Until the end of start-up period (day 115), the TSS and VSS in the reactor were still 1509 mg/L and 1345 mg/L, indicating the slow regrowth of sludge occurring in the reactor. However, the failing seemed to improve the performance of the reactor. After day 97, effluent NH_4-N, and NO_3-N concentrates were much lower than before. Effluent NH_4-N increased from 44.0 mg/L on day 97 to about 93.2 mg/L on day 104 and then decreases to about 47 mg/L on day 115, which is the end of start-up period. Similar concentration variations also applied to effluent NO_3-N that increased from 28.2 mg/L on day 97 to about 85.0 mg/L, on day 105, then decreased to 55.4 mg/L, on day 115. The ARRs were above 90% and ended with 94.7% on the last day of start-up period. During the 115 days of start-up period, cycle lengths were relatively long and unstable. The cycle length decreased from 46 h on day 76 to 25 h on day 115, while the HRT also decreased from 9.6 d to 5.2 d (Figure 3.5). The decreasing cycle length and HRT indicate the acceleration of the CANON reaction, which could be caused by the growth of both nitrifiers and anammox bacteria.

3.2 Sludge enrichment period

After successful start-up the reactor, the system was operated under steady condition for sludge enrichment, from day 115 to day 148. Mixed liquor volume was 350 L, feeding volume was 70 L, and reactor temperature was kept at 32 ºC. The reactor was continuously aerated with an airflow rate starting from 5 L/min on day 115. The aeration was increased to 7 L/min on day 125 and was further increased to 10 L/min on day 137. An increase in effluent nitrite concentrations from 0.92 mg/L to 1.75 mg/L was observed right after changing the airflow rate from 7 L/min to 10 L/min. The NO_2-N concentration kept increasing to about 9.4 mg/L during the following several days, indicating that the activity of AOB could be stimulated by increasing the aeration, thus generating surplus nitrite for the anammox reaction.

NH_3-N and NO_3-N concentrations in the effluent were kept relatively constant during the sludge enrichment period. The NH_3-N varied from 33.8 mg/L to 56.3 mg/L (Figure 3.1) while effluent NO_3-N changed from 37.8 mg/L to 60.2 mg/L (Figure 3.2). The ARR remained above 92%, and

varied from 92.8% to 95.7% during day 115 to day 148. The stable NH_3-N and NO_3-N concentrations in the effluent and the relatively unchanged ARRs indicated that the reactor could be considered as "stable running".

For the purpose of sludge enrichment, the settling time before decanting was kept at 10 min, allowing most of sludge to settle and remain in the reactor. Thus, the TSS and VSS in the effluent were relatively low. Effluent TSS was around 400 mg/L, while VSS was around 350 mg/L. Since sludge was maintained in the reactor, TSS and VSS in the reactor increased about 3 times during this period. TSS increased from about 1509 mg/L on day 115 to 4050 mg/L on day 148, while VSS increased from 1345 mg/L to 3175 mg/L. Red anammox granular sludge was observed in the reactor, which is obviously different from the rest of flocculent nitrifying sludge (Figure 3.6). In addition, the anammox granules trended to grow denser and bigger as shown in Figure 3.7. The average diameter (Φ) of anammox granular sludge increased slowly from 0.8 mm on 105 d to 2.0 mm on 174 d, and remained at 2.0 mm until day 308. Since the size of anammox sludge granule may be influenced by mixing speed (Arrojo *et al*., 2006), 2.0 mm might be considered as the maximum average diameter of anammox granule under a certain mixing speed (15 rpm) of this research project. Sludge granules with a diameter larger than 2.0 mm might break up due to shear forces. Since anammox granules settled much faster than flocculent nitrifiers, almost all anammox bacteria could be preserved in the reactor, while a part of nitrifiers could be lost through decanting (leading to a healthier balance in reactor). The sludge enrichment of anammox bacteria was considered as successful on day 148, at the end of this period.

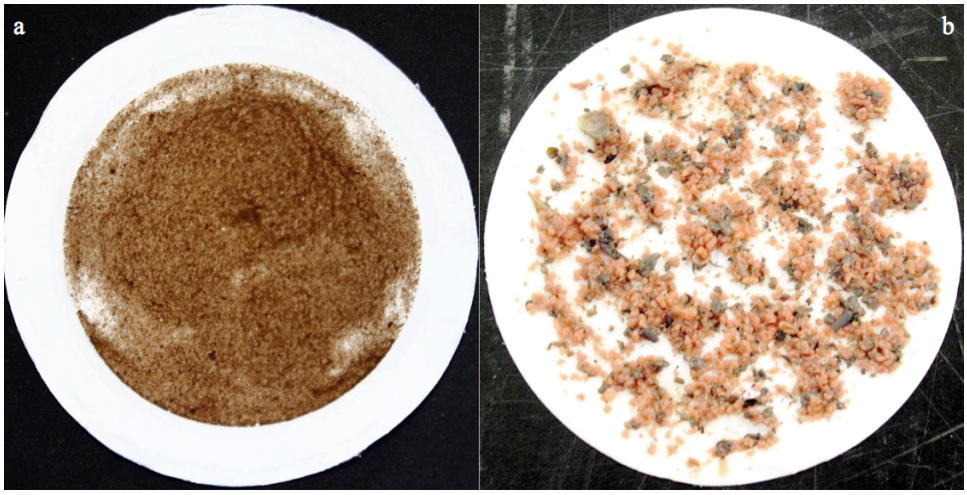

Figure 3.6 Picture of flocculent nitrifying sludge (a) and granular anammox sludge (b)

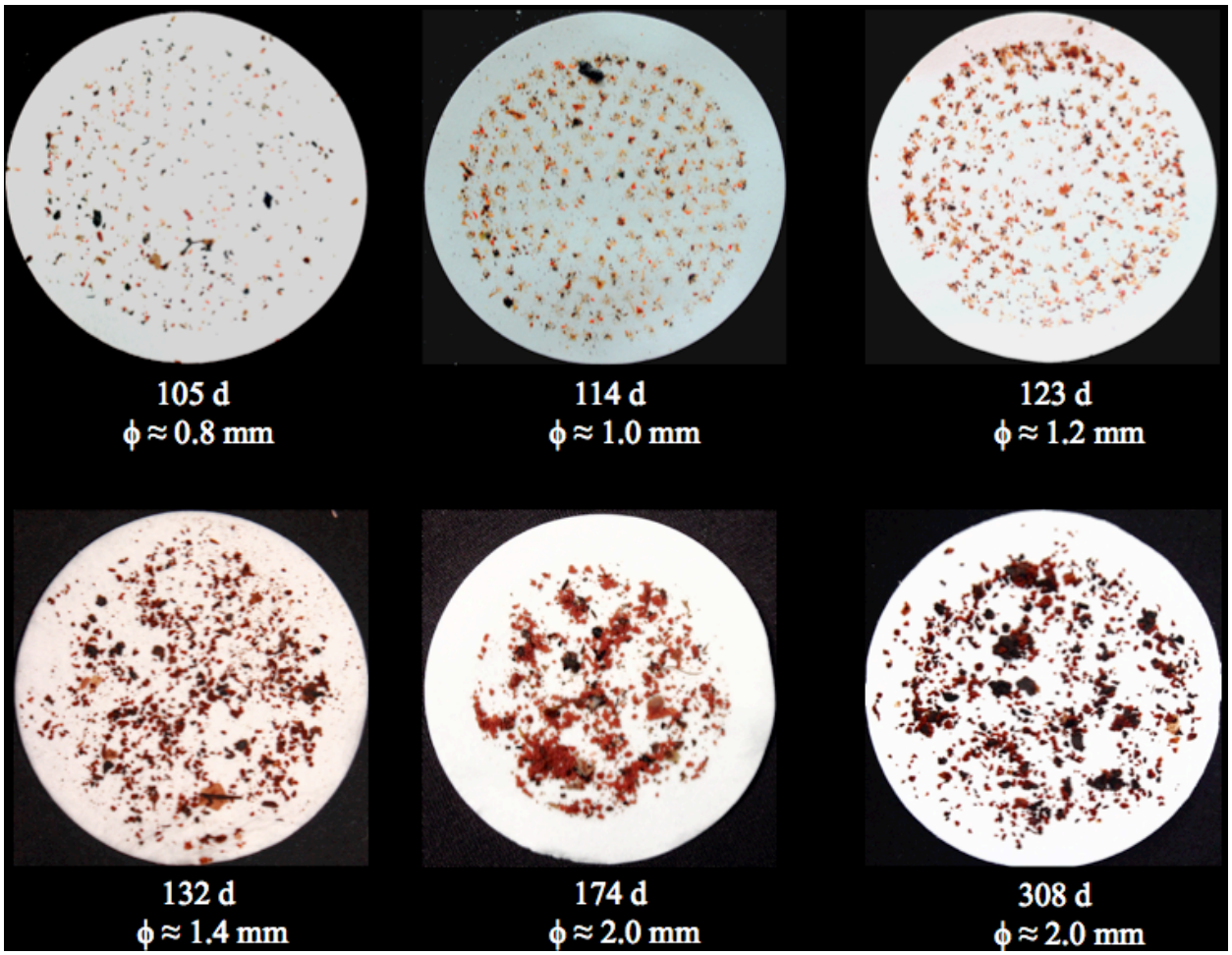

Figure 3.7 Picture of the size of granular anammox sludge

Due to enriched sludge, the cycle length reduced significantly from 25 h on day 115 to 10 h on day 148 (Figure 3.5). Due to the faster reaction speed, the HRT also decreased from 5.21 d on day 115 to 2.08 on day 148. In addition, the ammonia-nitrogen loading rate (ALR) increased from 0.17 kgN/m^3•d on day 115 to 0.45 kgN/m^3•d on day 148 (Figure 3.8). Without decreasing the ARR, higher ALR of the reactor indicated a higher treatment ability of CANON process. More centrate could thus be treated, within a certain period of time.

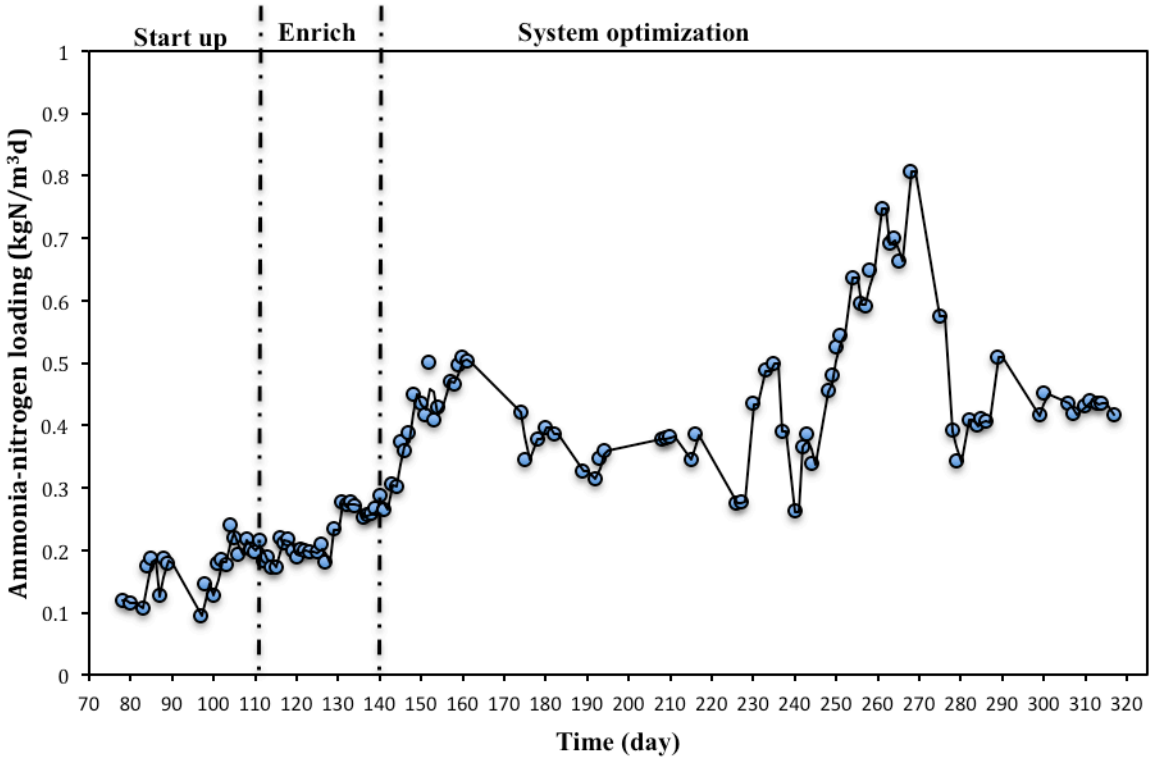

Figure 3.8 Variation of ammonia-nitrogen loading rate during reactor running period

3.3 System optimization period

From day 148 to day 317, the CANON SBR was tested under different environmental conditions, as summarized in Table 2.3. Under each condition, the reactor was allowed to run at least 5 cycles for taking enough samples. The environmental conditions during sludge enrichment period were adopted as an initial baseline for comparison. The initial conditions were set as: temperature 32 ºC, 70 L feeding, 1 min feeding time, 350 L reactor mixed liquid, 8 L/min airflow, 10 min settling time and pH set point of 6.0. Since the ARR under the baseline condition was already as high as 92.8%, other optimization conditions could not be expected to achieve significant ARR improvement. Reactor running under most conditions could achieve around 90% ARR, which was still much higher than other conventional biological ammonia removal processes.

It should be noted that on day 168 the system was moved to the J.A.M.E.S. WWTP and the change of centrate quality could influence on the performance of the reactor. Different from Lulu

Island WWTP, centrate from J.A.M.E.S. WWTP usually contains a 1~2 mm a sludge layer on the top of the clear centrate layer (Figure 3.9). Adding centrate with sludge layer directly into the CANON SBR might contaminate the reactor, since the added sludge may compete for oxygen and substrate with the original existed AOB and anammox bacteria. For this reason, the performance of the CANON SBR decreased significantly during the first month in the J.A.M.E.S. WWTP. The effluent NH_3-N increased from 29 mg/L on day 171 to about 205 mg/L on day 212 (Figure 3.1). The NO_3-N concentration in the effluent also increased from 22.1 mg/L on day 171 to about 132 mg/L on day 212 (Figure 3.2). In addition, the ARR of CANON SBR decreased drastically from 96.6% on day 171, to 78.2% on day 212 (Figure 3.1). This situation was a bit like the one occurring during day 76 to day 88, indicating the nitrite for ANAMMOX reaction was used up by NOB for nitrate generation, due to the outcompetition of anammox bacteria by NOB. The cycle length also increased for 20% from 10 h on day 168 to 12 h on day 212 (Figure 3.5), showing the decrease of the ANAMMOX reaction.

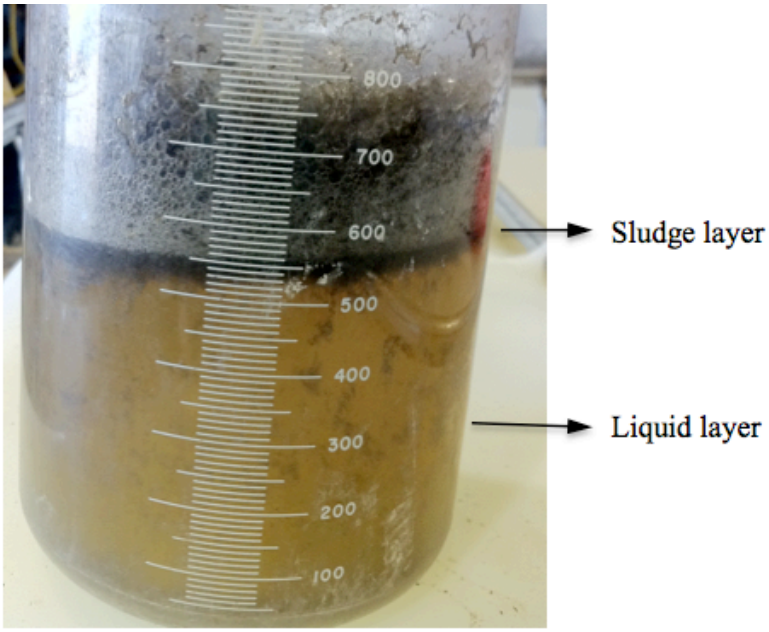

Figure 3.9 Centrate with sludge layer from J.A.M.E.S. WWTP

The situation of centrate contamination was not noticed until day 213. When feeding substrate was changed back to clear centrate, with the sludge layer being wasted, a significant improvement in reactor performance was then observed. The effluent NH_3-N concentration decreased continuously from about 205 mg/L on day 212 to 76.0 mg/L on day 226 (Figure 3.1),

while effluent NO_3-N also decreased from about 132 mg/L to 52.3 mg/L on day 226 (Figure 3.2). The ARR also increased to 91.7% on day 226 (Figure 3.1).

During the optimization period, TSS in the reactor varied from 2500 mg/L to 4500 mg/L, while VSS was usually 500 mg/L less than TSS (Figure 3.3). This was caused by adjusting the sludge settling time. The sludge settling time was changed stepwise from 10 min to 4 min. Reduced sludge settling time would decrease TSS and VSS in the reactor, by withdrawing more sludge through decanting.

3.4 Cycle test

Within the system optimization period, the reactor was running under variable environmental conditions. For more detailed study, the performance of the reactor under each condition, cycle test was conducted and cycle profiles were generated for each cycle. Data of cycle tests are summarized in Appendix A. As an example, the cycle profile for the baseline condition (32 ºC, 70 L feeding, 1 min feeding time, 350 L reactor mixed liquid, 8 L/min airflow, 10 min settling time and pH set point of 6) is presented in Figure 3.10.

