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CULTIVATION AND APPLICATION OF ANAMMOX ORGANISMS FOR HIGH NITROGEN WASTEWATER TREATMENT

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A Thesis Presented

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Nutchanat Chamchoi

Abstract

The study consists of four experimental phases to investigate the cultivation and application of Anammox organisms for high nitrogen wastewater. Experimental phase I focused on the enrichment of Anammox culture from conventional seed sludges which are from upflow anaerobic sludge blanket reactor (UASB), activated sludge, and anaerobic digester. Anammox cultures were gradually developed within four months under strictly control environment in the sequencing batch reactors (SBR). The time sequences were 7 h react, 30 min settle, and 15 discharge. The development was observed through the near perfect nitrite removal and an 80% ammonium conversion which was further confirmed by the Fluorescene in situ hybridization (FISH) using PLA46 and Amx820 probes and the scanning electron microscope examination. An inoculation of Anammox seed sludge can accelerate the start-up operation to be within two-month time.

Experimental phase II emphasized on factors affecting Anammox operation. The optimum NH_4^+ to NO_2^- ratio was found close to the stoichiometric value of 1:1.32. The deviation caused a poor system performance either the left-over of NH_4^+ or NO_2^- concentrations in the effluent. Inhibition of Anammox activity was observed at ammonium or nitrite concentrations over 120 mg N l^{-1} and moderately higher for phosphate concentration at 170 mg P l^{-1}. Optimum specific removal rates were obtained near 0.05 g N.(g MLSS. d)$^{-1}$ at reaction time of 24 to 48 hours and sludge concentration of 1,000 mg MLSS l^{-1}. Concurrent operations of Anammox and denitrification were observed in experimental phase III in both SBR and UASB reactors. COD concentration and COD to N ratio were found to affect Anammox reaction by allowing the out-completion for denitrification, especially at COD concentration of greater than 400 mg l^{-1} and COD to N ratio of over 2. A greater tolerance was observed in SBR reactor with double in COD to N ratio. High COD loading up to 8 g l^{-1} cause a wipe-out of Anammox organism and could not be recovered over time.

Experimental phase IV decribed a kinetic model development based on Anammox activity and possibly denitrification with the presence of COD in wastewater. The models were well described system performance especially during steady state conditions but not acclimatization. This was due to a shift of microbial communities in favor Anammox organisms in the early stage when conventional seed sludges were used. This might take up to 4 months of process adjustment and could be shorter if Anammox seed was used.

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List of Abbreviations

Anammox	Anaerobic ammonium oxidation
AS	Activated sludge
ASM	Activated sludge models
Canon	Completely autotrophic nitrogen removal over nitrite
COD	Chemical oxygen demand
COD to N	Chemical oxygen demand to nitrogen
DEAMOX	Denitrifying ammonium oxidation
FISH	Fluorescene in situ hybridization
HRT	Hydraulic retention time
MATLAB	Matrix laboratory
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
ODE	Ordinary differential equation
OLAND	Oxygen-limited autotrophic nitrification-denitrification system
RPM	Round per minute
SBR	Sequencing batch reactor
SD	Standard deviation
SEM	Scanning electron microscopy
Sharon	Single reactor system for high ammonium removal over nitrite
SRT	Solid retention time
TIN	Total inorganic nitrogen
UASB	Upflow anaerobic sludge blanket
$K_{d\ AN}$	Decay coefficient
K_{sCOD}	Half velocity constant for COD under the concurrent operation of Anammox and denitrification
K_{sNH}	Half velocity constant for ammonium in Anammox
K_{sNO2}	Half velocity constant for nitrite in Anammox
K_{sNO3}	Half velocity constant for nitrate in Anammox
S_{COD}	Influent COD concentration
S_{NH}	Influent ammonium
S_{NO2}	Influent nitrite
S_{NO3}	Influent nitrate
X_{AN}	Anammox biomass
r_{AN}	Reaction rate of Anammox process
μ_m	Maximum specific growth rate

Chapter 1

Introduction

1.1 Rationale of the Study

Typically, nitrogen in wastewater can be eliminated by nitrification and denitrification processes. The recent discover of the new process for nitrogen removal "Anaerobic Ammonium Oxidation" (Anammox) provides benefits for wastewater treatment systems. In this process, ammonium is oxidized to dinitrogen gas (N_2) by particular microorganisms, Anammox bacteria, with nitrite as an electron acceptor (van de Graaf et al., 1995). This process requires no additional organic carbon source and the biomass yield is very low, so that it produces little sludge. It is known that Anammox takes place under anaerobic conditions. Therefore, it can save up to 90% of operational costs as compared to conventional nitrification/denitrification (Jetten et al., 2001).

A sequencing batch reactor (SBR) has been reported to have a high efficiency for Anammox enrichment and a good performance for nitrogen removal. According to the study of Fux et al. (2002), Anammox-SBR achieved over 90% ammonium removal. An upflow anaerobic sludge blanket (UASB) reactor provides high solid retention and mass transfer rate which would minimize sludge washout during initial stage and allow good substrate conversions. Experiments of Schmidt et al. (2004) showed that it was an appropriate reactor for the Anammox process. Both reactors were selected for Anammox applications in this study.

However, the enrichment of Anammox seed by using the sludge from conventional wastewater treatment is not frequently done. The study will provide potential use in practice if we can apply the sludge from various wastewater treatments for the Anammox seed enrichment. Therefore, this study was performed to gain a better understanding of the Anammox enrichment and its applications, which were divided in four phases. The first phase focused on Anammox seed enrichment from various conventional sludges in the sequencing batch reactors. It is known that Anammox bacteria can exist in various types of wastewater treatment systems. A mixture of bacteria populations from activated sludge was initially selected for Anammox enrichment due to its popularity for wastewater treatment. As Anammox takes place under anaerobic conditions, the remaining sludges were from an anaerobic digester and a UASB reactor. Acceleration of the Anammox process with Anammox seeding was also observed during the first phase. The second phase of the study was to investigate the effect of operational parameters on Anammox-SBR. The performance of Anammox-SBR is mostly dependent on the operational parameters. In this study, the time sequence of SBR operation was varied to find out the optimum one for nitrogen removal. It included the investigation of the effect of shock load nitrogen and phosphorus on the Anammox reaction. The optimum sludge concentration and reaction time that provided the good specific removal rate was also investigated in the second phase. The effect of chemical oxygen demand to nitrogen, COD to N ratio, on the Anammox reaction was considered in the third phase of study. Low ratios of COD to N can lead to poor denitrification, whereas relatively high COD loading

can detrimentally affect nitrification (Tseng et al., 1998). Generally, wastewater contains organic matter which is normally expressed as BOD or COD. High COD may affect Anammox activity and, accordingly, the Anammox process. Therefore, the investigation of the effect of variation of influent COD to N ratio on the Anammox performance was carried out in lab-scale SBR and UASBs. Finally, a mathematical model for describing system behavior on nitrogen removal of Anammox-SBR and the application in UASB reactor was proposed in the fourth phase of study.

1.2 Objectives of the Study

1. To study Anammox enrichment from various conventional sludges in Thailand such as UASB sludge, activated sludge, and anaerobic digestion sludge.
2. To study the acceleration of Anammox seed sludge on system start-up and operation.
3. To find out the effect of operational parameters on Anammox-SBR and propose the optimum operational parameters for good performance of Anammox-SBR for high nitrogen removal.
4. To investigate the effect of COD on the Anammox process running on the SBR and UASB reactors.
5. To observe the co-removal of COD and nitrogen in SBR and UASB reactors and propose the applicable COD to N ratio for the concurrent operation of Anammox and denitrification.
6. To develop a mathematical model for describing system behavior on nitrogen removal of Anammox-SBR and the application in UASB reactor.

1.3 Scope of the Study

The study was separated into four phases, as follows:

Phase I: Anammox seed enrichment from conventional sludges and acceleration of the Anammox process using Anammox seeding - Lab-scale experiment

Phase II: Operational parameters effect on Anammox-SBR - Lab-scale experiment

Phase III: Co-removal of COD and nitrogen in Anammox-SBR and Anammox-UASB - Lab-scale experiment

Phase IV: Mathematical model describing system behavior on nitrogen removal of Anammox-SBR and the application in UASB reactor - Computer work

Chapter 2

Literature Review

2.1 Introduction

Nitrogen in wastewater treatment effluent can cause a serious problem to the receiving watercourse, such as eutrophication. Conventionally, treatment of nitrogen can be achieved by nitrification followed by a denitrification process. Recent findings indicate that nitrogen removal can also be accomplished through "ANaerobic AMMonium OXidation" (Anammox) under anaerobic conditions. The uncovered process can save up to 90% of operation cost as compared to typical nitrogen treatment processes (Jetten et al., 2001).

Nitrogen removal in terms of anaerobic ammonium oxidation (Anammox) has been discovered in a denitrifying fluidized bed reactor (Mulder et al., 1995). The Anammox system has been gradually unveiled ever since for its beneficial use for advanced wastewater treatment. These include the attempt to cultivate the Anammox biomass (van de Graaf et al., 1996; Strous et al., 1998; Fujii et al., 2002; Toh et al., 2002; and Jianlong and Jing, 2005), the study of physiology and characteristics of the bacteria, the interest to develop a microbial technique for detecting and identifying it (Neef et al., 1998; Schmid et al., 2000, 2001, and 2003), and system performance using various reactors (Strous et al., 1997a; Helmer et al., 2001; van Dongen et al., 2001a; Fux et al., 2002; Sliekers et al., 2003; Dapena-Mora et al., 2004a).

According to the Anammox stoichiometry shown in Equation 2.1, the theoretical rate of nitrite to ammonium consumption is 1.32, while the rate of nitrate formation to ammonium consumption is 0.26 (Strous et al., 1998). In this process, ammonium is oxidized to dinitrogen gas with nitrite as the electron acceptor. No organic carbon source is needed and the biomass yield is very low, so that it produces little sludge.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (2.1)$$

2.2 Sequencing batch reactor

The sequencing batch reactor (SBR) is one of the biological treatment systems, which has been successfully used to treat both domestic and industrial wastewater, and is suitable for low or intermittent flow conditions. The term sequencing batch reactor stems from the sequence of steps that the reactor goes through as it receives wastewater, treats it, and discharges it, since all steps are accomplished in a single tank (Grady et al., 1999). All SBR systems have five steps in common, which are carried out in sequence as follows: (1) fill, (2) react (aeration), (3) settle (sedimentation/clarification), (4) draw (decant), and (5) idle (Metcalf & Eddy, 2003). Each of these steps is illustrated in Figure 2.1 and described in Table 2.1.

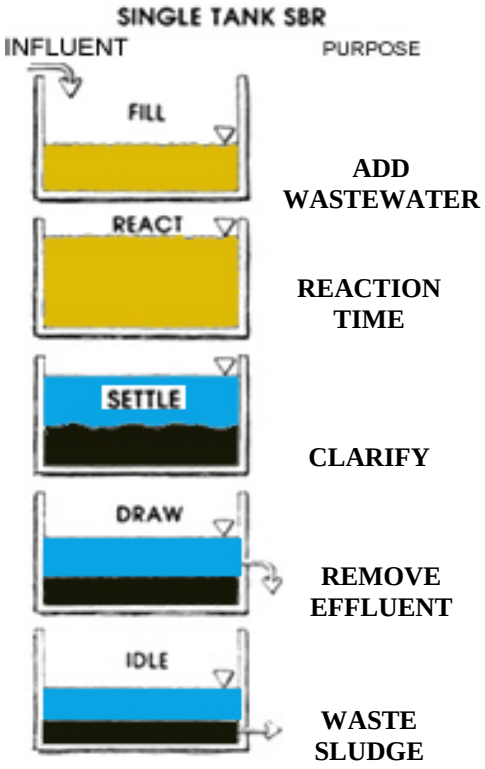


Figure 2.1 SBR operating cycles

Table 2.1 Description of operational steps for the sequencing batch reactor (SBR)

Operational step	Description
Fill	During the fill operation, volume and substrate (raw wastewater or primary effluent) are added to the reactor. The fill process typically allows the liquid level in the reactor to rise from 75% of capacity (at the end of the idle period) to 100%. When two tanks are used, the fill process may last about 50% of the full cycle time. During fill, the reactor may be mixed only or mixed and aerated to promote biological reactions with the influent wastewater.
React	During the react period, the biomass consumes the substrate under controlled environmental conditions.
Settle	Solids are allowed to separate from the liquid under quiescent conditions, resulting in a clarified supernatant that can be discharged as effluent.
Decant	Clarified effluent is removed during the decant period. Many types of decanting mechanisms can be used, with the most popular being floating or adjustable weirs.
Idle	An idle period is used in a multitank system to provide time for one reactor to complete its fill phase before switching to another unit. Because idle is not a necessary phase, it is sometimes omitted.

Source: Metcalf & Eddy (2003)

Time control sequence in the operation systems is an important issue in design of the SBR performance efficiency. Actually, reaction, i.e., biomass growth and substrate utilization, also occur during the fill period. This period is dependent on the influent flow rate. Reaction period is determined for special objectives in treatments. In the conventional SBR, generally, included with aeration, but the development is changed to variation operations depend upon the designer. The time allocated to react is sufficient to achieve the desired level of effluent quality. If the reactor is design for treating nutrients particularly nitrogen and phosphorus, the reaction period will be long to provide a complete reaction. After the reaction phase, there is some sludge, which requires time to settle. It can be noted that a long settle period may make the sludge bulk. One hour is usually designed for the settle period. After the settle finished, the clarified effluent is discharged from the reactor. An idle period is necessary in two stage-SBRs or continuous flow. Sometimes the idle period is determined together with the decant period.

2.3 Biological nitrogen treatment

In a wastewater treatment system, nitrogen can be removed by primary sedimentation and biological treatment, 20% each. The remaining 60% is generally discharged to the receiving waters (Tchobanoglous et al., 1998). The well-known mechanisms for biological nitrogen removal are nitrification followed by denitrification. Anammox is the new mechanism used to eliminate nitrogen in wastewater.

2.3.1 Nitrification

In nitrification, ammonia is oxidized in a two-step process: first to nitrite and then to nitrate. The biomass synthesis and overall reactions are expressed by Equation 2.2-2.6.

Oxidation of ammonium to nitrite:

$$NH_4^+ + 1.5O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2H^+ + H_2O \qquad (2.2)$$

Oxidation of nitrite to nitrate:

$$NO_2^- + 0.5O_2 \xrightarrow{\text{Nitrobacter}} NO_3 \qquad (2.3)$$

Overall ammonium oxidation reaction:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \qquad (2.4)$$

Biomass synthesis from ammonium:

$$4CO_2 + HCO_3^- + NH_4^+ + H_2O \longrightarrow C_5H_7O_2N + 5O_2 \qquad (2.5)$$

Overall oxidation and biomass synthesis from nitrification:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \longrightarrow 0.021\ C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O + 1.88H_2CO_3 \qquad (2.6)$$

Autotrophic organisms involved in these reactions are called nitrifying bacteria. Nitrifying bacteria are sensitive organisms and extremely susceptible to a wide variety of inhibitors. The factors affecting them, also affect the nitrification process and include:

(1) Temperature

The activity of nitrifying bacteria decreases at lower temperatures.

(2) Concentration of ammonia and nitrite

High concentrations of ammonia and nitrous acid can be inhibitory.

(3) The ratio of BOD_5 to TKN

The activities of nitrifying bacteria are limited at a ratio of 5 or higher.

(4) Concentration of dissolved oxygen

At a DO level in the range of 0.3-0.5 mg l^{-1}, the growth rate of nitrifying bacteria may be insufficient and nitrification may not occur. A minimum DO level of 2.0 mg l^{-1} is recommended for process design.

(5) pH

Nitrification will stop at a pH below 6.3. A pH in the range of 7.2-8.6 is suitable for nitrification.

Alkalinity is also consumed during the nitrification process (Equation 2.5 and 2.6). Approximately 7.1 g of alkalinity as $CaCO_3$ is required per gram of NH_4^+-N oxidized (Qasim, 1999).

Nitrification can be achieved in both suspended growth and attached growth processes. In the suspended growth process, nitrification can be achieved either in a separate suspended growth reactor following a conventional activated sludge treatment process, or in the same reactor used in the treatment of carbonaceous organic matter. In the same way, nitrification can be provided in attached growth reactors in both the same and in separate reactors. The wastewater treatment systems, which can accomplish nitrification, include trickling filters, rotating biological contactors, and submerged packed-bed reactors. In small systems, the single reactor is commonly used.

2.3.2 Denitrification

The biological conversion of nitrate to nitrogen gas is achieved in anoxic conditions, in which nitrate serves as the electron acceptor. Denitrification is carried out by denitrifying bacteria which obtain energy for growth from the conversion of nitrate to nitrogen gas. However, internal or external carbon sources are required for cell synthesis because the nitrified effluents are usually low in carbonaceous matter. Methanol is an example of one popular external carbon source. The biomass synthesis and overall reactions of denitrification are described in Equation 2.7-2.11.

Reduction of nitrate:

$$6NO_3^- + 2CH_3OH \longrightarrow 6NO_2^- + 2CO_2 + 4H_2O \quad (2.7)$$

Reduction of nitrite:

$$6NO_2^- + 3CH_3OH \longrightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^- \quad (2.8)$$

Overall reduction of nitrate:

$$6NO_3^- + 5CH_3OH \longrightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \quad (2.9)$$

Biomass synthesis:

$$3NO_3^- + 14CH_3OH + CO_2 + 3H^+ \longrightarrow 3C_5H_7O_2N + 19H_2O \quad (2.10)$$

Overall energy reaction and synthesis:

$$NO_3^- + 1.08CH_3OH + H^+ \longrightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \quad (2.11)$$

The variables affecting the denitrification process include:

(1) DO level

Denitrification process will be inhibited at a DO concentration of 0.1-0.2 mg l^{-1}.

(2) pH

The desired range of pH for denitrification is 6.5-8.0. Alkalinity is recovered by this reaction. It is approximately 3.57 gram per gram of NO_3^--N reduced (Qasim, 1999).

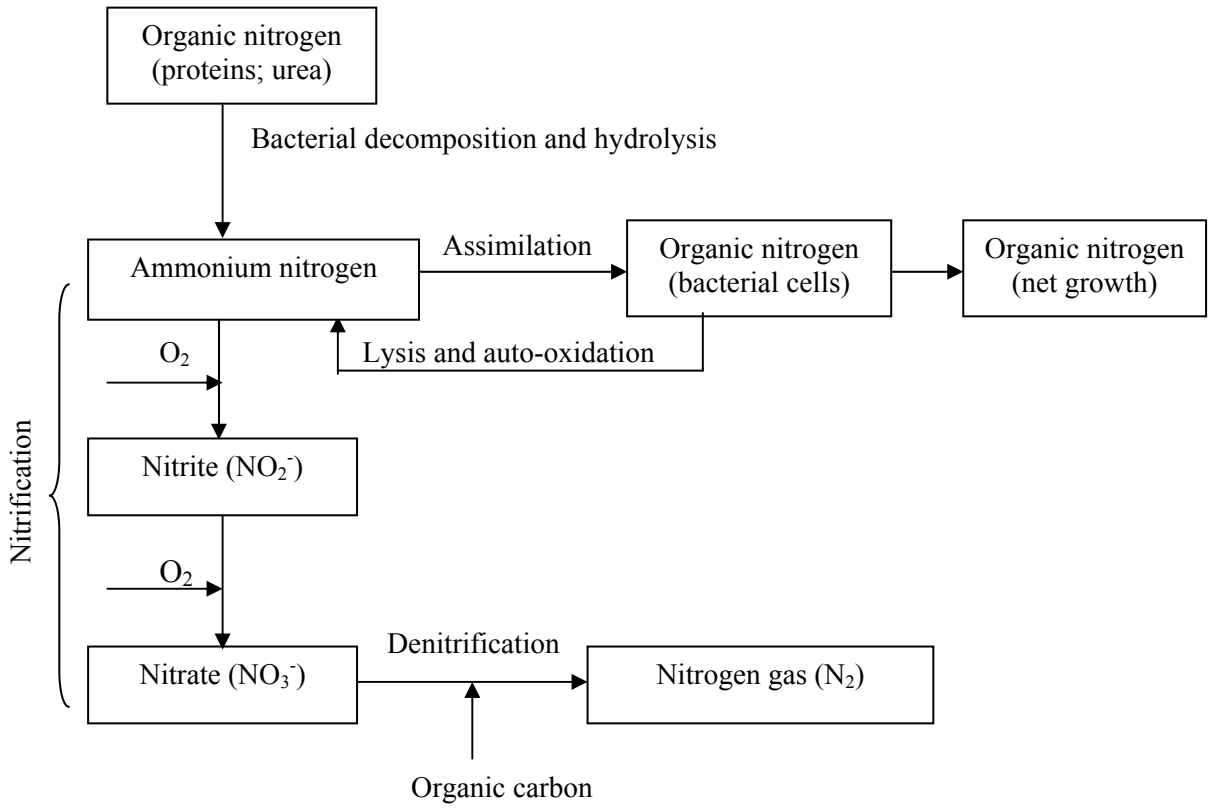
(3) Temperature

The growth rate of denitrifying bacteria decrease with a decrease in temperature.

Denitrification reactions can be achieved in both suspended growth and attached growth reactors. Suspended growth denitrification is usually carried out in a plug flow type of activated sludge system (Tchobanoglous et al., 1998). Denitrification can be classified as single sludge system and separate sludge system together. A single sludge system is the combination of carbon oxidation and nitrification/denitrification, which uses the incoming raw wastewater as the sole organic carbon source. A separate sludge system is the separate stage denitrification using a suitable external organic carbon source.

The overall denitrification rate in a single sludge system would be lower than in a separate sludge system. This is because the heterotrophic population in a single sludge system with internal recycle is alternated between anoxic and aerobic environments.

The two principal mechanisms, nitrification and denitrification for biological nitrogen removal are illustrated in Figure 2.2



Source: Tchobanoglous et al. (1998)

Figure 2.2 Definition sketch for the transformation of various forms of nitrogen in biological treatment processes

2.3.3 Combined Nitrification-Denitrification

Combined nitrification-denitrification can be achieved in a single reactor or a series of reactors that create aerobic and anoxic conditions. The advantages of this combination include:

(1) Reduction in the volume of air needed to achieve nitrification and BOD_5 removal.
(2) Elimination of the external organic carbon sources requirement.
(3) Elimination of intermediate clarifiers.
(4) Improved settling and process stability.

Many processes have been developed to achieve a combined nitrification-denitrification, such as oxidation ditch, bardenpho process, wuhrmanr process, ludzack-ettinger process, sequencing batch reactor, alternation aeration systems, and attached growth processes (Qasim, 1999).

2.4 Novel processes related Anammox

2.4.1 Partial nitrification

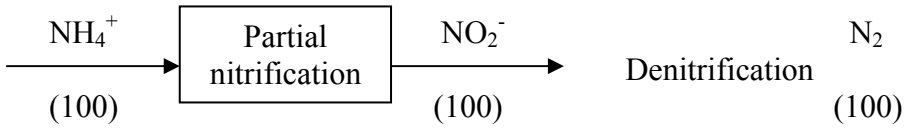
Partial nitrification is the oxidation of ammonium in wastewater to nitrite but not to nitrate as in Figure 2.3 and the Equation 2.12.

$$NH_4^+ + 1.5\ O_2 \longrightarrow NO_2^- + 2\ H^+ + H_2O \qquad (2.12)$$

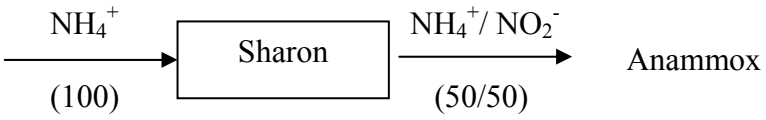
Partial nitrification is carried out by nitrifying bacteria. This process needs less aeration, and the subsequent denitrification consumes less COD, since only nitrite and not nitrate has to be reduced to molecular nitrogen (N_2). So, partial nitrification requires low C:N ratio. This is cost-effective because the external carbon source is not necessary.

To achieve partial nitrification, the subsequent oxidation of nitrite to nitrate must be prevented. It can be done in six principal ways. First, by using of the difference in activation energy between ammonia and nitrite oxidation. The activation energy of ammonia and nitrite oxidation is 68 and 44 KJ mol^{-1}, respectively (Schmidt et al., 2003). The high activation energy of ammonia oxidation makes the rate of partial nitrification more dependent on temperature. Then, activation energy brings about the creation of Sharon process. Second, by providing low oxygen concentrations, such as less than 0.4 mg l^{-1} or 5% air saturation (Schmidt et al., 2003). At this DO level and with surplus ammonium, nitrite oxidizers are unable to grow so that nitrite becomes the end product of the reaction. The third clue is the substrate concentration. However, it is not an operational parameter because it is the objective variable in terms of wastewater treatment. The remaining ways to achieve partial nitrification are also to provide appropriate conditions for inhibiting nitrite oxidizing microorganisms such as pH, temperature, and sludge retention time. The pH regulates the equilibrium between nitrite (NO_2^-) and nitrous acid (HNO_2). The pH values > 7 have the double effect of limiting the conversion of nitrite into nitrous acid and also inhibiting the ammonium oxidizers and providing surplus ammonia or free ammonia. Surplus ammonia will inhibit nitrite oxidizers. Thus, pH > 7 is the favourite for partial nitrification. pH and temperature also affect the formation of free ammonia by involving the ionization of ammonia (Jianlong et al., 2003). Temperature and sludge retention time, are also important operational parameters. At temperatures higher than 30 °C combined with pH values > 7, nitrite oxidizing organisms have slower growth rates than ammonium oxidizers (Pollice et al., 2002). These conditions provide the appropriate biomasses, which are capable of partial nitrification. Achieving the appropriate biomasses can be done by operating on the average cell residence time in the aeration tank such as the sludge age or sludge retention time (SRT). Temperature and sludge retention time, are considerations in the development of Sharon process for partial nitrification.

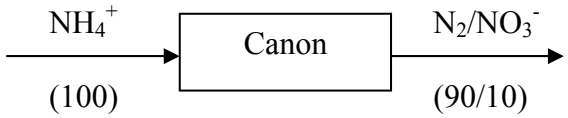
1a. Partial nitrification



1b. Partial nitrification (Sharon)



2. Canon



Source: Schmidt et al. (2003)

Figure 2.3 Flux diagrams of the partial nitrification (1a.), Sharon (1b.), and Canon (2.) (< number >) N-compound in % (values idealized; they may vary depending on process parameter) *In the presence of oxygen the supplemented NO_2 acts as regulatory signal (not as a substrate), inducing the denitrification activity of the aerobic ammonia oxidizers.

The main advantages of partial nitrification are the lower oxygen demand of up to 25% energy savings during aeration, reduced organic substrate requirements for heterotrophic denitrification of up to -40%, lower biomass production of up to -300%, and increased denitrification kinetics (Pollice et al., 2002). However, partial nitrification still has a problem. It is the accumulation of nitrite and its presumed toxic effect on the biomass even at relatively low concentrations of 10-30 mg NO_2^--N l^{-1} (Pollice et al., 2002).

2.4.2 Sharon

Partial nitrification can be applied in the Single reactor High activity Ammonia Removal Over Nitrite, so called Sharon process. It was developed at the Technical University of Delft. The principle is based on a short-circuit in the denitrification path way (Verstraete et al., 1998). It means that this process makes use of the different growth rates of ammonia and nitrite oxidizers at sufficiently high temperatures (more than 26 °C). The hydraulic retention time is lower than the growth rate of nitrite oxidizers but higher than ammonia oxidizers (about 1 day). In addition, the process has no sludge retention, so the nitrite oxidizers are not able to remain in the Sharon reactor and they are washed out. (Schmidt et al., 2003). The limitation of the Sharon process is that it is not suitable for all

wastewater due to its dependency on high temperature. But many wastewaters that contain high levels of ammonium also have a high temperature, such as sludge liquor. So, this is not a serious problem of the Sharon reactor. The point that should be considered is that there is no sludge retention and the hydraulic retention time in Sharon is fixed. So, the volumetric ammonium reactor loading depends on the ammonium concentration. It shows that the process costs will rise if the ammonium concentration decreases. Another interesting point of Sharon is that it still makes use of denitrification clue, which includes external carbon source adding such as methanol. This is supplied periodically while the aeration is switched off. Aeration is given for oxygen supply and to strip CO_2 from the reactor. The stripping of CO_2 is to control the pH. Both CO_2 stripping and methanol adding neutralize all protons formed in the reaction. Sharon had been scaled-up and applied successfully at the Rotterdam wastewater treatment plant to achieve the sludge liquor treatment. The 1,500-m^3 reactor operated for 2 years and treated 1,000 kg N d^{-1} (Schmidt et al., 2003)

2.4.3 Canon

Canon is an acronym for Completely Autotrophic Nitrogen removal Over Nitrite. The aerobic ammonia oxidizers and anaerobic ammonia oxidizers simultaneously oxidize ammonia to dinitrogen gas and give a small amount of nitrate (Figure 2.3). The equation below describes these reactions.

$$NH_3 + 0.85\ O_2 \longrightarrow 0.11\ NO_3^- + 0.44\ N_2 + 0.14\ H^+ + 1.43\ H_2O \qquad (2.13)$$

The process can be achieved in a single reactor, at oxygen limited condition, without the production of N_2O or NO (Sliekers et al., 2002). This concept can be viewed as the combination of partial nitrification and Anammox process in a single, aerated reactor (Schmidt et al., 2003). The performance of the Canon process depends on the competition among the involved organisms, such as nitrite oxidizers, ammonium oxidizers, and Anammox organisms. Nitrite oxidizers should be out competed by ammonium oxidizers and Anammox organisms. Since the autotrophic organisms involved in the Canon process have different growth rates and temperature coefficient, change of temperature can result in changing the process performance. The dissolved oxygen and ammonium surface load (ASL) are also identified as the key process factors governing the behaviour of a Canon process (Hao et al., 2002).

The Canon process appeared to be particularly suitable for the removal of ammonia from wastewater that does not contain enough organic material to support the conventional nitrification/denitrification process. In order to maintain the oxygen limitation in practice, the ammonia influx to such reactors is maintained higher than the oxygen influx. Since Canon requires only one reactor, it might be advantageous in terms of economics when the daily ammonium load is low. It can achieve NH_4^+ loading 2-3 kg N $m^{-3}_{reactor}$ day^{-1}, while Anammox process can removed in higher NH_4^+ loading as 10-20 kg N $m^{-3}_{reactor}$ day^{-1} (Schmidt et al., 2003). However, it would need process control to prevent nitrite build-up by oxygen excess.

Canon has not been purposefully tested at pilot or full-scale level, but is known to occur accidentally in sub-optimally functioning full-scale nitrification system (Schmidt et al., 2003). It has been tested extensively in laboratory-scale experiments, with the gas-

lift reactor and sequencing batch reactor. Relatively low N-conversion rates have been reached in laboratory-scale Canon sequencing batch reactors. It was evident that the gas liquid mass transfer of oxygen was the rate-limiting step in the reactors. However, from the studies of Sliekers et al. (2002), Canon process in SBR showed 85% conversion of ammonia to N_2 and the remaining 15% was recovered as NO_3^-, while the N_2O production was negligible at less than 0.1%. Gas-lift reactors are reported to have a relatively high gas-liquid mass transfer of oxygen (Sliekers et al., 2003).

2.4.4 OLAND

A new nitrogen removal process has been developed to treat these highly loaded wastewaters. The process in which NH_4^+ is autotrophically oxidized to N_2 with NO_2^- as the electron acceptor under oxygen-limited conditions is further referred to as oxygen-limited autotrophic nitrification denitrification (OLAND) (Kuai and Verstraete, 1998).

This autotrophic process consumes 63% less oxygen and 100% less biodegradable organic carbon compared to the conventional nitrification–denitrification process and has, therefore, a lower operating cost (Verstraete and Philips, 1998). The OLAND process was first described for a mixed culture of nitrifying bacteria (Kuai and Verstraete, 1998), but was afterwards examined in more detail in a mixed community biofilm of a lab-scale rotating biological contactor (RBC) (Pynaert et al., 2002a, b, 2003, 2004). A mature OLAND biofilm under high NH_4^+ loading rate consists primarily of two major groups of bacteria responsible for autotrophic N removal. The aerobic ammonium oxidizing bacteria (AerAOB, Nitrosomonas sp.) convert NH_4^+ to NO_2^- with oxygen as the electron acceptor (nitritation) and the anaerobic ammonium oxidizing bacteria (AnAOB, a close relative of Kuenenia stuttgartiensis) subsequently oxidize NH_4^+ with NO_2^- as the electron acceptor (Anammox) (Strous et al., 1998; Pynaert et al., 2003; Wyffels et al., 2003). The Schematic presentation of the OLAND concept is depicted in Figure 2.4.

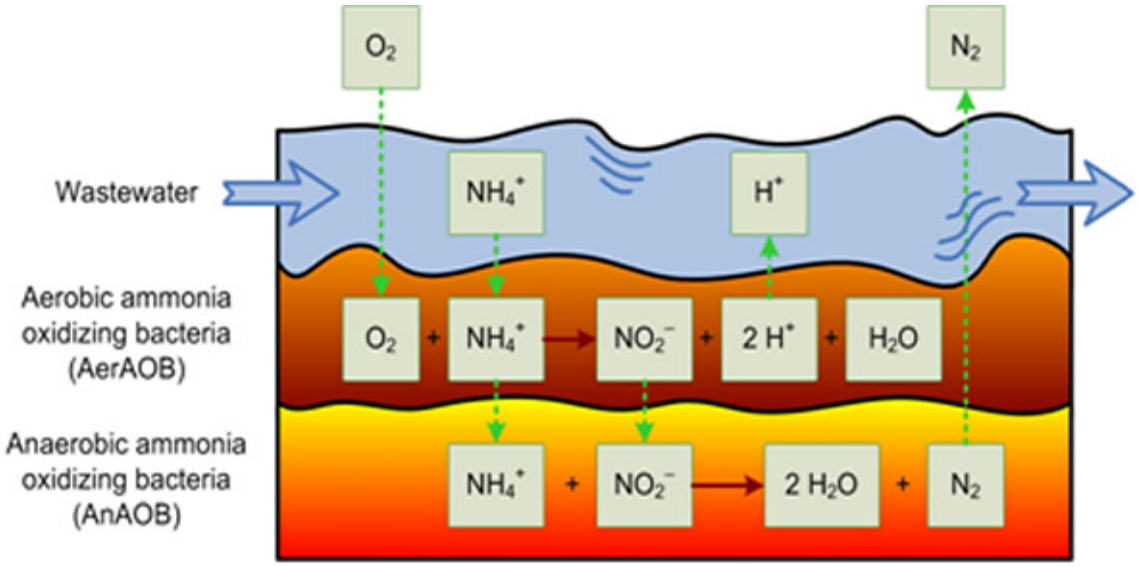
2.4.5 DEAMOX

A new process called DEAMOX (Denitrifying AMmonium OXidation) was proposed recently (Mulder, 2004) to realize the Anammox process under autotrophic denitrifying conditions. The principal flow diagram of this process, as well as the major biochemical reactions involved, is given in Figure 2.5.