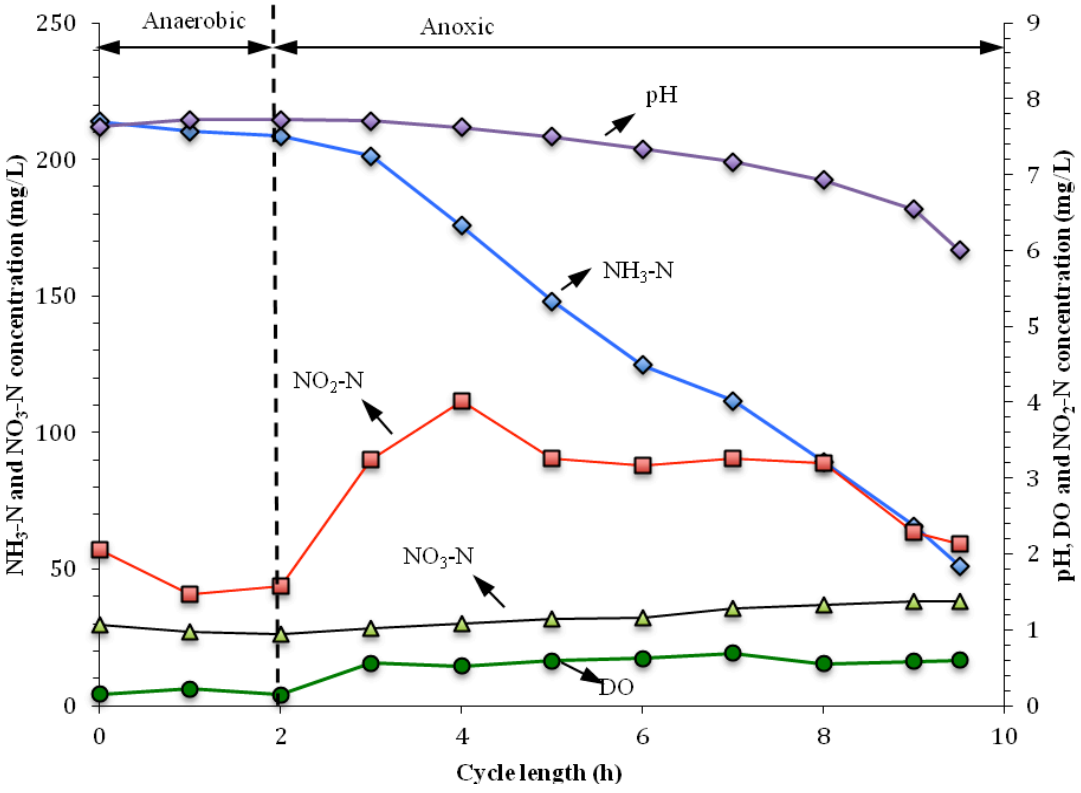

Figure 3.10 Cycle profile for cycle test of CANON SBR under baseline condition

The cycle profiles reflect the variations in DO level, pH values, NH_3-N, NO_2-N and NO_3-N concentrations within the reactor, from beginning to the end of a whole cycle. For example, in the profile of cycle test under baseline conditions (Figure 3.10), it shows that the cycle length of CANON SBR, under the baseline condition was 9.5 h. A whole cycle consisted of 2 hours of an anaerobic phase at the beginning, followed by 7.5 hours of anoxic aeration phase. DO levels were below 0.2 mg/L under the anaerobic phase, and increased to around 0.5 mg/L ~ 0.6 mg/L, when air was introduced. Right after feeding the centrate, the initial NH_3-N concentration in the reactor was about 214 mg/L and the initial pH was 7.63 due to the dilution. NH_3-N and pH remained unchanged during the first two hours of anaerobic phase. The two-hour anaerobic phase was designed to consume the remaining nitrite in the reactor. Thus, anammox bacteria could be protected from nitrite inhibition. As a result, the NO_2-N concentration was decreased from 2.1 mg/L to 1.57 mg/L, after the anaerobic phase. During this period of time, nitrite was converted to nitrate and nitrate was converted to nitrogen gas. Due to the denitrification process, NO_3-N concentration dropped for about 12% from 36.1 mg/L to 31.9 mg/L, after 2 hours. When aeration started and the CANON reaction was initiated, the NH_3-N concentration started to drop. After 7.5 hours of aeration, the NH_3-N concentration decreased to about 51.2 mg/L, and the pH decreased to the set point of 6.0. NO_2-N slightly increased to around 3.0 mg/L in the reactor, while NO_3-N kept increasing to 38.3 mg/L by the end of the cycle. The nitrate generation to ammonia reduction ratio was 0.08, which was lower than the theoretical ratio of 0.11 for CANON reaction (Equation 12).

4. Kinetics of CANON SBR

The CANON system requires the cooperation of both ammonia oxidation bacteria and anammox bacteria while inhibiting the competition between anammox bacteria and nitrite oxidation bacteria. A study on the effects of variety parameters that may influence on the performance of the reactions would help better control and optimize the reactor. In this section, the effects of temperature, aeration, ammonia loading, feeding rate, and sludge settling time were investigated, to the extent possible.

4.1 Effects of temperature

Temperature is usually critical for most biological reactions. The temperature for optimum growth of the nitrifying bacteria is between 30-35 ºC, while optimum temperature for anammox bacteria is around 35 ºC (Schmidt *et al.,* 2003; Zhang *et al*., 2008). However, in the real case of centrate treatment, centrate temperature may vary from 30 ºC to 37 ºC. If there is no extra heating device to heat the centrate during the process, temperature within the reactor could be even lower. Thus, the effects of temperature ranging from 26 ºC to 32 ºC were investigated and the results are presented in Figures 4.1 to Figures 4.5. Temperatures below 26 ºC are too low to maintain the advantage of AOB, to outcompete NOB (Khin and Annachhatre *et al*., 2004). Except for the difference in temperature, all cycle tests were done under the same condition: 70 L feeding in one minute, 70 L decanting with 10 min sludge settling time, 350 L reactor mixed liquid volume, continuously aeration with 8 L/min, and pH set point of 6.0.

Since the system was controlled by pH variation, the cycle length was directly determined by the speed of pH decreasing (or alkalinity consumption) in the reactor. In other words, ammonia-nitrogen concentration in the effluent was not controlled. As shown in Figure 4.1, cycle lengths were slightly different under temperature conditions of 26 ºC, 28 ºC, 30 ºC and 32 ºC. All four sets of cycle tests started at almost the same pH level of 7.6, which is reasonable due to the same volume of feeding and the stable pH of centrate. A pH of 6.0 was the set point, which means a cycle will be completed when the pH in the reactor dropped to 6.0. For cycles at 26 ºC, 28 ºC, 30 ºC and 32 ºC, time to accomplish the cycle was 9 h, 11 h, 10 h, and 9.5 h, respectively, including

the first two hours of anaerobic protection period. The cycle length at 26 ºC was the shortest, while the cycle length under 28 ºC was the longest. However, the differences were not significant.

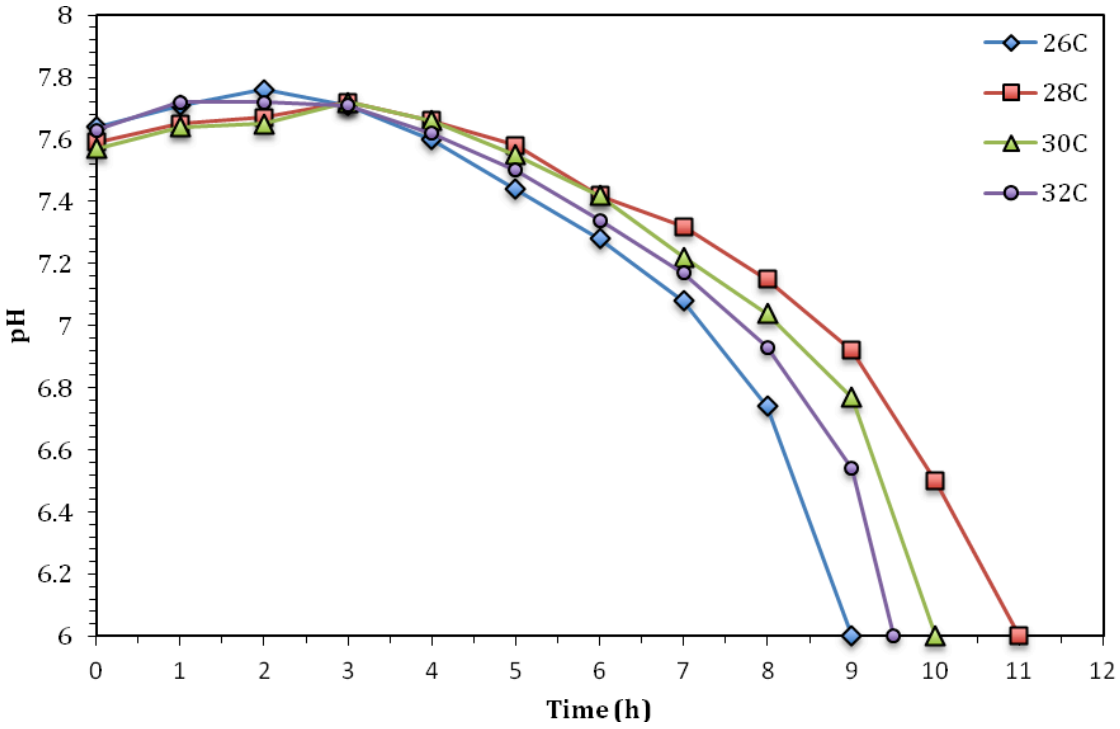

Figure 4.1 Comparison of the effects of temperature on pH variation in the reactor

Although the cycle length tested at 26 ºC was the shortest, the performance of ammonia removal was the worst. As shown in Figure 4.2, the NH_3-N in the reactor was reduced from about 223 mg/L to 79 mg/L, which only achieved 64% ammonia removal. The ammonia removal rate increased at higher temperature. The ARRs at temperature of 28 ºC, 30 ºC and 32 ºC were 68%, 70%, and 76%, respectively. Due to the increase in temperature from 28 ºC to 32 ºC, the ARR was increased significantly by 12%, from 64% to 76%. This was attributed to the activity of anammox bacteria were inhibited at lower temperature, while the influence of temperature on AOB activity was not that significant.

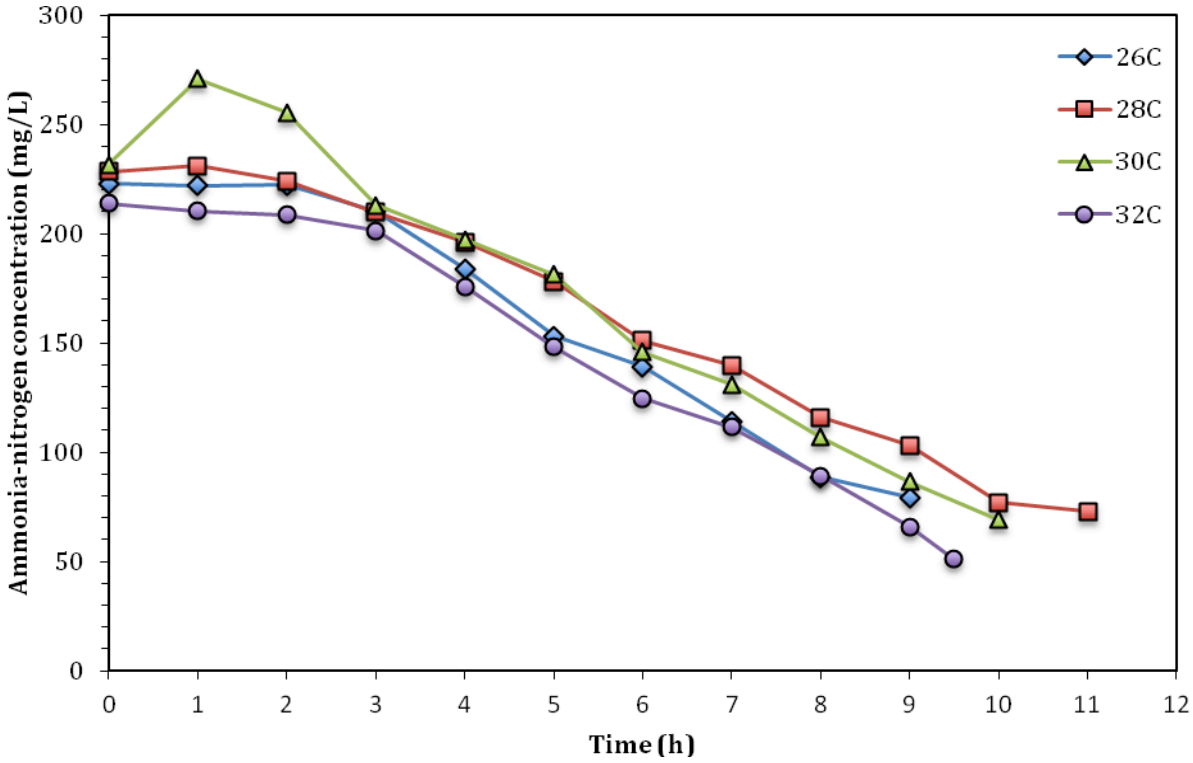

Figure 4.2 Comparison of the effects of temperature on ammonia concentration in the reactor

The inhibition of anammox bacteria activity can be observed in Figure 4.3, which presents the effects of temperature on nitrite concentrations in the reactor, within cycles. As shown in Figure 4.3, the effect of the two-hour anaerobic protection period was obvious. Although the initial concentrations at the beginning of the cycle might be as high as 13.4 mg/L at 26 ºC, nitrite-nitrogen concentrates dropped blow 2.0 mg/L after two hours of anaerobic phase. During the cycle, nitrite concentration would increase due to a partial nitrification process. At temperature of 32 ºC, the level of NO_2-N was relatively stable. The NO_2-N increased from 1.6 mg/L to the maximum level of 4.0 mg/L, and then decreased to 2.1 mg/L at the end of the cycle, indicating anammox bacteria were robust and were capable of utilizing most of nitrite in the reactor generated by AOB. However, the nitrite concentration in the reactor increased with temperature decreasing. At 26 ºC, NO_2-N concentration increased dramatically from 1.6 mg/L right after the anaerobic phase, to 15.7 mg/L at the end of the cycle. Compared with the relatively stable nitrite concentration at temperature of 32 ºC, the accumulated nitrite in the reactor at 26 ºC indicated that the activity of anammox bacteria was significantly inhibited at lower temperature; thus, nitrite generated by AOB could not be completely utilized by anammox bacteria. Although

nitrite levels at 26 ºC was still lower than the safety threshold value of 20 mg/L, more nitrite in the reactor would stimulate the growth of NOB and lead to more nitrate generation in the reactor, as shown in Figure 4.4. Due to the denitrification process within the anaerobic phase, nitrate concentrates were slightly reduced. At a temperature of 26 ºC, the nitrate-nitrogen increased from 31.9 mg/L, after the anaerobic phase, to 54.3 mg/L at the end of cycle, while NO_3-N increased from 26.2 mg/L to 38.3 mg/L, at a temperature of 32 ºC. At 26 ºC, the NO_3-N increased for 70.2%, which was much higher than the increased rate of 46.1%, at 32 ºC, since the activity of anammox was inhibited at 26 ºC; thus, some nitrites were utilized by NOB to generate nitrates.

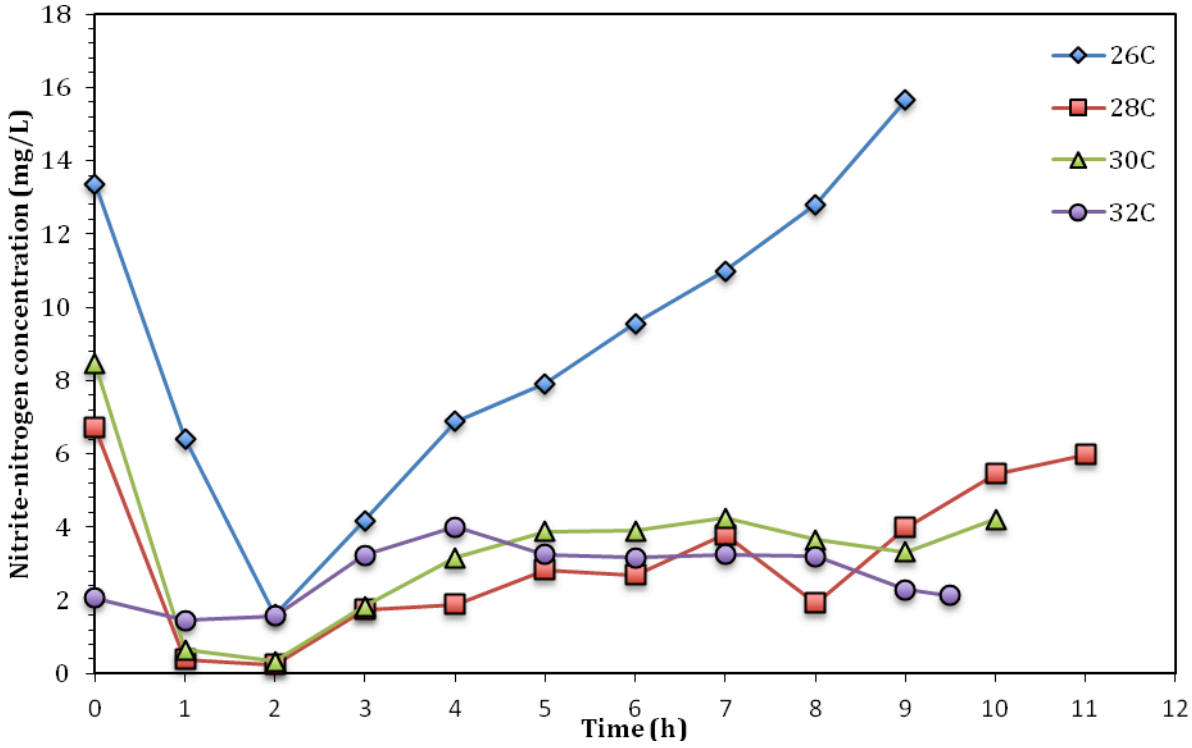

Figure 4.3 Comparison of the effects of temperature on nitrite concentration in the reactor

In addition, the ratio of nitrate production to ammonia consumption (N/A ratio) was higher at a lower temperature, as shown in Figure 4.5. The N/A ratio was 0.16, 0.14, 0.10 and 0.08, respectively at temperatures of 26 ºC, 28 ºC, 30 ºC and 32 ºC. Compared to a theoretical N/A ratio of 0.11 for the CANON process, the N/A ratio of 26 ºC was much higher, indicating partial nitrification and a more unbalanced anammox process at lower temperatures.

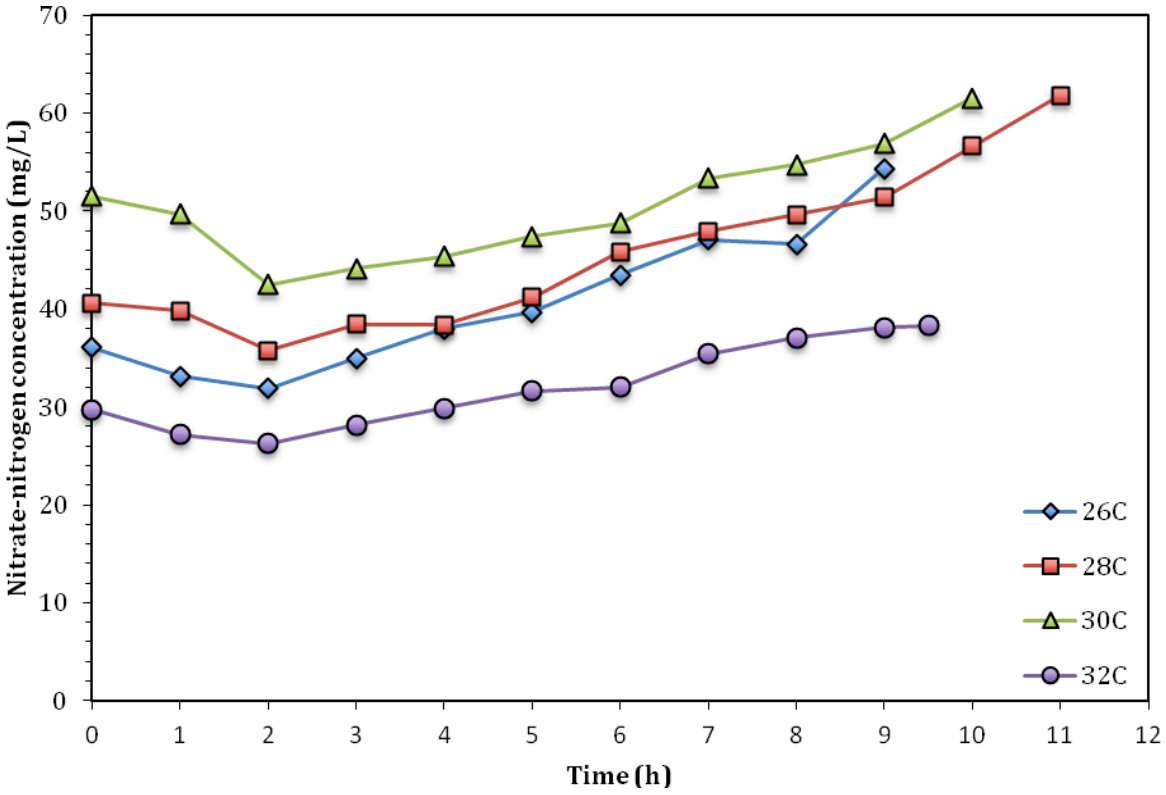

Figure 4.4 Comparison of the effects of temperature on nitrate concentration in the reactor

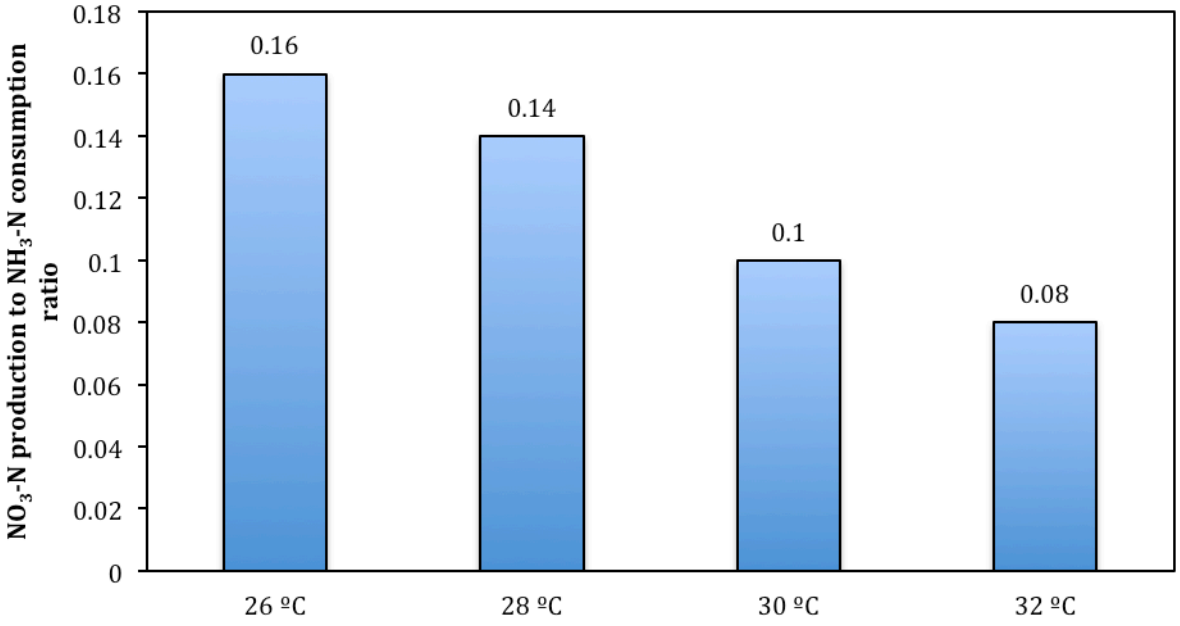

Figure 4.5 Comparison of the effects of temperature on the ratio of NO_3-N generation to NH_3-N consumption

From the discussions above, it can be concluded that temperature is a factor that can change the performance of the CANON SBR reactor. A higher operational temperature of 32 ºC was recommended for the CANON SBR, since the ammonia removal rate was the highest. Temperatures as low as 26 ºC would lower the ARR to about 12 %, from 76% at 32 ºC to 64% at 26 ºC. At the mean time, nitrite increased drastically at 26 ºC, possibly leading nitrite accumulation in the bulk liquid of the reactor and stimulating the outgrowth of NOB over anammox. The higher level of accumulated nitrite in the bulk liquid would also require longer anaerobic protection time to consume the nitrite, before the CANON process could be started. For full-scale devices, heating the reactor could be costly. However, the temperature of the centrate is around 38 ºC ~ 40 ºC. Increase the feeding volume of the high-temperature, on-site centrate could lift the temperature of the reactor and dilute accumulated nitrite in the bulk liquid, if there is no external heater.

4.2 Effects of aeration

Aeration is one of the most critical factors in the CANON process, since the reaction consumes 0.85 mol oxygen to convert 1 mol ammonia to 0.435 mol of nitrogen gas and 0.11 mol nitrate-nitrogen (Equation 12). Thus, insufficient aeration would slow the CANON reaction. However, excessive amount of oxygen supply would also stimulate the growth of AOB and generate superfluous nitrite that could not be used up by anammox bacteria, leading to nitrite accumulation and NOB reproduction. The effects of airflow rate of 10 L/min, 12 L/min, and 14 L/min were investigated and the results are presented in Figures 4.6 to Figures 4.10. Except for the aeration rate, all three sets of cycle tests were conducted under the same condition of 120 L feeding, 120 L decanting with 8 min sludge settling time, 350 L reactor mixed liquid, temperature of 32 ºC and pH set point of 6.0. In addition, before the continuous aeration, an hour of anaerobic protection period was set to remove possible accumulated nitrite from the reactor.

As shown in Figure 4.6, increasing the airflow rate from 10 L/min to 12 L/min significantly reduced the cycle length from 16 h to 14 h. However, a further increase in airflow rate from 12 L/min to 14 L/min made only a slightly difference, reducing the cycle length time from 14 h to 13.5 h. For all three sets of tests, the pH increased during the first hour of anaerobic protection period, since denitrification occurred in this period of time and generated extra alkalinity. When air was continuously introduced after the anaerobic period, the pH still increased for one or two

more hours. This was attributed to the air stripping effect that released parts of unionize ammonia and carbon dioxide from the bulk liquid phase of the reactor, leading to a slightly pH increase. After that, the pH started to decrease due to alkalinity consumption by the CANON process and ended at 6.0 as the set point.

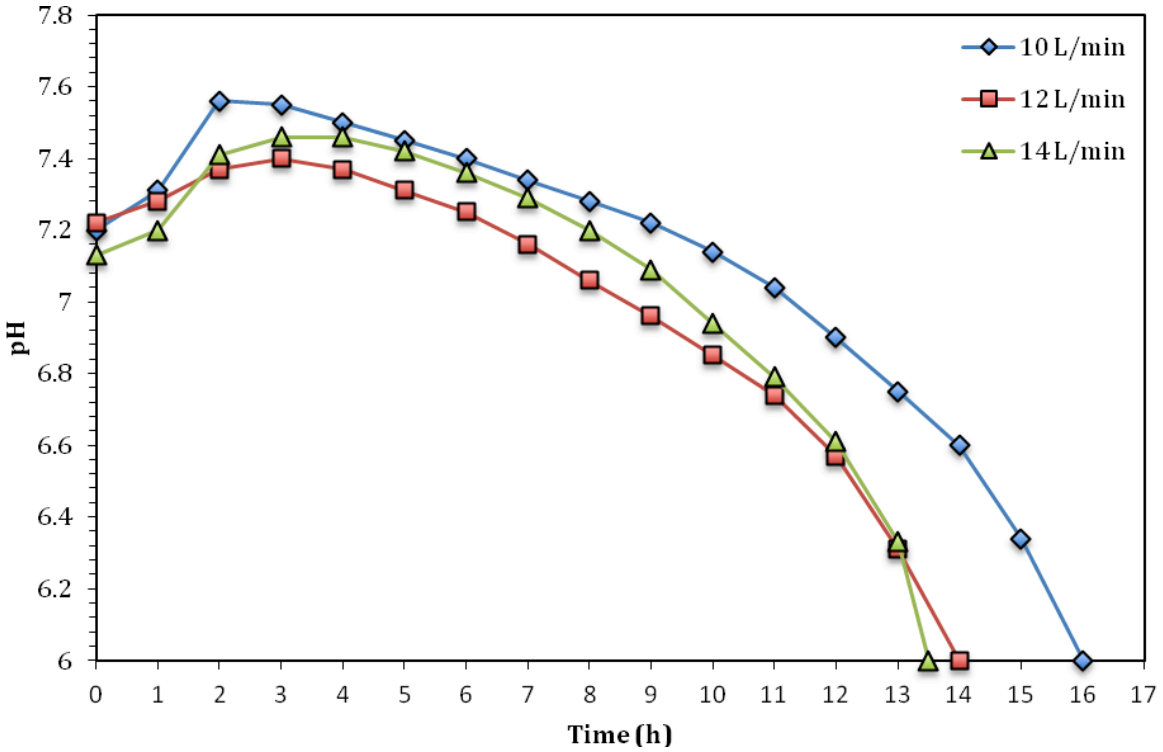

Figure 4.6 Comparison of the effects of airflow rate on pH variation in the reactor

Increasing the airflow rate also lead to the reduction of hydraulic retention time (HRT) of the reactor (Figure 4.7). HRT is a critical parameter, especially for the design of bioreactors. With a smaller HRT, a certain volume reactor can deal with a larger volume of wastewater. On the other hand, a certain amount of wastewater can be treated in a smaller volume of reactor. In this case, HRT reduced from 1.94 d to 1.76 d when the airflow was increased from 10 L/min to 14 L/min, indicating a 10% HRT reduction could be achieved at the cost of 40% increase in aeration. Reducing the HRT for 10% may lead to a 10% smaller reactor or 10% more treatment capacity, while the increase in 40% aeration may result in 40% more cost for aeration of the CANON system. Hence, a cost benefit optimization would be needed before a finial design package could be recommended.

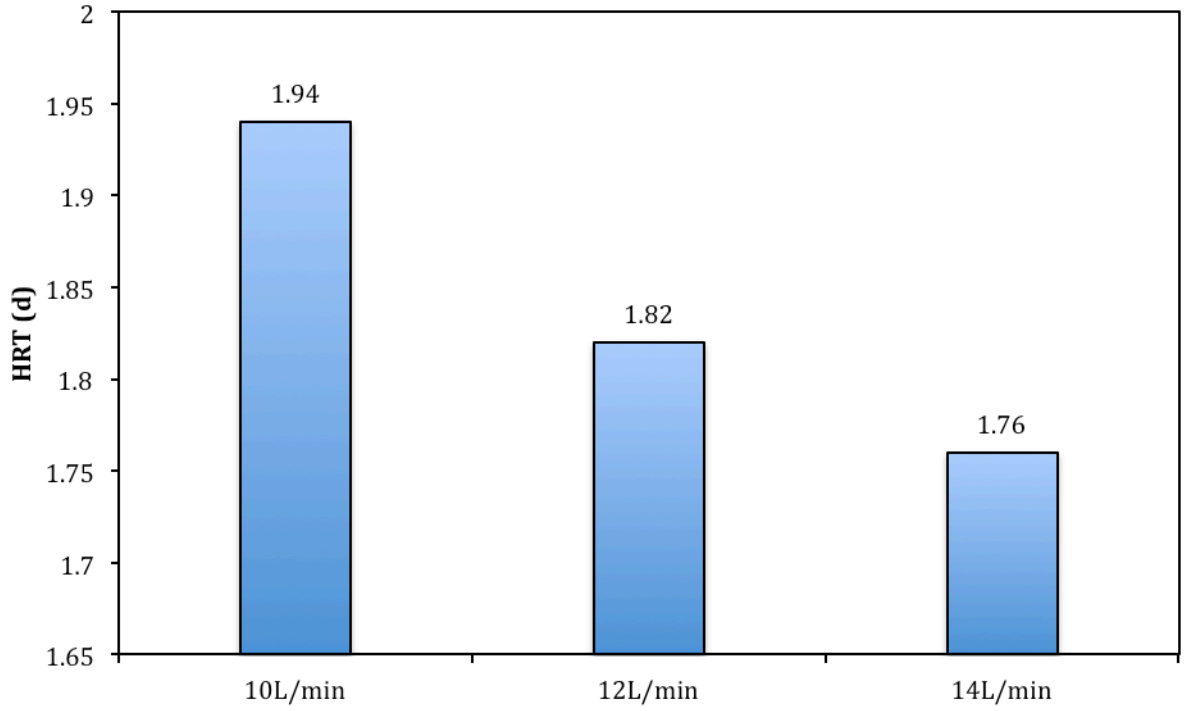

Figure 4.7 Comparison of the effects of airflow rate on the hydraulic retention time

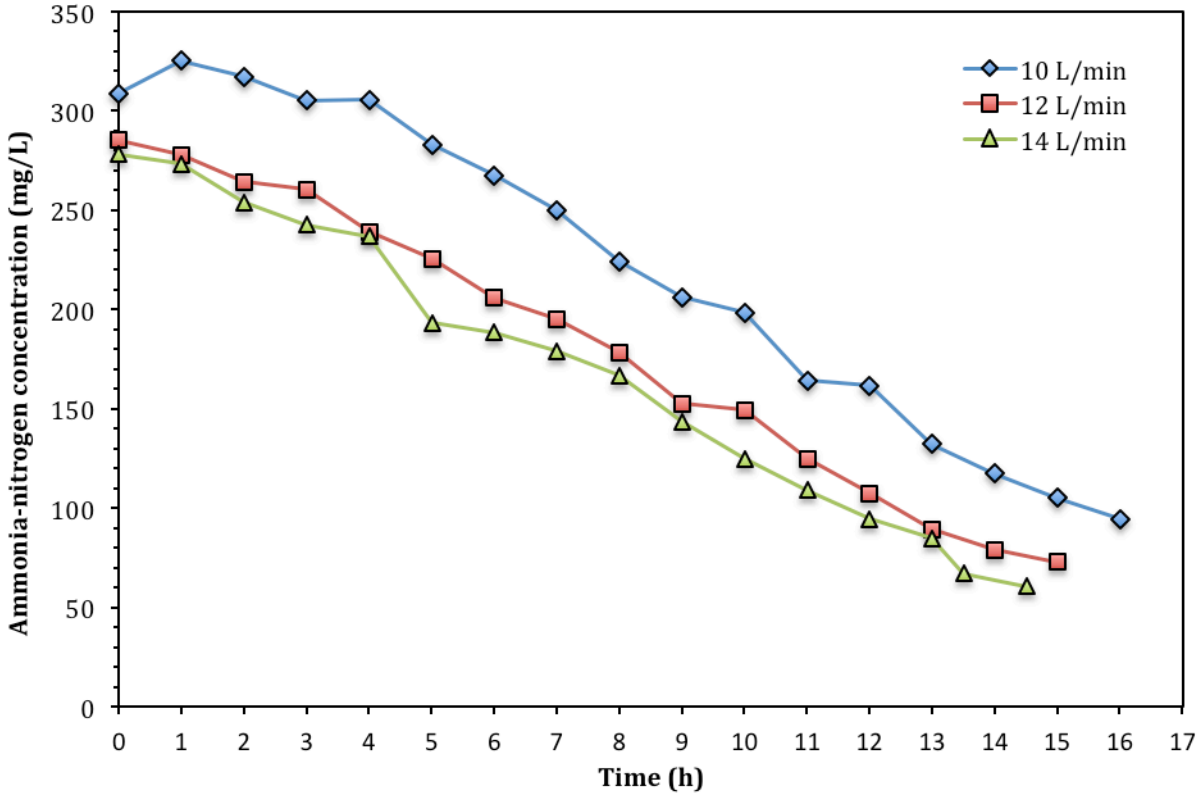

Figure 4.8 Comparison of the effects of airflow rate on the variation of ammonia-nitrogen in the reactor

For the performance of ammonia removal, the influence of airflow rate was not that significant (Figure 4.8). With 120 L feeding centrate, initial ammonia in the bulk liquid of the reactor was around 300 mg/L. With 10 L/min aeration, NH_3-N was reduced from about 308 mg/L to 94.2 mg/L. At 12 L/min, the NH_3-N was reduced from about 284 mg/L to 72.5 mg/L. With an airflow rate of 14 L/min, the NH_3-N was further reduced from about 278 mg/L to 60.4 mg/L. The ammonia removal rate was slightly increased along with the increase in airflow rate. The ARR of 10 L/min aeration was 69%. A 6% ARR improvement from 69% to 75% was achieved, when aeration was increased to 12 L/min. However, only a 3% ARR increase, from 75% to 78%, was achieved when aeration was further increased to 14 L/min.