The essential distinguishing characteristics of this innovative process compared to the current anammox applications are the following: (a) nitrite is produced mainly from nitrate using sulfide as an electron donor; and (b) the DEAMOX-reactor is partially fed directly with anaerobic effluent from the pre-treatment (nitrogen mineralization) step; the distribution ratio of anaerobic/aerobic flows is determined by the composition of the wastewater, especially by the electron donor concentrations (sulfide, ammonium).

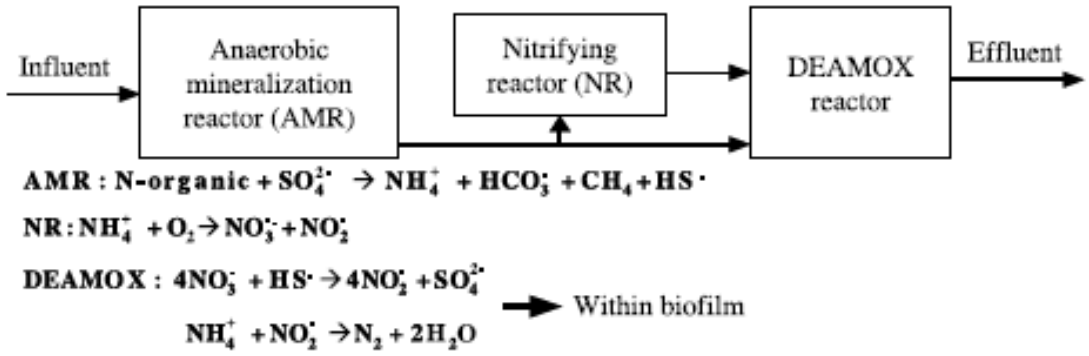
The DEAMOX process configuration has several major advantages, which are summarized below:

(a) no complex process control is required for the production of nitrite;
(b) the denitrifying conditions in the DEAMOX reactor will enhance the growth of granules stimulating the development of the Anammox process; and
(c) the absence of high nitrite levels, which may be toxic, reactive and result in the unwanted emission of NO_x gases, which are greenhouse gases.



Source: Verstraete et al. (2001)

Figure 2.4. Schematic presentation of the OLAND concept



Source: Kalyuzhnyi et al. (2006)

Figure 2.5 Flow diagram of the DEAMOX-process and major biochemical reactions involved

2.5 Anammox process description

The possible pathway of Anammox process (Figure 2.6) is the biological oxidation of ammonium with hydroxylamine as the electron acceptor by hydrazine hydrolase, the hydrazine forming enzyme (Jetten et al., 1999; van Niftrik et al., 2004). The hydrazine oxidizing enzyme, which has some similarity to hydroxylamine oxidoreductase, is the responsible enzyme for hydrazine oxidation to dinitrogen gas (Jetten et al., 1999; Schmidt et al., 2003; van Niftrik et al., 2004). The oxidation of hydrazine is supposed to generate four electrons that combine with five protons of nitrite reducing enzyme for initial reduction of nitrite to hydroxylamine (Kuenen and Jetten, 2001; Schmidt et al., 2003; van Niftrik et al., 2004). In theory, the mole ratio of ammonia and nitrite in Anammox catabolism is 1:1.3 (Strous et al., 1998), not 1:1. The excess 0.3 mol of nitrite is anaerobically oxidized to nitrate that yields the electron for CO_2 fixation or reduction to the level of oxidation of cell material for cell growth (Orhorn and Artan, 1994; van de Graaf et al., 1996; Strous et al., 1998; van Dongen et al., 2001a; Fux et al., 2002; Schmidt et al., 2002; Schmidt et al., 2003). This catabolism takes place inside the anammoxosome (van Niftrik et al., 2004).

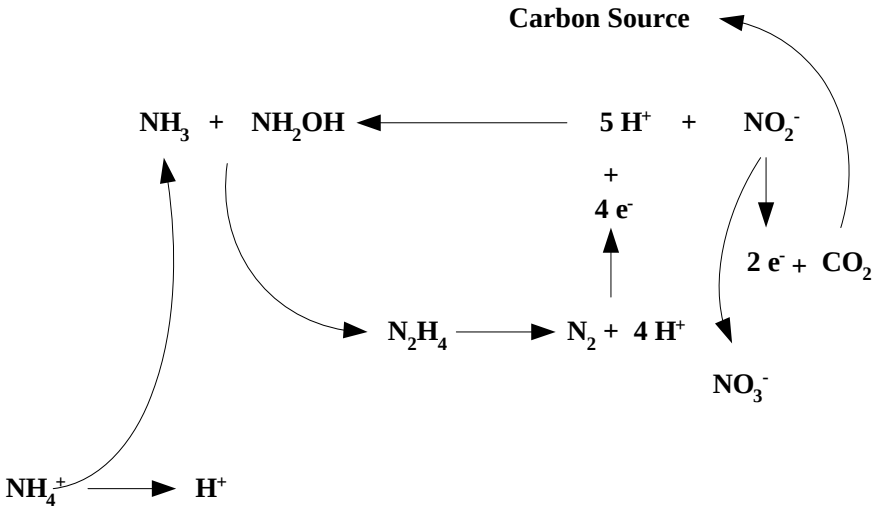


Figure 2.6 Mechanism of Anammox pathway

2.5.1 The Anammox bacteria

2.5.1.1 Characteristics

Anammox have been mediated by a group of planctomycete bacteria (Strous et al., 1999a). Two of which have been named provisionally; *Candidatus* 'Brocadia Anammoxidans', and *C.* 'Kuenenia Stuttgartiensis' (Schmidt et al., 2003), are an interesting group of bacteria with many rare or unique properties. Schmid et al. (2003) further purposed the new species of Anammox, *C.* 'Scalindua brodae', and 'Scalindua wagneri' discovered in a wastewater treatment plant treating landfill leachate in Pitsea, UK, and *C.* 'Scalindua sorokinii' detected in the water column of the Black Sea. A phylogenetic tree reflecting the relationships of the Anammox, other Planctomycetales, and

other reference organisms is shown in Figure 2.7. Based on this phylogenetic analysis, the discovered Anammox organisms branched deep in the *Planctomycetes* phylum.

Anammox are coccoid bacteria with a diameter of less than 1 μm (van Niftrik et al., 2004). They have a doubling time of 10-30 days and are physiologically distinct from the other known Planctomycetes: they are anaerobic chemolithoautotrophs. According to Schmidt (2003), Anammox bacteria have a doubling time of 11 days and the biomass yield of 0.13 g dry weight per g NH_3-N oxidized.

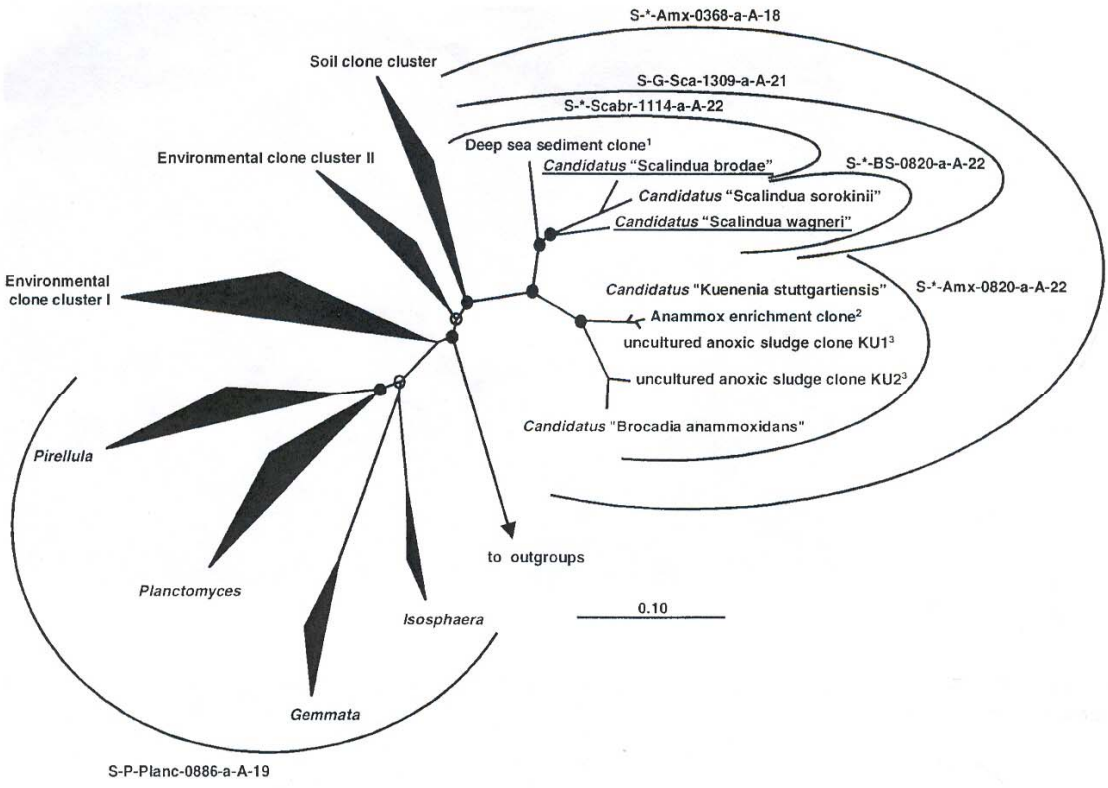


Figure 2.7 16S rRNA gene-based phylogenetic tree reflecting the relationship of Anammox organisms to other *Planctomycetes* and other reference organisms (Schmid et al., 2003)

Anammox bacteria plays a significant role for anaerobic ammonium oxidation not only in the wastewater treatment process. They have been reported recently as being responsible for the nitrogen cycle in a range of environments including marine sediments, sea ice and anoxic water columns (Dalsgaard et al., 2005). In terms of nitrogen removal, they can oxidize ammonium with nitrite as the electron acceptor to yield dinitrogen gas. Hydrazine (N_2H_4) and hydroxylamine (NH_2OH) are referred as intermediates of their reaction. The Anammox reaction takes place inside the Anammoxosome. Anammoxosome is an intracytoplasmic compartment bounded by a single ladderane lipid-containing membrane. The structure of the ladderane membrane lipids is unique in nature. Ladderane membrane lipids have so far been found only in Anammox bacteria. They contain one, two or both of two different ring-systems, X and Y (Figure 2.8). Ring-system X is composed of three cyclobutane moieties and one cyclohexane moiety substituted with an octyl chain,

which is ether-bound at its ultimate carbon atom to the glycerol unit. Ring-system Y is composed of five linearly concatenated cyclobutane rings substituted with a heptyl chain, which contains a methyl ester moiety at its ultimate carbon atom. All rings in ring-system X and Y are fused by *cis*-ring junction, resulting in a staircase-like arrangement of the fused rings, defined as ladderane (van Niftrik et al., 2004).

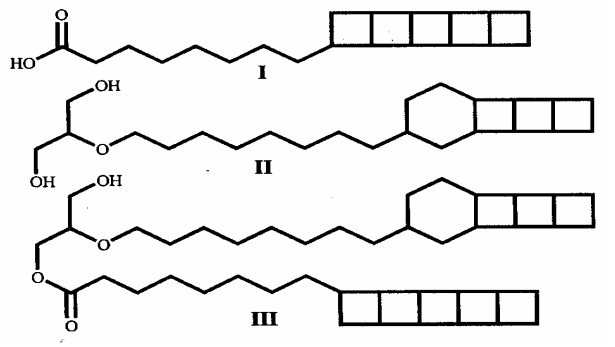


Figure 2.8 Structures of three characteristic ladderane lipids: I ladderane fatty acid-containing ring-system Y. II ladderane monoalkyl glycerol ether-containing ring-system X. III ladderane glycerol ether/ester containing both ring-systems, X and Y (van Niftrik et al., 2004).

The major characteristics of Anammox bacteria compared with the other two groups of bacteria capable of nitrogen removal are summarized in the Table 2.2.

2.5.1.2 Cell biology

Anammox are gram-negative planctomysises bacteria. Like other planctomycetes, these bacteria have a differentiated cytoplasm, with different membrane-bound 'organelles'. The inner three compartment cytoplasms are separated by a single bilayer membrane. The outer cytoplasm is the paryphoplasm that compart by the two outer membranes, i.e., the cell wall and the cytoplasmic membrane, and the inner intracytoplasmic membrane. The middle inside riboplasm is the cytoplasm that compart by outer intracytoplasmic and inner anammoxozome membrane. The inner cytoplasm, the anammoxozome, where supposedly the Anammox reaction takes place, is bounded by the anammoxozome membrane (Figure 2.9). The membrane surrounding the anammoxosome consists of ladderane lipids, unique in biology. Such lipids contain multiple concatenated cyclobutane rings as described above in the topic 2.6.1.

Table 2.2 The major characteristics of Anammox bacteria compared with Proteobacterial ammonia oxidizers and Aerobic nitrite oxidizers (Strous et al., 1998; Strous et al., 1999b; Jetten et al., 1999; Schmidt et al., 2003)

Major characteristics	Bacteria capable of nitrogen removal		
	Proteobacterial ammonia oxidizers	Aerobic nitrite oxidizers	Anaerobic ammonia oxidizers
1. Doubling time, d	30	-	11, 1.8[a]
2. Biomass yield, g dry weight (g NH_3-N)$^{-1}$	0.13 ± 0.019	0.036[b]	0.13
3. K_s, μM	20	-	≤ 5
4. pH	6.7 – 8.3	-	6.7 – 8.3
5. Temperature, °C	-	-	20 - 43°C
6. Maximum growth rate, h^{-1}	-	0.04	0.0027
7. Apparent activation energy, kJ mol^{-1}	70[c]	44	70[c]
8. Inhibitor / Irreversibly	Carbon compounds	Hydroxylamine Free ammonia[d] NO	Oxygen Nitrite[e] Phosphate[f] Free ammonia[g]
9. Ammonia consumption ratio	-	-	1 : 1.3

[a] reported by Isaka et al. (2005)
[b] g dry weight (g NO_2-N)$^{-1}$
[c] kJ (mol NH_3)$^{-1}$
[d] 1-5 mg l^{-1}
[e] at concentration in excess of 70 mg N l^{-1} for several days
[f] > 60 mg P l^{-1} for several days
[g] 10-150 mg l^{-1}

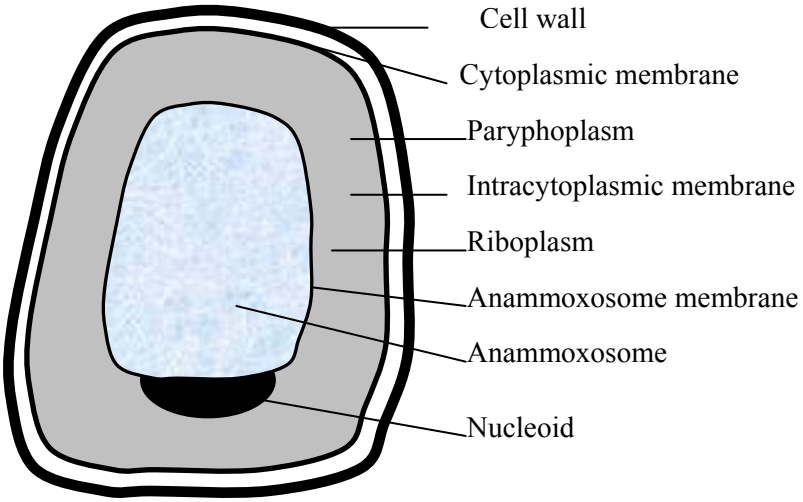


Figure 2.9 Cellular compartmentalization of Anammox bacteria (van Niftrik et al., 2004)

2.5.1.3 Kinetics and modelling

A few studies have been conducted on modelling of the Anammox process during start-up or long term dynamics. Koch et al. (2000) simulated the model using the data of a lab-scale batch experiment, and the maximal growth rate of Anammox bacteria of 0.081 d^{-1}, but the model did not fit the measured data. Hao et al. (2002) described a simulation work on the behavior of a partial nitrification-Anammox in biofilm process under different variation conditions of temperature, ammonium surface load, inflow, and dissolved oxygen concentrations. The simulation results showed that variable inflow or dissolved oxygen concentration negatively affect the nitrogen removal efficiency and the small range of dissolved oxygen concentration of ±0.2 g O_2 m^{-3} has no significant influence on the process performance. However, their results cannot be interpreted as being fully quantitatively correct because no verification with real experimental data was performed in the study. The modelling of Anammox in SBR by Dapena-Mora et al. (2004b) showed that the simulations can be used to predict the experimental data in relation to the nitrogenous compounds concentration and used to estimate the evaluation of Anammox and heterotrophic biomass in the reactor. The simulations also reveal that heterotrophs still remain in the system after the start-up of the reactor and can protect the Anammox organisms from a negative effect of the oxygen. A stoichiometric matrix expressed in Peterson matrix format and the additional Monod term used to describe an eventual inhibition of the Anammox organisms by oxygen were mentioned in the reported of Dapena-Mora et al. (2004b) as shown in Table 2.3 and Equation 2.14.

The kinetics of the Anammox for nitrogen removal in a fluidised bed reactor have been reported in terms of sludge specific activity and reactor capacity of 0.15 kg total-N $(kg\text{-}VSS)^{-1}$ d^{-1} and 1.5 kg total-N $m^{-3}_{reactor}$ d^{-1} (Strous et al., 1997b). The affinity constants for the substrates ammonium and nitrite are each less than 0.1 mg N l^{-1} (Strous et al., 1999). The maximal growth rate and endogenous respiration rate of Anammox in a biofilm reactor of 0.028 and 0.001 d^{-1} were referred to in the simulation work of Hao et al. (2002). Stoichiometric parameters of the Anammox process in different reactors, which

include gas-lift, SBR, and canon reactor in terms of nitrite consumed to ammonium consumed (mol/mol) and nitrate produced to ammonium consumed (mol/mol) are shown in Table 2.4. Nitrite to ammonium consumption ratios of 1.28, 1.11-1.45, 1.40-1.50, and 1.00-1.18 were recorded for gas-lift, SBR, fluidised bed, and fixed bed reactor, respectively. Fluidised bed fed with sludge digestion effluent provided a low ratio of 0.06-0.55. Nitrate produced to ammonium consumed was recorded for all reactors in the range of 0.20-0.31 whereas the lower ratio of 0.07 was obtained from gas-lift operated under oxygen limited condition (Table 2.4).

Table 2.3 Stoichiometric matrix for the Anammox process in Peterson matrix format (Dapena-Mora et al. (2004b)

Name: Symbol: Unit:	Ammonium S_{NH} gN m^{-3}	Nitrite S_{NO2} gN m^{-3}	Nitrate S_{NO3} gN m^{-3}	Nitrogen gas S_{N2} gN m^{-3}	Anammox X_{AN} gCOD m^{-3}	Slowly degradable substrate X_S gCOD m^{-3}	Inert X_i gCOD m^{-3}	Process rate ρ gCOD $m^{-3}d^{-1}$
Growth of X_{AN}	$-1/Y_{AN}-i_{nbm}$	$-(1.52 + 1/Y_{AN})$	1.52	$2/Y_{AN}$				ρ_{growth}
Decay of X_{AN}	$i_{nbm} - f_i\, i_{Xp}$				-1	$(1 - f_i)$	f_i	ρ_{decay}

$$\rho_{growth} = \mu_{AN} \cdot \frac{K_{O,AN}}{K_{O,AN} + S_O} \cdot \frac{S_{NO2}}{K_{NO2,AN} + S_{NO2}} \cdot \frac{S_{NH}}{K_{NH,AN} + S_{NH}} \cdot X_{AN} \qquad (2.14)$$

2.5.1.4 Inhibitory substances

Inhibitory substances and conditions that affect Anammox organisms include main substrates such as nitrite and ammonia, exogenous compounds, and environmental conditions. The Anammox process was completely inhibited by nitrite concentrations higher than 0.1 g N l^{-1}; however, the addition of trace amounts of either of the Anammox intermediates, 1.4 mg N l^{-1} of hydrazine or 0.7 mg N l^{-1} of hydroxylamine, restored activity completely (Strous et al., 1999b). Schmidt et al. (2003) further reveal that nitrite loading to Anammox process should not be overloaded because high nitrite concentrations for extended periods is detrimental to the Anammox bacteria. More than 70 mg NO_2-N l^{-1} is detrimental to *Candidatus* Brocadia anammoxidans, and more than 180 mg NO_2-N l^{-1} is harmful to *Candidatus* Kuenenia stuttgartiensis. Recently, Dapena-Mora et al. (2007) found that the nitrite concentration of 25 mM corresponded to the 50% inhibition concentration (IC_{50}). Free ammonia should not be more than 150 mg l^{-1} or can be varied in the range of 10-150 mg l^{-1} (Jetten et al., 1997). The present work of Dapena-Mora et al. (2007) reveal that ammonia concentration of 55 mM had no effect on the Anammox activity based on IC_{50} evaluation.

Table 2.4 Stoichiometric parameters of Anammox process combined in different reactors

Reactors	NO_2^- consumed to NH_4^+ consumed mol /mol	NO_3^- produced to NH_4^+ consumed mol /mol	References
Gas-lift	1.28	0.26	Dapena-Mora et al., 2004a
SBR	1.11	0.20	Dapena-Mora et al., 2004a
SBR	1.30	0.26	Strous et al., 1998
SBR	1.45	0.30	Sliekers et al., 2004
Gas-lift*	-	0.07	Sliekers et al., 2003
SBR	1.29	0.31	Fux et al., 2002
SBR	1.25	0.28	Sliekers et al., 2002
Fluidised bed	1.40-1.50	-	Strous et al., 1997b
Fixed bed	1.00-1.18	-	Strous et al., 1997b
Fluidised bed**	0.06-0.55	-	Strous et al., 1997b

* Under oxygen limited condition
** Feed with sludge digestion effluent

Exogenous compounds effect on the Anammox process mostly come from the industrial and domestic effluents, including COD, acetate, phosphate, nitrate, sulphide, salts, flocculant, allylthiourea, chloramphenicol, and methanol. An earlier study of van de Graaf et al. (1996) showed that the specific inhibitors of hydrazine, acetone, N-serve, allylthiourea) did not affect the Anammox activity, but acetylene inhibited the process by 87% compared with the control in the study. Experiments of Dong and Tollner (2003) showed that high concentration of COD was not suitable for treating with the Anammox process as Anammox was less competitive to other kinds of bacteria like anaerobes. Acetate of 25-50 mM inhibited the Anammox activity; the higher the concentration the higher the inhibiting effect (Dapena-Mora et al., 2007). Phosphate present in more than 60 mg P l^{-1} for several days inhibit Anammox activity and the Anammox process (Schmidt et al., 2003). However, the work of Dapena-Mora et al. (2007) showed that phosphate could be present in concentrations up to 15 mM without causing significant Anammox activity decrease. Nitrate concentration of 45-70 mM was also found to have no effect on the Anammox activity (Strous et al., 1999b; Dapena-Mora et al., 2007). The inhibitive effects on Anammox activity based on nitrogen gas production, evaluated by Dapena-Mora et al. (2007), further showed that 0.3 mM of sulphide results in increasing of inhibitive effect on the Anammox activity. The concentration of salts, NaCl below 150 mM did not affect the

activity while KCl and Na_2SO_4 had effect only at concentrations higher than 100 and 50 mM, respectively. Flocculant had no physical detrimental effect on the Anammox activity while allylthiourea was a very specific inhibitor to the nitrification but not Anammox, and chloramphenicol concentrations up to 1 g l^{-1} did not show inhibitive effects Anammox activity (Dapena-Mora et al., 2007). The process was made irreversible by methanol at the concentrations as low as 0.5 mM (Guven et al., 2005).

Thermal pretreatment has been reported to reduce both dinitrogen production and ammonium removal in the ammonium oxidation process. Chemical pretreatment by using HCl and NaOH increased dinitrogen production and ammonium removal by 45% and 55% over the control (Lin and Lee, 2002). Alkali addition was shown to be more efficient than acid addition in enhancing both values. The influence of oxygen on the Anammox process has been investigated by Strous et al. (1997a), due to this process being combined with a preceding partial nitrification step. Their study indicated that the Anammox process is inhibited reversibly by the presence of oxygen.

The environmental conditions of the process, such as pH and temperature, also affect Anammox bacteria and consequently affect the Anammox process. The pH should be in the range of 7.0-8.5 (Table 2.5). Temperatures suitable for Anammox bacteria, which play a dominant role in the process, can be varied from 28 to 37 °C (Table 2.4) (Jetten et al., 1997; Strous et al., 1997b; Helmer et al., 2001; van Dongen et al., 2001a; Dapena-Mora et al., 2004a).

In order to enrich the Anammox organisms, a medium containing ammonium, nitrite, and bicarbonate is needed since bicarbonate serves as the sole carbon source and also is used as a buffer. Moreover, the reactor used to achieve Anammox process should be well mixed to keep the redox potential in the denitrification zone and prevent the formation of toxic sulfide (Schmidt et al., 2003). Inhibitory substances of Anammox bacteria and affecting factors of the Anammox process are summarized in Table 2.6.

Table 2.5 Optimum pH and temperature for the Anammox process

pH	Temperature, °C	References
7.0 – 8.5	30 - 37	Jetten et al., 1997; van Dongen et al., 2001a
7.8 ± 3	32 ± 2	Strous et al., 1997b
8.0 ± 0.1	30	Dapena-Mora et al., 2004a
8.0	28	Helmer et al., 2001

Table 2.6 Inhibitory substances of Anammox bacteria and affecting factors of Anammox process

Inhibitory substances / Affecting factors of Anammox process	Effect to Anammox process	References
1. Nitrite concentration		
0.1 g N l^{-1}	Completely inhibited the process	Strous et al., 1999b
>70 mg NO_2^--N l^{-1}	Detrimental to Anammox bacteria	Schmidt et al., 2003
>180 mg NO_2^--N l^{-1}		
25 mM	Loss of Anammox activity	Dapena-Mora et al., 2007
2. Free ammonia		
>150 mg l^{-1}	Inhibit the process	Jetten et al., 1997
55 mM	No effect	Dapena-Mora et al., 2007
3. COD		
2.2-5.4 g l^{-1}	Not suitable for the process	Dong and Tollner, 2003
4. Acetate		
25-50 mM	Inhibit Anammox activity	Dapena-Mora et al., 2007
5. Phosphate		
>60 mg P l^{-1}	Inhibit the process	Schmidt et al., 2003
15 mM	No effect	Dapena-Mora et al., 2007
6. Nitrate		
70 mM	No effect	Strous et al., 1999b
45 mM	No effect	Dapena-Mora et al., 2007
7. Sulphide		
0.3 mM	Inhibit Anammox activity	Dapena-Mora et al., 2007
8. Salts		
8.1 NaCl		
<150 mM	No effect	Dapena-Mora et al., 2007
8.2 KCl		
>100 mM	Inhibit Anammox activity	Dapena-Mora et al., 2007
8.3 Na_2SO_4		
-	No effect	van de Graaf et al., 1996
>50 mM	Inhibit Anammox activity	Dapena-Mora et al., 2007
9. Flocculant	No effect	Dapena-Mora et al., 2007
10. Allylthiourea	Inhibit to nitrification but not Anammox	Dapena-Mora et al., 2007

Table 2.6 Inhibitory substances of Anammox bacteria and affecting factors of Anammox process (cont.)

Inhibitory substances / Affecting factors of Anammox process	Effect to Anammox process	References
11. Chloramphenicol		
-	No effect	van de Graaf et al., 1996
1 g l^{-1}	No effect	Dapena-Mora et al., 2007
12. Methanol		
0.5 mM	Irreversibly inhibit to the process	Guven et al., 2005
13. Thermal pretreatment	Reduce N_2 production	Lin and Lee, 2002
14. Chemical pretreatment	Increase N_2 production and ammonium removal	Lin and Lee, 2002
15. Oxygen	Reversibly inhibitor	Strous et al., 1997a
16. pH		
7.0-8.5	Optimum for the process	Jetten et al., 1997; Strous et al., 1997b; Helmer et al., 2001; van Dongen et al., 2001a; Dapena-Mora et al., 2004a
17. Temperature		
28-37 °C	Optimum for the process	Jetten et al., 1997; Strous et al., 1997b; Helmer et al., 2001; van Dongen et al., 2001a; Dapena-Mora et al., 2004a

2.5.1.5 Confirmation techniques

To confirm the active Anammox organisms in the environment or the wastewater treatment process, three techniques have been studied: the Fluorescene In Situ Hybridization (FISH), Scanning Electron Microscopy (SEM), and Hydroxylamine Test. FISH analysis was not only used to confirm or detect the Anammox organisms in a range of environments and biomass from wastewater treatment process, but also to study a new type of Anammox bacteria. In situ hybridization results of environmental samples from Neef et al. (1998) indicated widespread presence of planctomycetes in different ecosystems. Toh et al. (2002) revealed that the dominant cell type enriched in the experiment had a similar 16S rDNA sequence homology to that of the recently described Anammox organisms, *Candidatus* Brocadia anammoxidans. The hybridization of the biomass with the probe Pla46 confirmed the presence of bacteria of the order

Planctomycetales, and with the probe Amx820 specifically detected *C.* Brocadia anammoxidans (Third et al., 2005). Schmid et al. (2003) used FISH to identify the Anammox organisms and proposed the new species, which considerably extends the biodiversity of the Anammox lineage on the 16S rRNA gene level. The obtained probe design has proven beneficial for other researcher in many fields of study. Identification of such organisms as typical Anammox bacteria can be confirmed with scanning electron microscopy. Jianlong and Jing (2005) observed the morphology of the inner structure of the Anammox incorporated by granular sludge in an EGSB reactor using SEM (Figure 2.10). The Hydroxylamine Test has been used to prove that Anammox bacteria were responsible for nitrogen conversion in the SBRs (Figure 2.11) (van Dongen et al., 2001b).

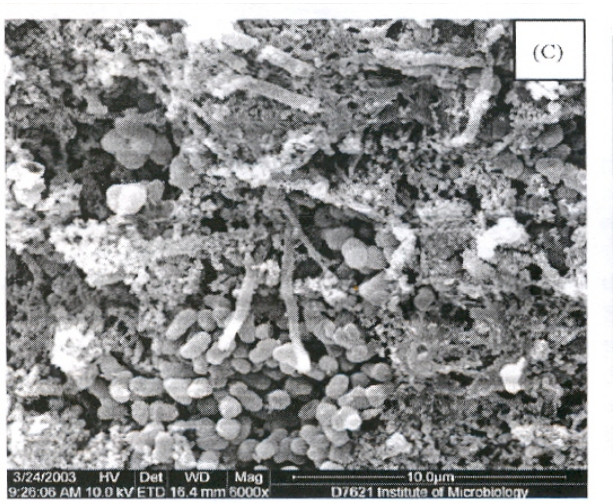


Figure 2.10 Scanning electron micrographs of an inner structure of Anammox incorporated by granular sludge (Jianlong and Jing, 2005)

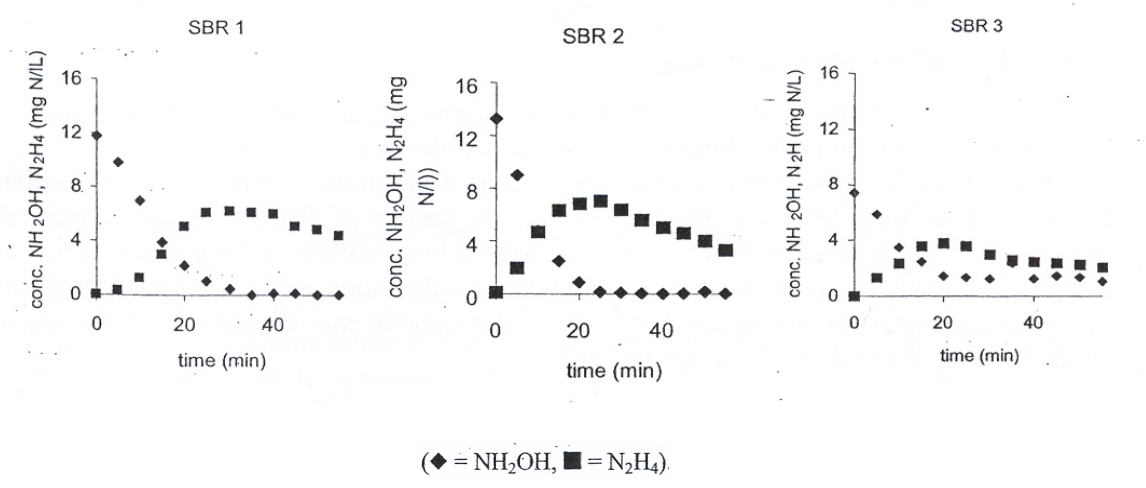


Figure 2.11 Results of hydroxylamine tests in SBR 1, 2, and 3 (van Dongen et al., 2001b)

2.5.2 Process applications

2.5.2.1 Wastewater

The Anammox process targets wastewaters that contain much nitrogen and few organic materials. Wastewater containing high nitrogen concentration are sludge digester effluent, piggery manure, poultry waste, landfill leachate, industrial wastewater (including food and ago-industry), pharmaceutical industry, tanneries, slaughterhouse waste processing, alcohol and starch production, and formaldehyde production. Septage, which is a product from septic tanks, contains high ammonia nitrogen and has been treated by Anammox process in a UASB reactor (Lin and Lee, 2002). Wastewater produced in food and ago-industry were treated using sludge digestion. The effluent from sludge digestors generally contains high ammonium concentrations up to 2 kg m^{-3} (Jetten et al., 1997; Strous et al., 1997b; Jetten et al., 1999). This kind of wastewater is suited for the Anammox process. Sharon effluent can be also used as influent for the Anammox process operating in both fluidized bed and SBR (Jetten et al., 1999; van Dongen et al., 2001a; Fux et al., 2002). Since the Anammox process has almost always been studied in lab-scale, the synthetic wastewater has also been used for easily controlling the process operation in various reactors (Table 2.7).