For nitrite in the reactor, a slight increase in nitrite concentration was observed when aeration was increased. For all three sets of tests, the nitrite concentration decreased drastically within the first hour of anaerobic protection period and then increased significantly when air was introduced. The nitrite concentration then kept decreasing after 2 ~ 3 hours of aeration, due to the activity of anammox bacteria. At the end of the cycle, nitrite concentrations increased again, probably because the activity of anammox bacteria was reduced at lower pH. Although nitrite production was stimulated by increased airflow, the overall nitrite concentrations were still kept at a low level. Even at an airflow rate of 14 L/min, the NO_2-N concentration would not be higher than 3.5 mg/L, which would not inhibit the performance of anammox bacteria. An hour of anaerobic period, before aeration, could consume most of accumulated NO_2-N in the bulk liquid.

Increasing the airflow rate had almost no effect on nitrate production in the reactor. Except for the first hour of anaerobic period, nitrate concentration increased continuously until the end of the cycle (Figure 4.10). With an airflow rate of 10 L/min, the NO_3-N increased from 43.4 mg/L to 74.4 mg/L. With an aeration of 12 L/min, the NO_3-N increased from 49.5 mg/L to 74.1 mg/L. While at 14 L/min aeration, NO_3-N increased from 47.9 mg/L to 70.8 mg/L. For all three sets of cycles, the nitrate production to ammonia consumption (N/A) ratios were 0.12, very close to the theoretical N/A ratio of 0.11 for the CANON process. The same N/A ratio of 0.12 for cycles with 10, 12 and 14 L/min of aeration indicated increasing airflow rate did not affect the balance of partial nitrification and ANAMMOX reaction.

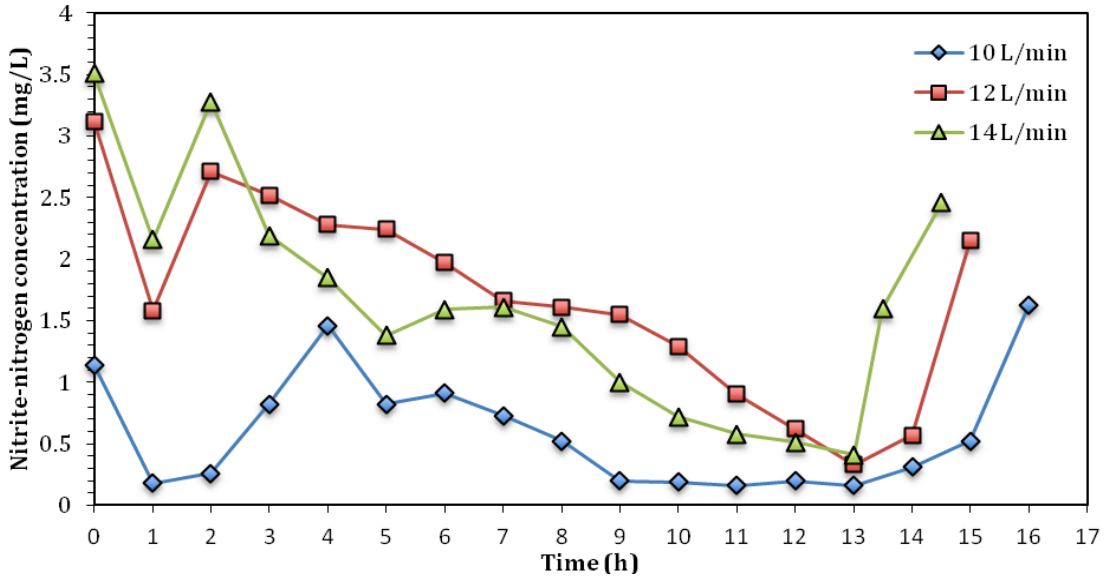

Figure 4.9 Comparison of the effects of airflow rate on the variation of nitrite-nitrogen

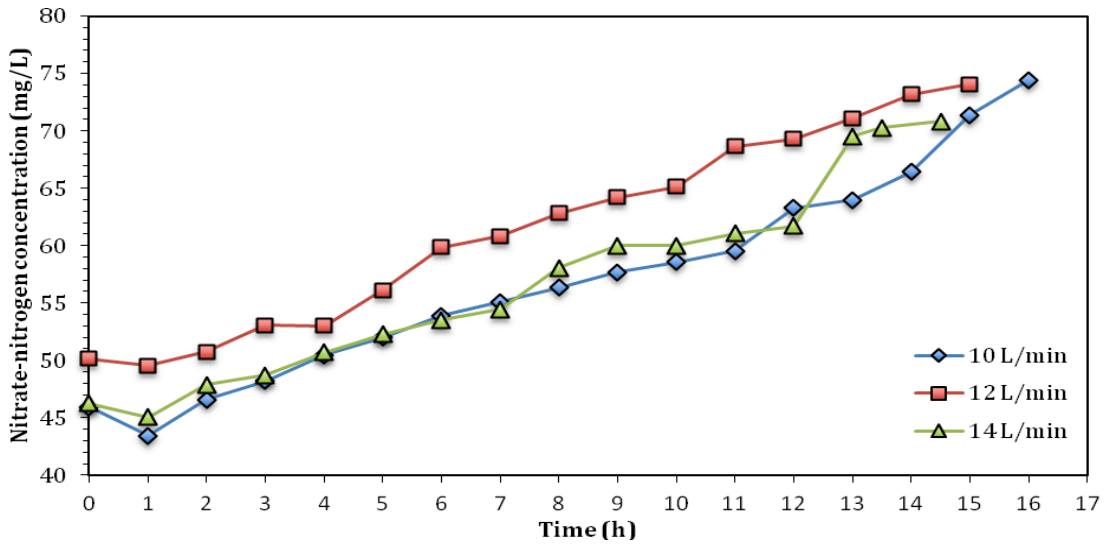

Figure 4.10 Comparison of the effects of airflow rate on the variation of nitrate-nitrogen

In conclusion, increasing the airflow rate could improve the performance of the reactor, since higher ARR and shorter HRT were achieved. However, the improvement was not that significant. Even at the cost of 40% more aeration, ARR could only be improved by 9%, while HRT could be reduced by 10%. Although the aeration requirement for the CANON process is 63% less than conventional biological ammonia removal process, as the effects of increasing the airflow rate to 14 L/min were still not impressive. Compared with an airflow rate of 10 L/min, it is more acceptable to maintain aeration at 12 L/min, for achieving 6% in ARR improvement and 6% HRT reduction, at a reasonable 20% more aeration cost.

4.3 Effects of ammonia loading

Ammonia is one of the main substrates for the CANON process, so increasing the initial concentration of ammonia in the reactor should be able to stimulate the CANON process. However, excessive amounts of ammonia could also inhibit the ANAMMOX process. As reported by Ni and Meng *et al*. (2011), 50% inhibition was observed under NH_3-N concentration of about 988 mg/L.

To investigate the effects of ammonia loading on the performance of the CANON SBR, the reactor was tested under three conditions of different feed to reactor liquid volume (F/R) ratios: F/R = 0.2 (70 L feeding centrate, 350 L reactor liquid), F/R = 0.34 (120 L feeding centrate, 350 L reactor liquid), and F/R = 0.5 (190 L feeding centrate, 380 L reactor liquid). Except for F/R ratios, all three sets of cycle tests were conducted under the same conditions: 32 ºC, 12 L/min continuously aeration with one hour anaerobic protection period at prior, pH set point of 6.0 and 4 min of sludge settling time.

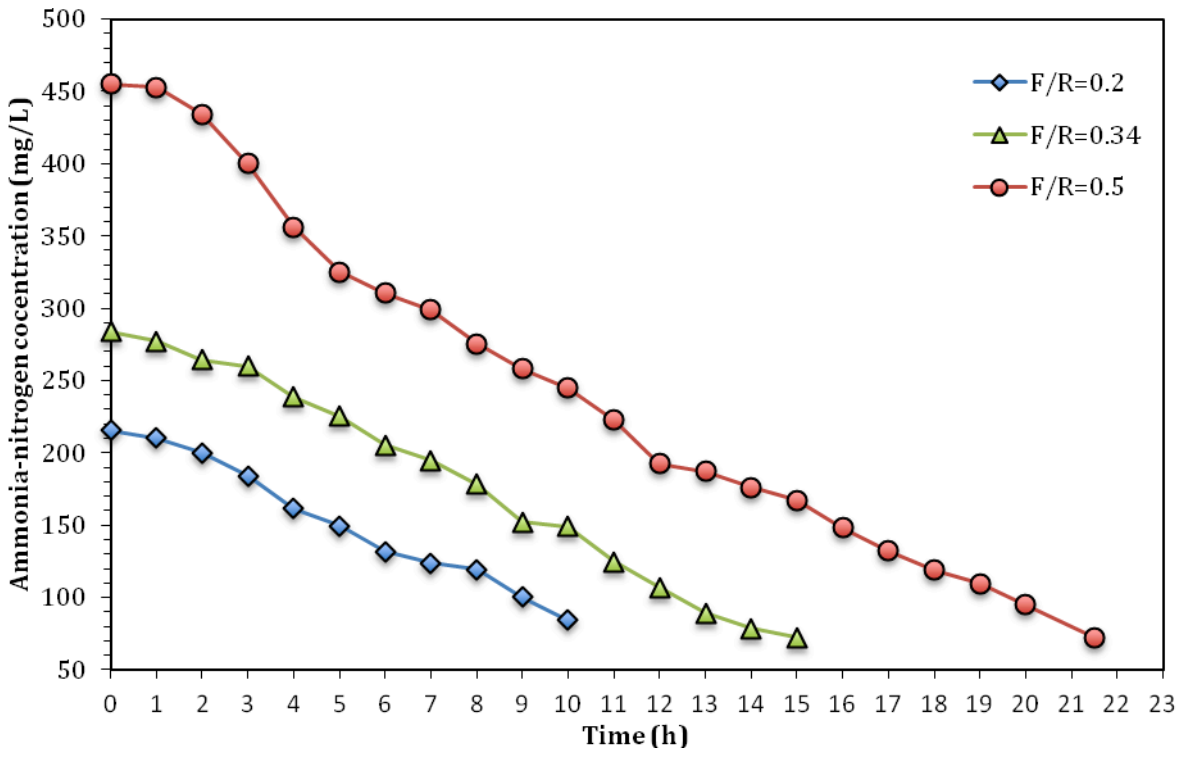

Figure 4.11 Comparison of the effects of ammonia concentrations on the variation of ammonia- nitrogen in the reactor within cycles

Due to the differences in F/R ratios, initial NH_3-N concentrations (NH_3-N_i) in the reactor were significantly different (Figure 4.11). A higher F/R ratio would lead to significantly higher initial NH_3-N concentrations in the reactor. NH_3-N concentrations started with about 215 mg/L, 289 mg/L, and 455 mg/L, respectively, for F/R ratio of 0.2, 0.34 and 0.5. The NH_3-N concentrations dropped smoothly to 84.1 mg/L, 72.5 mg/L and 72.4 mg/L respectively for cycles with NH_3-N_i of 215 mg/L, 285 mg/L and 455 mg/L. It should be noted that effluent NH_3-N concentrations did not change significantly, due to the increase of initial ammonia concentrations, indicating that the effluent NH_3-N concentration was independent of F/R ratio or initial NH_3-N concentration. However, a higher ammonia removal rate was observed at a higher initial NH_3-N, probably because the activity of anammox bacteria was increased at higher ammonia concentration. 61% NH_3-N removal was achieved at NH_3-N_i = 215 mg/L, while 72% NH_3-N was removed at NH_3-N_i = 285 mg/L. At NH_3-N_i =455 mg/L, the ARR was further improved to 84%.

At the same time, higher ARR was achieved at the cost of a longer cycle length (Figure 4.12) since the initial pH was also raised by an F/R increase. The initial pH level was 7.1 at NH_3-N_i = 215 mg/L, and was increased to 7.22 at NH_3-N_i = 285 mg/L. At NH_3-N_i = 455 mg/L, initial pH was even increased to 7.39. Obviously, longer cycle length time was required for the cycle with higher initial pH to reach the finial pH set point of 6.0. Differences of cycle length times, between different initial ammonia concentrations, were remarkable. The cycle length increased from 10 h to 14 h when NH_3-N_i was increased from 215 mg/L to 285 mg/L. When NH_3-N_i was further increased from 285 mg/L to 455 mg/L, the cycle length was extended to 21.5 h.

Although cycle length was the longest at NH_3-N_i of 455 mg/L, the HRT was actually the shortest, as shown in Figure 4.13. The HRT was 1.79 d for cycle with NH_3-N_i = 455 mg/L and 1.82 d with NH_3-N_i = 285 mg/L. The HRT was slightly reduced when NH_3-N_i was increased from 285 mg/L to 455 mg/L. Compared with NH_3-N_i = 285 mg/L and 455 mg/L, the HRT of the cycle under NH_3-N_i = 215 mg/L was as long as 2.08 d, indicating smaller ammonia treatment capacity of the reactor under lower initial ammonia concentration. The nitrogen removal rate (NRR) was the lowest with NH_3-N_i = 215 mg/L, which was 0.27 $kgN/m^3 \cdot d$. At NH_3-N_i = 285 mg/L, the NRR improved to 0.3 $kgN/m^3 \cdot d$. The maximum NRR of 0.36 $kgN/m^3 \cdot d$ was achieved at the highest NH_3-N_i of 455 mg/L.

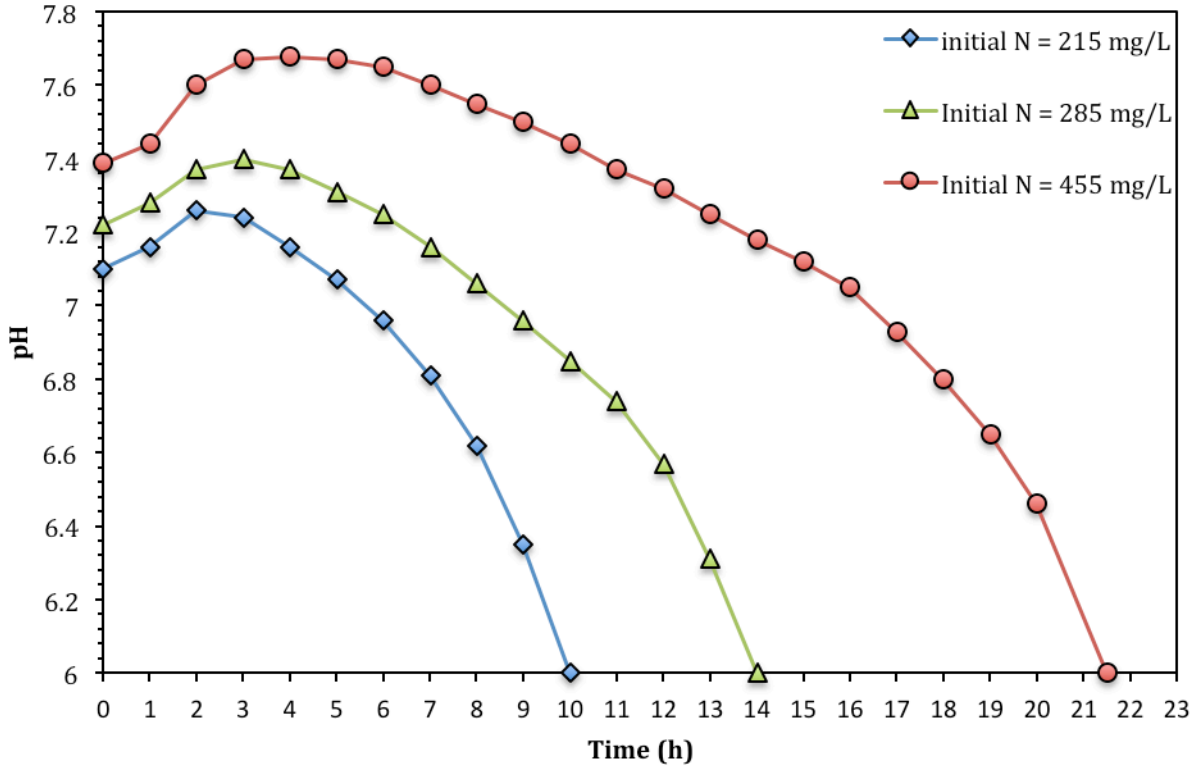

Figure 4.12 Comparison of the effects of ammonia concentration on the variation of pH in the reactor within cycles

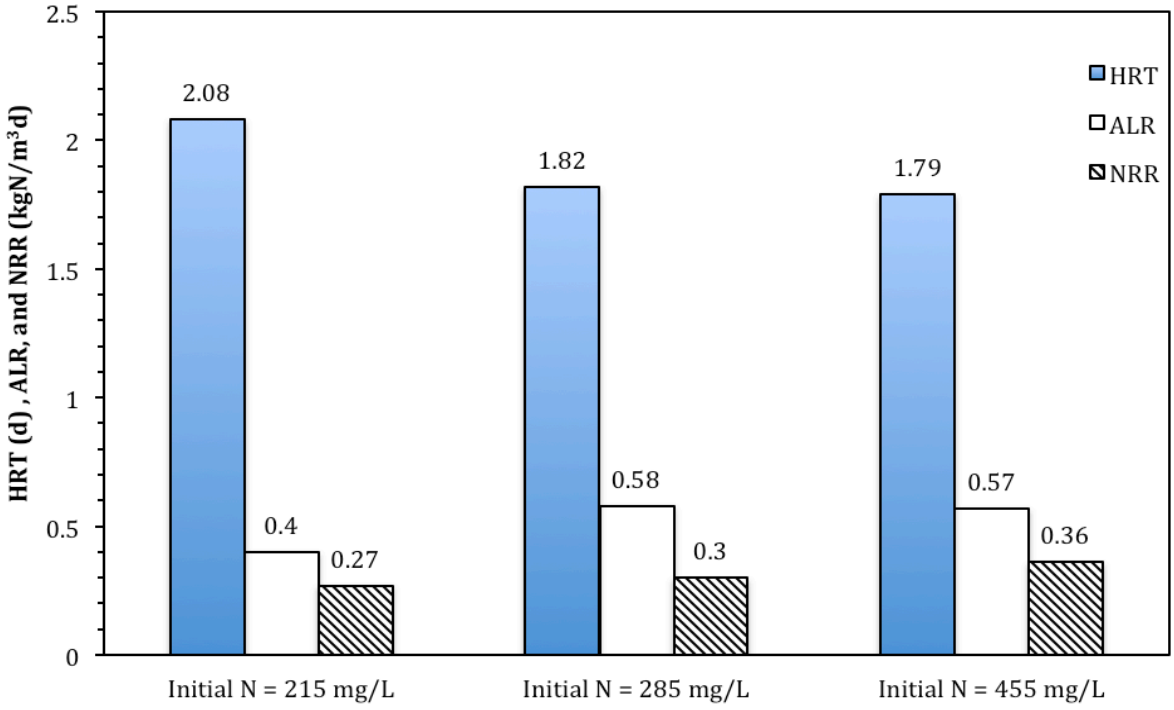

Figure 4.13 Comparison of the effects of ammonia concentration on the HRT, ALR and NRR

The improvement in nitrogen removal ability indicated that the CANON process was stimulated by higher initial NH_3-N concentration. More nitrite was generated during the CANON process at higher NH_3-N_i (Figure 4.14). At lower NH_3-N_i = 215 mg/L, nitrite increased from 0.2 mg/L to 1.1 mg/L after three hours of aeration. Nitrite-nitrogen increased from 1.6 m/L to 2.7 mg/L at NH_3-N_i = 285 mg/L and also increased from 1.4 mg/L to 4.0 mg/L at NH_3-N_i = 455 mg/L. The increasing nitrite concentration indicated that the partial nitrification process of AOB was stimulated by higher initial NH_3-N concentration. This stimulation effect can be explained by the Monod Equation:

$$\mu = \mu_{max}\frac{S}{K_s+S} \qquad [16]$$

Where, S is the substrate concentration, μ is specific growth rate, μ_{max} is the maximum specific growth rate and K_s is the Monod coefficient. Supplied by higher concentration substrate, both AOB and anammox bacteria should have higher specific growth rates that accelerate the process of partial nitrification and ANAMMOX. Anammox bacteria in the reactor could quickly consume the nitrite generated by AOB, thus no nitrite accumulation problem would occur, even at high initial ammonia concentration of 455 mg/L.