Table 2.7 Wastewater used for Anammox process in various reactors

Wastewater	Reactors	References
1. Septage	UASB	Lin and Lee, 2002
2. Sludge digester effluent	Sharon- Anammox Fluidized bed	Jetten et al., 1997; Strous et al., 1997b; Jetten et al., 1999
3. Food and ago-industry wastewater	Fixed-bed & fluidised-bed	Strous et al., 1997b
4. Poultry manure	Anaerobic digestion	Dong and Tollner, 2003
5. Synthetic wastewater	Fixed-bed	Strous et al., 1997b
	Fluidised-bed	Strous et al., 1997b
	SBR	Strous et al., 1998; Dapena-Mora et al., 2004a
	Gas-lift	Dapena-Mora et al., 2004a
6. Sharon effluent	SBR	van Dongen et al., 2001a; Fux et al., 2002

Poultry waste has been studied by using the Anammox process compared with the classical nitrogen removal process of nitrification followed with denitrification. However, the Anammox process was shown to be less competitive than the classical nitrogen removal in terms of poultry wastes treatment (Dong and Tollner, 2003). This raises the question of whether COD affects the Anammox process. Because poultry waste

also contains high COD concentration, when the Anammox process is used to accomplish nitrogen removal, it also needs to achieve COD removal at the same time. So, the question is whether the Anammox process can be used for COD and nitrogen removal at the same time and in the same tank. Nevertheless, the upper and lower limit of COD to nitrogen ratio in the influent, which will determine the success of nitrogen removal by Anammox process, is an interesting point for Anammox study. Recently, experiments of Waki et al. (2007) showed that the wastewater from animal waste treatment processes were suitable for Anammox treatment. However, the results did not show clearly an Anammox reaction, which suggests the presence of an inhibitory factor.

2.5.2.2 Cell cultivation

The Anammox cultures are very slowly growing microorganisms. According to the study of Jetten et al. (2001), the doubling time of Anammox is 10.6 days, while 11 days was reported by Schmidt et al. (2003). The advantage of this process is the low amount of excess sludge, while the disadvantage is the long start-up time (van Dongen et al., 2001a).

The very slow growing Anammox organism cannot be cultivated using conventional microbiological techniques (Fujii et al., 2002). To apply the Anammox process, the choice of reactor type is very important. It should be suited for the long term enrichment, cultivation and quantitative analysis (Strous et al., 1998). The biofilm or granular sludge reactor can be best for the Anammox process (Strous et al., 1997b; van Dongen et al., 2001a). Various reactors were successfully used to develop Anammox activity, such as a fluidized bed reactor (Mulder et al., 1995; van de Graaf et al., 1996), a rotating biological contractor (RBC) (Egli et al., 2001), a gas-lift reactor (Sliekers et al., 2003; Depena-Mora et al., 2004a), and a sequential batch reactor (SBR) (Strous et al., 1998; van Dongen et al., 2001b; Fux et al., 2002). Among them, the SBR was well accepted for the Anammox enrichment for its simplicity, efficient biomass retention, homogeneity of mixture in reactor, stability and reliability for a long period of operation, stability under substrate-limiting condition and high nitrogen conversion (Strous et al., 1998; Jetten et al., 1999; van Dongen et al., 2001b).

The various sources of biomass used for inoculation of the reactor were the Anammox seed sludge from previous Anammox studies (Strous et al., 1997b and 1998; Sliekers et al., 2002 and 2003; Depena-Mora et al., 2004a), the excess or recycle sludge from an activated sludge treatment plant (van Dongen et al., 2001a; Fux et al., 2002; Dong and Tollner, 2003), and the sludge from a laboratory scale fill and draw denitrification reactor (Fujii et al., 2002). A full-scale Anammox reactor can be enriched from activated sludge fed with sludge water, thus no large Anammox sludge quantity of lab-grown inoculum is needed (van Dongen et al., 2001a). The experiment conducted by Toh et al. (2002) revealed that the Anammox consortium was successfully enriched from municipal treatment plant sludges, but not from industrial coke-oven wastewater sludges.

Synthetic wastewater was also used for acclimatization or studies of Anammox process in pilot or research scale. The composition of synthetic wastewater included mineral medium as a nutrient; $KHCO_3$ 1.25, KH_2PO_4 0.025, $CaCl_2.2H_2O$ 0.3, $MgSO_4.7H_2O$ 0.2, $FeSO_4$ 0.00625, EDTA 0.00625 g l^{-1} and 1.25 ml of trace element solution; and NH_4Cl with $NaNO_2$ as substrate at various concentrations (Sliekers et al., 2002). The 10 mM of nitrate were fed during the startup period, before Anammox activity

was observed, to prevent sulphate from reducing to sulphide which is toxic to Anammox bacteria (van Dongen et al., 2001a). However, it was impossible to start up a full-scale Anammox process by synthetic wastewater. In such a case, effluent from partial nitrification process or sludge water can be used (van Dongen et al., 2001a). The gas mixture of helium or Ar/CO_2 was used to maintain the anaerobic condition and prevent the rapid increasing of pH in the reactor (van Dongen et al., 2001a).

2.5.2.3 Stability and performance

The Anammox process has been applied with various reactor types such as fixed bed, fluidized bed, SBR, gas-lift and Sharon. The efficiency of the Anammox process in terms of ammonium removal in each reactor type was in the range of 76-99% (Table 2.8). The combined Sharon-Anammox process has been successfully tested by using sludge digester effluent with a total nitrogen load of 0.8 kg N m^{-3} d^{-1} and the ammonium removal efficiency of about 76-90% (Jetten et al., 1997 and 1999). According to the study of Jetten et al. (1997) and Strous et al. (1997b), the pH of 7.0-8.5 and temperature of 30-37°C (Table 2.4) were optimum for the process. Experiments with a laboratory scale fluidized bed reactor showed that the Anammox biomass was capable of removing ammonium from the sludge digester effluent with the nitrogen conversion rate of 0.04-0.26 kg N_{tot} (kg SS)$^{-1}$ d^{-1}. The Anammox fluidized bed reactor with synthetic wastewater feeding could remove 5.1 kg N_{tot} $m^{-3}_{reactor}$ d^{-1} (Jetten et al., 1997). Strous et al. (1997b) reported that a fixed bed reactor fed with synthetic wastewater provided 88% ammonium removal, whereas a fluidized bed reactor fed with synthetic wastewater and sludge digestion effluent provided 84% and 82% ammonium removal, respectively. The maximum nitrogen conversion capacity was 0.7 kg NH_4^+-N $m^{-3}_{reactor}$ d^{-1} and 1.5 kg total N $m^{-3}_{reactor}$ d^{-1}(Strous et al., 1997b). The performance of Anammox process in moving-bed biofilm systems was studied by Helmer et al. (2001). The conditions suited for their study included two important parameters, such as pH value of 8 and the temperature of 28 °C, with the HRT of 8 h. It showed the ammonium removal efficiency of 98.9%. Fux et al. (2002) studied the feasibility of nitrogen removal from digester effluents using the sequencing batch reactor. Their results showed that the anaerobic ammonium oxidation was achieved with a nitrogen elimination rate of 2.4 kg N $m^{-3}_{reactor}$ d^{-1} and over 90% ammonium removal.

The stability of the Anammox process to achieve nitrogen removal from poultry manure compared with the classical nitrogen removal process of nitrification followed with denitrification was studied by Dong and Tollner (2003). Anammox could achieve only about 13-22% ammonium removal, while the total ammonium reduction was not proportional to the reduction of nitrite. This was due to Anammox organisms evidently developing at a very slow rate, and/or not being able to compete with the denitrifying organisms in the anaerobic digester for nitrite consumption. Dapena-Mora et al. (2004a) also studied the stability and performance of Anammox process in a gas-lift reactor and sequencing batch reactor. They found that the gas-lift reactor and SBR could be used to carry out the Anammox process for high NLRs up to 2.0 g l^{-1} d^{-1}. The problem stated in the study was that the reduction on the settling ability of the granular sludge appeared when the specific NLRs applied to each reactor exceeded the corresponding specific Anammox activity of the biomass. It caused the decreasing of the settling ability of the sludge due to nitrogen gas accumulation with the consequent washout form gas-lift reactor. In the case of the SBR, the problem occurred from the mechanism of effluent withdrawal. The maximum specific Anammox activity (MSAA) was 0.9 and 0.44 g g^{-1} d^{-1}, respectivelt for biomasses

from the gas-lift reactor and the SBR. The authors noted that flotation of biomass occurred most likely due to a granule density decrease caused by dinitrogen gas accumulation inside the granules and an apparent breakage of the granules. Therefore, the process requires a balance between gas production and release.

Table 2.8 Ammonium removal efficiency of Anammox process in different reactor types

Reactor	Inlet	Ammonium removal efficiency, %	References
Sharon	Sludge digestion effluent	76-90	Jetten et al., 1997 and 1999
Fluidized bed	Sludge digestion effluent Synthetic wastewater	82-88 84	Jetten et al., 1997; Strous et al., 1997b
Fixed bed	Synthetic wastewater	88	Strous et al., 1997b
Moving bed biofilm	Synthetic wastewater	98.9	Helmer et al., 2001
SBR	Sludge digestion effluent Synthetic wastewater	90 78	Fux et al., 2002; Dapena-Mora et al., 2004a
Gas-lift	Synthetic wastewater	88	Dapena-Mora et al., 2004a

2.5.3 Concluding remarks

To apply the Anammox process, the choice of reactor type is very important. It should be suited for the long time enrichment, cultivation and quantitative analysis. The SBR was well accepted for the Anammox enrichment for its simplicity, efficient biomass retention, homogeneity of mixture in reactor, stability and reliability for a long period of operation, stability under substrate-limiting condition and high nitrogen conversion (Strous et al., 1998; Jetten et al., 1999; van Dongen et al., 2001b). The upflow anaerobic sludge blankets (UASB) reactor is currently suited for anaerobic digestion and may be suitable for Anammox operation as it offers integral sludge retention, high space-loading, low footprint and good resistance to shocks and toxins (Schmidt et al., 2004).

As Anammox organisms cannot be cultivated using conventional microbiological techniques, enrichment using various conventional sludges will provide benefit for practical use and full-scale process operation.

To accelerate the Anammox process, the Anammox seeding may be necessary. Suitable environments and optimum substrate are also important for Anammox growth.

The suitable environment to support the Anammox growth include a temperature of 30-37 ºC and a pH of 7-8.5 (Jetten et al., 1997). Substrate needed for Anammox growth includes ammonium and nitrite. Nitrate can also be added in the feed to reduce the use of nitrite by co-existing bacterial group in the system, denitrifying bacteria. The environmental parameters influencing Anammox activity and enrichment should be further investigated for better understanding the process.

The effects of COD on the Anammox reaction should be investigated since the Anammox process may be used for COD and nitrogen removal at the same time and in the same tank. The upper and lower limit of COD to nitrogen ratio in the influent, which will provide the succession of nitrogen removal by Anammox process, is an interesting point for further Anammox study.

Three techniques can be used for confirmation of the successful Anammox enrichment: Fluorescene In Situ Hybridization (FISH), Scanning Electron Microscopy (SEM), and the Hydroxylamine Test.

Chapter 3

Methodology

3.1 Experimental phase Ia : Anammox seed enrichment using conventional sludges

3.1.1 Influent and seed sludge

The synthetic wastewater consisted of mineral media which include $NaHCO_3$, KH_2PO_4, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, $FeSO_4$, EDTA, trace element solution I (including EDTA and $FeSO_4$), and trace element solution II (including EDTA, $ZnSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, and H_3BO_3) (Table 3.1). The formula were based on the previous studies (van de Graaf et al., 1996; Sliekers et al., 2002; Dapena-Mora et al., 2004a). This mineral medium was added with nitrite and ammonium solution to support Anammox activity in the form of $NaNO_2$ and $(NH_4)_2SO_4$, respectively. The quantities of nitrite and ammonium were varied with the growth of Anammox organism during the experiment. The fresh synthetic wastewater was prepared daily to avoid the change in feed composition due to biological activity or any other interfering factors. The NH_4^+-N to NO_2^--N ratio in the feed was maintained at 1:1.5. This ratio was found to be appropriate for optimum NO_2^--N removal in this study. It was slightly higher than the previous reported optimum ratio of 1:1.32 (Strous et al., 1998). The pH in the synthetic wastewater was in the range of 7.7-8.4.

A mixed culture of microorganisms from excess sludges of UASB, activated sludge process and anaerobic sludge digester was used as seed sludge. These were taken from the wastewater treatment plant of Boonrod Brewery company, Asiangame plant in Thammasat University, and Nongkham wastewater treatment plant, respectively. Acclimatization was obtained by feeding the sludge with synthetic wastewater for several months and the reactor performance was monitored. The conditions of the SBR reactor were varied according to the optimum growth of Anammox organism. The initial concentration of biomass expressed as mixed liquor suspended solid (MLSS) was 1,500 mg l^{-1}.

3.1.2 Reactors

The SBR was used for Anammox enrichment and the configuration is depicted schematically in Figures 3.1 and 3.2. The reactors were cylindrical shape made of acrylic plastic, covered with a water jacket for temperature controlling. The working volume of the reactor was 7 liters. The reactors were provided with cover to maintain anaerobic environment and drilled at appropriate positions for sludge and samples collections.

Table 3.1 The composition of synthetic wastewater used during the first phase of experiment (Sliekers et al., 2002; Dapena-Mora et al., 2004a)

Composition	Quantity (g l^{-1})
$NaHCO_3$	1.25
KH_2PO_4	0.025
$CaCl_2.2H_2O$	0.3
$MgSO_4.7H_2O$	0.2
EDTA (Trace element solution I)	0.00625
$FeSO_4$ (Trace element solution I)	0.00625
Trace element solution II[a] (ml l^{-1})	1.25
$NaNO_2$	0.42
$(NH_4)_2SO_4$	0.37

Trace element solutions prepared according to van de Graaf et al. (1996).
[a] It consists of EDTA 15 g, $ZnSO_4.7H_2O$ 0.43 g, $CoCl_2.6H_2O$ 0.24 g, $MnCl_2.4H_2O$ 0.99 g, $CuSO_4.5H_2O$ 0.25 g, $Na_2MoO_4.2H_2O$ 0.22 g, $NiCl_2.6H_2O$ 0.19 g, and H_3BO_3 0.014 g.

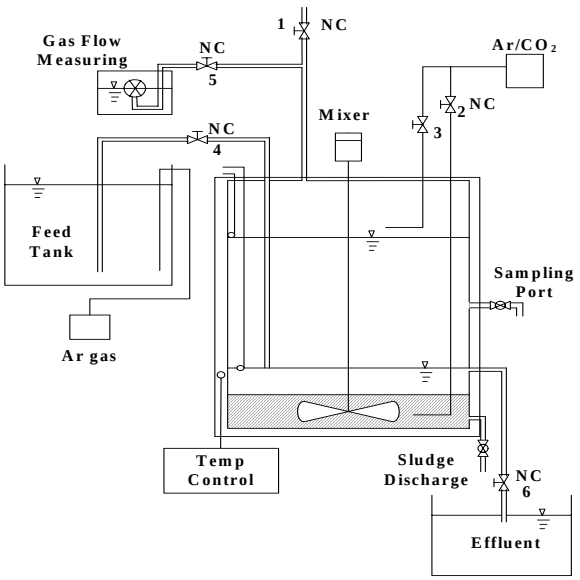


Figure 3.1 SBR reactor configuration for Anammox enrichment

Figure 3.2 Experimental Anammox-SBR reactors

3.1.3 Experimental procedure

Three similar reactors were seeded with different sludges from UASB (S1), activated sludge (S2), and anaerobic digestion reactors (S3). The reactors were flushed with Ar/CO_2 (95/5%) gas mixture to maintain anaerobic condition during the experiment and support CO_2 for Anammox bacteria. The time sequence in a cycle was maintained based on the following time steps, react 5-7 h, settle 30 min, and discharge 15 min. The water jacket was used to control the temperature of the liquid inside the reactors to be within the range of 33-34 ^{o}C. This temperature range was referred as an optimum temperature for Anammox cultivation (Jetten et al., 1997; Strous et al., 1997b; van Dongen et al., 2001a).

Anaerobic condition in the feeding tank was maintained by feeding Ar gas 99.99% to expel dissolved oxygen in the synthetic wastewater. Within the reactors, Ar/CO_2 95/5% was used to flush the air above the water column. This also provided dissolving CO_2 for supporting the growth of Anammox bacteria, which are autothrophs.

Experimental scheme of the first phase of experiment is depicted in Figure 3.3.

3.1.4 Sampling and analysis

Due to slow growth of the Anammox culture, samplings were performed every three days for monitoring the treatment conditions, effluent quality, and bacterial growth. Water samples were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Parameters analyzed included chemical parameters such as ammonium, nitrite, nitrate, and pH. The biomass concentration was observed as MLSS and mixed liquor volatile suspended solids (MLVSS). Ammonium was measured by using the titrimetric method, nitrite was analyzed by using the colorimetric method, and nitrate was analyzed by using the ultraviolet spectrophotometric screening method (Table 3.2).

PHASE I

Anammox seed enrichment from conventional sludges

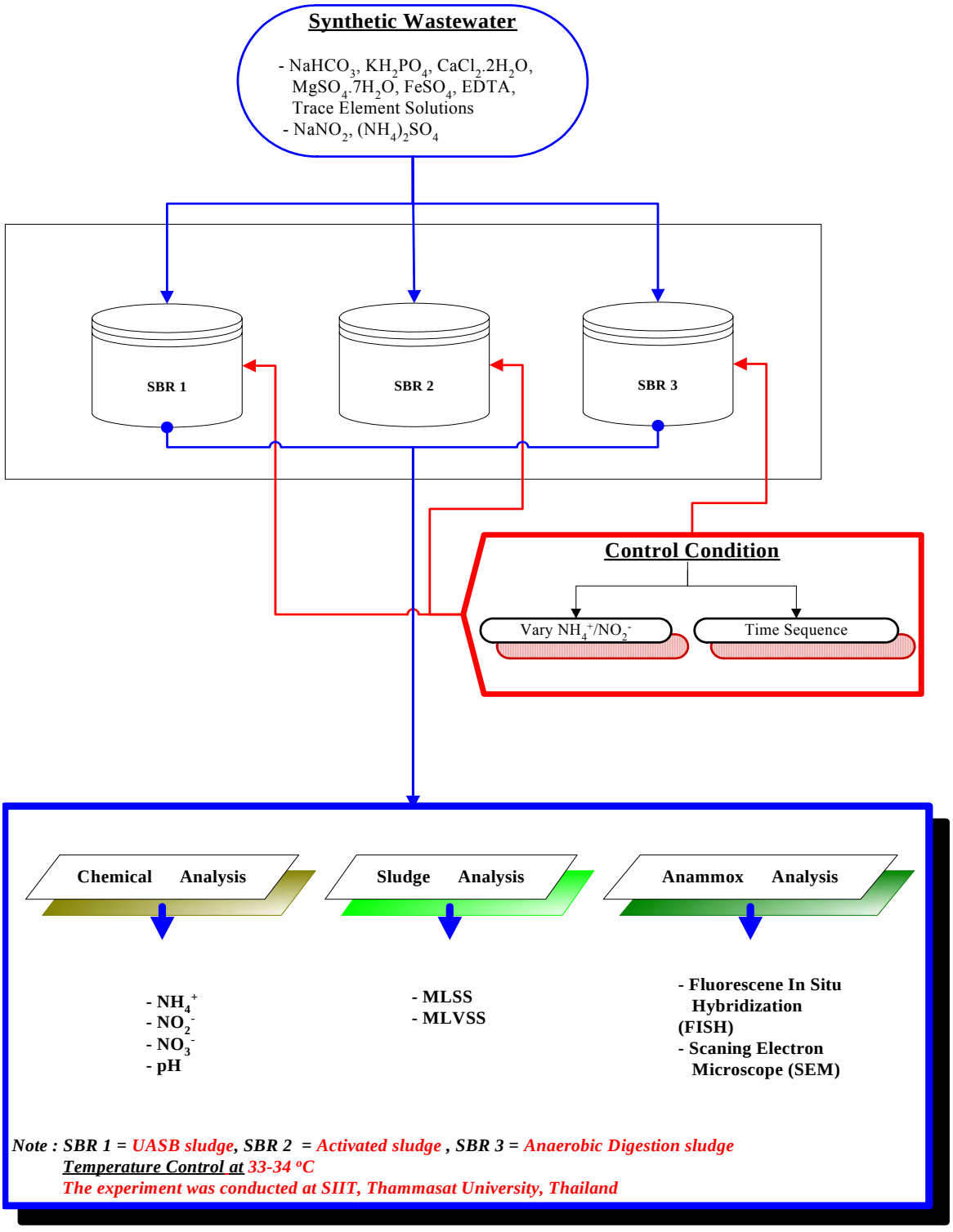


Figure 3.3 Experimental scheme of the first phase of the study

Table 3.2 Parameters, frequency of sampling and method of analyses for experimental phase I

Parameters	Frequency of sampling in phase I	Analytical method
NH_4^+	Every three days	Titrimetric Method
NO_2^-	Every three days	Colorimetric Method
NO_3^-	Every three days	Ultraviolet Spectrophotometric Screening Method
pH	Every three days	pH meter
MLSS	Once a month / steady state	Dried at 103-105 °C
MLVSS	Once a month / steady state	Ignited at 550 °C
FISH	During steady state condition	16 S rRNA gene probe, PLA46 and Amx820 probes
SEM	During steady state condition	Using scanning electron microscope

3.1.5 Concept of the molar ratio in the Anammox process

According to the Anammox stoichiometry, a molar ratio of NH_4^+ to NO_2^- consumption is 1.32 and NO_3^- production to NH_4^+ consumption is 0.26. This molar ratio was used to indicate when Anammox process was achieved in the experiment.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$

3.1.6 SEM observation and FISH analysis

Sludge samples were analyzed by FISH technique and SEM to confirm the existence of Anammox culture. The 16S rRNA gene probes used for in situ hybridization were Amx820 (Schmid et al., 2005) and PLA46 (Neef et al., 1998). Both gene probes were labeled with Cy3 and fluorescine. They were ordered from Thermo Electron (Ulm, Germany). The physiological characteristics of biomass were observed using the SEM manufactured by JEOL model, JSM-5410LV. Specimens for SEM were prepared by fixing with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 h. After that specimens were rinsed twice in a buffer for 10 min/each and once in distilled water for 5 min, followed by dehydration with a graded series of ethanol, and dried at a critical point, then mounted and coated.

3.2 Experimental phase Ib : Acceleration of Anammox process in SBR using Anammox seeding combined with conventional sludge

Acceleration of Anammox process start-up was achieved by inoculating of a combination of Anammox seeding and conventional sludge to the two parallel sequencing batch reactors fed with synthetic medium as proposed by van de Graaf et al. (1996); Sliekers et al. (2002); Dapena-Mora et al. (2004a). The nitrite and ammonium added in the synthetic medium were 90-110 and 70-80 mg N. l^{-1}, respectively. The reactor (Figure 3.2) had 7 liters working volume enclosed by a water jacket for controlling the temperature of the liquid inside the reactor to be within the range of 33-34 °C. The mixing inside the reactor was achieved by a two-blade stirrer. Ar/CO_2 (95/5%) gas mixture was flushed to maintain anaerobic condition and support CO_2 for Anammox bacteria. Seed sludge used in this study was taken from the excess sludge of activated sludge process combined with the existing Anammox biomass from a previous experiment with the blending composition of approximately 2,500 and 500 mg MLSS. l^{-1}, respectively. The following time sequence in SBR cycle was maintained: react 7 h, settle 30 min, and discharge 15 min. Steady state condition was obtained by observing the conversion of nitrite and ammonium in the reactor.

3.2.1 FISH analysis

Cultivated sludge samples were analyzed by FISH technique to confirm the existence of Anammox culture. The 16S rRNA gene probes used for in situ hybridization were PLA46 (Neef et al., 1998) and Amx820 (Schmid et al., 2005). The gene probes were labeled with Cy3 and fluorescine and were ordered from Thermo Electron (Ulm, Germany). FISH analysis procedure was applied from the method of Hugenholtz et al. (2001).

3.2.2 Sampling and analysis

Water samples taken from the reactors were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Analyzed parameters included chemical parameters such as ammonium, nitrite, nitrate, pH and COD. The titrimetric method, colorimetric method, ultraviolet spectrophotometric screening method, and closed reflux method were used for the measurement of NH_4^+, NO_2^-, NO_3^-, and COD, respectively. Biomass concentration was measured as MLSS and MLVSS.

3.3 Experimental phase II : Operational parameters affecting Anammox-SBR

The Anammox-SBR reactors were continually operated from experimental phase I (Figure 3.2). This experiment was designated to find out the suitable time sequence, NH_4^+:NO_2^- ratio, and the quantity of PO_4 inhibited to the Anammox process. The quantity of NO_2^- and NH_4^+ inhibited to the Anammox process was also investigated in this experiment.

Batch studies were carried out to find the optimum sludge concentration and reaction time leading to a good specific removal rate. The effects of COD on the Anammox reaction was also investigated. The experimental scheme for the second phase of experiment is depicted in Figure 3.4.

3.3.1 Operational variables

General conditions of Anammox-SBR operation were maintained as in phase I but the operational parameters which are expected to affect the process were varied. They are reaction time, NH_4^+:NO_2^- ratio, COD:N ratio, concentration of PO_4^{3-}, NO_2^-, COD, and NH_4^+, and sludge quantity. The details of each operational parameter are described below.

3.3.1.1 Time sequence

Time sequences emphases on reaction time were varied as 71, 47, 23, 11, and 7 h. Settle and discharged period were set after trial runs at 30 and 15 min, respectively.

3.3.1.2 NH_4^+:NO_2^- ratio

NH_4^+:NO_2^- ratios were varied to find out an appropriate range to cultivate Anammox bacteria by selecting reaction time that provided the highest ammonium removal efficiency (7 h) in phase I. The ratios were varied as follows: 1:1, 1:1.1, 1:1.2, 1:1.3, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1.2:1, 1.6:1, and 2:1. The concentrations of NH_4^+ and NO_2^- which may inhibit the Anammox process were also investigated.

3.3.1.3 COD:N ratio

Selected COD:N ratios used for preliminary study of Anammox combined with denitrification process were provided as 0.5:1, 1:1, 1:1.5, and 1:2. This also was intended to investigate the COD concentration that may affect the Anammox reaction. The study was conducted by exploring of the influent containing such COD:N ratios to the SBR 1, which indicated the co-existing filamentous with Anammox bacteria as described in 4.1.2.1. The presented biomass concentration in the reactor was 1,100 mg MLSS l^{-1}. Additional investigations were carried out using batch experiments and further observed in experimental phase III.

3.3.1.4 PO_4^{3-} concentration

PO_4^{3-} concentrations were varied to observe the effect on process performance. Concentrations were varied as follows: 30, 45, 60, 70, 175, and 215 mg P l^{-1}.

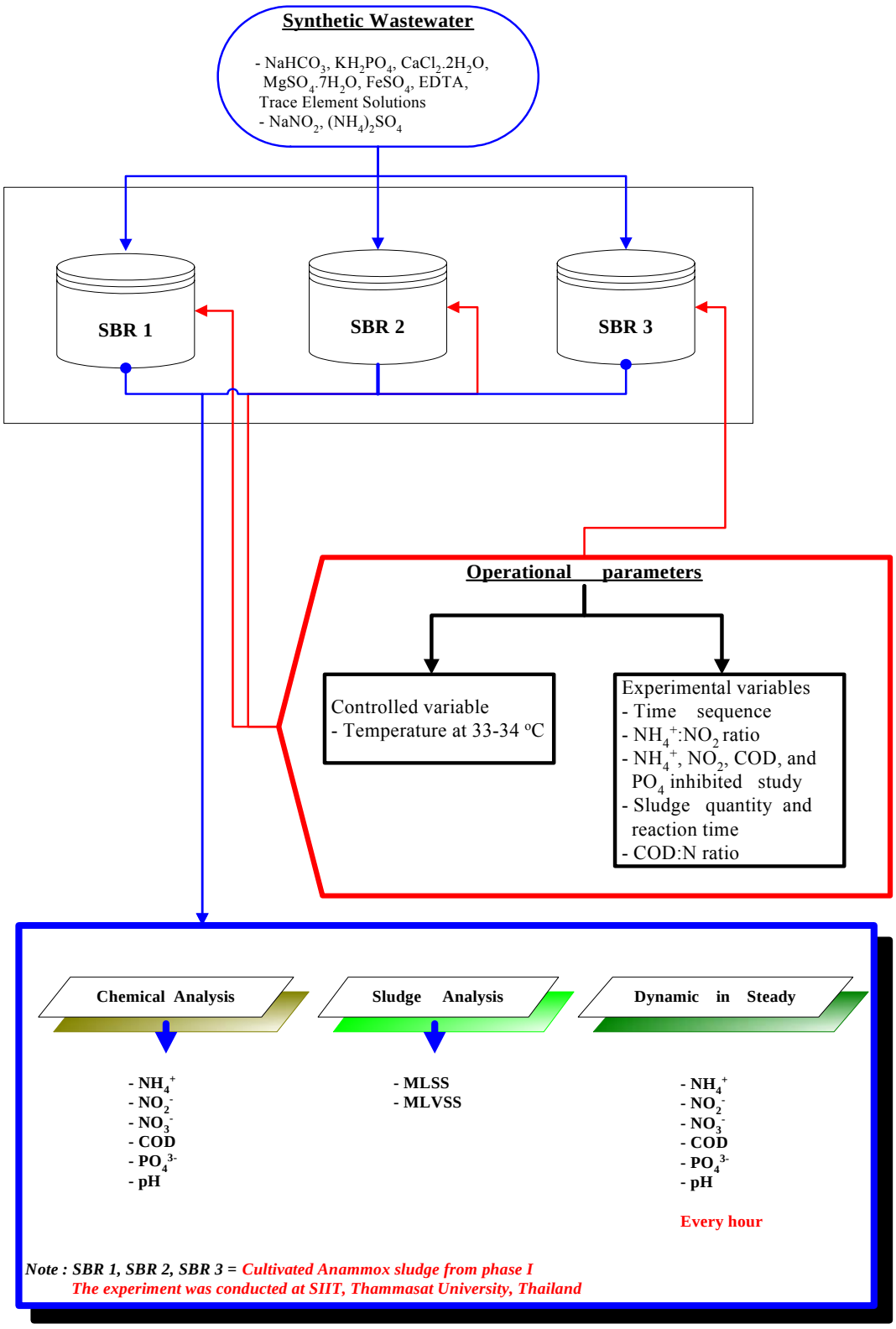


Figure 3.4 Experimental scheme of the second phase of experiment

3.3.1.5 Batch study on optimum sludge quantity and reaction time

Batch tests were run in the screwed-glass bottles with a working volume of 250 ml. The Anammox sludge was taken from the Anammox-SBR and transferred to bottles which contained synthetic medium (van de Graaf et al., 1996; Sliekers et al., 2002; Dapena-Mora et al., 2004a), and the bottles were flushed with N_2 gas for 15 min, and shaken at 145 rpm.

Optimum reaction time and sludge quantity for high nitrogen removal efficiency was observed at the reaction time of 7, 24, 48, and 72 h with the Anammox MLSS of approximately 500, 1000, 1500, 2000, and 2500 mg l^{-1}. The nitrogen removal efficiency in each reaction time interval was observed for the optimum sludge quantity used.

3.3.1.6 Batch study on effect of COD:N ratio on Anammox activity and degree of denitrification

Batch tests were performed to investigate the combined function of the two bacteria: denitrifying and Anammox. The variation of effluent nitrogen species in relation to the influent COD:N ratio was observed every hour. Two COD concentrations at approximately 80 and 180 mg l^{-1} were investigated by spiking glucose into the synthetic medium, making the COD:N ratio of 0.6:1 and 1.3:1. The amount of NO_2^--N, NH_4^+-N, and NO_3^--N in the feed was in the range of 70-80, 55-60, and 9.0-11.7 mg l^{-1}, respectively. The obtained workable ratio was then used in a later experimental phase III for a study of co-removal of COD and nitrogen in SBR and UASB.

3.3.2 Parameter measurements, sampling, and analyses

Water samples, both from intermittent fed reactors and batch studies, were analyzed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Parameters analyzed included chemical parameters such as ammonium, nitrite, nitrate, phosphate, pH and COD. The titrimetric method, the colorimetric method, the ultraviolet spectrophotometric screening method, the ascorbic acid method, and the closed reflux method were used for the measurement of NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, and COD, respectively. Sludge concentration was observed as MLSS and MLVSS. The detail of all analytical methods and the frequency of sampling are summarized in Table 3.3.

Table 3.3 Parameters, frequency of sampling and method of analyses used in experimental phase II

Parameters	Frequency of Sampling in Phase II	Analytical Method
NH_4^+	Once a week during initial stage and every hour during steady state conditions	Titrimetric method
NO_2^-	Once a week during initial stage and every hour during steady state conditions	Colorimetric method
NO_3^-	Once a week during initial stage and every hour during steady state conditions	Ultraviolet spectrophotometric screening method
COD	Once a week during initial stage and every hour during steady state conditions	Closed reflux method
PO_4^{3-}	Once a week during initial stage and every hour during steady state conditions	Ascorbic acid method
pH	Once a week during initial stage and every hour during steady state conditions	pH meter
MLSS	Once or twice per month	Dried at 103-105 °C
MLVSS	Once or twice per month	Ignited at 550 °C
FISH	During steady state conditions	16 S rRNA gene probe, PLA46 and Amx820 probes

3.4 Experimental phase IIIa : Co-removal of COD and nitrogen in Anammox-SBR

Experimental phase IIIa was conducted at the Institute of Environment and Resources, Technical University of Denmark.