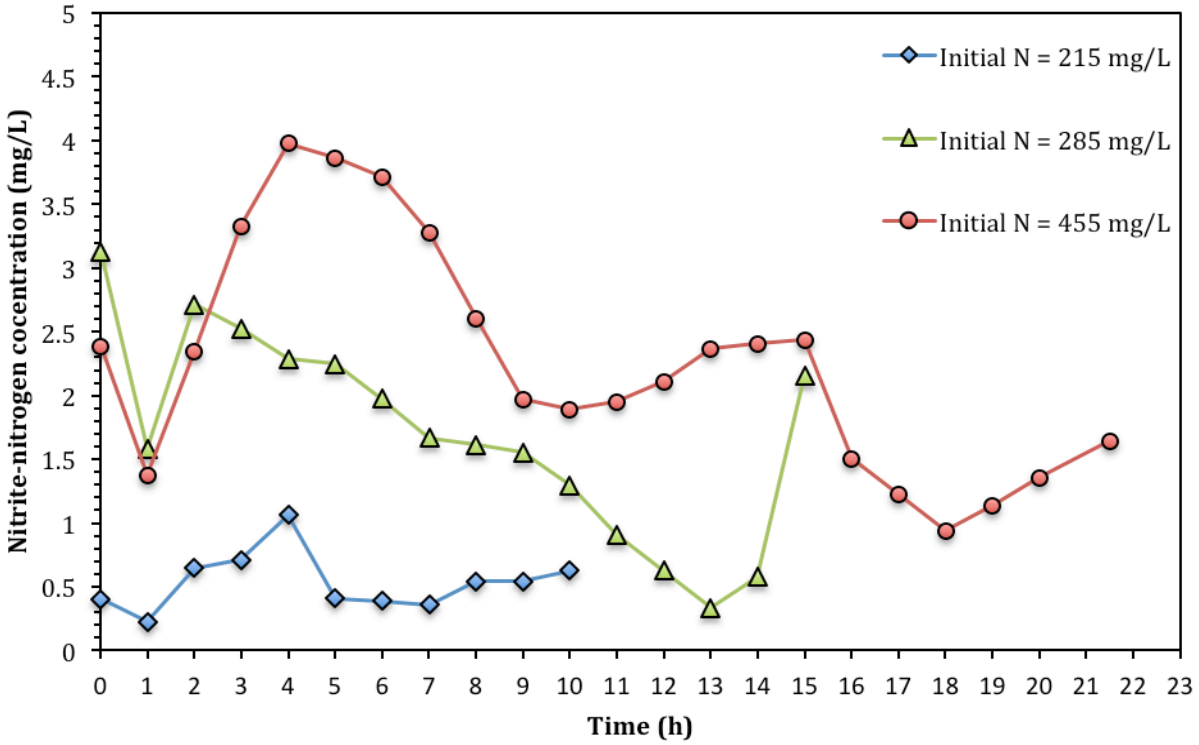

Figure 4.14 Comparison of the effects of ammonia concentration on the variation of nitrite-nitrogen in the reactor within cycles

For nitrates in the reactor, more nitrates would also be generated at higher initial ammonia concentrations (Figure 4.15). 18.3 mg/L nitrate-nitrogen was generated at NH_3-N_i = 215 mg/L while 24.1 mg/L nitrate-nitrogen was generated at NH_3-N_i = 285 mg/L, and more nitrates, at 45.6 mg/L, were produced at higher NH_3-N_i = 455 mg/L. Most of the nitrates produced at higher initial ammonia concentration should come from the CANON reaction (Equation 12), since the nitrate production to ammonia consumption (N/A) ratio were 0.12 for both cycles with NH_3-N_i of 285 mg/L and 455 mg/L, which were very close to the theoretical N/A ratio of 0.11. It indicated that the NOB was inhibited at a surplus ammonia condition, which was also shown by Schmidt *et al*. (2003). At lower NH_3-N_i = 215 mg/L, the N/A ratio was increased to 0.17, indicating some nitrite might be used by NOB for extra nitrate production at lower ammonia concentration conditions.

Thus, the CANON SBR could achieve better performance with higher, initial ammonium concentration. At NH_3-N_i = 455 mg/L, the reactor had the lowest HRT of 1.79 d and highest NRR = 0.36 kgN/m^3•d.

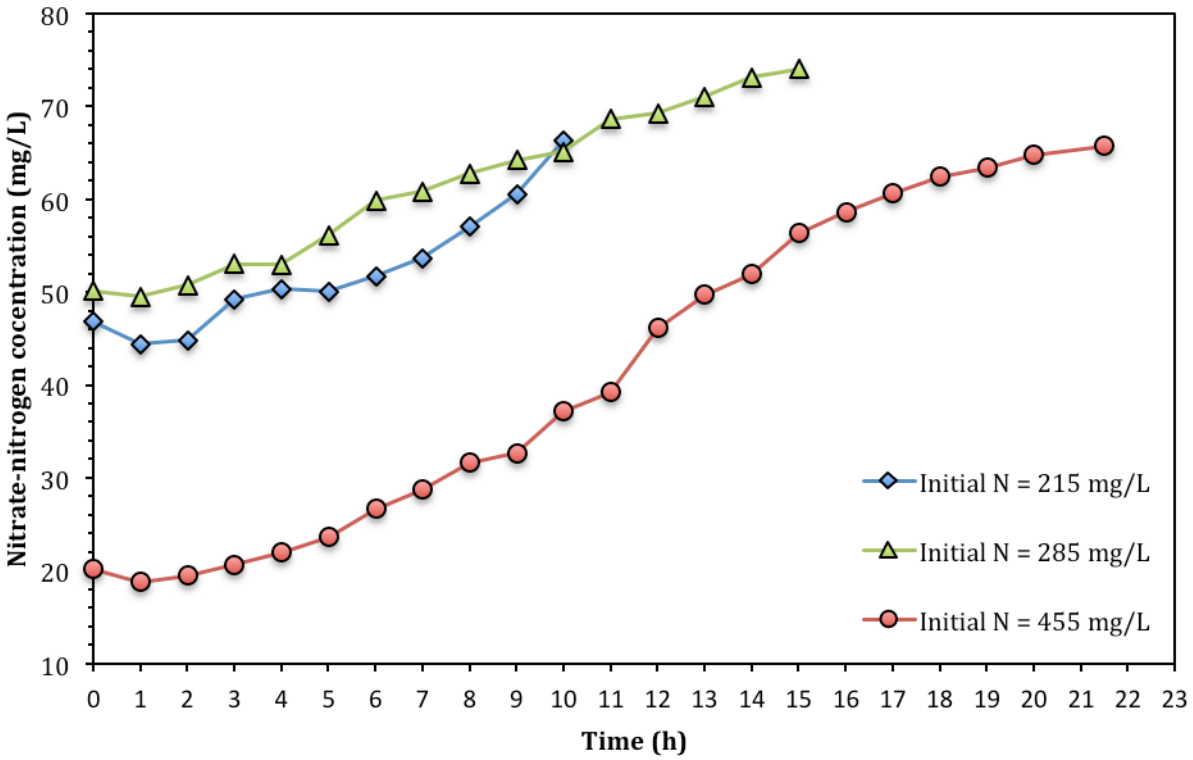

Figure 4.15 Comparison of the effects of ammonia concentration on the variation of nitrate- nitrogen in the reactor within cycles

4.4 Effects of feeding rate

The effects of feeding rate (FR) on the performance of the CANON SBR was investigated under the following conditions: 190 L feeding, 380 L reactor mixed liquid, temperature of 32 ºC, 12 L/min continuously aeration flow rate, one hour anaerobic protection period, pH set point of 6.0 and 4 min of sludge settling time. Two FR strategies were tested separately: 1) FR = 500 mL/min, so that the feeding period of 190 L centrate took 380 min (or 6.3 h), 2) FR = 190 L/min, so that the feeding 190L of centrate was rapidly completed within one minute.

The cycle lengths for both cycles with FR = 500 mL/min and FR = 190 L/min were 21.5 h, indicating the reaction speed was almost the same for two tested cycles. For the cycle with FR = 190 L/min, that 190L of centrate was fed to the reactor within one minute, and the pH in the reactor was increased from 6.0 to 7.39. Due to the denitrification process over the anaerobic period and air stripping by aeration, the pH was further increased from 7.39 to 7.68 on the first 4 hours of cycle. The pH then kept dropping and ended with 6.0, due to the alkalinity consumption by the CANON process. For the cycle with FR = 500 mL, the centrate was slowly pumped into the reactor within 380 min (or 6.3 h); this lead to a pH increase from 6.0 at the beginning of the cycle to 7.65 over 6 hours. As presented in Figure 4.16, the pH decreasing curve of the cycle with FR = 500 mL/min almost matched the pH curve of FR = 190 L/min, after 6 hours of reaction, when centrate feeding was completed. This proves that the CANON reaction was not influenced by substrate feeding rate.

For the performance of ammonia removal within the two cycles, effluent ammonia-nitrogen concentrations were also very close to each other as presented in Figure 4.17. NH_3-N concentration was reduced from about 455 mg/L to 72.4 mg/L for the cycle with FR = 190 L/min. For the cycle with FR = 500 mL/min, NH_3-N concentration increased from 69.3 mg/L to about 338 mg/L, during the first 6 hours of feeding, and decreased to 70.2 mg/L at the end of cycle, which was only 2.2 mg/L less than the effluent NH_3-N concentration of the fast feeding one. This means that the feeding rate had almost no effect on effluent NH_3-N concentration or nitrogen removal ability of the CANON SBR.

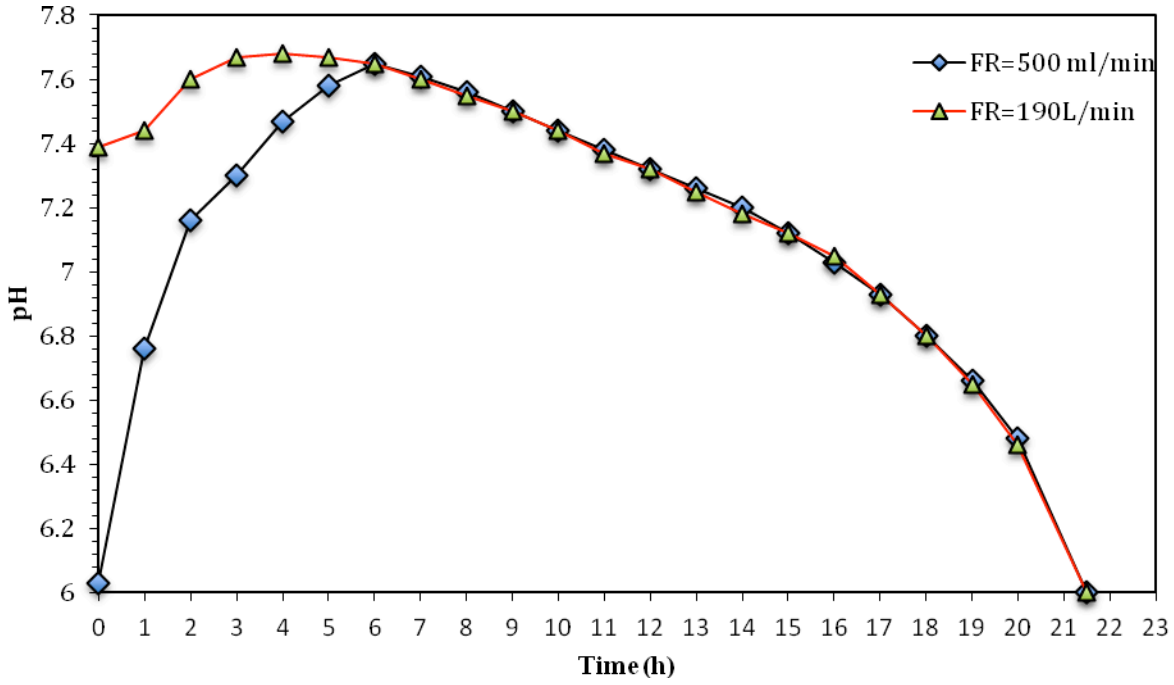

Figure 4.16 Comparison of the effects of feeding rate on the variation of pH in the reactor

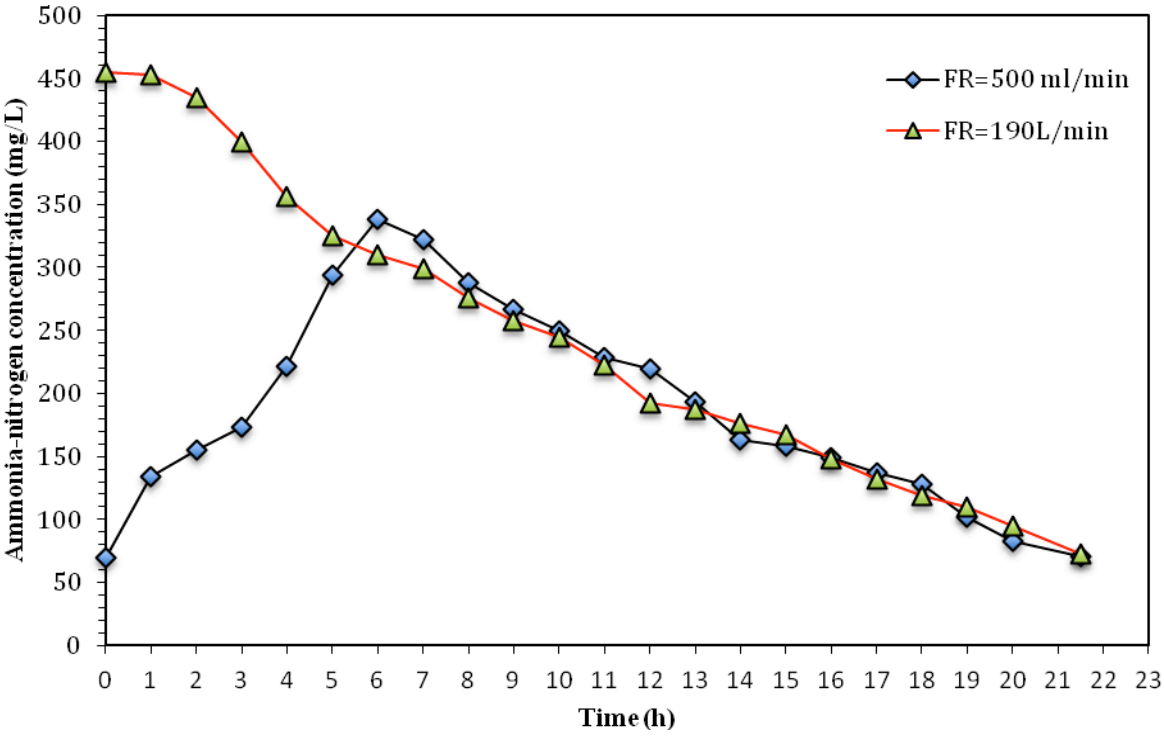

Figure 4.17 Comparison of the effects of feeding rate on the variation of ammonia-nitrogen concentration in the reactor within cycles

As for nitrite generation in the reactor, NO_2-N concentrations were much more stable during the slow feeding cycle with FR = 500 mL/min, than in the fast feeding cycle with FR = 190 L/min. As shown in Figure 4.18, NO_2-N concentrations varied around 2.0 mg/L within cycle of FR = 500 mL/min and ended at 1.8 mg/L. For the cycle with fast feeding rate of 190/min, NO_2-N concentration was increased drastically from 1.4 mg/L to 4.0 mg/L after first three hours of aeration. This was probably because of the partial nitrification process, where, AOB converted ammonia to nitrite and was stimulated at higher initial ammonia concentrations in the reactor. However, due to consumption of anammox bacteria, nitrite concentration decreased to 1.6 mg/L at the end of the cycle. Thus, no nitrite accumulation would occur in the reactor.