3.4.1 Experimental set-up for shock loading of COD (first operation) and co-removal of Anammox and denitrification study (second operation)

3.4.1.1 Seed sludge

Enriched Anammox sludge cultivated from activated sludge was used to start up the Anammox process in SBR. The sludge was previously used and kept under starvation conditions for approximately a month prior to reuse. The concentration was approximately 1,500 mg MLSS l^{-1}.

3.4.1.2 Feed preparation

Feed composition is shown in Table 3.4. For the first operation, a shock loading study, the COD was prepared using the diluted pig manure slurry in concentration of approximately 8,000 mg l^{-1}. For the second operation, a co-removal investigation, synthetic COD was prepared from fat milk in a range of 135-600 mg l^{-1}. The fat milk used was produced in Denmark. It includes protein 3.4%, carbohydrate 4.8%, and fat 3.5%. At the beginning of the start-up process, ammonia, nitrite, and nitrate concentration of 70, 100, and 40 mg N l^{-1} were explored to the reactor. Due to the improper nitrogen conversion for the Anammox being observed, the concentration of ammonium and nitrite in the feed was then decreased proportionally. After that it was maintained constantly through the experiment at approximately 40 and 50 mg N l^{-1}. Nitrate was added in the feed to reduce the potential competition of heterotrophs over Anammox organisms, which was in the range of 25-50 mg N l^{-1} and 50-70 mg N l^{-1} for the first and second operation in this phase, respectively. The pH of the feed ranged between 7.0 and 8.9 without deliberate control. The anaerobic condition of the feed was provided by flushing nitrogen gas 7 min before being fed to the reactor.

3.4.1.3 Reactor set-up

The laboratory-scale SBR (Figure 3.5) was made of acrylic plastic with total volume of 1.1 liters. The effective volume for liquid retention was 0.8 liter. The reactor was operated under control mesophilic temperature of 33-35 ºC and equipped with mechanical stirrers (80-90 rpm). It was operated in a batch mode with a time cycle of 2 days reaction, 30 min settling, and 5 min decanting. Liquid inside the reactor was flushed again with nitrogen gas for 10 min at the beginning of the operation cycle to maintain an anaerobic condition.

3.4.2 Anammox activity study and confirmation techniques

Anammox activity was tested in a batch study using 100 ml vials, with 10 ml Anammox biomass taken from the reactor, MLVSS of approximately 300 mg l^{-1}, and 60 ml feed solution. Initial ammonia, nitrite, and nitrate concentration was 40, 50, and 30 mg N l^{-1}, respectively, with pH value of 8.2. The vials were flushed with N_2 gas for 15 min and then kept at 37 ºC incubation for 24 h. The Anammox activity was observed by monitoring of nitrogen species concentration every two hours for a day. The oxygen-limited autotrophic nitrification-denitrification (OLAND) sludge taken from the microbial ecology laboratory, Ghent university, Belgium, was used to compare an activity with the Anammox.

Anammox confirmation was carried out using a hydroxylamine test and fluorescence in-situ hybridization (FISH) analysis. The hydroxylamine test was conducted to detect the active Anammox biomass in the reactor. Biomass taken from the reactor was injected to 100 ml vials containing 2.1 μM anaerobically prepared hydroxylamine solution and kept in the 37 °C incubation. Concentrations of hydroxylamine and hydrazine were measured every 10 min for half an hour.

FISH technique was used to identify most bacteria in the sample and confirm the existence of Anammox microbes using the gene probe EUB 338 (5'- GCT GCC TCC CGT AGG AGT -3') (Daims et al., 1999) and Amx 820 (5'- AAA ACC CCT CTA CTT AGT GCC C -3') (Schmid et al., 2005), respectively.

Table 3.4 Composition of feed used in experimental phase IIIa

Composition	Concentration (mg l^{-1})	
	First operation (day 21-65)	Second operation (day 66-97)
$NaHCO_3$	1,250	1,250
KH_2PO_4	25	25
$CaCl_2 \cdot 2H_2O$	300	300
$MgSO_4 \cdot 7H_2O$	200	200
EDTA (Trace element solution I)	6.25	6.25
$FeSO_4$ (Trace element solution I)	6.25	6.25
Trace element solution II[a]	1 ml l^{-1}	1 ml l^{-1}
$(NH_4)_2SO_4$	40-160 (as N)	40 (as N)
$NaNO_2$	50-100 (as N)	50 (as N)
$NaNO_3$	30-50 (as N)	50-70 (as N)
COD	20-8,000[b]	135-600[c]

Trace element solutions prepared according to van de Graaf et al. (1996).

[a] It consists of EDTA 15 g, $ZnSO_4.7H_2O$ 0.43 g, $CoCl_2.6H_2O$ 0.24 g, $MnCl_2.4H_2O$ 0.99 g, $CuSO_4.5H_2O$ 0.25 g, $Na_2MoO_4.2H_2O$ 0.22 g, $NiCl_2.6H_2O$ 0.19 g, and H_3BO_3 0.014 g.

[b] Shock loading of COD from diluted pig manure slurry of 8,000 mg l^{-1} was added once at the 21st day of operation. After that the concentration was diluted when the new cycle started with the feed containing no COD content.

[c] COD produced from fat milk ($TKN_{fat\ milk}$ = 6.50 g l^{-1} (SD 1 %); measured by E&R laboratory, DTU)

Figure 3.5 Experimental Anammox-SBR

3.4.3 Sampling and analysis

Samples were taken from the sampling port of the SBR after reaction periods of one day at the beginning and two days after steady state condition was achieved. Steady state conditions in this study were obtained by observing the conversion of ammonia and nitrite in the reactor when their removal is above 80% and does not vary by more than 10%. Analyzed parameters included ammonium, nitrite, nitrate, pH, and COD. The ammonium, nitrite, and nitrate were determined by colorimetric method using the spectroquant NOVA 60, Merck. The pH was measured using glass electrode connected to the 692 pH/Ion Meter, Metrohm. The COD was determined using the closed reflux method (APHA, 1998). The biomass concentration was observed as MLSS and MLVSS by drying the sample at 105 °C for 24 h and then igniting it at 550 °C for 2 h, respectively. Hydroxylamine and hydrazine were analyzed in the batch study according to the procedure of Frear and Burrell (1955) and Watt and Chrisp (1952), respectively. Biomass fixation, slice preparation, and hybridization conditions were performed according to the method of Hugenholtz et al. (2001). Hybridized slices were viewed under an epifluorescence microscope Axioskop by Carl Zeiss, Oberkochen, Germany, with the photographs magnification of 1000X.

3.5 Experimental phase IIIb : Co-removal of COD and nitrogen in Anammox-UASB

Experimental phase IIIb was conducted at the Institute of Environment and Resources, Technical University of Denmark.

3.5.1 Reactor and feed preparation

Three parallel 200-ml laboratory-scale UASB reactors (Figure 3.6) were inoculated with 40 ml granules from anaerobic granular sludge, MLSS of 15 g l^{-1} and 40 ml Anammox seed sludge, approximately 200 mg MLSS l^{-1}. The Anammox sludge was from an ongoing SBR reactor enriched for over three months. The UASB reactor was selected due to its high solid retention and mass transfer rate which would minimize sludge washout during initial stage and allow good substrate conversions. All reactors were acclimatized for about 2 weeks prior to monitoring.

The reactors were continuously fed with synthetic wastewater at a mesophilic temperature of 35 ºC and a hydraulic retention time of 2 days. Influent ammonium, nitrite, and nitrate concentrations were 40, 50, and 50 mg N l^{-1}, respectively. Nitrate concentration was later increased to observe the competitiveness of denitrifying bacteria to Anammox organisms. The composition of influent substrate and trace elements was prepared according to the earlier study and van de Graaf et al. (1996) (Table 3.5). COD concentration in wastewater was originated from fat milk and prepared in ranges of 100-200, 200-300, and 300-400 mg l^{-1}. This is to simulate an alike COD in actual wastewater. The fat milk used was produced in Denmark. It includes protein 3.4%, carbohydrate 4.8%, and fat 3.5%. The feed was flushed with nitrogen gas for 10 min before use to maintain anaerobic condition in the reactors. Influent pH varied in a range of 8.0 to 8.5 without deliberate control.

Figure 3.6 Experimental Anammox-UASBs

3.5.2 Anammox confirmation

Two Anammox confirmation techniques were used, namely, a Fluorescence in-situ hybridization (FISH) analysis and a hydroxylamine test. A FISH analysis was carried out to identify most bacteria and confirm the existence of Anammox microbes using the gene probe EUB 338 mixed (Daims et al., 1999) and Amx 820 (Schmid et al., 2005), respectively. A hydroxylamine test was conducted to detect the active Anammox biomass in the reactors.

Table 3.5 Composition of feed used in experimental phase IIIb

Composition	Concentration (mg l^{-1})
$NaHCO_3$	1,250
KH_2PO_4	25
$CaCl_2 \cdot 2H_2O$	300
$MgSO_4 \cdot 7H_2O$	200
EDTA (Trace element solution I)	6.25
$FeSO_4$ (Trace element solution I)	6.25
Trace element solution II[a]	1 ml l^{-1}
$(NH_4)_2SO_4$	40 (as N)
$NaNO_2$	50 (as N)
$NaNO_3$	50-120 (as N)
COD[b]	100-400

Trace element solutions prepared according to van de Graaf et al. (1996).

[a] It consists of EDTA 15 g, $ZnSO_4.7H_2O$ 0.43 g, $CoCl_2.6H_2O$ 0.24 g, $MnCl_2.4H_2O$ 0.99 g, $CuSO_4.5H_2O$ 0.25 g, $Na_2MoO_4.2H_2O$ 0.22 g, $NiCl_2.6H_2O$ 0.19 g, and H_3BO_3 0.014 g.

[b] COD produced from fat milk ($TKN_{fat\ milk}$ = 6.50 g l^{-1} (SD 1 %); measured by E&R laboratory, DTU)

3.5.3 Sampling and analysis

Samples were taken from a sampling port on top of the reactors every day and reduced to be every two days when a steady state condition was attained, as indicated by a constant rate of nitrite removal. Steady state conditions were defined as those occurring after at least three turnovers of the hydraulic detention time, and when the reactor effluent quality does not vary by more than 10%. Analyzed parameters included ammonium, nitrite, nitrate, pH, and COD. The ammonium, nitrite, and nitrate were determined by colorimetric method using the spectroquant NOVA 60, Merck. The pH was measured using glass electrodes connected to the 692 pH/Ion Meter, Metrohm. The COD was determined using the closed reflux method (APHA, 1998). Hydroxylamine and hydrazine were analyzed in the batch study. Hydroxylamine was measured according to the procedure of Frear and Burrell (1955). Hydrazine was determined following the method of Watt and Chrisp (1952). Hybridization conditions for FISH analysis were from Hugenholtz et al. (2001) which were 40% formamide at 46 ºC. FISH images were obtained using an epifluorescence microscope Axioskop by Carl Zeiss, Oberkochen, Germany, with the photographs magnification of 1000X.

3.6 Experimental phase IV : Mathematical model describing system behavior on nitrogen removal of Anammox-SBR and the application in UASB reactor

The steps for finding out an Anammox-SBR model were composed of a literature review of the commonly used kinetics and the reported assumption models, stoichiometric calculation and model development. The models were constructed with the typical assumptions of mass balance in a batch system. The obtained equations were solved by using MATLAB 6.5; Rung-Kutta method. The predicted model curves were compared with the experimental results from the first, second, and third phases of the experiment. Predicted model curves from simulation results included an estimation of Anammox biomass and systems performance on nitrogen removal during the enrichment in the first phase, inhibited ammonia and nitrite concentration studied in the second phase, and the concurrent operation of Anammox and denitrification in the third phase of experiment. The flow diagram of overall steps in the fourth phase of experiment is shown in Figure 3.7.

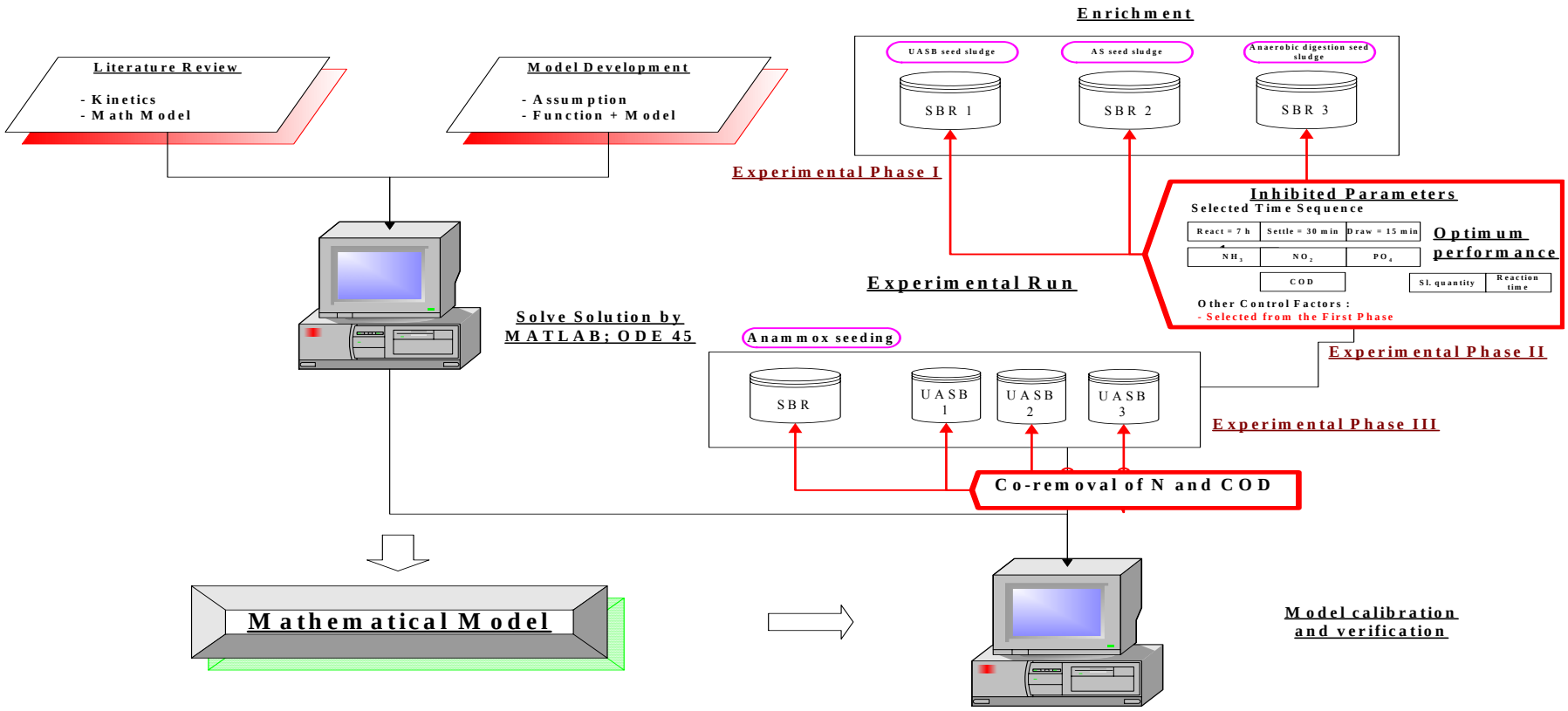


Figure 3.7 Experimental scheme of the fourth phase of experiment

Chapter 4

Results and Discussion

4.1 Experimental phase Ia : Anammox seed enrichment using conventional sludges

4.1.1 Early operation stage of Anammox seed enrichment

4.1.1.1 Reaction time cycle

In the early stage of Anammox acclimatization, the long reaction time cycle was conducted to try to acclimatize the sludge. Reaction time cycles for sludge S1 (Anammox seed sludge and UASB seed sludge in the later operation when no Anammox reaction was observed), S2 (activated seed sludge), and S3 (anaerobic digestion seed sludge) are shown in Table 4.1. During 85 days of operation, eleven cycles were taken for sludge S1 and S2, while twenty-one cycles were taken for sludge S3. The changing of reaction time cycle depended on the effluent nitrogen species that closed to the zero value or showed stable conditions for treatment.

Table 4.1 Reaction time cycle in the early stage of acclimatization

Cycle	Reaction time (days)	
	S1, S2	S3
1	17	17
2	6	6
3	3	3
4	3	3
5	20	4
6	12	4
7	9	6
8	6	3
9	3	3
10	3	3
11	3	3
12		3
13		3
14		3
15		3
16		3
17		3
18		3
19		3
20		3
21		3

4.1.1.2 Experimental results of sludge S1

Sludge S1 was the presumably Anammox sludge taken from Denmark that was replaced by the sludge from the UASB reactor at the 64th day of operation due to the sludge showing no activity, which might result from it being kept under starvation condition prior to use. The experimental results indicating influent and effluent nitrogen species and pH is shown in Figure 4.1. The stoichiometric calculation of the Anammox process are shown in Table 4.2 as follows.

Monitoring results of influent and effluent nitrogen species from the reactor seeded with sludge S1 in the first 2 cycles, 17 and 6 days of reaction time, showed that the effluent ammonium concentration rose above the influent concentration, while the effluent pH value dropped below the influent value. This could be due to the occurrence of ammonification process that converted the organic nitrogen in the sludge to ammonium (equilibrium with ammonium in aqueous). The conversion of organic nitrogen to ammonium consisted of two steps, such as the transformation of organic nitrogen to amino acid and the deamination to ammonia (Pansawat, 2001). It, therefore, resulted in the decreasing of effluent pH as described earlier.

The effluent ammonium concentrations recorded during cycle 3-5, reaction time of 3, 3, and 20 days, were very close to the influent concentrations. The effluent nitrite concentration rose during cycle 3-4, after that it decreased. However, it is still a higher value than the influent concentration. The fluctuation of pH value was observed during cycle 3-5. It was lower than the influent value during cycle 3 but after that it showed higher value than the influent in cycle 4 and the value decreased again in cycle 5. The nitrate concentration increased a small amount during these cycles. It demonstrated that during cycle 3-4, the denitrification process proceeded resulting in the conversion of nitrate to nitrite and simultaneously produced OH^- as the by-product. Thus, the increasing of pH value could be observed. During cycle 5, the concentration of nitrite and pH value decreased while nitrate concentration increased. This might come from the nitrification that converted the nitrite to nitrate and simultaneously produced H^+ as the by-product. It indicated a possible leakage of oxygen into the reactor during the operation process. While, the ammonification process was still probably occurring in the system.

From Table 4.2, the mole ratio of NH_4^+:NO_2^-:NO_3^- in cycle 5 was 1:1.4:0.53. It was completely deviated from the stoichiometric ratio of Anammox process, (1):(1.32):0.26, which indicates that the decrease of 1 and 1.32 mole of ammonium and nitrite with the increase of 0.26 mole of nitrate will be observed. Whereas, the increase of ammonium, nitrite, and nitrate concentration was observed from the experiment during these cycles (Figure 4.1). Therefore, the Anammox process could not be initiated and nitrification was the main process during this time.

The effluent quality obtained from cycle 6-7 (12 and 9 days of reaction time) showed a better reactor performance as the effluent concentrations of ammonium and nitrite decreased below the influent values. A similar trend was observed for the effluent pH value, while the effluent concentration of nitrate rose above influent concentration during cycle 6, after that it decreased below the influent value.

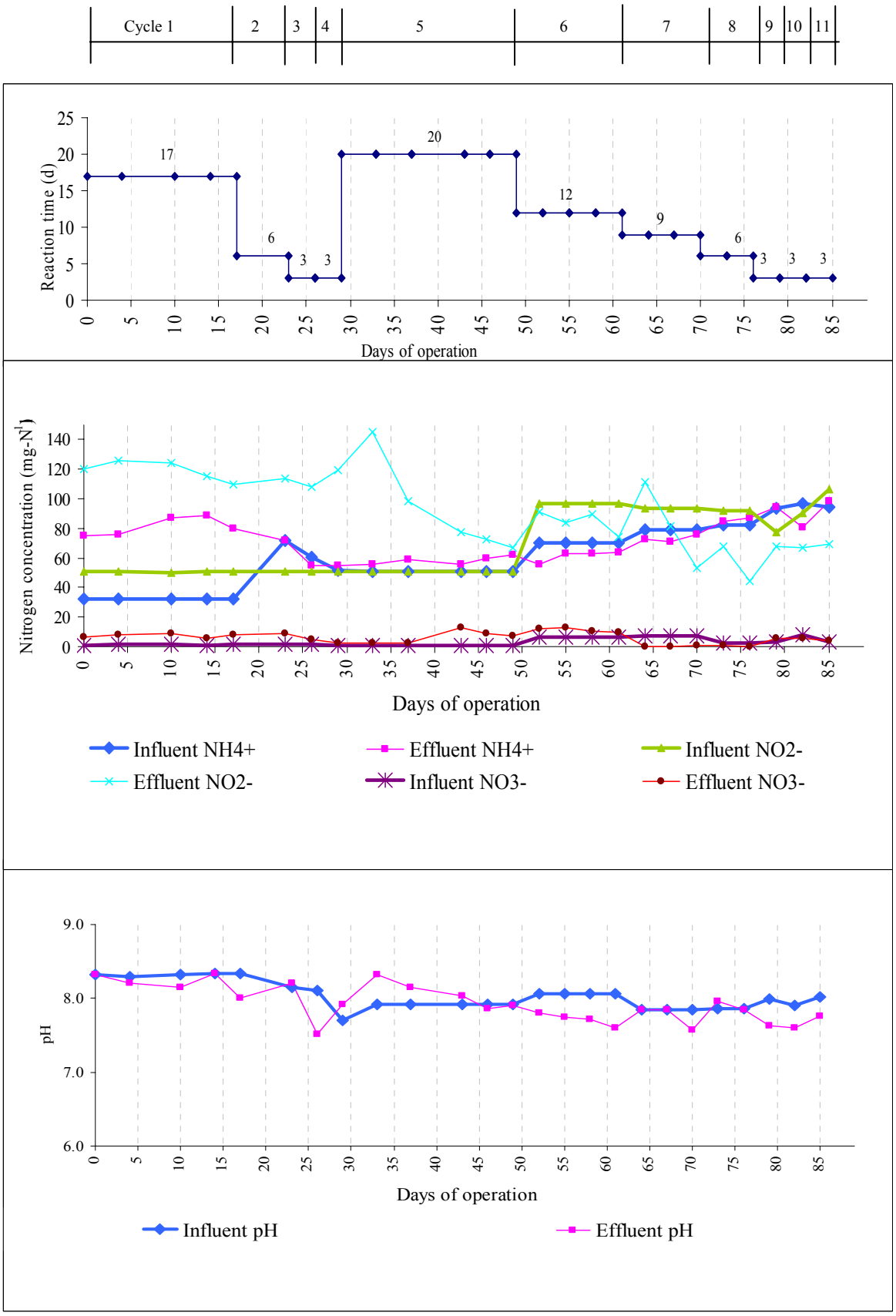


Figure 4.1 Experimental results of sludge S1 in the early stage of acclimatization

At the 64th day of operation, there was a replacement of Anammox sludge by the sludge from UASB reactor. The reason for this replacement was the results of the lower change of ammonium, nitrite, nitrate, and pH when compared with experimental reactor S2 and S3. The Anammox sludge used at the beginning of this experiment might be the old cell and it was non-active as it was kept under starvation condition prior to use. The results of sludge S1 during cycle 1-6 showed a very low activity, which obviously can be seen from the lower change of nitrogen species when compared with the other reactors. During cycle 6-7, the nitrification might proceed as shown by the decreasing of nitrite but increasing of nitrate concentration. The acclimatization of new sludge was proceeded later which resulted in the consumption of nitrate being observed consequently.

From Table 4.2, the average mole ratio of NH_4^+:NO_2^-:NO_3^- in cycle 6-7 was (1):(7.34):(0.65), showing the decrease of ammonium, nitrite, and nitrate occurred from nitrification and denitrification processes. Thus, the Anammox process could not be initiated in these cycles either.

The results from cycle 8-11 showed the tendency of the low change between influent and effluent parameters of ammonium and nitrite. The probable reason of these results might come from the zero dissolved oxygen provided in the synthetic wastewater (fresh synthetic wastewater was fed with Ar gas before fed to reactors; used Ar gas feeder at the 64th day of experiment). Then, the nitrification could not proceed. But the slow activity of Anammox organisms resulted in the low change of ammonium and nitrite concentration in the system.

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during the early stage of acclimatization of sludge S1 through the experiment was 0.14:(6.81):0.41. Considering the effluent nitrogen profile in Figure 4.1, the increase of ammonium, nitrate and the decrease of nitrite, the processes that occurred in the reactor 1 were the ammonification, nitrification and denitrification, while the Anammox process showed the minor function. The fluctuation of mole ratio through this experiment is shown in Figure 4.2.

Table 4.2 Stoichiometry of sludge S1 in the early stage of acclimatization

Cycle	Reaction	NH_4^+-N (mg l^{-1})		ΔNH_4^+	NO_2^--N (mg l^{-1})		ΔNO_2^-	NO_3^--N (mg l^{-1})		ΔNO_3^-	pH		NH_4^+ :	NO_2^- :	NO_3^-
	time (days)	influent	effluent		influent	effluent		influent	effluent		influent	effluent	(1)	(1.32)	0.26
5	20	50.56	62.16	11.60	51.03	67.24	16.21	0.94	7.04	6.10	7.96	7.90	1	1.40	0.53
6	12	70.32	63.84	-6.48	96.55	74.53	-22.02	6.54	9.36	2.82	8.06	7.60	(1)	(3.40)	0.43
7	9	79.20	75.60	-3.60	93.61	52.96	-40.65	7.34	1.10	-6.24	7.85	7.57	(1)	(11.29)	(1.73)
8	6	82.56	87.36	4.80	91.94	44.10	-47.84	2.45	0.29	-2.16	7.86	7.84	1	(9.97)	(0.45)
9	3	93.36	94.08	0.72	77.82	67.39	-10.43	2.86	5.80	2.94	7.99	7.63	1	(14.48)	4.08
10	3	96.96	80.64	-16.32	90.02	66.96	-23.06	8.33	5.39	-2.94	7.90	7.60	(1)	(1.41)	(0.18)
11	3	94.24	98.56	4.32	106.21	69.39	-36.82	3.05	3.76	0.71	8.02	7.76	1	(8.52)	0.16
Average													0.14	(6.81)	0.41

Note :

Δ : Difference between effluent and influent (effluent - influent)

(...) : Negative value

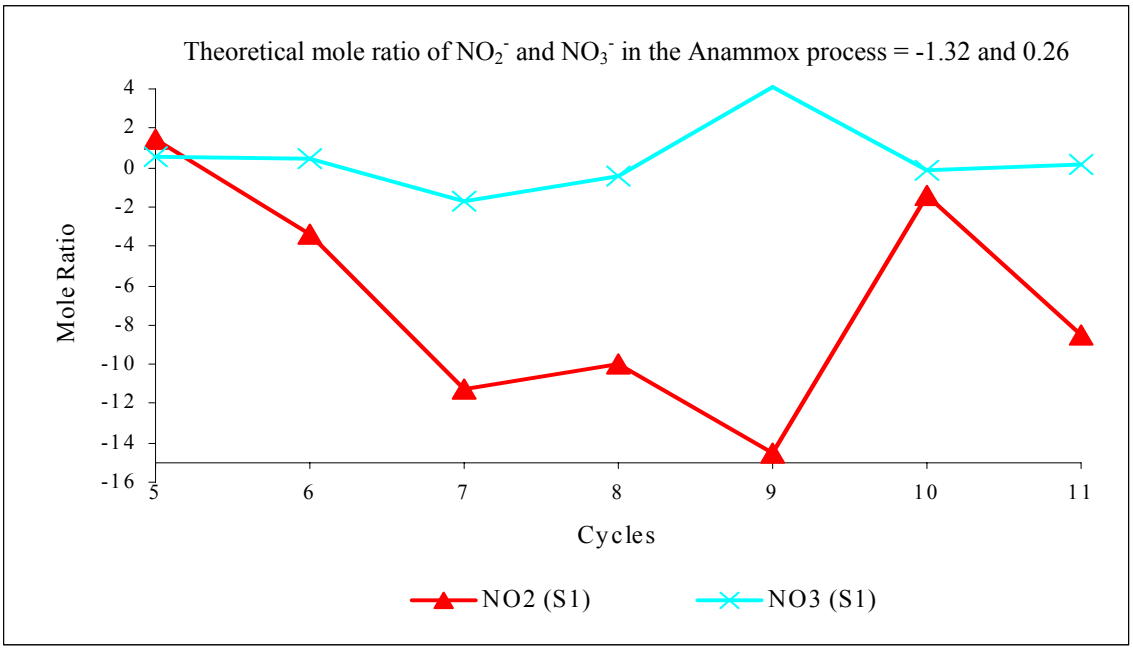


Figure 4.2 Mole ratio of sludge S1 in the early stage of acclimatization

4.1.1.3 Experimental Results of Sludge S2

Sludge S2 used for Anammox enrichment was the activated sludge. The experimental results of nitrogen species and pH during 85 days of operation are shown in Figure 4.3. The stoichiometric calculation of the Anammox process is shown in Table 4.3.

The effluent ammonium concentrations observed during the first 2 cycles, 17 and 6 days of reaction time, were higher than the influent concentrations, while the effluent pH dropped below the influent value. This result was similar to the S1 experiment in that the ammonfication was the main process in the early stage of acclimatization.

The results of cycle 3-5 (3, 3, and 20 days of reaction time, respectively) showed that the effluent ammonium concentration was close to the influent concentration. The nitrite concentration decreased until it was lower than the influent concentration, while the effluent concentration of nitrate increased. The effluent pH value dropped during cycle 3-4, after that it increased. However, it decreased again after cycle 5. This pointed out that nitrification and denitrification were the main processes in this period. The limited oxygen condition for nitrification came from the air leakage during reactor repair and set-up combined with the remained dissolved oxygen in the fresh feed of synthetic wastewater, while the organic carbon for denitrification came from the old cell biomass. The limited dissolved oxygen in synthetic wastewater had been improved since the 64 th day of experiment by using an Ar gas feeder.

From Table 4.3, the mole ratio of NH_4^+:NO_2^-:NO_3^- in cycle 5 was 1:(8.74):11.3, which shows the increase of ammonium and nitrate with the decrease of nitrite. It indicates the occurrence of ammonification and nitrification in the system. Thus, the Anammox process did not occur during these cycles.

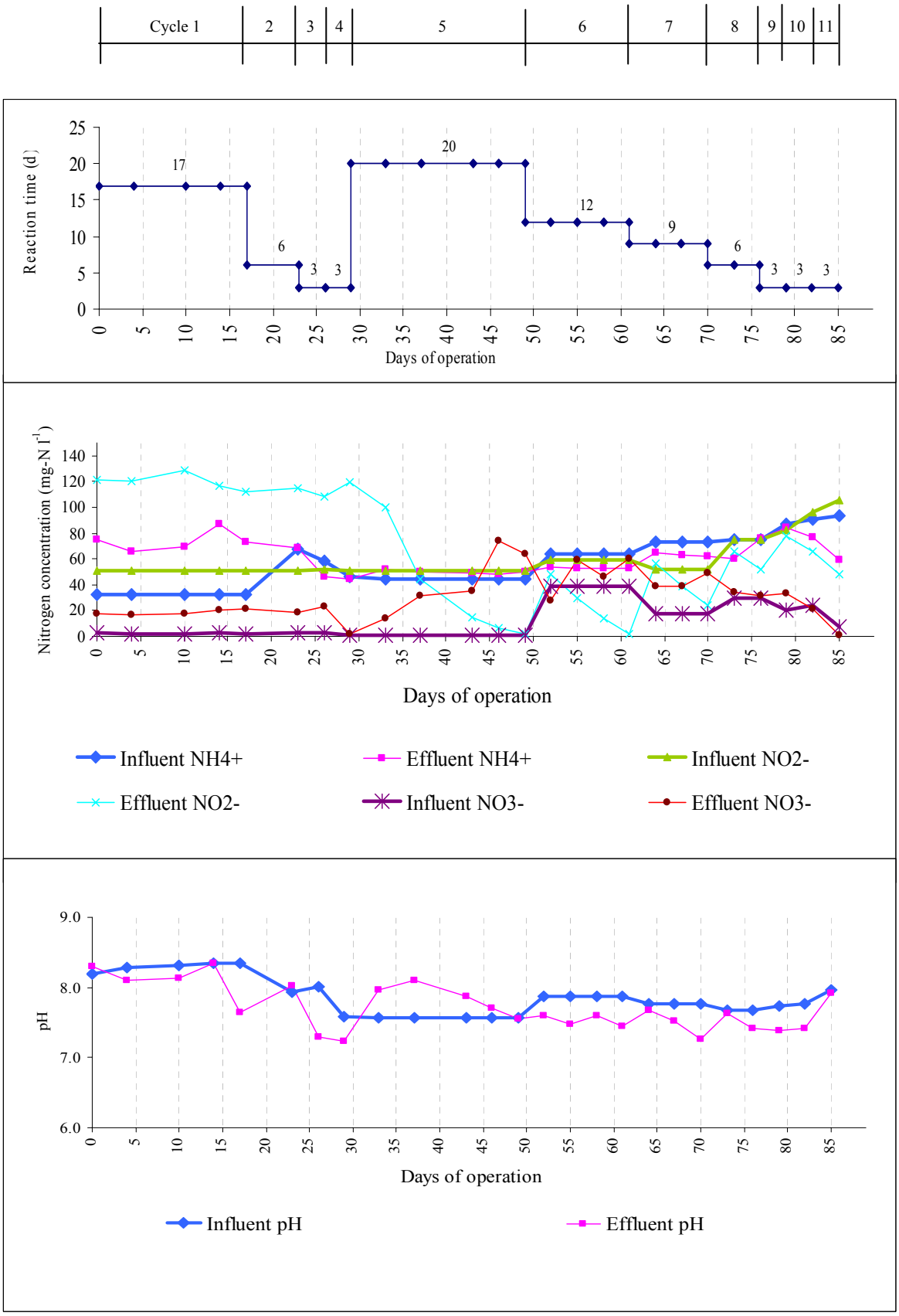


Figure 4.3 Experimental results of sludge S2 in the early stage of acclimatization

Table 4.3 Stoichiometry of sludge S2 in the early stage of acclimatization

Cycle	Reaction time (days)	NH_4^+-N (mg l^{-1})		ΔNH_4^+	NO_2^--N (mg l^{-1})		ΔNO_2^-	NO_3^--N (mg l^{-1})		ΔNO_3^-	pH		NH_4^+ :	NO_2^- :	NO_3^-
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	(1)	(1.32)	0.26
5	20	44.80	50.40	5.60	51.03	2.10	-48.93	0.94	64.24	63.30	7.57	7.56	1	(8.74)	11.30
6	12	63.60	53.20	-10.40	59.33	1.53	-57.8	39.23	27.51	-11.72	7.87	7.45	(1)	(5.56)	(1.13)
7	9	73.12	61.60	-11.52	51.90	24.10	-27.8	17.71	49.14	31.43	7.77	7.26	(1)	(2.41)	2.73
8	6	74.56	75.60	1.04	75.45	51.67	-23.78	29.90	31.18	1.28	7.68	7.41	1	(22.86)	1.23
9	3	86.64	84.00	-2.64	82.14	77.67	-4.471	20.51	32.94	12.43	7.74	7.38	(1)	(1.69)	4.71
10	3	91.20	77.28	-13.92	95.90	65.67	-30.23	23.84	21.10	-2.74	7.76	7.41	(1)	(2.17)	(0.20)
11	3	93.28	59.36	-33.92	105.84	47.96	-57.88	7.54	1.31	-6.23	7.97	7.92	(1)	(1.71)	(0.18)
Average													(0.43)	(6.45)	2.64

Note :

Δ : Difference between effluent and influent (effluent - influent)

(...) : Negative value

The effluent concentrations of ammonium, nitrite and pH observed during cycle 6-7 were lower than the influent concentrations, which indicates the tendency of ammonium and nitrite consumption in the system. The effluent nitrate concentration rose above the influent concentration through these cycles.

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during cycle 6-7 was (1):(3.99):0.8. This means that the reduction of ammonium and nitrite was observed while the increment of nitrate occurred. It could be explained that the nitrification converted ammonium contained in the influent and old cell biomass to nitrite and then nitrate.

The last 4 cycles of the experiment (cycle 8-11) showed a small change between influent and effluent concentration of nitrogen species. The effluent pH was still lower than the influent value. The probabe reason of this result comes from the limited dissolved oxygen in the system and synthetic wastewater. However, the nitrification still proceeded under the limited condition, which resulted in the reduction of the accumulation of ammonium and nitrite in the reactor.

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during cycle 8-11 was (1):(7.11):1.39. This showed the reduction of ammonium and nitrite with the increment of nitrate. However, the obtained ammonium reduction was not proportional to the reduction of nitrite and the production of nitrate as in the theoretical Anammox process. This might result from the nitrification as indicated earlier but the attempt to provide the strictness zero organic carbon and limited dissolved oxygen in the reactor probably allowed the opportunity for the Anammox process to proceed. The fluctuation of mole ratio through the S2 experiment is shown in Figure 4.4.

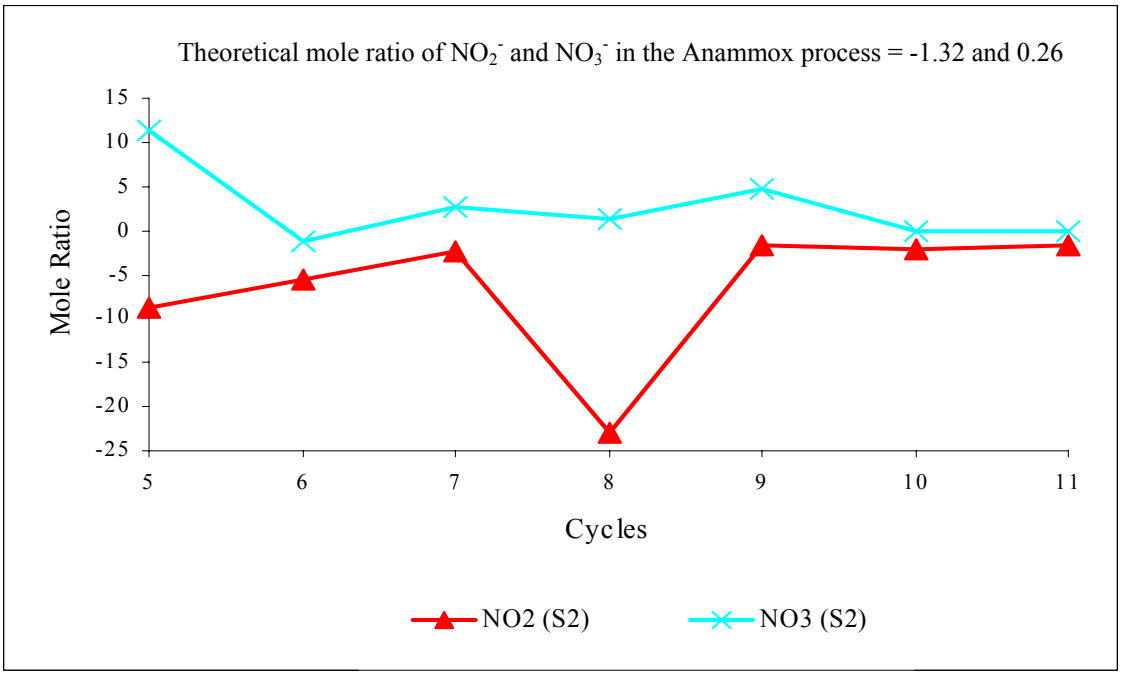


Figure 4.4 Mole ratio of sludge S2 in the early stage of acclimatization

4.1.1.4 Experimental Results of Sludge S3

Sludge S3 used for Anammox enrichment was the anaerobic digestion sludge. The experimental results during 85 days of operation are shown in Figure 4.5. The stoichiometry calculation of the Anammox process is shown in Table 4.4.

During the first 4 cycles, reaction time of 17, 6, 3, and 3 days, the effluent ammonium concentration was higher than the influent concentration, especially the first 14 days of cycle 1, but after that it showed a lower concentration. This could be explained by the use of active biomass of anaerobic digestion, which required a short time to decrease the ammonium concentration by nitrification under the oxygen-limited condition of the experiment. The lower value of effluent pH obviously shows that ammonium was decreased by the nitrification process.

The results of cycle 5-9 (4, 4, 6, 3, and 3 days of reaction time, respectively) showed the decreasing of ammonium, nitrite, and pH with the increasing of nitrate. This pointed out that nitrification mainly occurred during this period. The denitrification was the minor process, which can be seen from the increasing of effluent pH value during the 29^{th}-33^{rd} day in cycle 5-6. The conditions that supported nitrification and denitrification came from the oxygen-limited condition and the available organic carbon from old cell biomass.

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during cycle 6-9 was (1):(2.14):1.77, which shows the reduction of ammonium and nitrite with the increase of nitrate. As denitrification probably occurred during this period, about one mole of nitrite reduced and one mole of nitrate would be increased. The difference between mole ratio of nitrite and nitrate of 2.14 and 1.77 indicated that the denitrification process converted a part of nitrite to dinitrogen gas. The mole value of ammonium was half of the mole value of nitrite and nitrate, meaning that additional ammonium in the system came from the old cell biomass.

The results in cycle 10-15 (3 days reaction time in each cycle) showed the contrary tendency with cycle 5-9. This could be explained by the limited oxygen and organic carbon conditions provided in this period, which resulted in the limited activity from nitrification and denitrification. The monitoring of effluent pH value that decreased lower than the influent value, supported that during this period the nitrification and denitrification processes still occurred but less than in the earlier cycles.

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during cycle 10-15 was (1):(0.73):(0.61), which shows the reduction of ammonium, nitrite, and nitrate. This confirmed the occurrence of nitrification and denitrification processes.

The last 6 cycles of this experiment, cycle 16-21 (3 days reaction time in each cycle) showed the positive tendency of the Anammox process occurred as there was the consumption of ammonium and nitrite in the system. It could be seen from the effluent ammonium and nitrite concentrations that below the influent concentrations, while the effluent nitrate concentrations increased higher than the influent concentrations. The effluent pH value was still lower than the influent value.

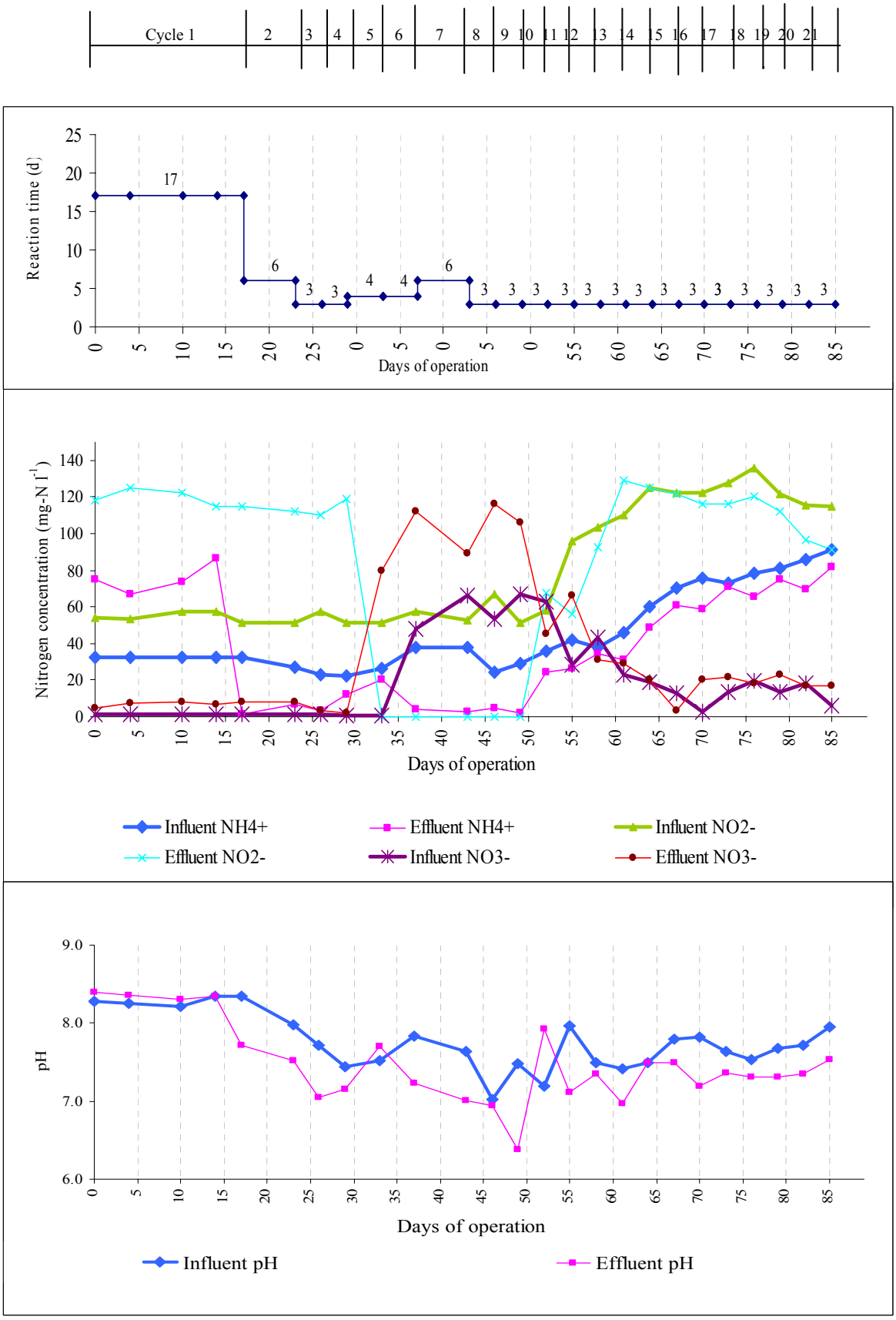


Figure 4.5 Experimental results of sludge S3 in the early stage of acclimatization

Table 4.4 Stoichiometry of sludge S3 in the early stage of acclimatization

Cycle	Reaction time (days)	NH_4^+-N (mg l^{-1})		ΔNH_4^+	NO_2^--N (mg l^{-1})		ΔNO_2^-	NO_3^--N (mg l^{-1})		ΔNO_3^-	pH		NH_4^+ :	NO_2^- :	NO_3^-
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	(1)	(1.32)	0.26
5	4	26.24	20.16	-6.08	51.03	0.02	-51.01	0.94	79.76	78.82	7.52	7.70	(1)	(8.39)	12.96
6	4	37.92	3.92	-34.00	57.28	0.004	-57.28	47.75	112.00	64.25	7.83	7.23	(1)	(1.68)	1.89
7	6	37.76	2.80	-34.96	52.37	0.001	-52.37	66.52	89.14	22.62	7.64	7.01	(1)	(1.50)	0.65
8	3	24.16	5.04	-19.12	66.94	0.22	-66.72	53.11	112.00	58.89	7.02	6.94	(1)	(3.49)	3.08
9	3	29.28	2.24	-27.04	51.15	0.01	-51.14	66.70	105.88	39.18	7.48	6.38	(1)	(1.89)	1.45
10	3	36.08	24.64	-11.44	58.13	67.81	9.68	63.02	45.47	-17.55	7.19	7.93	(1)	0.85	(1.53)
11	3	41.92	26.32	-15.60	96.02	56.10	-39.92	28.68	66.29	37.61	7.97	7.11	(1)	(2.56)	2.41
12	3	37.60	34.72	-2.88	103.49	92.39	-11.10	43.20	31.18	-12.02	7.49	7.35	(1)	(3.85)	(4.17)
13	3	46.24	30.80	-15.44	109.94	129.10	19.16	22.78	29.14	6.36	7.41	6.97	(1)	1.24	0.41
14	3	60.32	48.72	-11.60	124.80	125.24	0.44	18.64	20.16	1.52	7.49	7.50	(1)	0.04	0.13
15	3	70.56	60.48	-10.08	122.47	121.81	-0.66	12.64	3.43	-9.21	7.80	7.49	(1)	(0.07)	(0.91)
16	3	75.36	58.80	-16.56	122.59	116.10	-6.49	2.90	20.57	17.67	7.82	7.19	(1)	(0.39)	1.07
17	3	72.96	71.12	-1.84	128.02	116.10	-11.92	13.57	21.80	8.23	7.64	7.36	(1)	(6.48)	4.47
18	3	78.56	65.52	-13.04	135.73	120.53	-15.20	19.52	18.50	-1.02	7.53	7.31	(1)	(1.17)	(0.08)
19	3	80.88	75.04	-5.84	121.49	112.39	-9.10	13.27	23.14	9.87	7.68	7.31	(1)	(1.56)	1.69
20	3	86.08	69.44	-16.64	115.74	96.67	-19.07	18.24	16.61	-1.63	7.72	7.35	(1)	(1.15)	(0.10)
21	3	91.04	81.76	-9.28	114.70	91.24	-23.46	6.26	16.82	10.56	7.95	7.53	(1)	(2.53)	1.14
Average													(1)	(1.64)	0.72

Note :

Δ : Difference between effluent and influent (effluent - influent)

(...) : Negative value

The average mole ratio of NH_4^+:NO_2^-:NO_3^- during the last 6 cycles, cycle 16-21, was (1):(2.21):1.37. This means that the reduction of ammonium and nitrite occurred whereas the nitrate increased. The average mole ratio through the S3 experiment was (1):(1.64):0.72, which showed similarity to the mole ratio of the Anammox process more than those obtained from sludge S1 and S2. The fluctuation of mole ratio through this experiment is shown in Figure 4.6.

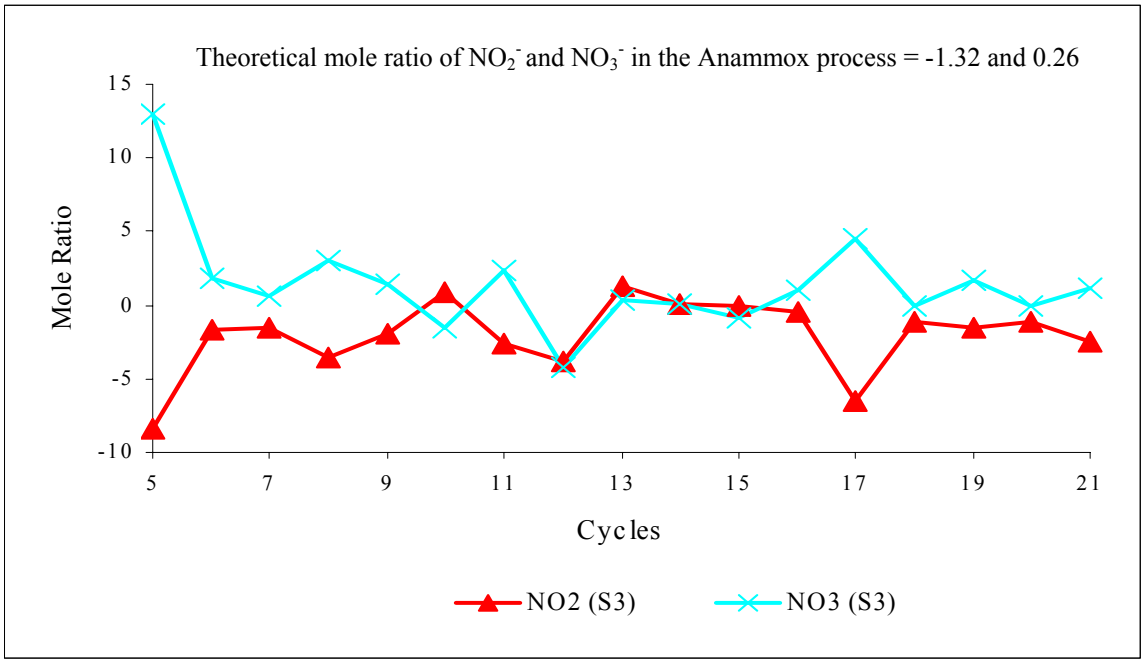


Figure 4.6 Mole ratio of sludge S3 in the early stage of acclimatization

4.1.1.5 Concluding remarks for early enrichment period

Sludge S1: the presumably Anammox seed sludge showed no activity after operated for 63 days, probably due to a long storage under starvation condition prior to acclimatization in the reactor causing the die-off of Anammox organisms. The results showed the slow change of ammonium, nitrite, and nitrate concentrations. The main process in this experiment was the nitrification. The low activity caused the replacement of the UASB sludge in the reactor S1 at the 64th day of operation. The results after the 64th day showed the acclimatization of UASB sludge for enrichment of Anammox organisms.

Sludge S2: activated seed sludge occurred ammonification initially. After that the nitrification and denitrification were the main processes for nitrogen conversion. During the middle of the experiment, denitrification was inhibited by the deficiency of organic carbon. There was a conversion of ammonium, nitrite, and nitrate, causing a decrease in concentration and the mole ratio of NH_4^+:NO_2^-:NO_3^- had the tendency to the Anammox process. From the condition of limited or zero oxygen condition, the Canon or Anammox process could proceed in this reactor.

Sludge S3: anaerobic digestion seed sludge possessed a similar tendency as the sludge S2. Three processes might involve in nitrogen conversion, namely, ammonification, nitrification, and denitrification process and finally, was the tendency of the Anammox to be proceeded. The difference between sludge S2 and S3 was that the faster proceeded step occurred in the reactor seeded with sludge S3. This could be because the active sludge S3 used in this study was familiar to the high nitrogen compound and anaerobic condition so that it had the faster proceeded step.

4.1.2 Reactor performance through the entire study of Anammox enrichment

Anammox acclimatization was continuing from the early stage in the SBR with a time sequence of 7 h react, 30 min settle, and 15 min discharge. A significant removal of ammonium and nitrite was initially observed after three months of operation and a near complete removal of nitrite was obtained within four months. The start-up period was considered shorter than that in the study of van de Graaf et al. (1996) for approximately seven months of cultivation using an attached growth system.

The ammonium, nitrite, and nitrate concentration profiles for sludge S1, S2, and S3 are presented in Figures 4.7, 4.8, and 4.9, respectively. The nitrogen removal efficiency for the three reactors was calculated through the entire period of study of 150 days to show development of Anammox activity (Figure 4.10).

4.1.2.1 Ammonium concentration

The SBR reactors were operated with the average influent ammonium oncentration of 47.4, 45.7, and 44.7 mg N l^{-1} for sludge S1, S2, and S3, respectively. Based on the obtained results, the ammonium concentration profile could be divided into three phases. The initial phase of operation (approximately 5-7 weeks) when the effluent ammonium concentrations were higher than the influent concentrations. This phenomenon occurred in all reactors. During this phase the change in environment of seed sludge might cause the turnover of bacteria. Thus, the former dormant bacteria might be killed, causing cell lysis and breakdown of organic nitrogen to ammonium. As a result, ammonium concentration increased. The postulation was well supported by the loss of MLVSS in the system without experiencing high sludge washout in the effluent. Based on a rough estimation of 10% nitrogen in cell, this would be accounted from 44 to 124 mg N l^{-1}. The unusual increase of nitrite and nitrate were also observed during this period as demonstrated in Figure 4.7-4.9. Both nitrite and nitrate were products of ammonium oxidation under oxic condition. Since there was no oxygen supplied and there might only be slight leakage of oxygen in the early stage of experiments, such increments were unclear. The reduction of indigenous organisms which are mostly non-Anammox culture and the favorable environment for the growth of Anammox culture led to a selection of microbial population in the system in favoring of Anammox bacteria. The phenomenon seemed to be necessary for the enhancement of Anammox culture and process performance, thereby initiating Anammox activity. The mean solid retention time (SRT) at this stage was reported at 42 d with SD of 14 d.

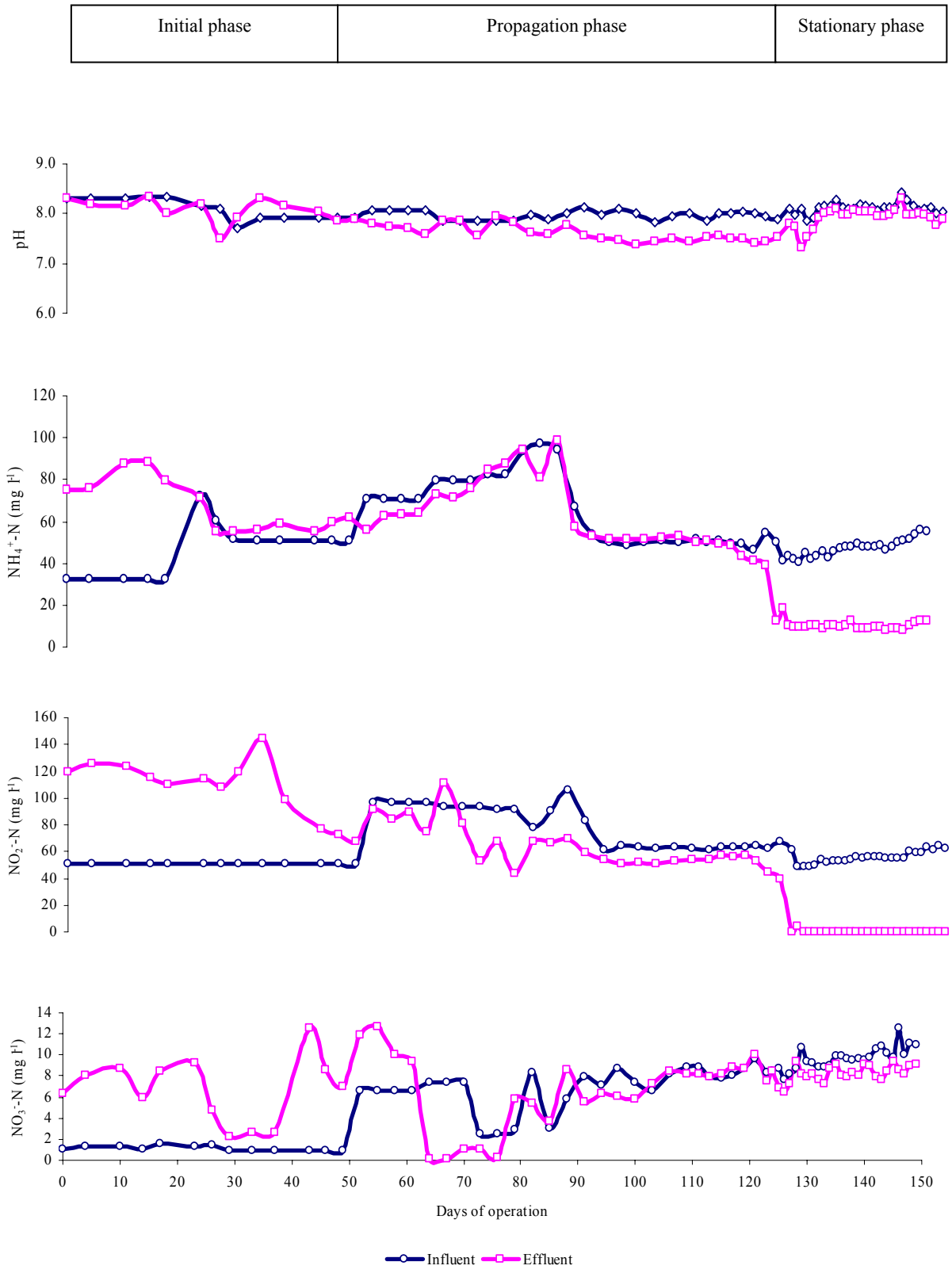


Figure 4.7 Profile of nitrogen removal during 150 days of operation for the reactor seeded with UASB sludge

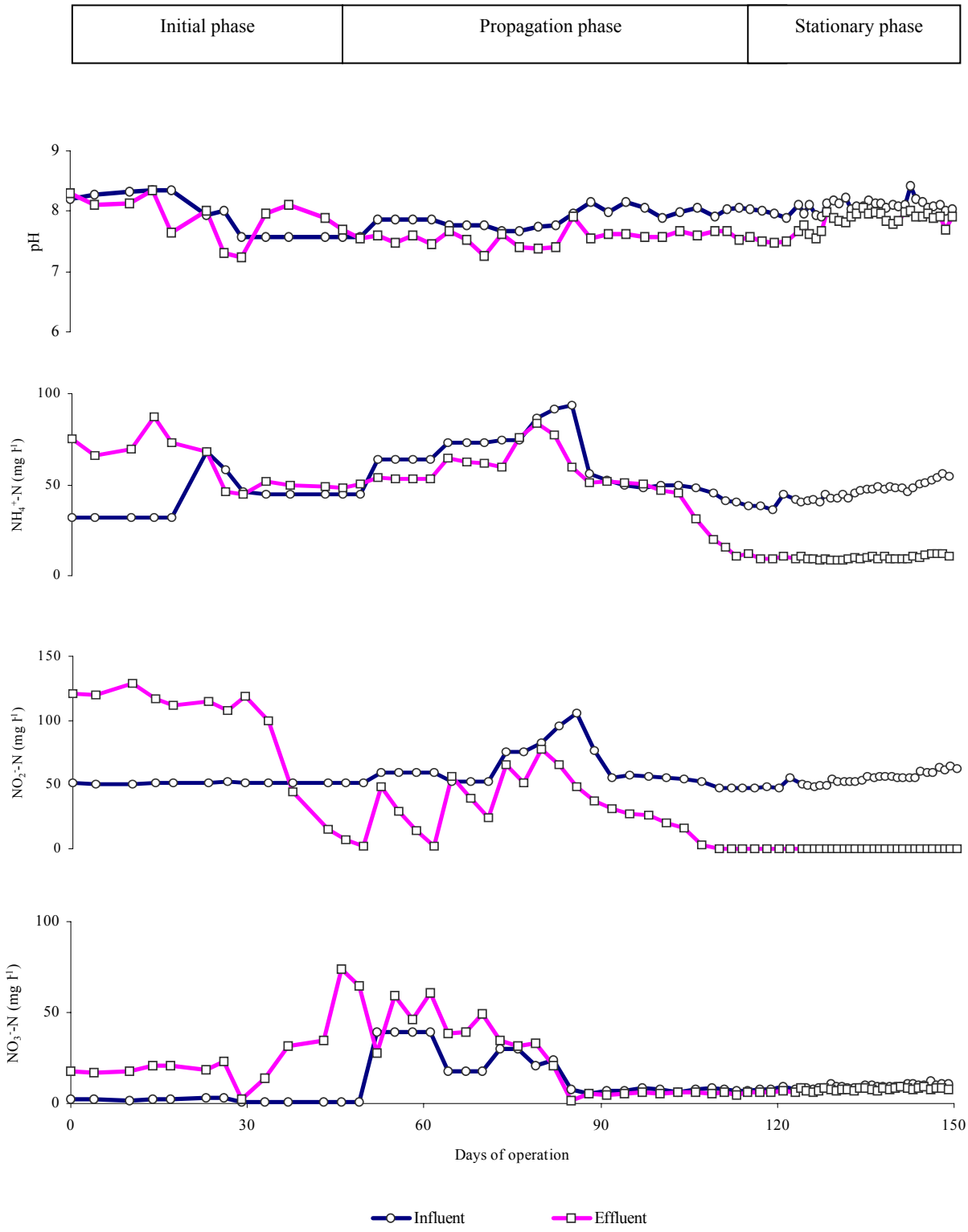


Figure 4.8 Profile of nitrogen removal during 150 days of operation for the reactor seeded with activated sludge

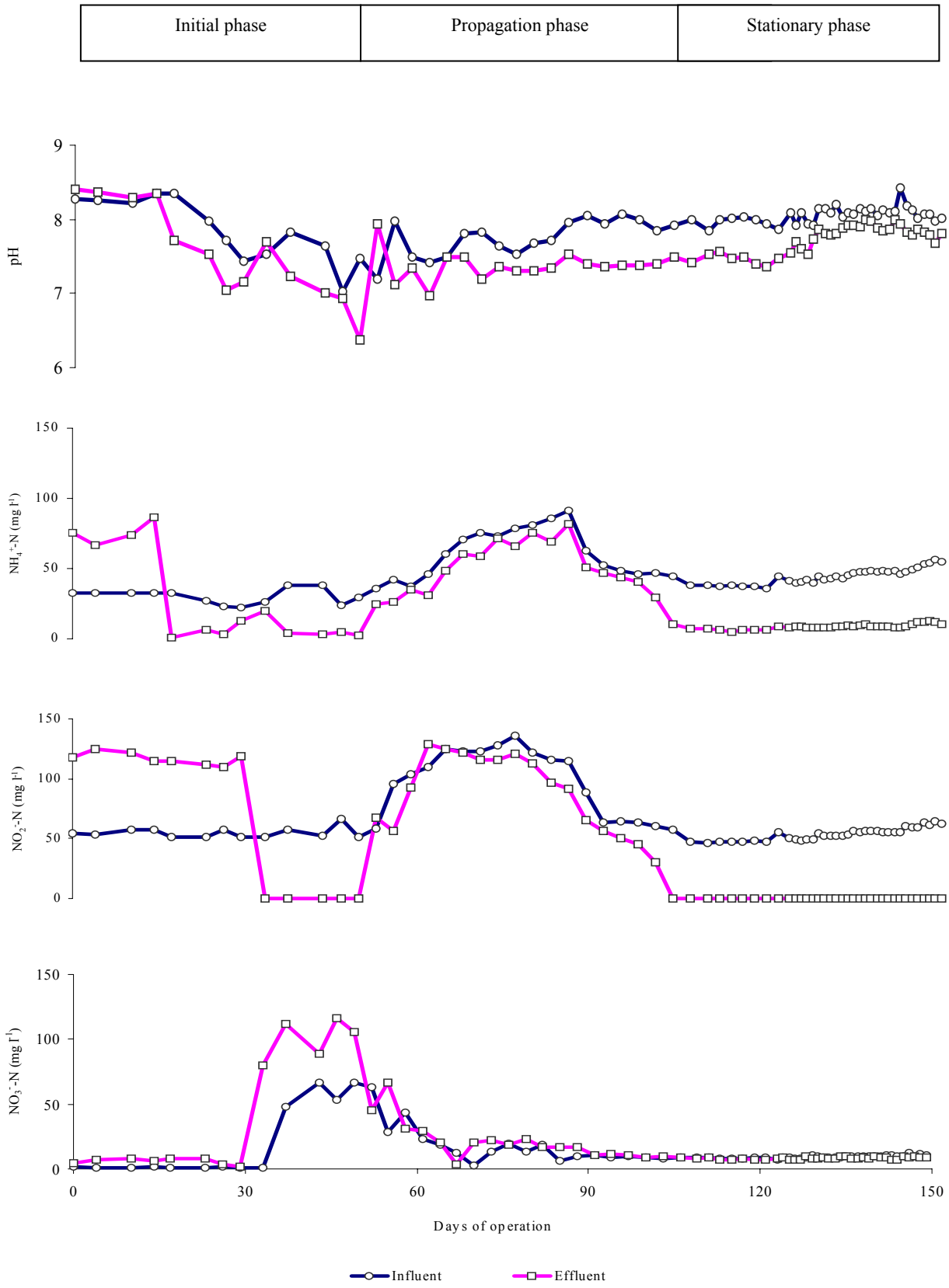


Figure 4.9 Profile of nitrogen removal during 150 days of operation for the reactor seeded with anaerobic digestion sludge

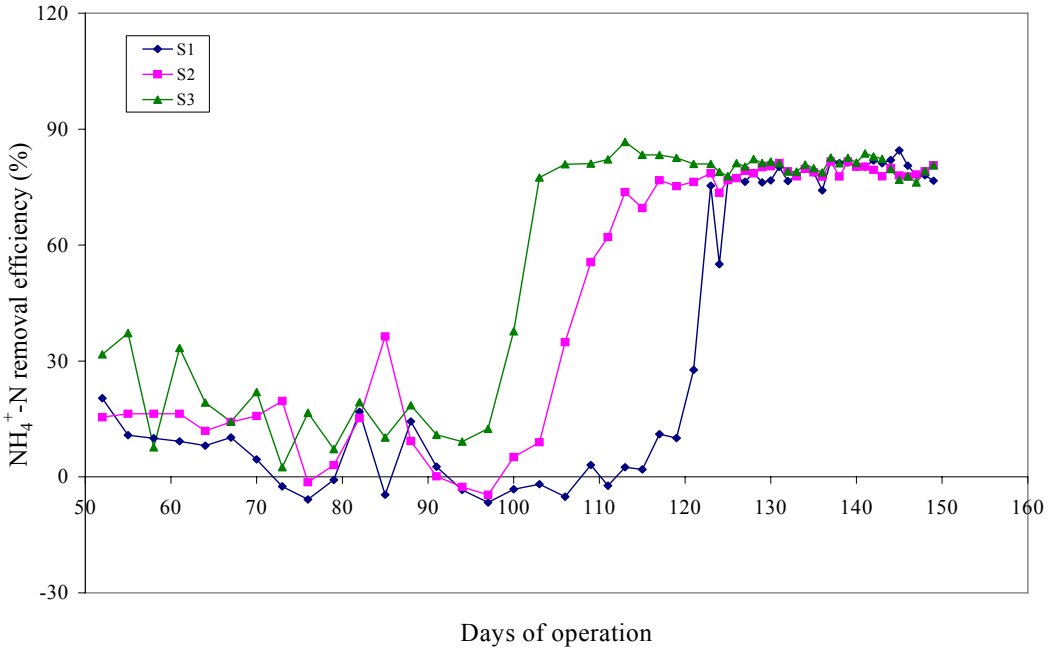


Figure 4.10 Nitrogen removal efficiency of cultivated Anammox sludge in each reactor

The second phase of propagation, the exhaustion of organic substrate from cell lysis occurred resulting in the reduction of sludge digestion activity and the increase of Anammox population in favoring of the provided substrate. This phase of cultivation lasted much longer, approximately 10 to 12 weeks. Ammonium nitrogen reduction reported at the end of the propagation phase was 28%, 35%, and 38% for sludge S1, S2, and S3, respectively (Appendix B). A longer propagation phase was clearly corresponded to a slow growing of Anammox bacteria. During propagation phase, the SRT dropped significantly to be as low as 8 d.