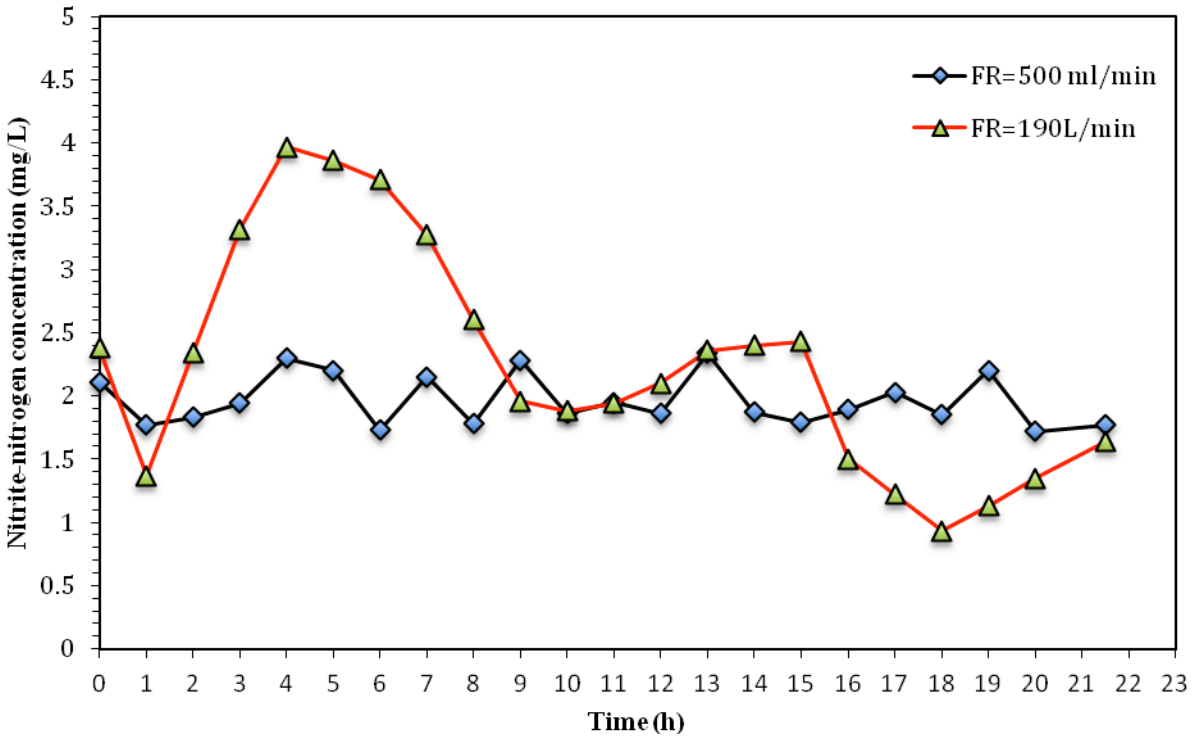

Figure 4.18 Comparison of the effects of feeding rate on the variation of nitrite-nitrogen concentration in the reactor within cycles

The variation of nitrate concentrations within cycles is shown in Figure 4.19. The effect of feeding rate on nitrate generation was also not obvious. With a feeding 190 L of centrate at 190 L/min, NO_3-N in the reactor was instantly diluted to 20.2 mg/L, and then slowly increased to 65.8 mg/L at the end of cycle. The N/A ratio was 0.12, which was very close to the theoretical N/A ratio of 0.11, indicating almost all nitrate was generated by the CANON process. With a slow feeding rate of 500 mL/min, NO_3-N concentration was slowly diluted from 64.4 mg/L to

32.0 mg/L over 9 hours, and then increased to 64.4 mg/L at the end of the cycle, which is very close to the effluent NO_3-N concentration of cycle feed with FR = 190 L/min.

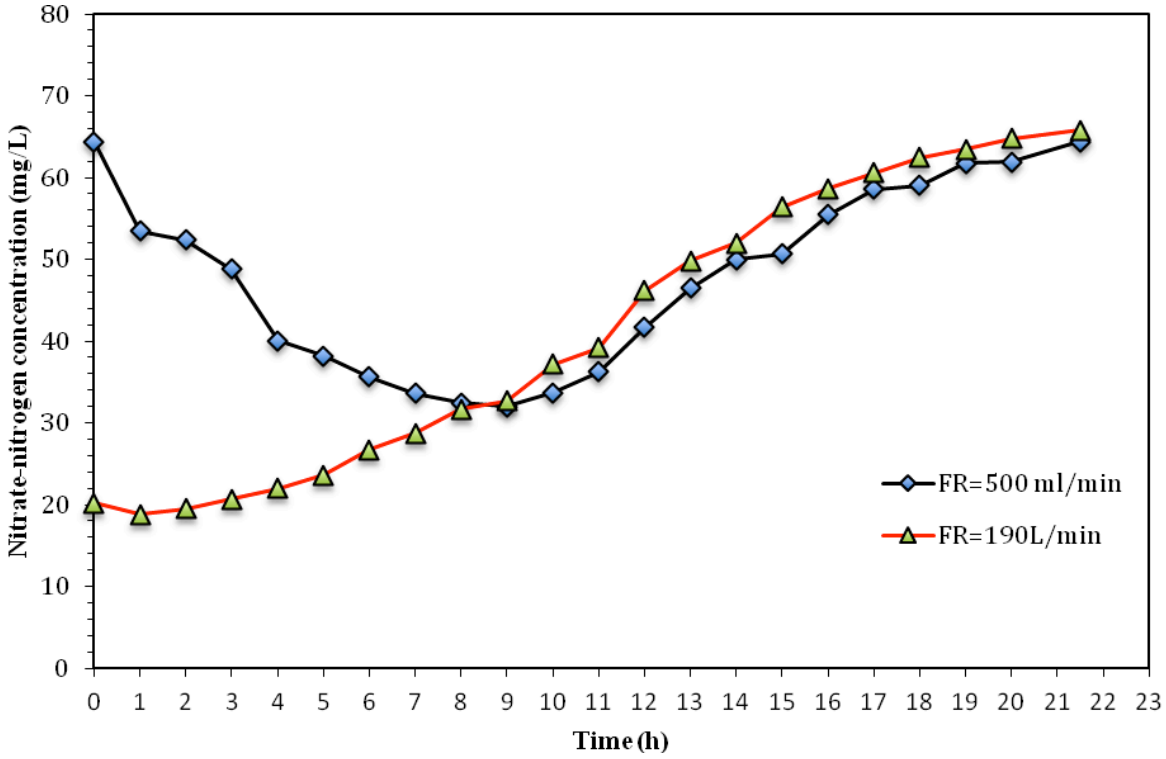

Figure 4.19 Comparison of the effects of feeding rate on the variation of nitrate-nitrogen concentration in the reactor within cycles

It could therefore be concluded that feeding rate had almost no effect on the overall performance of CANON SBR. Regardless of fast feeding rate (190 L/min) or slow feeding rate (500 mL/min), both cycles were completed within the same time, meaning the HRTs of both cycles were the same. In addition, ammonia, nitrite and nitrate concentrations in the effluents of both cycles were very close to each other. The concentration variation curves also matched to some degree, indicating the reaction speed of the CANON process was actually not affected by centrate feeding speed. However, nitrite concentrations were more stable at lower FR = 500 mL/min than higher FR = 190 L/min. A slow feeding rate might be more optimal for a CANON SBR, running under conditions like lower temperature or higher aeration airflow rate, that may tend to produce higher concentration peaks of nitrite within cycles and cause nitrite accumulation problems.

4.6 Effects of sludge settling time

Since anammox bacteria are slow growth bacteria and, thus, must remain in the reactor, the sludge settling time should be long enough for most of anammox sludge granules to settle to the bottom of the reactor. At the same time, nitrifiers grow much faster than anammox bacteria. Thus, the over populated AOB and NOB should be wasted through decanting at the end of each cycle. Adjusting the sludge settling time could effectively change TSS and VSS of both the effluent and reactor mix liquor. Three different sludge settling time settings of 10 min, 8 min, and 4 min were tested over the periods of sludge enrichment and system optimization. Settling time shorter than 4 min would lead to the loss of anammox granular sludge, due to insufficient settling of anammox.

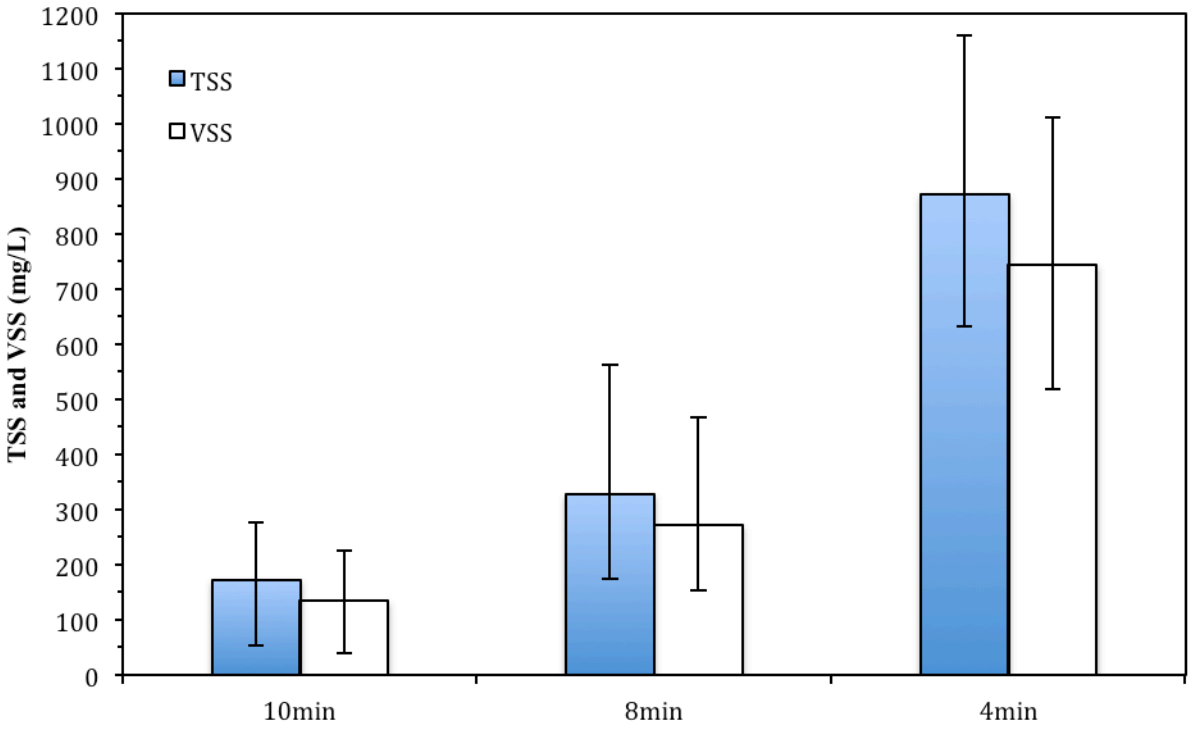

Figure 4.20 Comparison of the effects of sludge settling time on TSS and VSS in the effluent

As shown in Figure 4.20, average TSS and VSS in the effluent increased (an expected result) with the reduction of sludge settling time, indicating more nitrifiers would be wasted through decant with longer sludge settling time. With a sludge settling time of 10 min, a minimum amount of solids were wasted. Effluent TSS ranged from 53.3 mg/L to about 276 mg/L, with an

average value of about 172 mg/L. Most solids in the effluent were volatile, since the VSS concentrates were close to TSS, ranging from 40 mg/L to about 226.7 mg/L, with an average value of about 133 mg/L. With a sludge settling time of 8 min, average TSS and VSS in the effluent was slightly increased to about 328 mg/L and 271 mg/L, respectively. For the shortest sludge settling time of 4 min, effluent TSS and VSS increased drastically to about 873 mg/L and 743 mg/L, respectively.

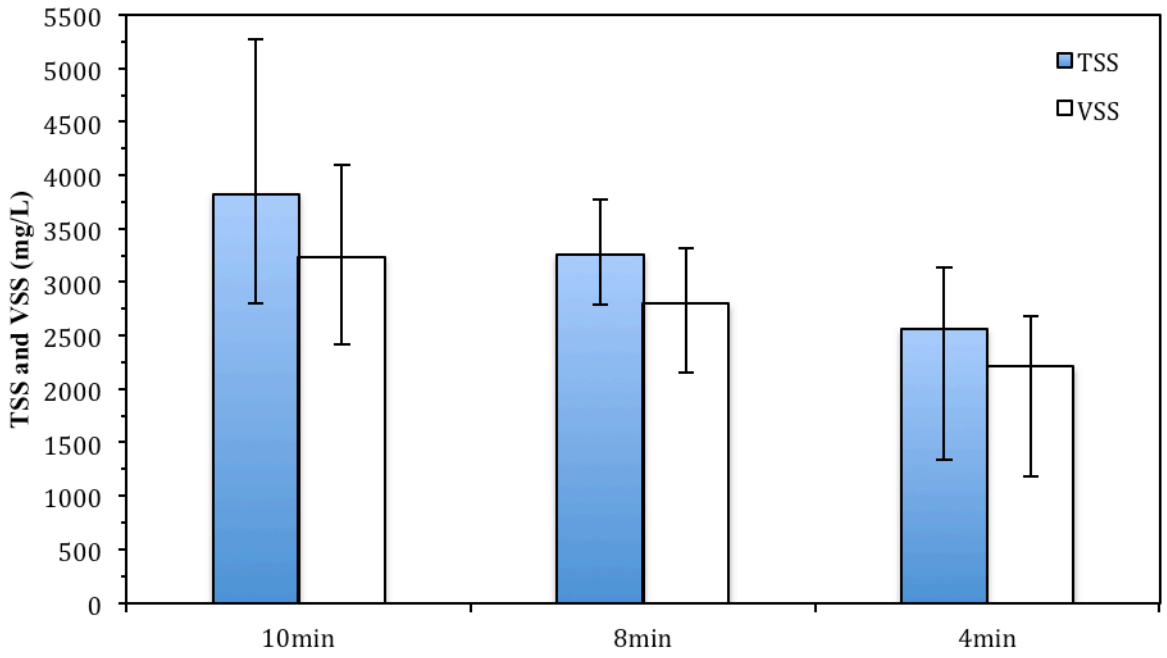

Figure 4.21 Comparison of the effects of sludge settling time on TSS and VSS in the reactor

Sludge settling time also affected TSS and VSS in the reactor. As shown in Figure 4.21, TSS and VSS in the reactor decreased with sludge settling time reduction. At a sludge settling time of 10 min, TSS and VSS concentrations in the reactor were maintained at the highest levels. TSS varied from 2800 mg/L to 5275 mg/L, with an average value of 3828 mg/L and VSS ranged from 2425 mg/L to 4100 mg/L with average value of 3231 mg/L. Since almost no sludge was wasted at a sludge settling time of 10 min, sludge enrichment could be achieved within the shortest of time. With a sludge settling time of 8 min, TSS and VSS in the reactor were slightly reduced to 3256 mg/L and 2797 mg/L, respectively. The average TSS and VSS concentration in the reactor were further reduced to 2560 mg/L and 2212 mg/L with a sludge settling time of 4 min. Since most of granular anammox sludge settled within 4 min and could be maintained in the reactor, the reduction of TSS and VSS was achieved by wasting nitrifiers.

5. Summary and Conclusions

In this research, a 400 L CANON SBR pilot system was built and tested for treating on-site, digester centrate with high ammonium concentrations. The reactor was able to maintain over 90% ammonia removal rates during the test period of 318 days. Concentrations of nitrite-nitrogen in the effluent of the reactor were kept below 5.0 mg/L, which was a safety level that would not inhibit the growth of anammox bacteria.

The reactor was inoculated with digested sludge. Mature anammox sludge was later added for a faster start up. It took 115 days to achieve a complete start-up of the reactor with an ammonia removal rate (ARR) of 94.7% and HRT of 5.2 d. The start-up period could have been much shorter if a failure event had not occurred in the reactor. Since the growth rate of anammox bacteria was low, TSS and VSS concentration was still as low as 1509 mg/L and 1345 mg/L until the end of the start-up period. Thus, a sludge enrichment period was necessary to increase TSS and VSS concentration in the reactor for better reactor performance. After 33 days of sludge enrichment, TSS and VSS levels increased to 4050 mg/L and 3175 mg/L, respectively. At the same time, red granular anammox sludge was observed in the reactor, which was good for sludge settling and resistance to shock loading. Due to the granulation of anammox sludge, the ammonia loading rate (ALR) was increased from 0.17 $kgN/m^3 \bullet d$ to 0.45 $kgN/m^3 \bullet d$, without ARR decreasing. During the same period of time, the cycle length and HRT of the reactor was reduced to 10 h and 2.08 d, respectively.

It was found out that the quality of on-site centrate, especially the sludge solids concentration in the centrate, was critical to affect the performance of the reactor. Solids in the centrate would introduce extra digested sludge into the reactor, and disturb the existing balance among bacteria groups within the reactor. Continuously feeding centrate, with a 1~2 mm sludge layer, reduced ARR significantly from 96.6% to 78.2%, since anammox bacteria were outcompeted by exogenous bacteria coming from sludge in the centrate. The ARR was recoverable when the sludge layer was removed from the feeding centrate. After 13 days of continuous feeding of clear centrate, the ARR increased to 91.7%, since the digested sludge was probably washed out from the reactor through decanting. Thus, for centrate containing too many sludge solids, pre-treatment for sludge removal might be necessary before centrate could be fed to the CANON SBR.

Through process kinetic studies of the CANON SBR under variety environmental conditions, aeration was found to be one of the most important factors that control the reactor performance. Higher ARR and lower HRT of the reactor could be achieved through increasing the airflow rate. Compared to an aeration airflow rate of 10 L/min, a 40% airflow increase, from 10L/min to 14 L/min, would lead to a 9% ARR increase and a 10% HRT reduction.

Temperature was also found to be a critical control factor for the CANON SBR. The highest ARR in the CANON SBR was achieved at 32 ºC, compared with lower tested temperatures of 30 ºC, 28 ºC and 26 ºC. At the lowest tested temperature of 26 ºC, ARR was reduced by 12 % and peak nitrite concentrations of 15.7 mg/L was observed in the reactor. The additional nitrite , which could not be completely consumed by anammox bacteria, might be utilized by NOB for nitrate production, stimulating the growth of NOB and disturbing the balance among microorganisms in the reactor.

Except for raising the temperature of the reactor, better CANON SBR performance was also observed at higher initial ammonium concentration in the reactor. Among tested initial ammonia concentrations of 215 mg/L, 285 mg/L and 455 mg/L, a cycle with NH_3-N_i = 455 mg/L achieved the highest NRR of 0.36 kgN/m^3•d and lowest HRT of 1.79 d; this indicated that both nitrification and ANAMMOX process were stimulated and more nitrogen could be removed under higher initial ammonia concentrations.