The last phase of cultivation, a stationary phase, allowed the optimum removal of ammonia in the system. Based on the provided conditions, approximately 80% ammonium removal efficiency was achieved in this study. Previous studies indicated the ammonium removal efficiencies of 84%, 88%, and 40% for fluidized bed (Stous et al., 1997b), fixed bed (Strous et al., 1997b), and expanded granular sludge bed reactors (Jianlong and Jing, 2005) fed with synthetic wastewater, respectively. The deviation of the system performances might be dependent on a different concentration of ammonium contained in the influent and the characteristic uniqueness of each presumably cultivated Anammox culture, which also provided different ammonium consumption in each reactor. The average ammonium removal efficiency during a stationary phase of this study was 79%, 78%, and 81% for seed sludge S1, S2, and S3, respectively (Figure 4.10). At this point, with the effluent pH of 7.3-8.3 (Figure 4.7-4.9), a steady system performance was obtained, allowing continuous operation of the system with satisfactory performance. The cultivation stage facilitated the retention of sludge leading to an increase of SRT to be approximately 46 d.

4.1.2.2 Nitrite concentration

The average influent nitrite concentrations of 55.9, 54.4, and 53.9 mg N l^{-1} were applied on the seed sludge S1, S2, and S3, respectively. The effluent nitrite concentrations of all reactors during the initial phase showed higher value than the influent concentrations. However, the effluent nitrite concentrations decreased with time from the conversion of nitrite to nitrate under initial oxygen limited condition.

The effluent nitrite concentrations during a propagation phase, starting around day 50 to about days 121, 106, and 100 for seed sludge S1, S2, and S3, respectively, were close to the influent concentrations. After this period the effluent nitrite concentrations were near zero with the removal efficiency of greater than 98% indicating the stationary phase of operation.

The average nitrite consumptions of 55.9, 54.4, and 53.9 mg N l^{-1} during the stationary phase of seed sludge S1, S2, and S3, were determined together with the ammonia consumption to calculate for the ratio of NH_4^+ to NO_2^- consumption. The obtained ratios were 1:1.5, 1:1.53, and 1:1.5 for sludge S1, S2, and S3, respectively. These ratios are slightly higher than the previous reported values of 1:1.32 (Strous et al., 1998) and 1:1.37 (Helmer et al., 2001). The excess utilization of nitrite was suspected to be due to other bacterial activities in the system which were still unclear and subject to further clarification.

4.1.2.3 Nitrate concentration

The average effluent nitrate concentrations were about 8.2, 7.5, and 8.4 mg N l^{-1} for seed sludge S1, S2, and S3, respectively. The nitrate concentration profile was similar in all reactors. The overshooting of effluent nitrate concentration was observed during initial and early propagation phases, during the fifth to ninth weeks. The phenomenon was suspected to relate to the cell lysis and oxidation as indicated earlier. During the mid-propagation and stationary phases, the effluent concentrations relatively synchronized with the concentrations in the feed suggesting that there was no biological activity leading to the production of nitrate.

The obtained average ratio of ammonium utilization to nitrate production was 1:0.04 which is deviated from the Anammox stoichiometry ratio of 1:0.22±0.02 (van de Graaf et al., 1996). The finding supported the co-existence of other microbial activities as stated earlier. The concurrent reactions of ammonium oxidation and nitrate reduction were reported by Mulder et al. (1995) and Jianlong and Jing (2005) in the fluidized bed reactor.

4.1.3 Stoichiometry of cultivated Anammox sludge in each reactor through the entire study

The stoichiometry of Anammox activity during steady state of nitrogen removal (120-150 days of operation) expressed as an average mole ratio of NH_4^+:NO_2^-:NO_3^- was (1):(1.5):(0.04), (1):(1.53):(0.05), and (1):(1.5):(0.03) for reactor 1 seeded with UASB sludge, reactor 2 seeded with activated sludge, and reactor 3 seeded with anaerobic digestion sludge, respectively (Appendix A).

The obtained stoichiometry of Anammox activity in each reactor was different from the ratio of Anammox, (1):(1.32):0.26 presented by Strous et al. (1998). The assumptions of this different ratio might come from the reduction of nitrate by a co-existing bacterial group, denitrifying bacteria. The mole ratio of nitrite per ammonium consumption of 1.5 in all three reactors shows that the excess mole of nitrite, 0.2, was oxidized to nitrate for carbon oxidation in Anammox activity. The oxidation of nitrate by a co-existing bacterial group resulted in the disappearance of nitrate in all reactors. Then, some extra quantity of nitrite was oxidized to nitrate under anoxic condition for sustain the equilibrium condition in the reactor.

4.1.4 Confirmation of the acquired Anammox seed

4.1.4.1 SEM observation

Sludge samples were collected for observation only if there was a successful operation demonstrated through ammonium and nitrite removals. This primarily occurred after three months of operation under a strictly controlled environment. Figure 4.11 shows the photographs of the cultivated sludge obtained form each reactor.

It can be seen that the flocs of cultivated sludge were mostly spherical shape with a smooth surface, presumably Anammox organisms. There were also other microbes that co-existed with cocci bacteria, such as filamentous and short-rod bacteria. In reactor 1 with seed sludge from UASB, there were both filamentous and spherical shaped bacteria (Figure 4.11a, b). Whereas in reactor 2 and 3 with seed sludge from activated sludge and anaerobic digestion sludge, the main types of bacteria were spherical shaped and short-rod shaped bacteria (Figure 4.11c, d, e, f). The observation, together with monitoring results, revealed the existence of Anammox activity in all three reactors seeded with different sludges. The result of SEM observation was subject to confirmation using FISH analysis.

Various bacterial morphologies found in the sludge also indicated a harmony of Anammox culture with other organisms. The presence of filamentous bacteria in reactor 1 (UASB seed sludge) played a negative role in system performance as indicated in the latter section. The cause of filamentous bacteria formation was unclear since all systems were maintained under the same control environment.

4.1.4.2 FISH analysis

FISH was performed with the 16S rRNA targeting oligonucleotide probes PLA46 and Amx820 (Figure 4.12-4.14). PLA46 targets the group of *Planctomycetales* bacteria whereas Amx820 targets the anaerobic ammonium-oxidizing bacteria, *C. Brocadia anammoxidans* and *C. Kuenenia stuttgartiensis*.

In all reactors, a dominant population developed and hybridized with both PLA46 and Amx820 probes. As found through SEM observation, the SBR reactor seeded with UASB sludge appeared to be different from others with less Anammox population (Figure 4.12c, d). The finding was well-supported by the SEM observation and the resultant system performance, which was lower than other seed sludges. The remaining reactors, which were seeded with activated sludge and anaerobic digestion sludge, were quite similar in characteristics and performance. After long-term operation, however, Anammox activities were observed similarly for all reactors.

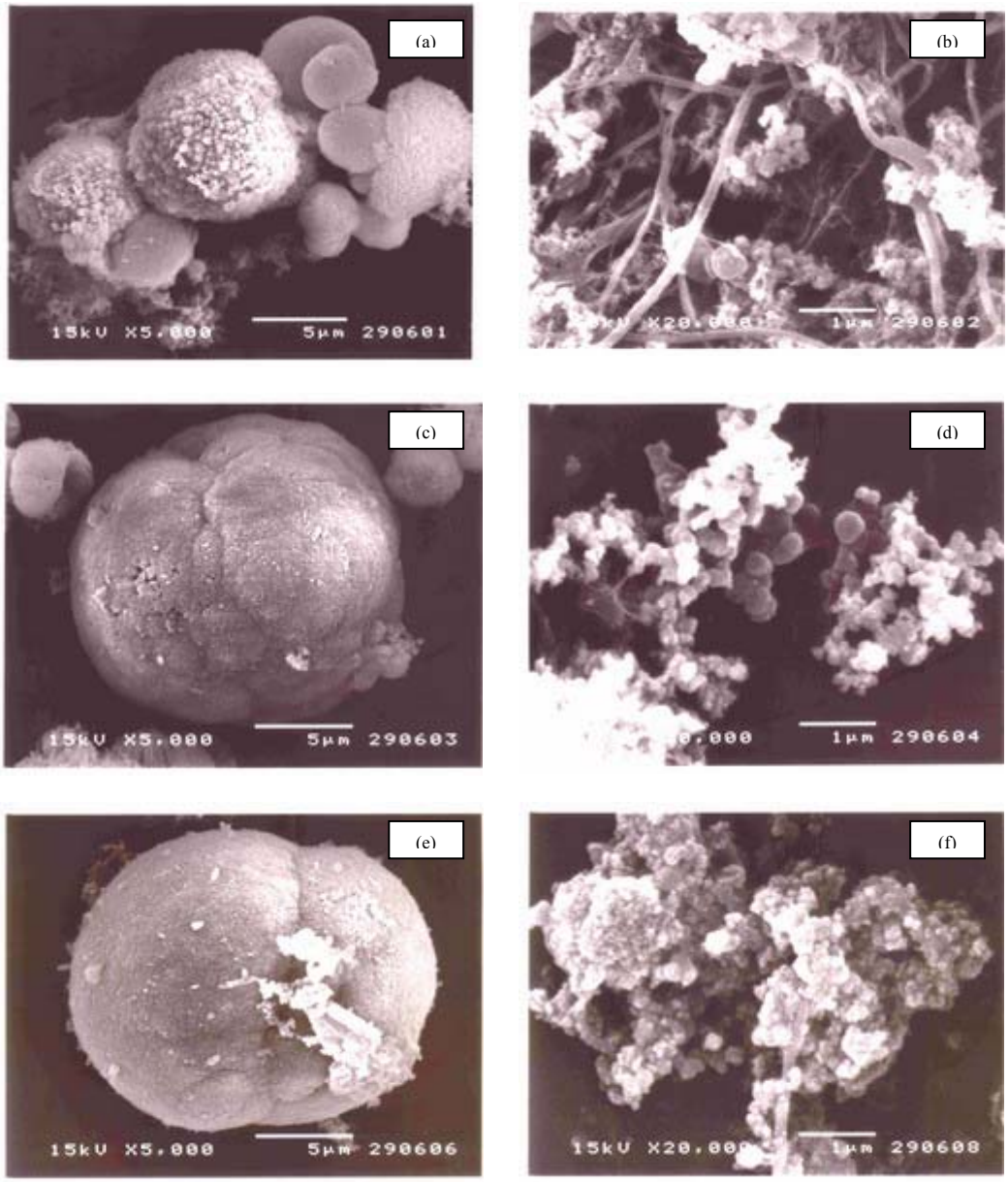


Figure 4.11 Scanning electron micrographs of sludge cultivated in SBR
(a) and (b) Morphology of cultivated sludge from UASB seed sludge,
(c) and (d) Morphology of cultivated sludge from Activated seed sludge,
(e) and (f) Morphology of cultivated sludge from Anaerobic digestion seed sludge

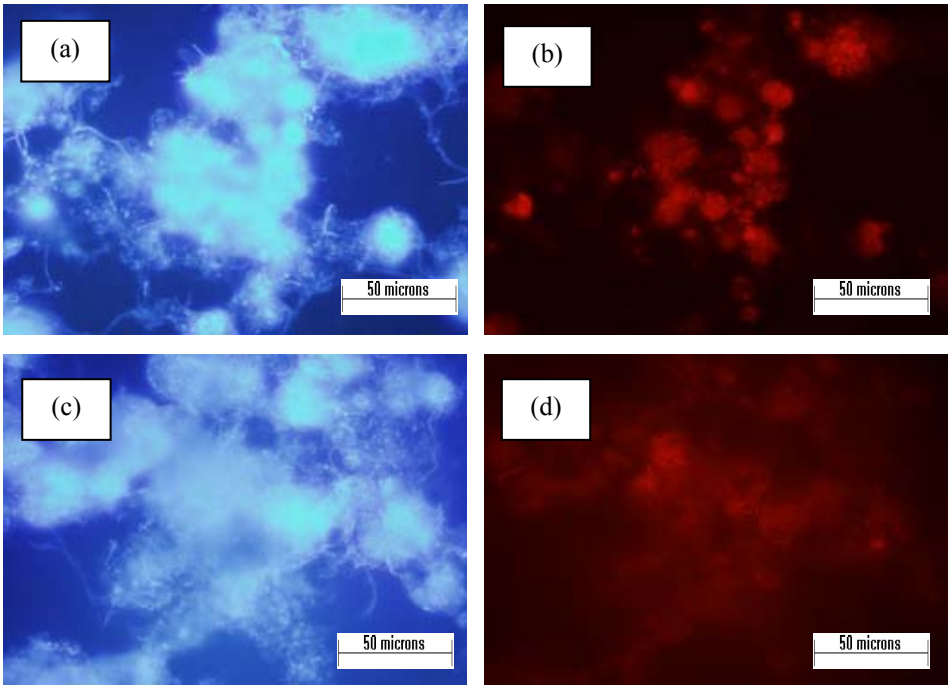


Figure 4.12 FISH analysis of biomass from SBR reactor seeded with UASB sludge, (a) and (b) with PLA46 probe (c) and (d) with Amx820 probe

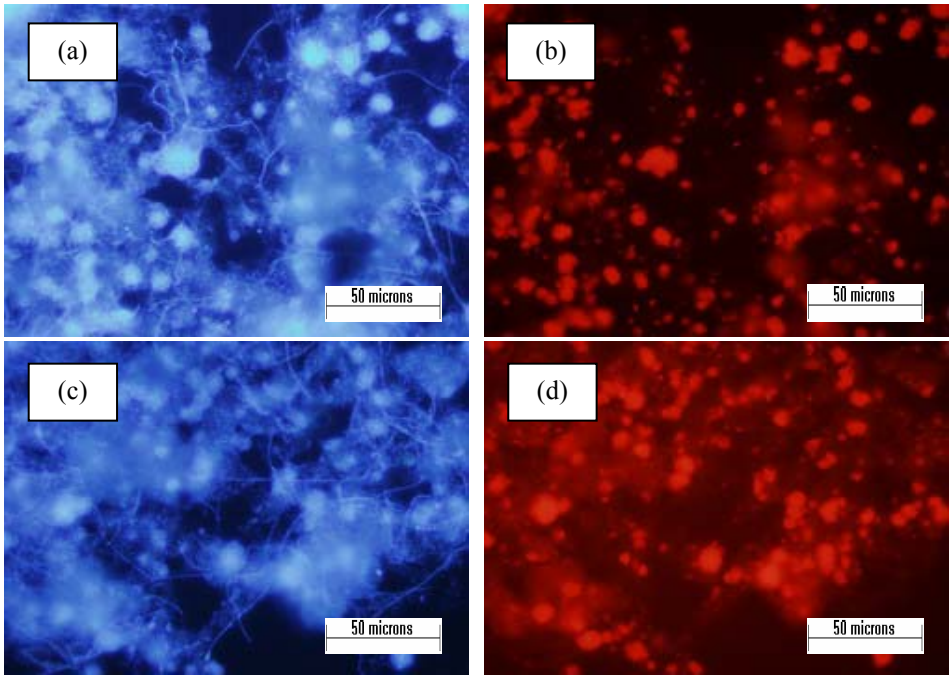


Figure 4.13 FISH analysis of biomass from SBR reactor seeded with activated sludge, (a) and (b) with PLA46 probe (c) and (d) with Amx820 probe

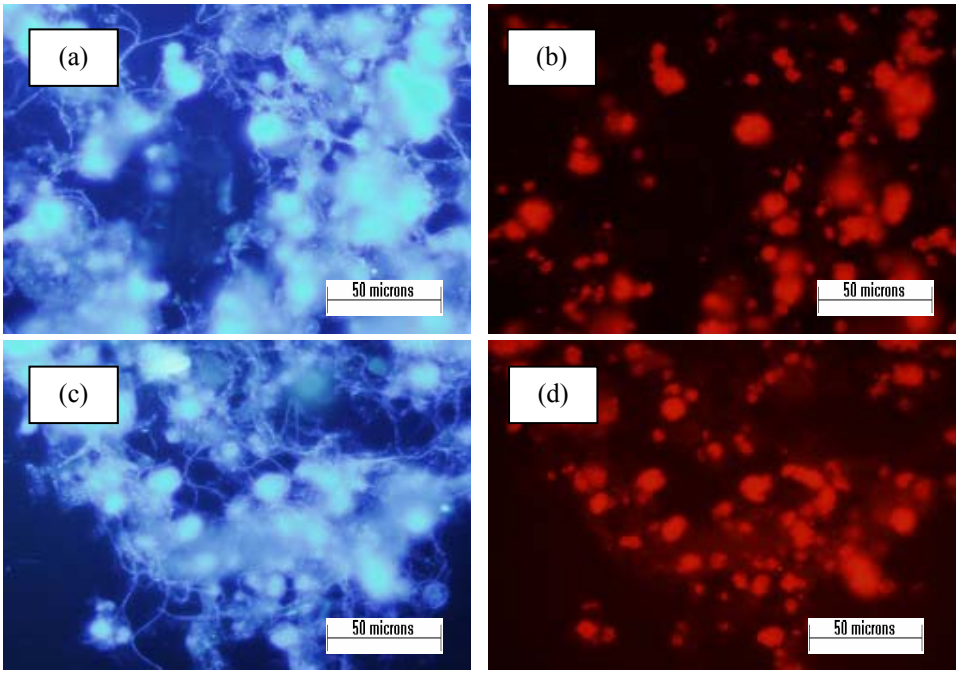


Figure 4.14 FISH analysis of biomass from SBR reactor seeded with anaerobic digestion sludge, (a) and (b) with PLA46 probe (c) and (d) with Amx820 probe

4.2 Experimental phase Ib : Acceleration of Anammox process in SBR using Anammox seeding combined with conventional sludge

4.2.1 Process performance

It was shown that the cultivation of Anammox sludge could be obtained within two months of operation with Anammox seeding (Figure 4.15 and 4.16) and four months without Anammox seeding (Figure 4.10).

Acclimatization of Anammox was accomplished using the synthetic wastewater containing ammonium and nitrite added intermittently to the SBR, starting with a long reaction time of 4 d. During the acclimatization period the ammonium and nitrite concentration in the synthetic wastewater was kept constant. To increase the nitrogen-loading rate, the hydraulic retention time was decreased by decreasing the reaction time. After a month of acclimatization, the reaction time was gradually decreased to 2 d and 1 d, consequently as a significant ammonium removal was observed. When steady state conversion for nitrogen removal was achieved, after 53 days of acclimatized period, the reaction time was decreased and kept constant at 7 h. Significant ammonium and nitrite conversion was observed with the removal efficiency of above 95% for both reactors (Figure 4.16). The obtained nitrite to ammonium ratio was around 1.5 (Figure 4.17). This ratio was similar to the value found in the earlier study without Anammox seed. It was slightly higher than the theoretical value and the previous reported values of 1:1.32 (Strous et al., 1998). This might come from the activity of the other bacterial groups, which thrived in the anoxic/anaerobic environment in the reactor. The nitrate reduction was also detected concurrent to the ammonium oxidation. It is in agreement with the ammonium oxidation

with nitrate reduction found in the fluidized bed reactor (Mulder et al., 1995; Jianlong and Jing, 2005). Without the addition of COD, still, there is the possibility of an endogenous denitrification by utilizing storage substances of the released COD from decaying biomass.

4.2.2 FISH analysis

The existence of Anammox community in the bioreactors was confirmed by the FISH analysis. The probe PLA46 (Neef et al., 1998) was used to see the group of *Planctomycetales* bacteria. A more specific probe, Amx820 (Schmid et al., 2005), which targets the anaerobic ammonium-oxidizing bacteria, *Candidatus Brocadia anammoxidans* and *Candidatus Kuenenia stuttgartiensis*, was also employed. Figure 4.18 shows that the developed biomass was found to hybridise with both probes PLA46 and Amx820.

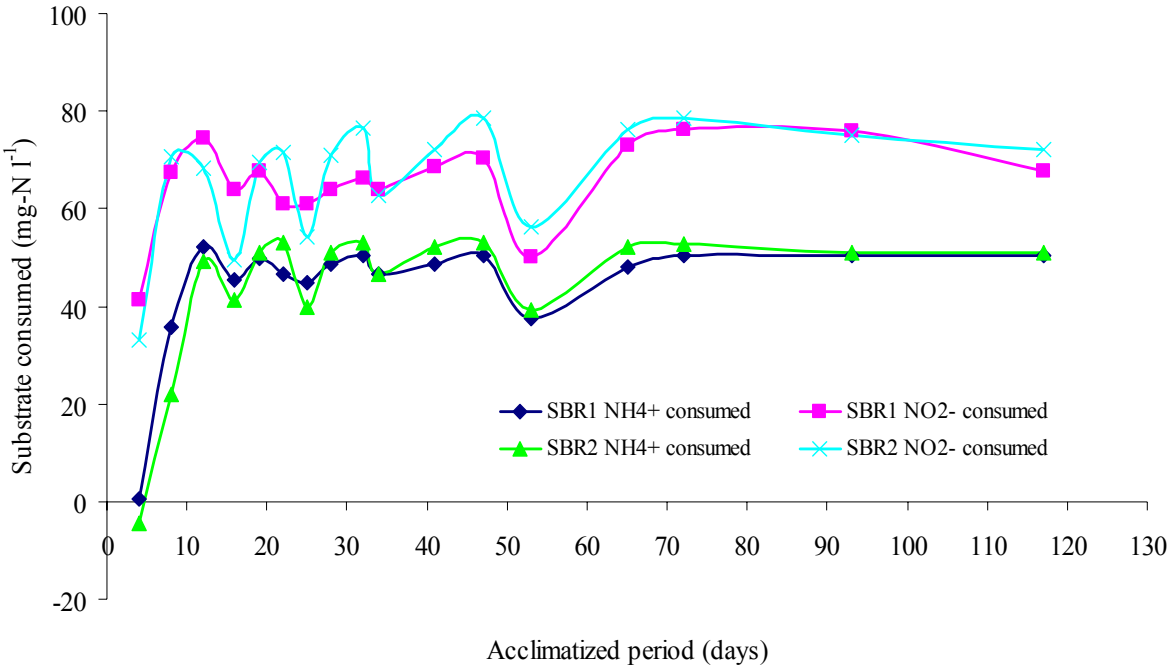


Figure 4.15 Nitrogen consumption during the acceleration of Anammox process in SBR

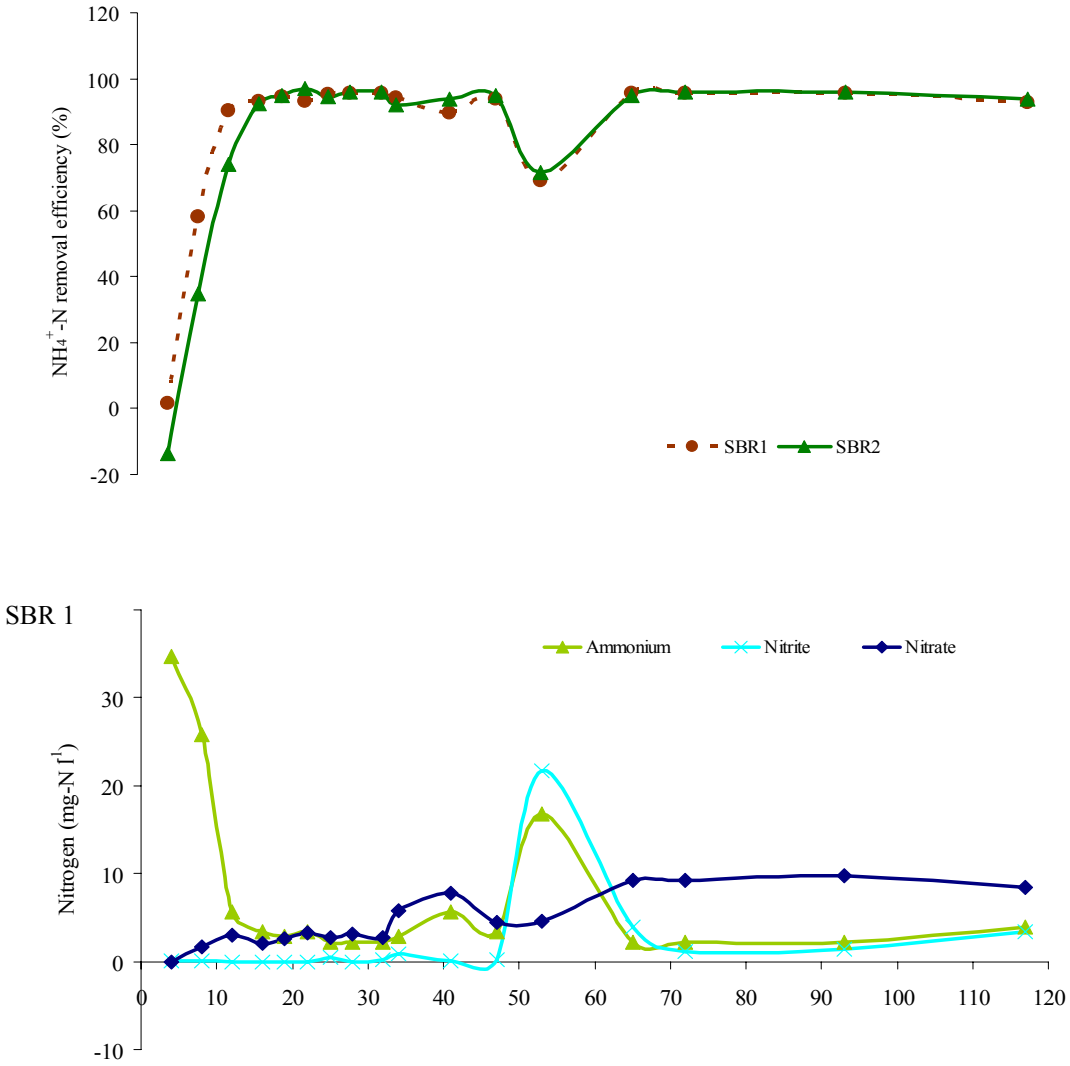


Figure 4.16 Nitrogen species in the effluent, removal efficiency, and the dilution rate during the acceleration of Anammox process in SBR

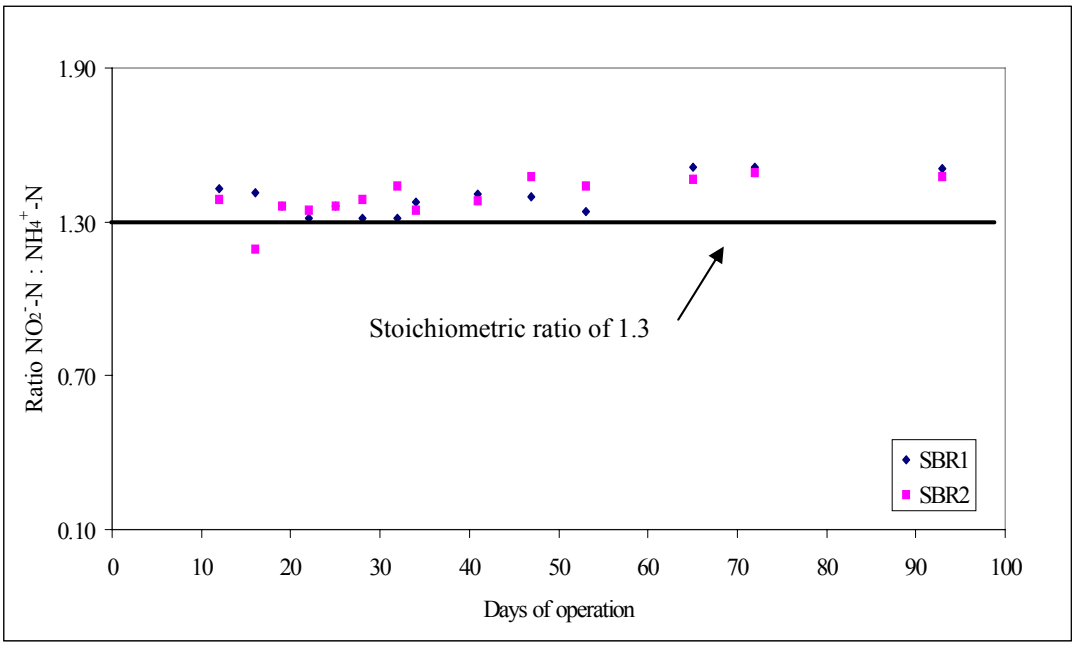


Figure 4.17 Ratio of nitrite to ammonium during the Anammox acclimatization period in SBR 1 and SBR 2. The dense line indicates the optimal nitrite to ammonium ratio for the theoretical Anammox process.

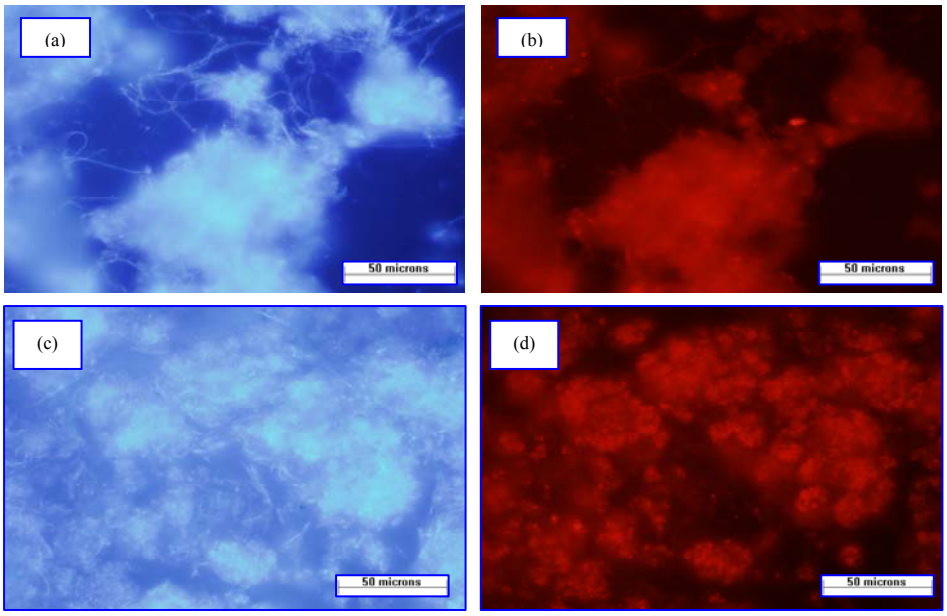


Figure 4.18 FISH analysis of the obtained sludge from SBR 1 seeded with activated and Anammox sludge (a) and (b) with PLA46 probe, (c) and (d) with Amx820 probe

4.3 Experimental phase II : Operational parameters affecting on Anammox-SBR

4.3.1 Time sequence of Anammox-SBR

From the start-up toward steady state conditions, reaction time was varied at 71, 47, 23, 11, and 7 h. Settle and discharge periods were tested for an appropriate run time. A good settle period should provide a good quiescence of liquid while the discharge period offers rapid discharge without sludge washout from the reactor. The run time of 30 and 15 min, were selected for settle and discharge, respectively. Figure 4.19 depicts the overall time sequences with emphasis on reaction time for efficient ammonium and nitrite removals through the entire period of Anammox enrichment using conventional sludges.

The higher efficiency of ammonium removal of more than 80% with complete nitrite consumption was achieved within 7 h of reaction time. This was recorded after 5 months of operation. It indicated the development of Anammox activity related to the biomass concentration in the system. The longer the reaction time, the longer the time until nitrite deficiency. When the new substrate was added to the system, the Anammox might take longer time to acclimatize with the substrate, which resulted in the longer acclimatized period, accordingly. The experiment showed that the optimum reaction time was between 4-7 h for NH_4^+:NO_2^- consumption ratios of 1:1 to 1:1.6 as explained in 4.3.2. The relation between reaction time and sludge quantity was further postulated in the batch study described in 4.3.5.

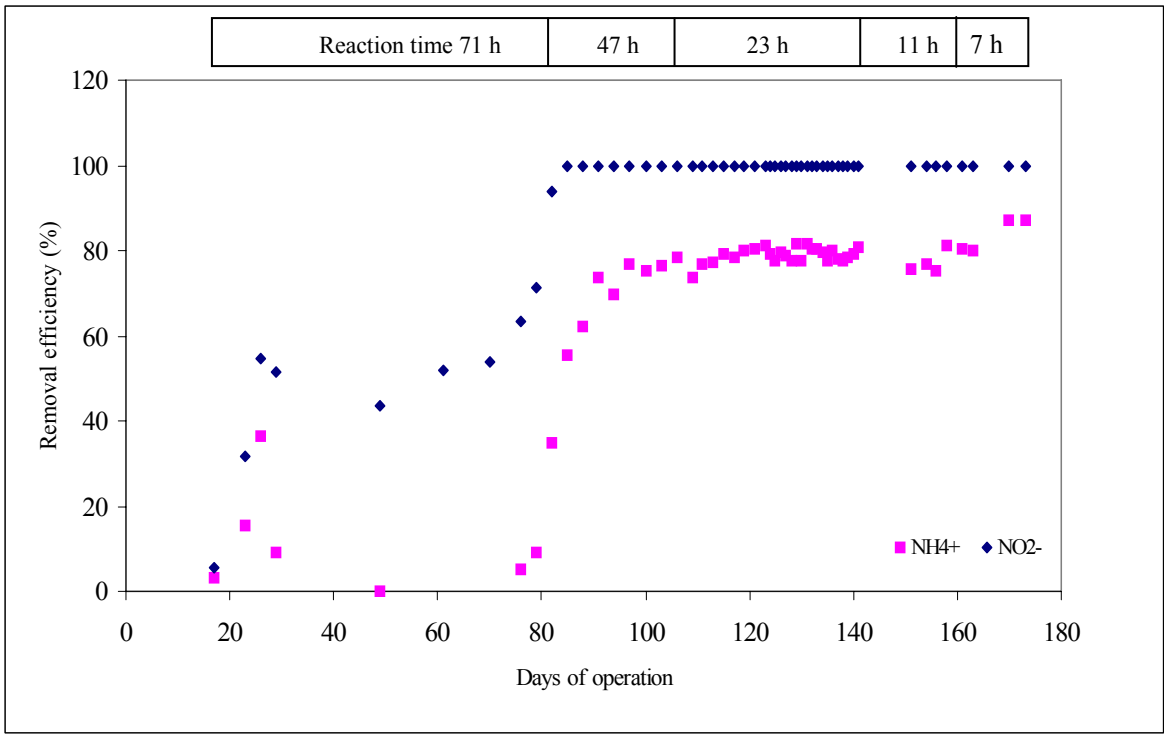


Figure 4.19 System performance under different reaction times

4.3.2 NH_4^+:NO_2 ratio

The NH_4^+:NO_2^- ratios used in experimental phase II were varied to find out which ratio was appropriate to the obtained cultivated Anammox bacteria and to study the NH_4^+ and NO_2^- concentrations effect on the Anammox reaction. Time sequence used for the batch reactor included 7 h react, 30 min settle, and 15 min discharge. This sequence was selected from the time sequence that provided the highest ammonium removal efficiency as obtained earlier. The ratios were varied, such as 1:1, 1:1.1, 1:1.2, 1:1.3, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1.2:1, 1.6:1, and 2:1. However, the obtained optimum ratio for Anammox enrichment in the SBR was observed at 1:1.5. In each ratio, when the process reached the steady state conditions for nitrogen removal, the profile of nitrogen species was monitored every hour for 7 h, which is equivalent to the reaction time used in the SBR. The time course of nitrogen species during steady state conditions in each NH_4^+:NO_2^- ratio is shown in Figure 4.20. The effluent nitrate concentrations showed synchronization with the initial concentrations in all experiments.

The NH_4^+:NO_2^- ratio of 1:1 showed the deficiency of nitrite to sustain the Anammox process. Within three hours of reaction time, the nitrite concentration was completely exhausted. Thus, the Anammox activity was stopped after this time.

The NH_4^+:NO_2^- ratio of 1:1.1 showed the exhaustion of nitrite after the seventh hour of reaction time. The effluent ammonium concentration was lower than that of the NH_4^+:NO_2^- ratio 1:1. However, it was not the optimum ratio because some amount of ammonium concentration still remained in the system.

The NH_4^+:NO_2^- ratio of 1:1.2, 1:1.3, and 1:1.5 demonstrated the exhaustion of nitrite after five hours of reaction time. The effluent ammonium concentration showed a lower value when compared with the above described ratios, indicating the higher Anammox performance. The profile of nitrogen species of the ratio 1:1.6 had the same tendency as these ratios but it showed an excess amount of nitrite used for the reaction.

The NH_4^+:NO_2^- ratio of 1:1.7, 1:1.8, 1.6:1, and 2:1 demonstrated an inhibitive effect to the Anammox reaction as was obviously seen from the non-decreasing of ammonium and nitrite concentration in the system.

The NH_4^+:NO_2^- ratio of 1.2:1 was not recommended for performing the Anammox process as it showed the exhaustion of nitrite in the fifth hour of reaction time, while a high amount of ammonium concentration still remained in the system. Thus, nitrite should be a limiting substrate for Anammox process operation in this study.

The concentration profiles (Figure 4.20), suggest that for the lower ratios of NH_4^+:NO_2^- under 1:1.2, ammonium was inadequate, but these lower ratios can be used to acclimatize the Anammox biomass without adverse effect to the Anammox reaction. However, this might extend the enrichment period of Anammox biomass. According to the monitoring nitrogen species profiles of NH_4^+:NO_2^- ratios of 1:1.7, 1:1.8, 1.6:1, and 2:1, it could be concluded that the NH_4^+ and NO_2^- concentrations of more than 115 and 120 mg N l^{-1} inhibited the Anammox process. Then, nitrite would be a limiting substrate in the Anammox process. The obtained inhibited nitrite concentration agreed with the value reviewed by Schmidt et al. (2003), i.e., the concentrations of above 70 and 180 mg N l^{-1} irreversibly inhibited *Candidatus* Brocadia anammoxidans and *Candidatus* Kuenenia stuttgartiensis, respectively.

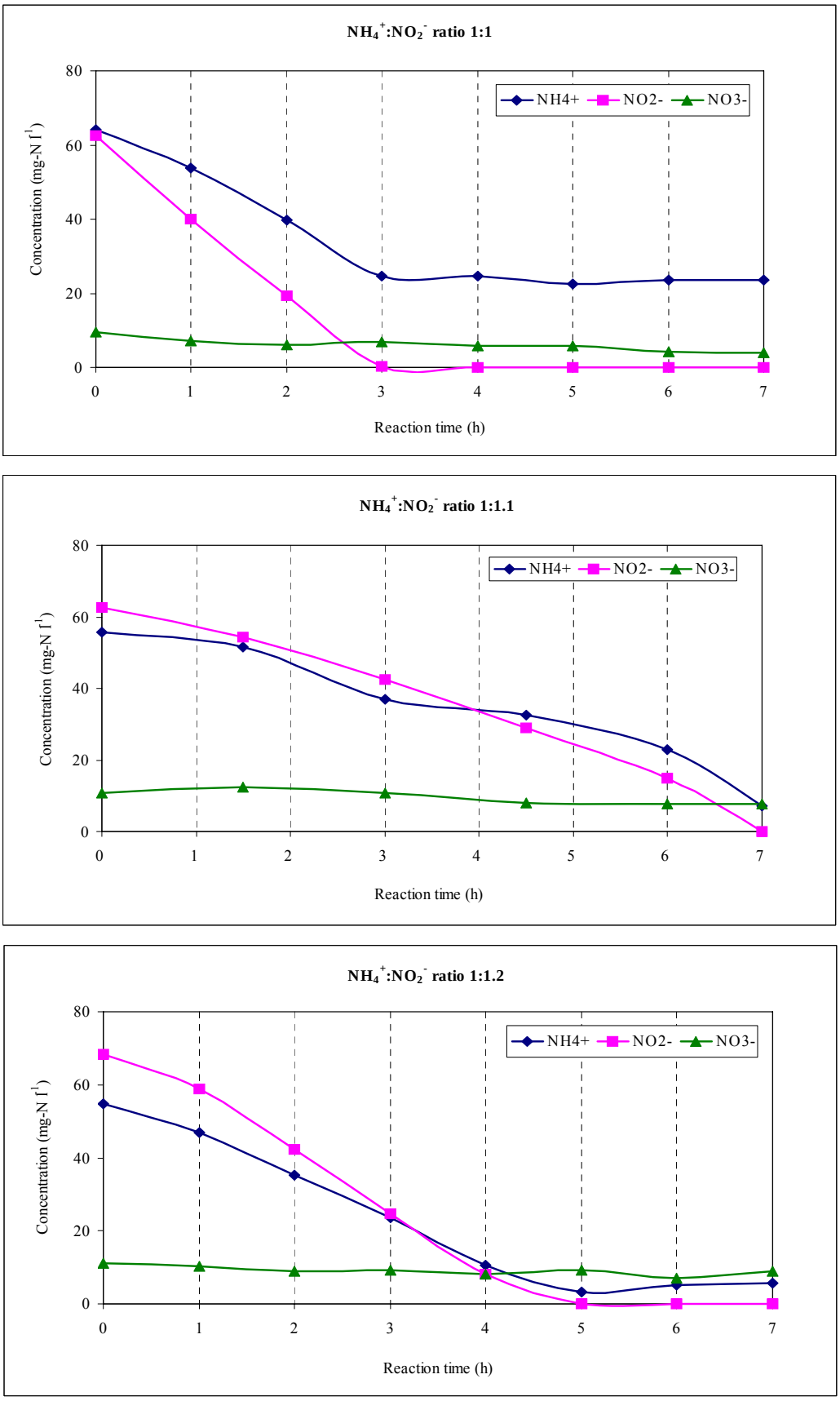


Figure 4.20 Profiles of the nitrogen species in the reactor operating at different NH_4^+:NO_2^- ratio

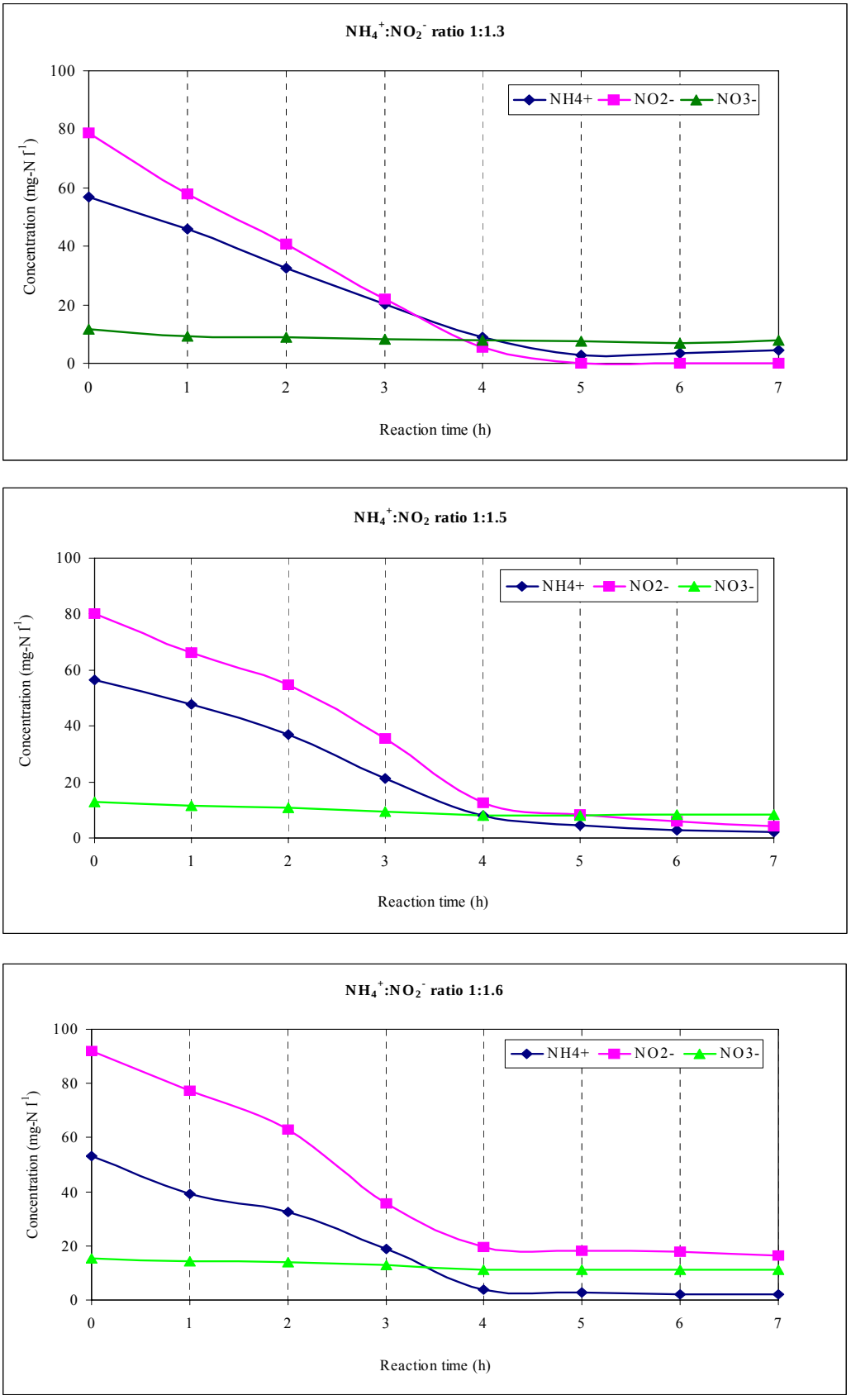
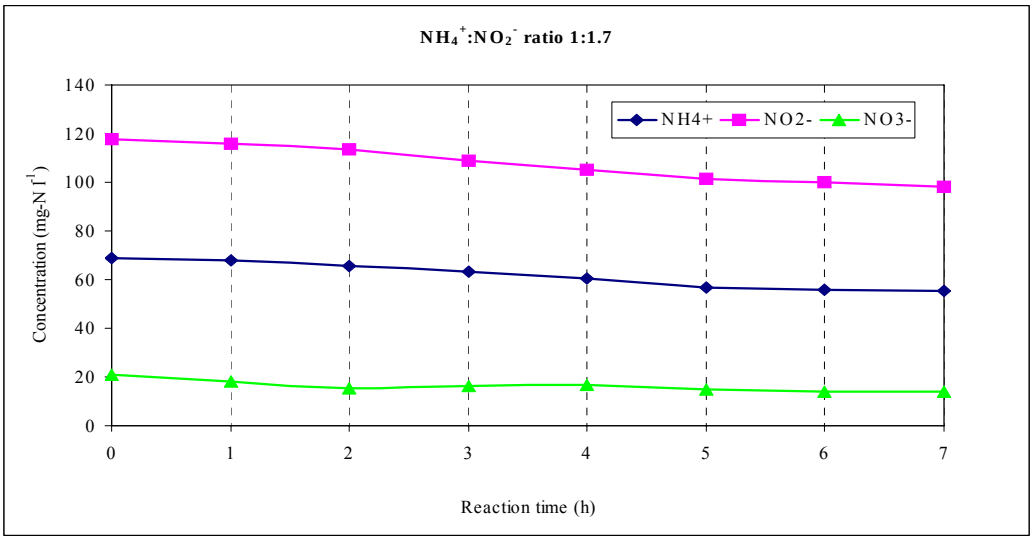
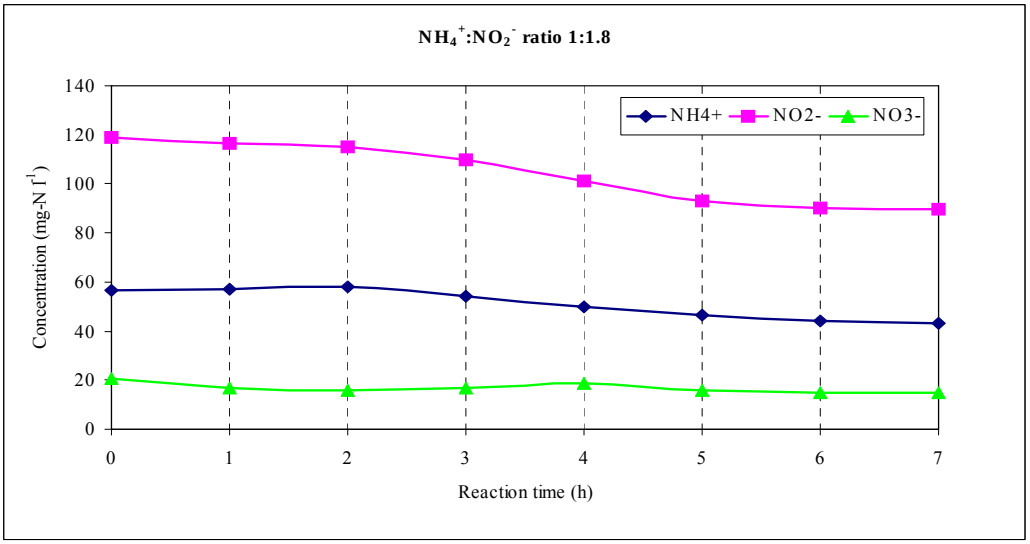


Figure 4.20 Profiles of the nitrogen species in the reactor operating at different NH_4^+:NO_2^- ratio (cont.)





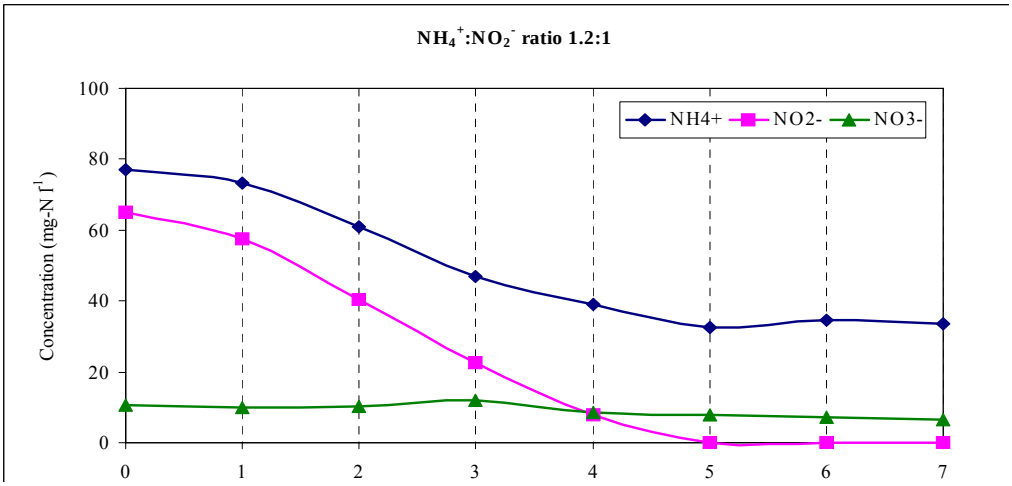


Figure 4.20 Profiles of the nitrogen species in the reactor operating at different NH_4^+:NO_2^- ratio (cont.)

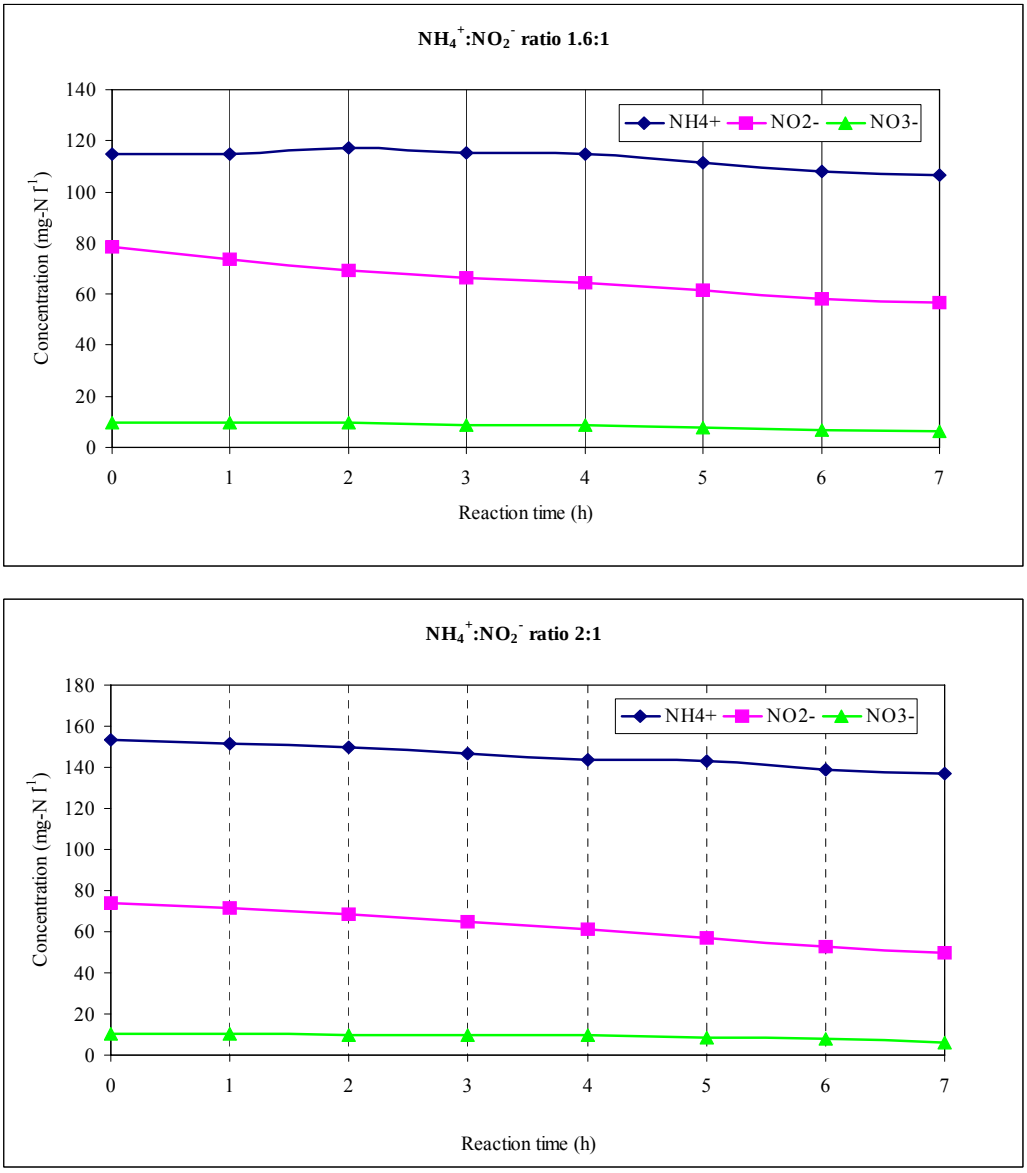


Figure 4.20 Profiles of the nitrogen species in the reactor operating at different NH_4^+:NO_2^- ratio (cont.)

4.3.3 COD:N ratio and COD concentrations

Selected COD:N ratios used for preliminary study of Anammox in combination with denitrification processes were provided as 0.5:1, 1:1, and 1:1.5 as described in 3.3.1.3. The concentration profiles of nitrogen species and COD during steady state conditions for each COD:N ratio are presented in Figure 4.21.

The initial COD:N ratio of 0.5:1 did not show the adverse affect to the Anammox reaction as the ammonium and nitrite still were consumed within six hours of operation. However, the COD consumption was less compared to the other COD:N ratios.

The COD:N ratio of 1:1 demonstrated that the high carbon composition tends to stimulate the activity of the other co-existing bacterial group, denitrifying, to reduce nitrate which was exhausted in the first hour of operation. Similarly, the COD concentration was rapidly reduced within the first hour, indicating that the COD reduction depended on the reduction of nitrate. Ammonium oxidation did not occur as shown by the constant value of ammonium concentration, whereas nitrite was exhausted during the third hour of operation. The COD concentration was gradually reduced after the first hour to be almost constant within the third hour of operation. It indicated the superiority of denitrifying over Anammox for nitrite consumption.

The COD:N ratio of 1:1.5 showed a slight consumption of ammonium within three hours of reaction, while the nitrate and nitrite were completely consumed within two and four hours, respectively. The lowest COD concentration was observed after six hours of reaction. Similar to results obtained from the COD:N ratio of 1:1, this experiment showed the superiority of denitrifying over Anammox for nitrite consumption.

The longer delaying time of ammonium exhaustion was observed in this experiment when compared with that obtained from operating without COD content. The time course of ammonium reduction was extended, while the COD reduction occurred within 3-4 hours related to the decreasing of nitrate and nitrite in the system. The complete consumption of nitrite and nitrate was observed through this study. Both ratios of 1:1 and 1:1.5 showed the trend to inhibit the Anammox reaction, which can be seen from the extended period of ammonium exhaustion. However, the results were unable to indicate the appropriate ratio for the concurrent operation of Anammox and denitrification. Further studies in 4.3.6, 4.4, and 4.5 were used for confirmation and clarification.

4.3.4 PO_4^{3-} concentration

Variations of PO_4^{3-} concentrations were added to the Anammox-SBR for observing the amount of PO_4^{3-} effect on the Anammox reaction. Levels started from PO_4^{3-} concentration of approximately 30 mg P l^{-1} and increased to 45, 60, and 70 mg P l^{-1} consequently. After that the shock load of PO_4^{3-} concentration, 175 and 215 mg P l^{-1} was conducted. The concentration profiles of nitrogen species and PO_4^{3-} during the steady state conditions are shown in Figure 4.22.

The inhibition of phosphate to the Anammox process did not occur at the inhibited phosphate study (1)-(4), 30-70 mg P l^{-1}, as they did not affect the ammonium and nitrite consumption in the experiment. The complete consumption of nitrite was observed within 3-4 hours of operation, while the concentration of phosphate showed nearly constant value through the experiment. The higher influent phosphate concentration in the study (5) and (6), 170 and 215 mg P l^{-1} obviously showed the inhibited effect to the Anammox reaction, resulting in the non-decreasing of ammonium and nitrite (Figure 4.22).

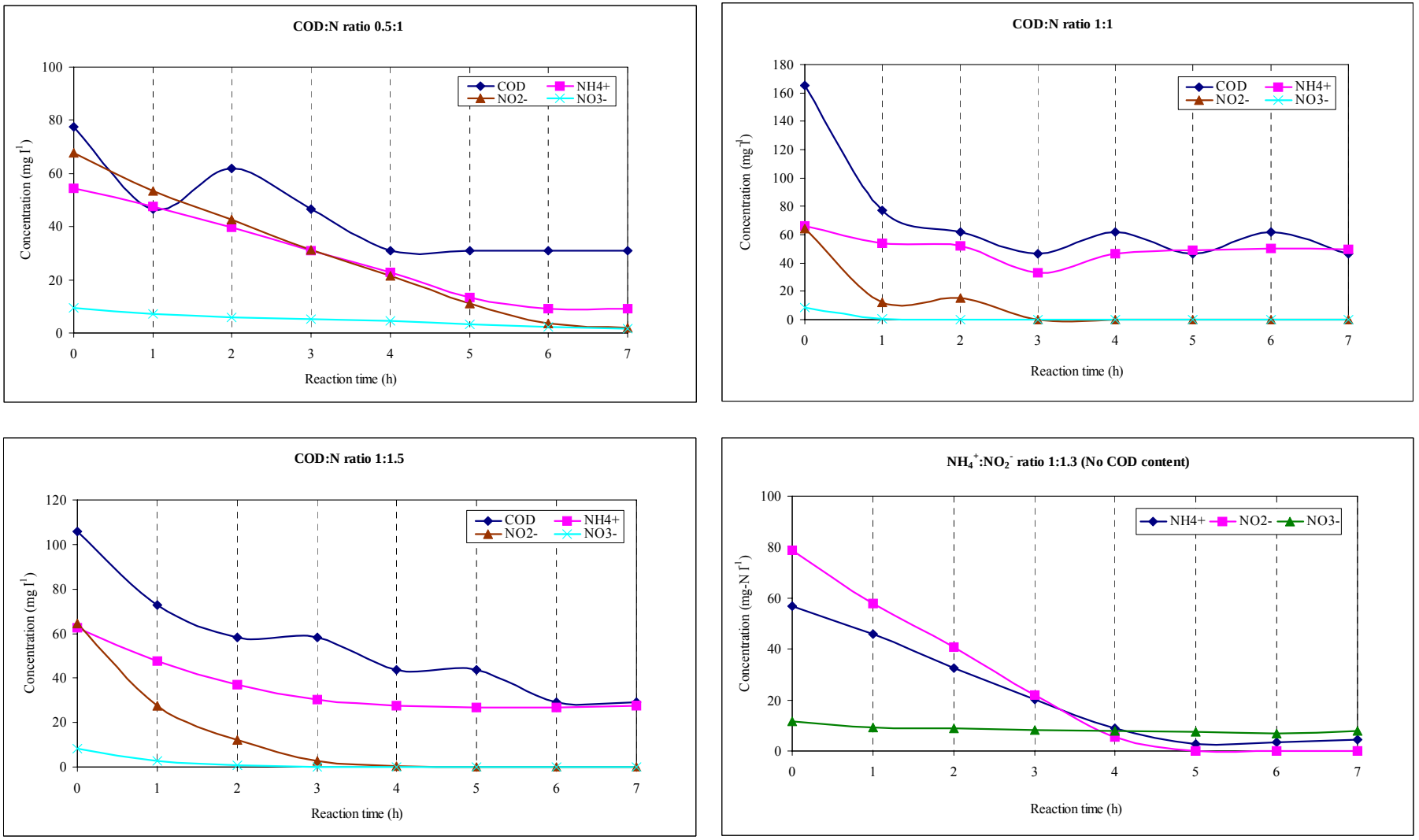


Figure 4.21 Concentration profiles in the reactor operating at different COD:N ratio

The obtained inhibited concentration of phosphate found in this study was in the middle of the range from the previous reports. The phosphate concentration of above 155 mg P l^{-1} was reported as a threshold for complete inhibition of Anammox type metabolisms, *Candidatus* Brocadia anammoxidans (van de Graaf et al., 1996). A difference in tolerance for phosphate between two well-known Anammox species was recorded. The concentrations of above 60 and 600 mg P l^{-1} were irreversible to *Candidatus* Brocadia anammoxidans and *Candidatus* Kuenenia stuttgartiensis, respectively (Schmidt et al., 2003). However, a contradictory conclusion was drawn by Egli et al. (2001) and Dapena-Mora et al. (2007) who reported no inhibition effect of phosphate to the Anammox, at the concentration up to 620 and 465 mg P l^{-1}, respectively.