The effect of feeding rate was also studied but no significant difference was observed. The cycle length or HRT was the same, no matter if the feeding rate was 190 L centrate at 190 L/min or at 500 mL/min, indicating that the feeding rate did not affect the reaction speed. In addition, the effluent ammonia and nitrate concentrates were similar for both cycles tested under the two different feeding rates. However, the reactor under a fast feeding rate of 190 L/min, tended to generate more nitrite than a slow FR = 500 ml/min. A high peak concentration of nitrite was observed during the cycle under FR = 190 L/min, while nitrite concentrations remained relatively constant (around 2.0 mg/L) during the cycle with FR = 500 mL/min. Thus, when the CANON SBR was operated under low temperature or high aeration rate conditions, that tended to generate high peak concentrations of nitrite, the operational strategy of a slow feeding rate might be preferred.

Changing the sludge settling time of the CANON SBR was found to be an effective biomass wasting strategy for solid concentrations (TSS and VSS) control in the reactor. Adjusting the sludge settling time from 4 min to 10 min could effectively control the amount of nitrifying bacteria in the reactor, without losing the anammox granular sludge. For the purpose of reactor start-up and sludge enrichment, the sludge settling time could be set at 10 min or longer, allowing most of the sludge to remain in the reactor. During the sludge enrichment period, with a sludge settling time of 10 min, relatively high TSS over 5000 mg/L and VSS over 4000 mg/L was achieved in the reactor. With a sludge settling time of 4 min, during system optimization period, TSS and VSS in the reactor dropped below 2500 mg/L.

6. Recommendations for Future Research

Since anammox is novel technology in nitrogen removal field, more research needs to be done to make it more applicable. Following are some recommendations for future research:

- Since anammox bacteria grow slowly, establishing proper kinetic models for CANON SBR process could be helpful to do better system optimization.

- The function of bacteria in the CANON SBR, including anammox bacteria, ammonia oxidation bacteria, and nitrite oxidation bacteria, could be further investigated. Knowing how to control the activities of these bacteria in the CANON SBR could be very helpful.

- Future research could also try to combine the CANON SBR with other treatment facilities, like MBR, to achieve further nitrogen removal and phosphorus removal.

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Appendices

Appendix A: Data of reactor performance

Date	Day	Inf NH4, mg/L	Inf NO2, mg/L	Inf NO3, mg/L	Eff NH4, mg/L	Eff NO2, mg/L	Eff NO3, mg/L	Re TSS, mg/L	Re VSS, mg/L	Cycle length, h
25-Aug-11	76	1107.675	0.414	0.285	116.225	0.158	111.892			
26-Aug-11	77									
27-Aug-11	78	1141.5	0.378	0	130.875	0.158	135.592	2230	1875	46
28-Aug-11	79									
29-Aug-11	80	1107.975	0.386	0	152.525	0.159	129.791	2128.6	1957.1	46
30-Aug-11	81	1042.65	0.419	0	163.475	0.161	153.739	3000	1928.6	
31-Aug-11	82									
1-Sep-11	83	1068.45	0.556	0.239	195.675	0.163	168.037	1855.6	1355.6	48
2-Sep-11	84	1093.725	0.4	0	196.975	0.164	175.836	2414.3	2142.9	30
3-Sep-11	85	1028.225	0.419	0	194	0.175	179.925			26.5
4-Sep-11	86									
5-Sep-11	87	1008.625	0.375	0	206.225	0.178	189.572			38.3
6-Sep-11	88	994.275	0.42	0	213.475	0.17	186.78	1500	1200	25.5
7-Sep-11	89	967.425	0.468	0	199.175	0.549	182.351	1980	1730	26
8-Sep-11	90									22
9-Sep-11	91									34
10-Sep-11	92									
11-Sep-11	93									
12-Sep-11	94									
13-Sep-11	95							1045.45	809.09	
14-Sep-11	96									
15-Sep-11	97	924.5	0.048	0.254	44.01	0.55	28.18	593.3	513.3	47
16-Sep-11	98	955.4	0.275	0.026	60.31	0.58	45.11			31.5
17-Sep-11	99									
18-Sep-11	100	953.2	0.244	0.089	71.1	0.62	55.91	782.1	667.9	36
19-Sep-11	101	890.45	0.288	0.018	88.4	0.65	40.5	675	600	24
20-Sep-11	102	1020.1	0.198	0.022	85.2	0.96	72.58	954.54	763.63	26.5
21-Sep-11	103	917.35	0.247	0.004	83.86	0.52	73.99	1250	1183.3	25
22-Sep-11	104	1049.2	0.204	0.14	93.2	0.3	79.97	1245.45	1127.3	21
23-Sep-11	105	1002.4	0.14	0.325	90.13	0.32	84.99			22
24-Sep-11	106	949.35	0.106	0.062	84.49	0.15	83.31			23.5
25-Sep-11	107									24
26-Sep-11	108	1083	0.227	0.464	76.25	0.38	78.73	1572.7	1463.6	24
27-Sep-11	109	1007.45	0.273	0.381	79.97	0.38	75.64			24
28-Sep-11	110	1010.15	0.329	0.428	74.63	0.36	73.95	1909.1	1709.1	24.5
29-Sep-11	111	1036.8	0.434	0.697	60.86	0.59	59.41			23
30-Sep-11	112	840.05	0.226	0.026	74.06	0.28	67.29	1791.6	1616.7	22
1-Oct-11	113	885.9	0.151	0.089	60.66	0.37	65.94			22.5
2-Oct-11	114	901.1	0.124	0.14	53.39	0.39	59.39			25
3-Oct-11	115	900.35	0.316	0.05	47.46	0.46	55.44	1509.1	1345.5	25
4-Oct-11	116	873.25	0.543	0.3	46.55	0.27	48.08			19
5-Oct-11	117	789.5	0.729	0.27	42.75	0.33	48.86	1623.1	1438.5	18
6-Oct-11	118	859.3	0.177	0.1	48.14	0.39	50.69			19
7-Oct-11	119	789.7	0.082	0	47.33	0.39	52.72	1007.1	900	19

Date	Day	Inf NH4, mg/L	Inf NO2, mg/L	Inf NO3, mg/L	Eff NH4, mg/L	Eff NO2, mg/L	Eff NO3, mg/L	Re TSS, mg/L	Re VSS, mg/L	Cycle length, h
8-Oct-11	120	790.8	0.083	0.02	36.97	0.4	48			20
9-Oct-11	121	840.5	0.087	0.08	43.23	0.67	45.67			20
10-Oct-11	122	830.5	0.119	0.37	38.19	0.4	44.07	2133.3	1766.6	20
11-Oct-11	123	779.05	0.097	0.04	39.69	0.38	43.65			19
12-Oct-11	124	782.75	0.088	0	36.52	0.4	49.04	2200	2069.2	
13-Oct-11	125	778.5	0.106	0	33.86	0.4	45.23			19
14-Oct-11	126	825.85	0.082	0	36.54	0.39	47.53	2277.8	1966.7	19
15-Oct-11	127	713.1	0.123	0.03	32.66	0.38	48.6			19
16-Oct-11	128									
17-Oct-11	129	923.25	0.313	0.063	42.08	0.46	39.04	2985.7	2671.4	19
18-Oct-11	130	936.4	0.142	0.063	43.84	0.34	41.3			
19-Oct-11	131	977.55	0.148	0	45.94	0.46	47.14	2750	2375	17
20-Oct-11	132	907.65	0.437	0	57.34	0.83	53.32			16
21-Oct-11	133	923.6	0.611	0.023	52.78	0.44	60.22	3062.5	2637.5	16
22-Oct-11	134	904.7	1.409	0.04	46.24	0.35	53.89			16
23-Oct-11	135									
24-Oct-11	136	894.6	0.232	0	40.38	0.92	37.76	3033.3	2677.8	17
25-Oct-11	137	856.5	0.318	0	41.73	1.75	33.12			16
26-Oct-11	138	779.9	0.169	0.086	46.76		52.09	3055.6	2577.8	14.5
27-Oct-11	139	805.5	0.271	0.095	35.61	2.11	51.88			14.5
28-Oct-11	140	868.45	0.072	0.034	38.09	5.64	45.49	2666.7	2311.1	14.5
29-Oct-11	141	802.75	0.767	0			53.23			14.5
30-Oct-11	142									
31-Oct-11	143	793.8	2.712	1.345	43.13	0.14	40.73	3155.6	2777.8	12.5
1-Nov-11	144	784.5	3.279	0.119	38.96	0	39.59			12.5
2-Nov-11	145	971.95	0.05	0.01	68	0.38	40.95			12.5
3-Nov-11	146	935.85	0.078	0	56.33	0	33.31			12.5
4-Nov-11	147	972.05	0.095	0	52.83	0.04	30.16	4050	3175	12
5-Nov-11	148	936.15	0.154	0	67.06	0	35.01			10
6-Nov-11	149									
7-Nov-11	150	905.25	0.376	0	77.89	2.22	52.74	4975	3975	10
8-Nov-11	151	867.05	0.285	0.06	46.89	2.96	31.5			10
9-Nov-11	152	1095.5	0.339	0	60.82	1.94	43.29	5275	4100	10.5
10-Nov-11	153	892.1	0.642	0	52.34	1.41	40.36			10.5
11-Nov-11	154	937.9	0.302	0	56.44	1.68	35.95	3666.7	3058.3	10.5
12-Nov-11	155									
13-Nov-11	156									
14-Nov-11	157	931.9	0.531	0.155	61.2	6.75	37.87	3942.1	3545.5	9.5
15-Nov-11	158	921	0.651	0.04	62.51	5.13	41.72			9.5
16-Nov-11	159	983.75	0.191	0.006	58.84	3.93	39.49	2441.7	1983.3	9.5
17-Nov-11	160	1006.6	0.214	0.032	60.18	3.07	40.44			9.5
18-Nov-11	161	994.45	0.16	0	48.79	2.43	30.36	3450	2912.5	9.5
19-Nov-11	162									
20-Nov-11	163									
21-Nov-11	164									
22-Nov-11	165									
23-Nov-11	166									
24-Nov-11	167	851	0.171	3.994	9.57	1.38				
25-Nov-11	168									
26-Nov-11	169									
27-Nov-11	170									
28-Nov-11	171	856.1	0.487	3.348	28.97	2.04	22.06	3800	3225	

Date	Day	Inf NH4, mg/L	Inf NO2, mg/L	Inf NO3, mg/L	Eff NH4, mg/L	Eff NO2, mg/L	Eff NO3, mg/L	Re TSS, mg/L	Re VSS, mg/L	Cycle length, h
29-Nov-11	172	820.1	0.377	2.584	38.43	2.01	29.08			
30-Nov-11	173									
1-Dec-11	174	921.4	0.512	2.404	54.05	2.06	44.31	2800	2480	10.5
2-Dec-11	175	791.8	0.766	2.283	49.24	2.67	38.42			11
3-Dec-11	176									
4-Dec-11	177									
5-Dec-11	178	865.4	2.752	2.614	58.32	2.25	49.22	2255.6	2022.2	11
6-Dec-11	179									
7-Dec-11	180	907.2		2.945	57.36	5.9	50.79	2887.5	2425	11
8-Dec-11	181									
9-Dec-11	182	885.5		2.927	58.81	6	51.27	2455.6	2066.7	11
10-Dec-11	183									
11-Dec-11	184									
12-Dec-11	185	809.75	1.932	0.498	63.45	0.84	55.44	3314.3	2700	
13-Dec-11	186									
14-Dec-11	187	735.3	1.437	0.165	55.75	0.91	50.21			
15-Dec-11	188									
16-Dec-11	189	816.15	0.923	0.091	57.58	0.71	48.57	3242.9	2871.5	12
17-Dec-11	190									
18-Dec-11	191									
19-Dec-11	192	786.4	0.632	0.01	56.59	0.52	48.42	2215.4	1976.3	12
20-Dec-11	193	829.35	0.573	0.076	63.86	0.58	57.39			11.5
21-Dec-11	194	825.1	0.766	0.1	68.21	3.29	58.66	3600	3077.8	11
22-Dec-11	195									
23-Dec-11	196	841.65	0.682	0.15	61.26	0.57	55.02			
24-Dec-11	197									
25-Dec-11	198									
26-Dec-11	199									
27-Dec-11	200	865.05	0.964	0.107	77.37	0.73	67.87	2633.3	2300	
28-Dec-11	201									
29-Dec-11	202									
30-Dec-11	203									
31-Dec-11	204	902.95	0.717	0.003	71.26	0.62	62.04			
1-Jan-12	205									
2-Jan-12	206	929.75	0.225	0.002	144.12	0.87	127.78	4316.7	3683.3	
3-Jan-12	207									
4-Jan-12	208	945.8	0.805	0.153	165.17	0.35	126.62			12
5-Jan-12	209	946.7	0.35	0.091	184.29	0.33	128.57			12
6-Jan-12	210	955.3	0.419	0.067	188.82	0.39	128.42	4532	3990	12
7-Jan-12	211									
9-Jan-12	212	941.55	1.026	0.258	205.15	0.39	132.29			
10-Jan-12	213									
11-Jan-12	214	961.55	0.094	0.058	209.86	0.44	119.57			
12-Jan-12	215	791	0.501	0.038	109.87	0.26	140.22			11
13-Jan-12	216	841.75	0.221	0.036	110.36	0.33	119.49	4250	3700	10.5
14-Jan-12	217									
15-Jan-12	218									
16-Jan-12	219									
17-Jan-12	220									
18-Jan-12	221									
19-Jan-12	222									
20-Jan-12	223									
21-Jan-12	224									

Date	Day	Inf NH4, mg/L	Inf NO2, mg/L	Inf NO3, mg/L	Eff NH4, mg/L	Eff NO2, mg/L	Eff NO3, mg/L	Re TSS, mg/L	Re VSS, mg/L	Cycle length, h
22-Jan-12	225									
23-Jan-12	226	918.3	0.055	0.19	75.99	0.12				16
24-Jan-12	227	865.85	0.087	0.137	134.36	0.13	109.26			15
25-Jan-12	228									
26-Jan-12	229									
27-Jan-12	230	995.7	0.642	0.157	151.92	0.18	104.5	4037.5	3275	11
28-Jan-12	231									
29-Jan-12	232									
30-Jan-12	233	965.85	0.042	0	133.04	0.12	87.72	3187.5	2762.5	9.5
31-Jan-12	234									
1-Feb-12	235	1040.6	0.118	0.065	136.28	0.075	87.7			10
2-Feb-12	236									10.5
3-Feb-12	237	974.1	0.1	0.778	130.87	0	71.7	2850	2583.3	12
4-Feb-12	238									
5-Feb-12	239									
6-Feb-12	240	873.05	0.082	0.074	126.29	0.47	94.94	3180	2800	16
7-Feb-12	241									
8-Feb-12	242	887.45	2.853	0.062	129.12	0.13	96.94			15
9-Feb-12	243	876.15	0.659	0.229	136.82	0.04	103.26			14
10-Feb-12	244	821.95	1.005	0.07	111.8	0.06	84.09	2790	2160	15
11-Feb-12	245									
12-Feb-12	246									
13-Feb-12	247									
14-Feb-12	248	884	0.058	0.151	90.95	0.22	68.3	3620	3320	12
15-Feb-12	249	856.15	0.387	0.119	125.27	0.07	96.55			11
16-Feb-12	250	851.7	0.086	0.101	123.135	0.84	95.77			10
17-Feb-12	251	881.85	0.423	0.037	128.73	0.43	96.4	3500	3100	10
18-Feb-12	252									
19-Feb-12	253									
20-Feb-12	254	1031	0.183	0.126	124.9	0.48	77.35	4500	3885.7	10
21-Feb-12	255									
22-Feb-12	256	1082.8	0.107	0.107	110.66	1.54	81.4			15
23-Feb-12	257	1078.95	0.355	0.075	118.14	2.49	82.21			15
24-Feb-12	258	1101.4	0.841	0.099	117.15	2.42	82.36	4611.1	3944.4	14
25-Feb-12	259									
26-Feb-12	260									
27-Feb-12	261	1089.5	0.513	0.282	103.1	2.3	74.32	3777.7	3266.7	12
28-Feb-12	262									
29-Feb-12	263	1007.5	0.112	0.112	89	3.13	70.91			12
1-Mar-12	264	1020.5	0.128	0.128	96.05	0.39	71.75			12
2-Mar-12	265	966.75	0.295	0.236	87.86	2.92	68.42	3144.4	2388.9	12
3-Mar-12	266									
4-Mar-12	267									
5-Mar-12	268	1177	0.452	0.102	88.99	2.85	68.76			12
6-Mar-12	269									
7-Mar-12	270	1101	0.205	0.603	57.63	3.07	44.37			
8-Mar-12	271	914.45	1.836	0.346	55.93	2.43	43.87			
9-Mar-12	272	922.1	0.715	0.299	58.68	2.31	53.15	4050	3570	
10-Mar-12	273									
11-Mar-12	274									
12-Mar-12	275	1029.75	0.428	0.428	68.8	2.58	59.11			21.5
13-Mar-12	276									
14-Mar-12	277									24

Date	Day	Inf NH4, mg/L	Inf NO2, mg/L	Inf NO3, mg/L	Eff NH4, mg/L	Eff NO2, mg/L	Eff NO3, mg/L	Re TSS, mg/L	Re VSS, mg/L	Cycle length, h
15-Mar-12	278	782.4	1.46	0.37	90.36	3.465	67.56			24
16-Mar-12	279	740.63	1.33	0.762	75.25	3.7	77			26
17-Mar-12	280									
18-Mar-12	281									
19-Mar-12	282	816.2	0.46	0.26	72.39	2.316	73.07	3688.9	2911.1	24
20-Mar-12	283									
21-Mar-12	284	798.6	0.44	0.247	74	2.202	70.13			24
22-Mar-12	285	785.55	0.43	0.226	74.81	2.167	70.99			23
23-Mar-12	286	778.1	0.61	0.273	63.27	2.984	62.2	3143.7	2681.25	23
24-Mar-12	287									
25-Mar-12	288									
26-Mar-12	289	891.7	0.2	0	82.6	0.92		2561.5	2184.6	21
27-Mar-12	290									
28-Mar-12	291	859.8	0.8	0.27	72.55	0.92				
29-Mar-12	292	862.6	4.5	0.38	65.675	0.8				
30-Mar-12	293	889.5	2.2	0.38	72.475	0.8		2550	2280	
31-Mar-12	294									
1-Apr-12	295									
2-Apr-12	296	927.6	0.24	0	80.55	1.06				
3-Apr-12	297									
4-Apr-12	298	808.55	1.803	0.278	75.27	1.165	66.01	3030.7	2653.8	
5-Apr-12	299	831.75	1.201	0.278	76.7	1.7	63.91			24
6-Apr-12	300	864.05	1.145	0.246	72.02	1.115	66.785			23
7-Apr-12	301									
8-Apr-12	302									
9-Apr-12	303	798.5	0.187	0	83.34	0.885	77.78	3228.6	2700	
10-Apr-12	304									
11-Apr-12	305	816.1	0.908	0.141	75.13	1.5	70.755			
12-Apr-12	306	780.45	2.415	0.364	70.91	1.51	70.79			21.5
13-Apr-12	307	748.55	3.559	0.523	60.41	1.125	54.01	2811.7	2425.3	21.5
14-Apr-12	308									
15-Apr-12	309									
16-Apr-12	310	788.65	0.811	0.099	67.75	0.075	50.885			22
17-Apr-12	311	805.75	1.153	0.06	75.02	0.39	60.43			22
18-Apr-12	312									
19-Apr-12	313	795.95	1.187	0.317	73.08	0.3	56.52			22
20-Apr-12	314	798.45	0.924	0.346	63.74	0.285	51.225	1953.3	1733.3	22
21-Apr-12	315									
22-Apr-12	316									
23-Apr-12	317	765.1	2.815	0.106	69.11	0.245	57.2			22

Appendix B: Data of cycle tests for system optimization

Test No.	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2011-10-26	0	30.7	0.13	7.59	618	162.32	0.06	24.03
T=32 °C	1	31.1	0.46	7.58	580.6	146.52	0.64	27.31
Decant V= 70L	2	31.3	0.39	7.5	550	137.15	0.49	28.69
Air flow = 8L/min	3	31.6	0.38	7.41	500	128.12	0.64	28.68
	4	31.8	0.33	7.33	391.7	114.01	0.41	30.54
	5	32	0.45	7.22	335	101.04	0.52	31.71
	6	32.2	0.51	7.13	295.8	85.16	0.31	31.22
	7	32.3	0.42	6.99	250	73.53	0.44	34.1
	8	32.4	0.47	6.86	210	65.59	0.33	33.91
	9	32.5	0.38	6.57	125.7	50.79	0.47	36.02
	10	32.6	0.4	6.28	77.5	41.5	0.46	36.94
	10.5	32.6	0.48	6	54	37.13	0.43	38.37s
2011-10-31	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
T=30 °C	0	28.7	0.59	7.7	695	180.78	0.715	25.085
Decant V= 70L	1	28.9	0.65	7.66	655.6	193.58	0.82	30.05
Air flow = 8L/min	2	29.1	0.51	7.57	483.3	158.22	0.94	28.345
	3	29.3	0.82	7.45	418.2	130.42	0.705	30.13
	4	29.5	0.77	7.32	362.5	113.27	0.68	31.46
	5	29.6	0.74	7.17	300	89.06	0.52	30.55
	6	29.6	0.68	6.98	226.3	76	0.645	35.57
	7	29.7	0.71	6.69	140	59.8	0.05	37.225
	8	29.8	0.94	6.22	70	45	0.815	41.12
	8.5	29.8	0.8	6	44	42.31	1.555	42.405
2011-11-05	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
T=28 °C	0	27.3	0.13	7.59	780	228.3	6.71	40.615
Decant V= 70L	1	27.7	0.13	7.65	890	230.97	0.38	39.79
Air flow = 8L/min	2	27.7	0.13	7.67	766.7	224.05	0.25	35.72
	3	27.8	0.86	7.72	706.25	209.75	1.75	38.435
	4	27.9	0.89	7.66	600	196.23	1.89	38.39
	5	28	0.8	7.58	552.6	178.1	2.83	41.18
	6	28.1	0.79	7.42	440.9	150.96	2.7	45.84
	7	28.2	0.83	7.32	386.4	139.65	3.79	47.92
	8	28.3	0.88	7.15	284.6	115.76	1.92	49.6
	9	28.3	0.91	6.92	222.6	103.12	3.98	51.36
	10	28.3	1.24	6.5	102.5	77.01	5.46	56.63
	11	28.3	3.92	6	48	72.79	5.975	61.77
2011-11-06	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
	0	29.1	0.16	7.57	720	231.68	8.465	51.505
T=30°C	1	29.3	0.13	7.64	840	270.7	0.645	49.715
Decant V= 70L	2	29.3	0.14	7.65	770	255.37	0.335	42.485
Air flow = 8L/min	3	29.6	0.81	7.72	660	212.88	1.84	44.105
	4	29.7	0.79	7.66	600	197.21	3.16	45.37
	5	29.8	0.68	7.55	517.6	181.23	3.875	47.39
	6	29.8	0.76	7.42	425	145.71	3.9	48.75
	7	29.9	0.64	7.22	330.4	131.12	4.24	53.33
	8	29.9	0.7	7.04	240	106.83	3.655	54.71
	9	29.9	0.79	6.77	150	86.65	3.325	56.91
	10	29.9	3.02	6	56	68.95	4.215	61.54

Test No.	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2011-11-11	0	31.2	0.12	7.6	820	161.87	1.45	20.28
T=32°C	1	31.4	0.11	7.64	918.2	211.69	1.49	25.39
Decant V= 70L	2	31.5	0.09	7.65	820	255.08	1.68	29.38
Air flow = 8L/min	3	31.7	0.55	7.72	620	150.34	1.55	18.34
	4	31.8	0.64	7.69	547.7	173.83	2.13	25.83
	5	31.9	0.68	7.63	500	185.69	2.12	32.5
	6	32	0.61	7.53	430.8	189.08	2.29	40.31
	7	32.1	0.58	7.39	354.5	121.42	1.99	32.38
	8	32.2	0.41	7.22	270.4	100.68	1.45	31.74
	9	32.2	0.44	6.97	126.5	119.94	1.29	50.48
	10	32.2	0.45	6.57	100	60.68	1.36	36.94
	10.5	32.2	0.9	6	50.6	47.39	1.49	37.93
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2011-11-15	0	24.3	0.17	7.64	742.9	222.8	13.36	36.05
T=26°C	1	24.7	0.15	7.71	700	222.08	6.42	33.13
Decant V= 70L	2	24.9	0.14	7.76	700	222.26	1.61	31.88
Air flow = 8L/min	3	25.2	0.59	7.71	633.3	210.3	4.17	34.95
	4	25.4	0.57	7.6	565	184	6.89	37.95
	5	25.6	0.66	7.44	405	153.17	7.91	39.7
	6	25.7	0.65	7.28	332	139.17	9.56	43.49
	7	25.7	0.74	7.08	266.7	114.26	10.99	47.05
	8	25.7	0.75	6.74	112	88.69	12.8	46.58
	9	25.7	1.74	6	56	79.37	15.66	54.33
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2011-11-17	0	27.3	0.15	7.63	—	213.96	2.06	29.72
T=32°C	1	28.3	0.22	7.72	—	210.24	1.46	27.14
Redo one	2	28.6	0.14	7.72	—	208.48	1.57	26.24
Decant V= 70L	3	29.3	0.56	7.71	—	201.23	3.24	28.17
Air flow = 8L/min	4	29.9	0.52	7.62	—	175.76	4.01	29.85
	5	30.4	0.59	7.5	—	148.07	3.26	31.59
	6	30.8	0.62	7.34	—	124.71	3.17	31.99
	7	30.9	0.69	7.17	—	111.59	3.26	35.43
	8	31.1	0.55	6.93	—	89.27	3.2	37.06
	9	31.2	0.58	6.54	—	65.78	2.29	38.12
	9.5	31.2	0.6	6	—	51.18	2.13	38.27
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-02-02	0	30.3	0.14	7	—	203.83	0.46	52.23
T=32°C	1	30.8	0.09	7.05	—	197.27	0.16	43.24
Air flow = 10L/min	2	31	0.42	7.15	—	191.74	0.42	44.39
Decant V= 70L	3	31.2	0.4	7.16	—	188.84	1.08	45.82
	4	31.3	0.38	7.13	—	174.66	0.64	46.89
	5	31.3	0.36	7.08	—	160.26	0.43	47.44
	6	31.3	0.38	7	—	135.04	0.6	48.41
	7	31.3	0.32	6.88	—	111.08	0.5	50.21
	8	31.4	0.43	6.73	—	108.43	0.84	51.76
	9	31.6	0.45	6.58	—	92.06	0.38	52.88
	10	31.6	0.47	6.31	—	82.55	0.33	53.46
	11	31.6	0.58	6	—	73.99	0.34	54.68
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-02-09	0	27.6	0.16	7	—	322.71	2.735	86.905
T=32°C	1	28.1	0.15	7.01	—	296.39	3.105	84.885
Increase decant volume to 90L	2	28.8	0.22	7.18	—	293.2	2.83	88.92
Air flow = 10L/min	3	29.2	0.2	7.2	—	277.86	2.645	93.53
	4	29.9	0.2	7.16	—	242.39	2.455	94.315

Test No.	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
	5	30.2	0.36	7.11	—	238.62	2.495	95.435
	6	30.4	0.24	7.05	—	222.79	5.015	96.795
	7	30.8	0.23	6.97	—	215.04	4.91	103.92
	8	31	0.31	6.86	—	198.06	2.88	108.63
	9	31.1	0.17	6.72	—	177.11	1.535	112.275
	10	31.2	0.21	6.56	—	163.58	1.29	117.055
	11	31.3	0.32	6.39	—	151.63	0.34	118.58
	12	31.3	0.18	6	—	137.36	0.19	122.84
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-02-15	0	28	0.17	7	—	282.175	1.81	72.78
T=32	1	28.7	0.11	7.04	—	288.225	3.45	69.99
Increase air flow to12 L/min	2	29	0.38	7.21	—	269.7	2.44	74.61
Decant volume = 90L	3	29.2	0.35	7.24	—	249.525	2.88	72.91
	4	29.4	0.2	7.18	—	228.675	3.44	76.13
	5	29.8	0.17	7.13	—	221.55	4.13	80.88
	6	30	0.24	7.07	—	202.275	4.17	81.45
	7	30.5	0.17	6.97	—	185.425	5.89	83.52
	8	30.9	0.18	6.85	—	175.825	3.97	86.41
	9	31.3	0.24	6.69	—	160.85	2.81	87.96
	10	31.4	0.27	6.49	—	142.325	2.41	93.02
	11	31.4	0.39	6	—	116.75	0.8	95.96
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-02-23	0	27.8	0.04	7.22	1005	284.97	3.12	50.13
	1							
Air flow = 12 L/min	2	28.4	0.36	7.37	—	264.21	2.71	50.76
T=32	3	28.9	0.25	7.4	—	259.89	2.52	53.04
Decant volume = 120L	4	29.5	0.3	7.37	—	238.77	2.28	53
	5	30	0.32	7.31	730	225.26	2.24	56.13
	6	30.3	0.23	7.25	—	205.54	1.97	59.84
	7	30.6	0.18	7.16	—	194.81	1.66	60.84
	8	30.8	0.29	7.06	—	178.14	1.61	62.8
	9	31	0.14	6.96	403.3	152.25	1.55	64.21
	10	31.2	0.25	6.85	—	148.99	1.29	65.14
	11	31.2	0.29	6.74	—	124.52	0.9	68.66
	12	31.3	0.34	6.57	—	106.91	0.62	69.3
	13	31.4	0.3	6.31	—	89.02	0.33	71.1
	14	31.5	0.16	6	68	78.49	0.57	73.17
	15	31.5	0.23	5.5	30	72.51	2.15	74.06
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-03-01	0	26.2	0.17	7.13	944	278.03	3.51	46.24
T=32	1	26.7	0.16	7.2	1092	272.91	2.16	45.07
Air flow = 14 L/min	2	27.3	0.36	7.41	1002	253.93	3.28	47.87
Decant volume = 120L	3	27.8	0.25	7.46	950.6	242.44	2.19	48.69
	4	28.2	0.38	7.46	873.3	236.53	1.85	50.69
	5	28.5	0.45	7.42	810	193.09	1.38	52.29
	6	28.9	0.33	7.36	740	188.17	1.59	53.5
	7	29.3	0.31	7.29	650.3	178.99	1.61	54.45
	8	29.5	0.27	7.2	564.5	166.57	1.45	58.05
	9	29.8	0.39	7.09	470	143.14	1	59.98
	10	30.2	0.54	6.94	360	124.5	0.72	59.98
	11	30.5	0.49	6.79	284	108.68	0.58	61.05
	12	30.8	0.33	6.61	200	94.34	0.51	61.69
	13	30.8	0.42	6.33	132.6	84.7	0.41	69.53
	13.5	30.8	0.28	6	64.6	66.71	1.6	70.28
	14.5	30.8	0.38	5.5	38	60.38	2.46	70.82

Test No.	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-03-12	0	26.8	0.08	7.39	1500	455.15	2.38	20.2
T=32	1	27.3	0.09	7.44	—	452.74	1.37	18.81
Decant volume = 190L	2	27.9	0.43	7.6	—	434.4	2.34	19.53
Air =12L/min	3	28.3	0.45	7.67	—	399.84	3.32	20.65
	4	28.8	0.48	7.68	—	356.23	3.97	21.97
	5	29	0.53	7.67	—	324.98	3.86	23.6
	6	29.4	0.47	7.65	—	310.35	3.71	26.68
	7	29.9	0.42	7.6	—	299.03	3.27	28.77
	8	30.1	0.54	7.55	—	275.43	2.6	31.67
	9	30.2	0.51	7.5	—	258.15	1.96	32.69
	10	30.4	0.47	7.44	823	244.88	1.88	37.15
	11	30.5	0.45	7.37	—	222.55	1.94	39.23
	12	30.5	0.48	7.32	—	192.6	2.1	46.18
	13	30.6	0.47	7.25	—	187.33	2.36	49.78
	14	30.8	0.42	7.18	—	175.95	2.4	52
	15	30.8	0.36	7.12	423.3	167.2	2.43	56.38
	16	30.9	0.42	7.05	—	148.25	1.5	58.64
	17	30.9	0.35	6.93	—	132.3	1.22	60.63
	18	31	0.4	6.8	—	118.75	0.93	62.43
	19	31	0.44	6.65	—	109.75	1.13	63.47
	20	31.2	0.42	6.46	—	94.99	1.35	64.78
	21.5	31.2	0.63	6	66.7	72.38	1.64	65.82
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-03-28	0	30.5	0.09	6.03		69.34	2.11	64.39
Decant volume = 190L	1	30	0.13	6.76		134.49	1.77	53.44
Air = 12L/min	2	29.8	0.27	7.16		154.86	1.83	52.4
Feeding rate= 500 ml/min	3	29.5	0.29	7.3		173.54	1.94	48.82
	4	29	0.24	7.47		221.89	2.3	40.06
	5	29.2	0.31	7.58		293.86	2.2	38.2
	6	29.6	0.44	7.65		338.56	1.73	35.61
	7	30.1	0.48	7.61		322.23	2.15	33.64
	8	30.5	0.44	7.56		288.35	1.78	32.45
	9	30.5	0.38	7.5		266.29	2.28	31.95
	10	30.6	0.39	7.44		249.22	1.86	33.7
	11	30.7	0.42	7.38		228.12	1.95	36.24
	12	30.8	0.48	7.32		219.87	1.86	41.7
	13	31	0.49	7.26		193.36	2.34	46.45
	14	31.1	0.37	7.2		163.27	1.87	49.99
	15	31.2	0.44	7.12		158.69	1.79	50.65
	16	31.2	0.46	7.03		149.2	1.89	55.49
	17	31.2	0.38	6.93		137.43	2.03	58.58
	18	31.2	0.42	6.8		127.66	1.85	59.01
	19	31.2	0.38	6.66		101.88	2.2	61.73
	20	31.2	0.33	6.48		83.06	1.72	61.91
	21.5	31.2	0.39	6		70.24	1.77	64.39
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-05-01	0	29.5	0.1	7.1		215.4	0.4	46.82
T=32	1	29.8	0.08	7.16		210.33	0.22	44.385
Airflow=12L/min	2	30	0.63	7.26		200.2	0.64	44.81
V=70L	3	30.3	0.51	7.24		184	0.705	49.22
	4	30.5	0.61	7.16		161.59	1.06	50.34
	5	30.7	0.48	7.07		149.42	0.405	50.035
	6	30.9	0.41	6.96		131.69	0.382	51.71
	7	31	0.52	6.81		123.95	0.355	53.59
	8	31.1	0.6	6.62		119.38	0.54	57.01
	9	31.2	0.61	6.35		100.21	0.54	60.5

Test No.	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-05-05	0	30.3	0.14	7	——	203.83	0.46	52.23
T=32°C	1	30.8	0.09	7.05	——	197.27	0.16	45.24
Air flow = 10L/min	2	31	0.42	7.15	——	191.74	0.42	44.39
Decant volume = 70L	3	31.2	0.4	7.16	——	188.84	1.08	45.82
	4	31.3	0.38	7.13	——	174.66	0.64	46.89
	5	31.3	0.36	7.08	——	160.26	0.43	47.44
	6	31.3	0.38	7	——	135.04	0.6	48.41
	7	31.3	0.32	6.88	——	111.08	0.5	50.21
	8	31.4	0.43	6.73	——	108.43	0.84	51.76
	9	31.6	0.45	6.58	——	97.02	0.38	52.88
	10	31.6	0.47	6.31	——	92.06	0.33	53.46
	11	31.6	0.58	6	——	89.43	0.34	54.68
	Time (h)	Temp (°C)	DO (mg/L)	pH	ALK (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
2012-05-10	0	30	0.13	7.2		308.82	1.14	45.94
T=32	1	30.2	0.14	7.31		325.22	0.18	43.43
Air=10L/min	2	30.5	0.52	7.56		316.87	0.26	46.59
Decent volume =120L	3	30.8	0.48	7.55		305.19	0.82	48.22
	4	31	0.46	7.5		305.25	1.46	50.48
	5	31	0.49	7.45		282.66	0.82	52.01
	6	31.1	0.5	7.4		267.43	0.91	53.89
	7	31.1	0.44	7.34		249.67	0.73	55.1
	8	31.2	0.48	7.28		224.15	0.52	56.32
	9	31.2	0.55	7.22		206.02	0.2	57.7
	10	31.2	0.53	7.14		198.31	0.19	58.55
	11	31.3	0.52	7.04		164.06	0.16	59.55
	12	31.6	0.62	6.9		161.31	0.2	63.26
	13	31.8	0.69	6.75		132.11	0.16	63.97
	14	32	0.7	6.6		117.22	0.31	66.42
	15	32	0.65	6.34		105.01	0.52	71.38
	16	32	0.75	6		94.2	1.63	74.43