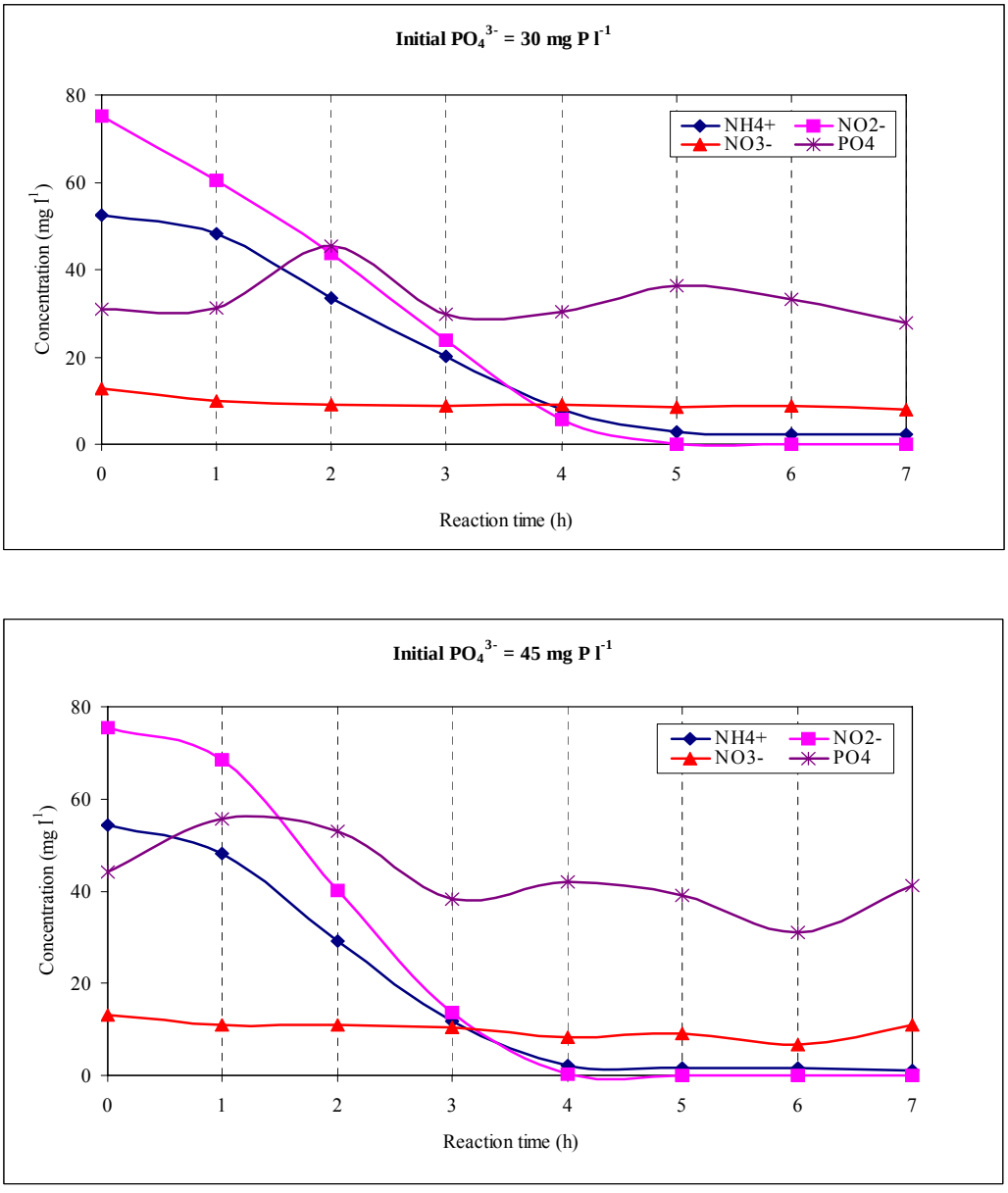


Figure 4.22 Concentration profiles in the reactor during various PO_4^{3-} concentration applications

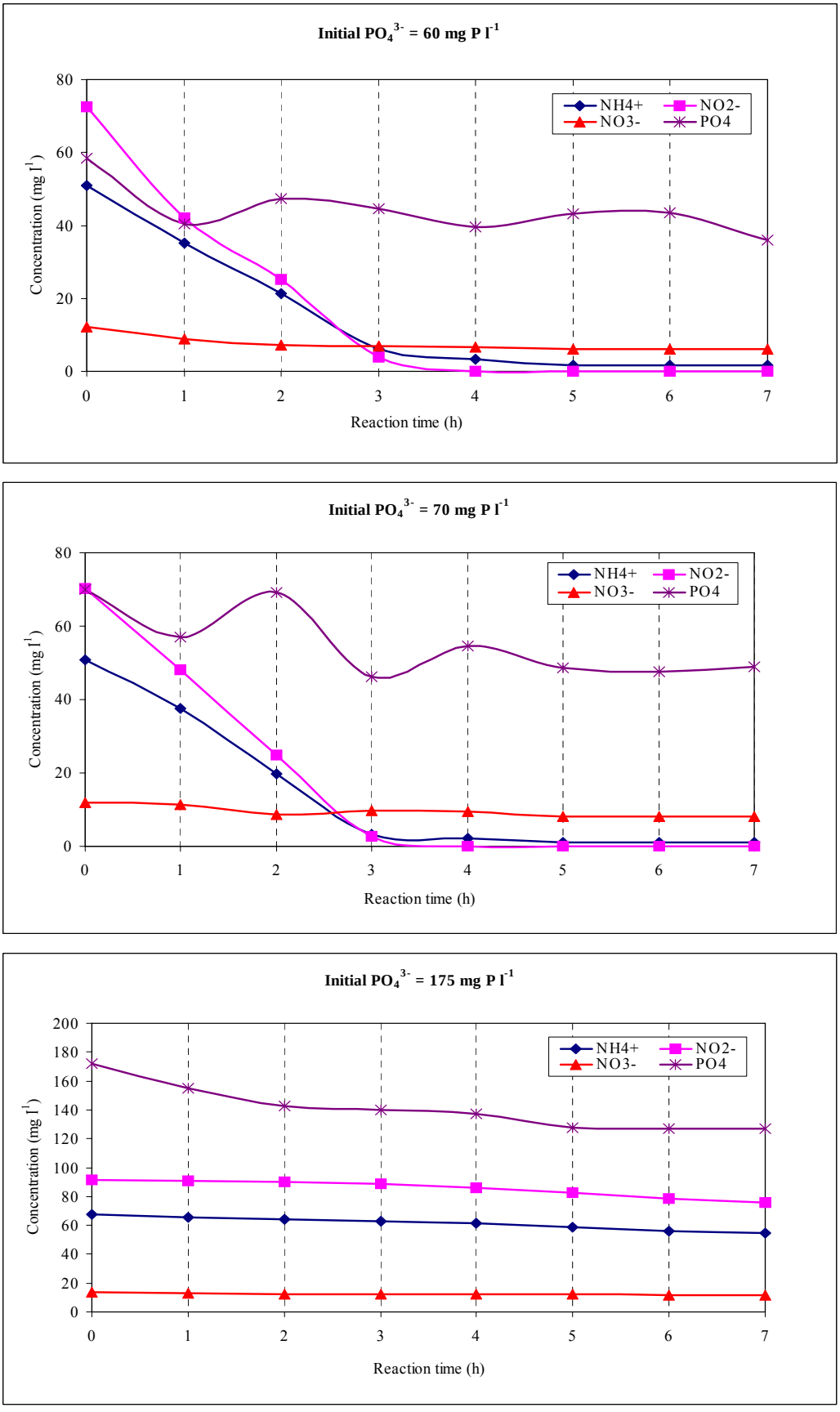


Figure 4.22 Concentration profiles in the reactor during various PO_4^{3-} concentration applications (cont.)

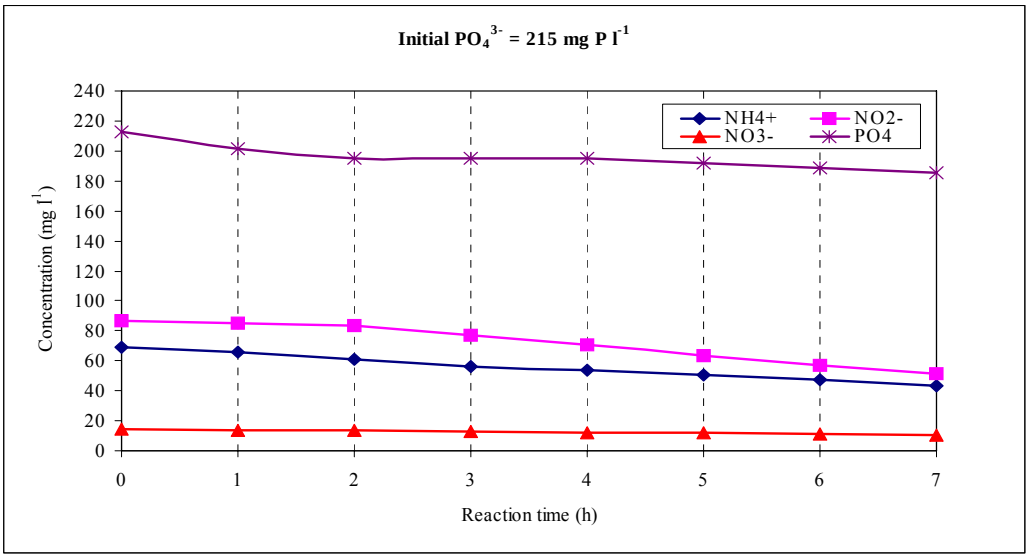


Figure 4.22 Concentration profiles in the reactor during various PO_4^{3-} concentration applications (cont.)

4.3.5 Evaluation of optimum reaction time and seeded sludge quantity

As both reaction time and sludge quantity play a significant role in bio-conversion, their effects on process performance were investigated through batch tests. The results from batch tests confirmed the ammonium to nitrite nitrogen ratio of 1.5 which is similar to the reactor operations but greater than the former study of Strous et al. (1998) with the ratio of 1:1.32. This was indicated through the regression analysis between ammonium and nitrite consumption during experimentation (Figure 4.23) (Appendix C). The lower ammonium to nitrite nitrogen ratio was obtained for sludge concentration of 500 mg MLSS l^{-1} which might be due to a lower Anammox activity as related to biomass concentration.

Figure 4.24 depicts the specific removal rates which were affected by variations of reaction time and sludge concentration. Clearly, both ammonium and nitrite demonstrate a similar effect from the two variables. At the reaction time of 24 to 72 h, the specific removal rates decreased with the increase in sludge concentration from 1,000 to 2,500 mg MLSS l^{-1}, indicating that the increase in sludge concentration beyond the optimum reaction time (or vice versa) may not be beneficial to the process performance. As obtained in this study, the optimum specific removal rate was achieved at the sludge concentration of 1,000 mg MLSS l^{-1} for both reaction times of 24 and 48 h, while the greater and lower sludge concentration resulted in the reduction of specific removal rates. At a shorter reaction time (e.g., 7 h), a greater sludge concentration is necessary. A gradual increase of specific removal rates was obtained with an increase in sludge concentration from 500 to 2,500 mg MLSS l^{-1}, indicating that in this study the optimum reaction time for Anammox reaction can be achieved with the reaction time of 7 h providing that sufficient sludge concentration is provided.

However, for reactor dimensioning, the volumetric load is also important aspect. The results from calculation based on volumetric load indicated the optimum volumetric load of ammonium and nitrite at the same sludge concentration of 1,000 mg MLSS l^{-1} for both reaction times of 48 and 24 h.

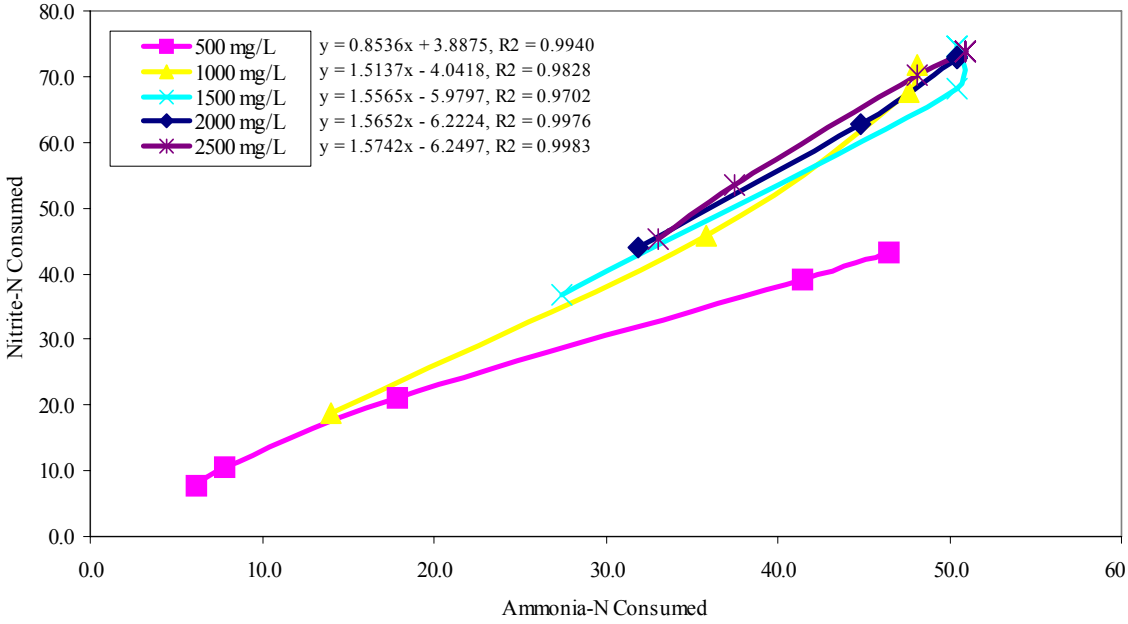


Figure 4.23 NH_4^+-N and NO_2^--N consumed at different sludge quantity

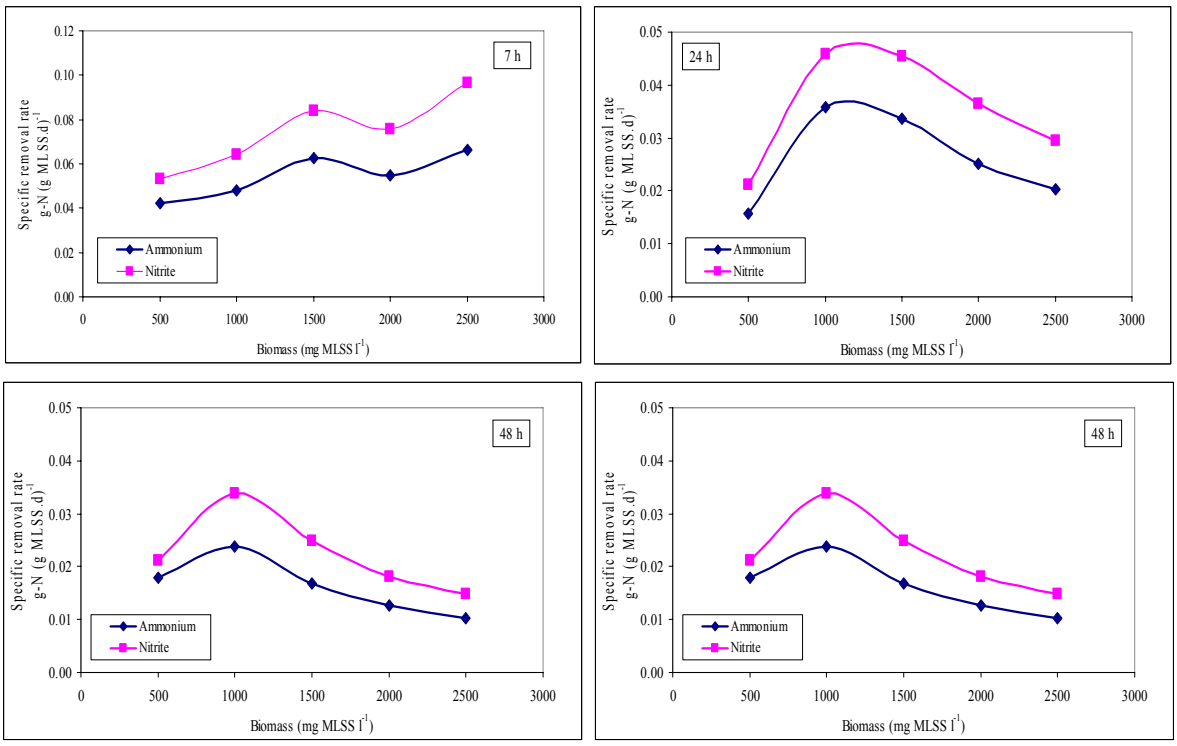


Figure 4.24 Specific NH_4^+-N and NO_2^--N removal rates at different reaction time and sludge quantity

4.3.6 Impact of COD on the Anammox reaction

Anammox is well-suited for the wastewater containing high nitrogen contents and low COD:N ratio. Nevertheless, it is still unclear about the level and role of COD in facilitating the competition of heterotrophic organisms and its inhibition to the Anammox process. It would be beneficial if both processes, denitrification and Anammox, could function concurrently. Batch studies were investigated based on COD:N ratio of 0.6 (low) and 1.3 (high). The concentration of Anammox sludge and reaction time used in this study were 2,000 mg l^{-1} and 7 h, respectively.

Time course profiles of nitrogen species concentrations at no COD content, low (0.6) and high (1.3) influent COD:N ratios are shown in Figure 4.25. The influent COD:N ratio had no effect on the nitrate and nitrite profiles, but strongly influenced the ammonium profile. It is possible that at a high influent COD:N ratio, complete denitrification was achieved. However, at a low influent COD:N ratio, limited denitrification, but nearly complete oxidation of ammonium with nitrite was observed. Hence, the heterotrophs might out-compete the autotrophs like Anammox when the satisfied organic substrate was provided. Therefore, at a high COD:N ratio, the denitrification might show more stimulation and better performance than the Anammox process.

Variation of effluent nitrogen species and the removal efficiency of COD and ammonium in relation to influent COD:N ratio are shown in Figure 4.26. Low influent COD:N ratios of 0.6 resulted in complete Anammox represented as low effluent total inorganic nitrogen (TIN), $NH_4 + NO_2 + NO_3$ concentration, and also the satisfied ammonium removal of 84% and the COD removal of 60% was observed. Operation at a high influent COD:N ratio of 1.3, which produced relatively low effluent nitrate and nitrite but high ammonium concentration, indicated the satisfied supplementation of organic substrate and also showed the higher competitive quality of heterotrophs, denitrifying bacteria. COD could be utilized by denitrifying bacteria to reduce nitrite and nitrate to form dinitrogen gas. This can be seen from the high COD removal of 82% and the low ammonium removal of 59% (Figure 4.26)

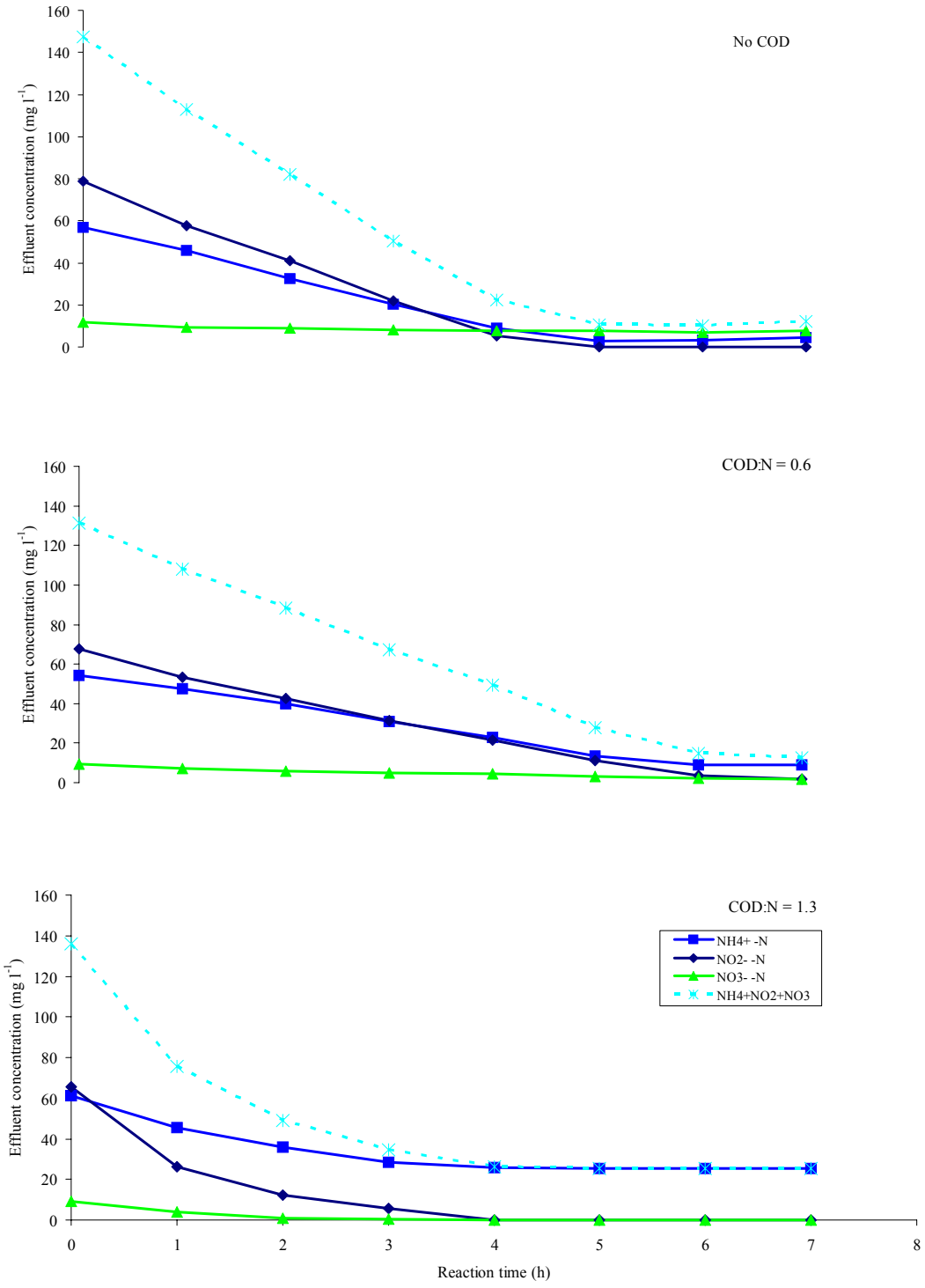


Figure 4.25 Time course profiles of nitrogen species concentrations at various degrees of COD in the feed

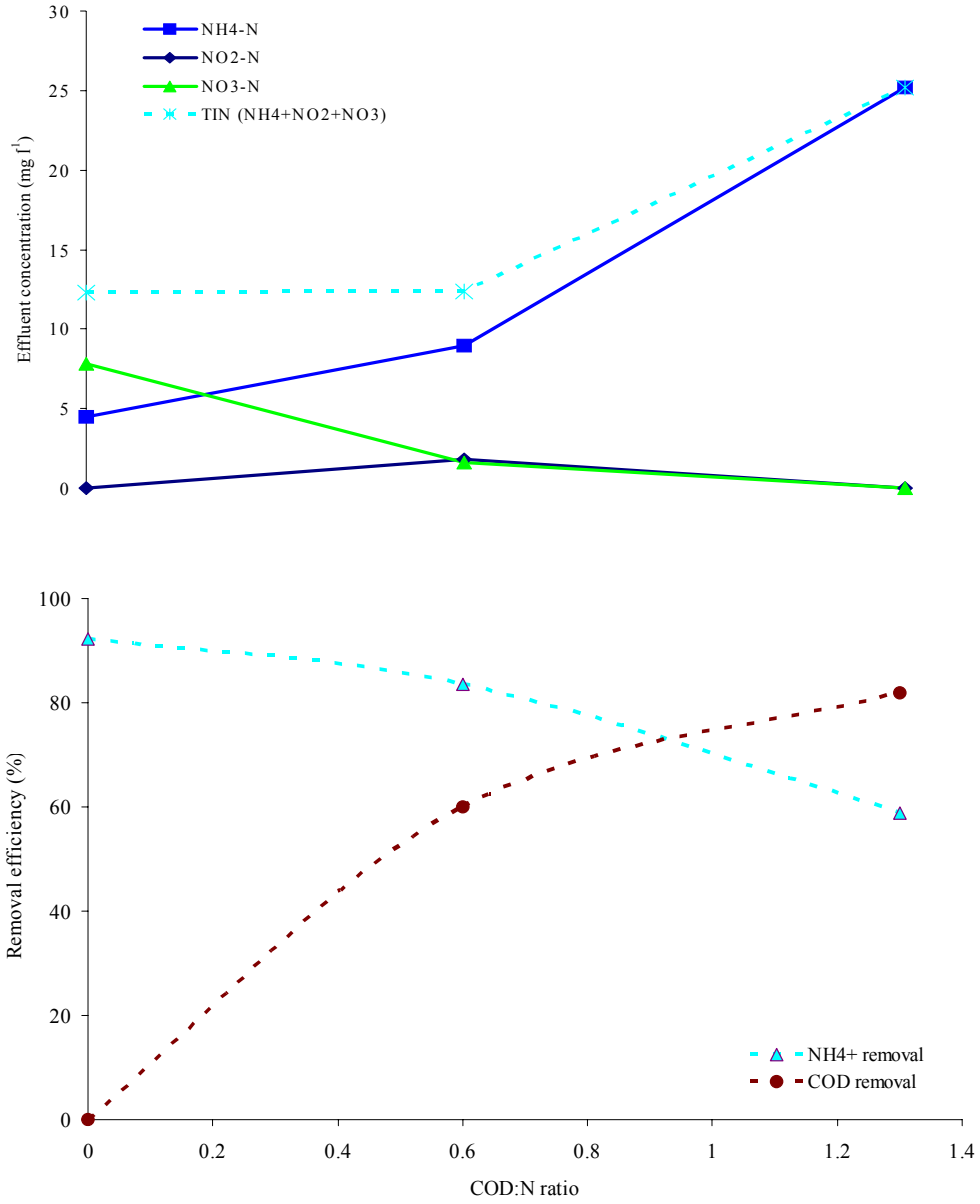


Figure 4.26 Effect of influent COD:N ratio on nitrogen species concentration in the Anammox process

4.4 Experimental phase IIIa : Co-removal of COD and nitrogen in Anammox-SBR

4.4.1 Start-up of Anammox process with Anammox seed under starvation

The SBR was operated with the initial one month starvation Anammox seeding of 1,500 mg MLSS l^{-1} with the influent ammonium, nitrite, and nitrate concentration as described in 3.4.1 and Table 3.4. Starvation condition was conducted when Anammox seed was kept under 4 °C without any nutrient. System performance and pH condition during the start-up period are shown in Figure 4.28. The results showed the improper nitrogen conversion occurred in the reactor during the first 11 days when 70, 100, and 40 mg N l^{-1} of ammonium, nitrite, and nitrate were explored, respectively. Then, a decreasing of ammonium and nitrite concentrations contained in the feed to 40 and 50 mg N l^{-1} was tried to re-start-up the Anammox process, while the nitrate concentration was maintained constant. The results showed a significant amount of ammonium conversion of 90% with complete consumption of nitrite. The steady state condition was achieved after 13 days of operation, indicated by the standard deviation (SD) of ammonium and nitrite during 13 to 19 days to be 10 and 5, respectively.

The mole ratio of nitrite to ammonium consumed was calculated after steady state condition was achieved, after 13 days. The mole ratio was 1.09-1.40 (SD 0.1). A greater variation was obtained as compared to stoichiometric ratio found by Strous et al. (1998) and Dapena-Mora et al. (2004a). This may be due to other co-existing microbial activities for nitrite consumption using organics from cell lysis. The average Anammox activity in terms of specific nitrogen removal rate during 19 days of operation was 0.07 kg N (kg-VSS d^{-1}) with SD of 0.04. Nevertheless, there was no nitrate production, but instead, consumption during this period, which was in the range of 0.11-0.33 mole NO_3^- consumed to NH_4^+ consumed. Nitrate was presumably consumed by the co-existing bacterial group, denitrifying bacteria, as experienced in the former experiment causing the reduction of nitrate in this study. Similar speculation was reported in the previous study of Mulder et al. (1995) and Jianlong and Jing (2005). This occurred due to the cell lysis during the early period of operation.

4.4.2 Effect of COD shock loading and process recovery

The effect of system performance and its recovery after shock loading was explored by spiking the diluted pig manure slurry at COD loading of 8 g l^{-1}, at the day 21st. The results are shown in Figure 4.28. It shows the rapid inhibition of ammonium consumption in the reactor with the decreasing of ammonium removal efficiency of 95% to 17%. Only nitrite and nitrate were completely consumed, resulting from heterotrophic microbial activation in the reactor. The COD was mainly consumed by nitrate-denitrification, and it was also partially consumed by nitrite-denitrification. This means that the denitrifying bacteria can out-compete the Anammox organism for nitrite utilization if there is an external carbon source in the feed solution. Therefore, in this part of the study, nitrate became the limiting substrate in the reactor. Anammox performance would be improved if there was sufficient nitrate for the co-process denitrification. Under this condition, only nitrate would be used for denitrification and nitrite would be used for the Anammox process. This assumption was investigated in the next part of the study when the increasing of nitrate contains in the feed was carried out. However, even if sufficient nitrate is available, the COD to nitrogen ratio is still an important aspect for consideration

to achieve the Anammox process. As a result, the Anammox process could not recover after one month even though the influent without COD content was fed as normal reactor operation. As a gradual dilution was obtained after 4 days from a continuous feed to the reactor, ammonium consumption was then gradually increased from 9% to 30%. However, the system performance as observed earlier could not be recovered. Additional Anammox seed was subsequently supplemented to the reactor on day 49, 52, and 56 for 20, 40, and 40 ml, which was equivalent to MLSS concentrations of approximately 100, 200, and 200 mg l^{-1}, respectively. The ammonium consumption was then increased to be above 90% with complete nitrite consumption after day 57 (Figure 4.28). After that it showed steady state reactor performance indicated as average ammonium consumption of 91% with SD of 9.2.

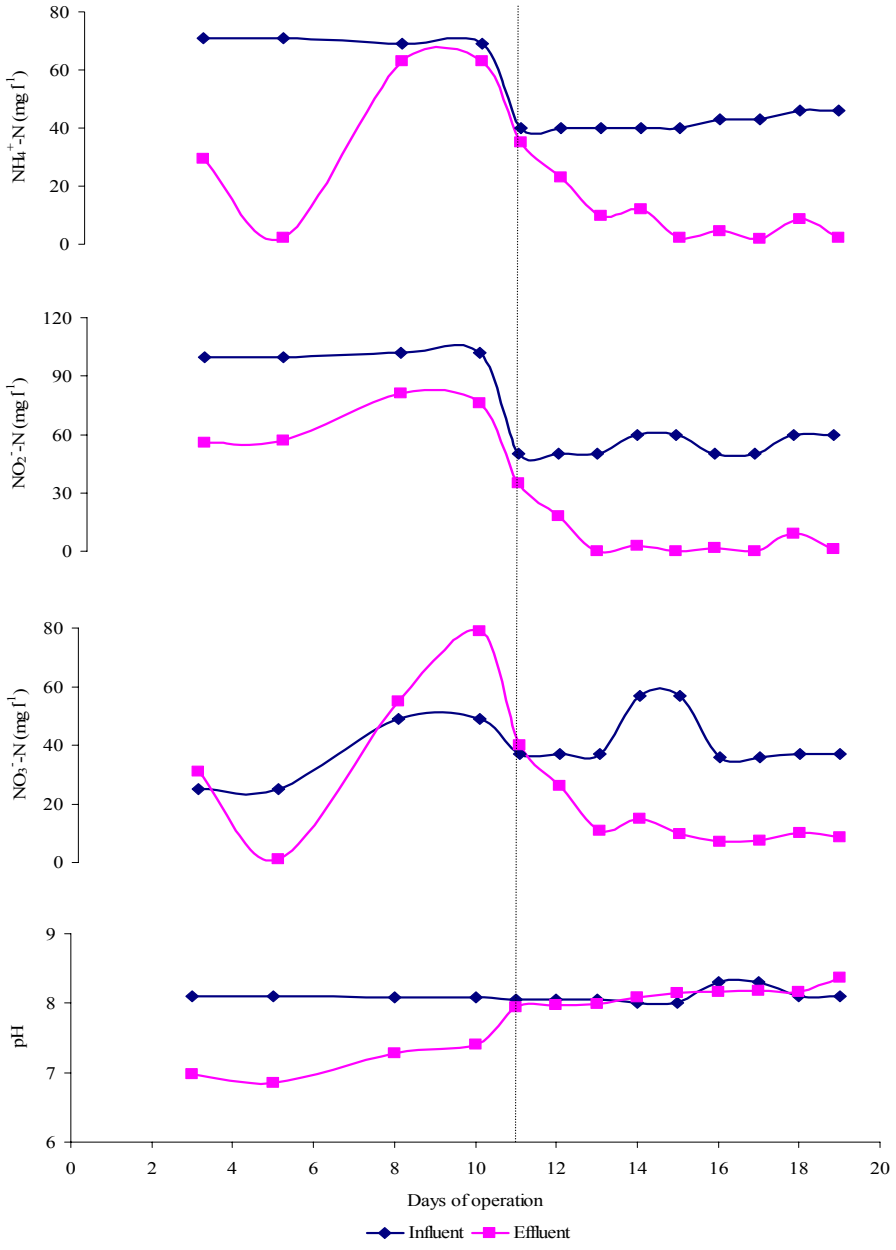


Figure 4.27 System performance and pH condition during the start-up period with starved Anammox seed

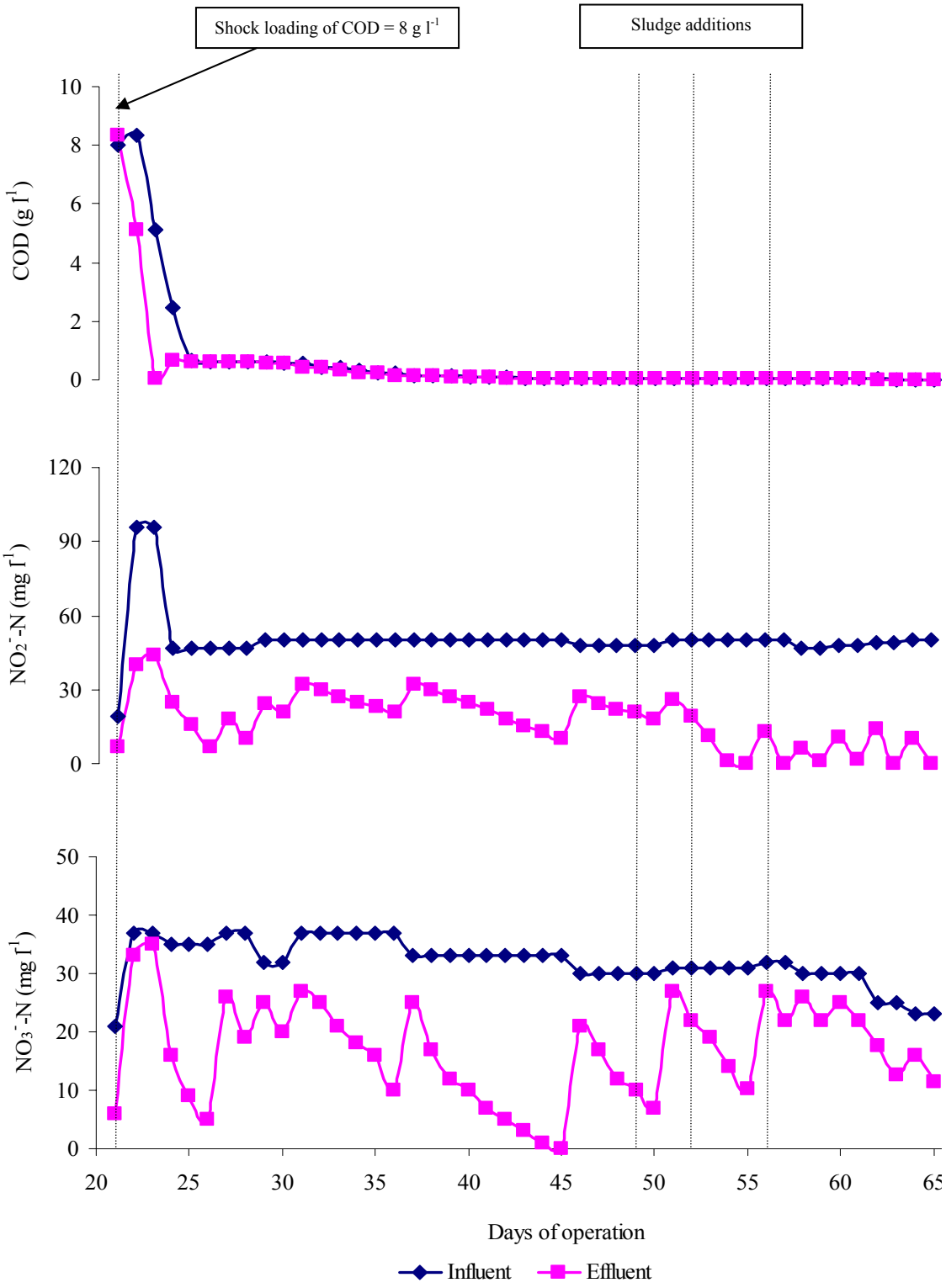


Figure 4.28 Effect of COD shock loading to the Anammox reaction; effluent concentrations and removal efficiency

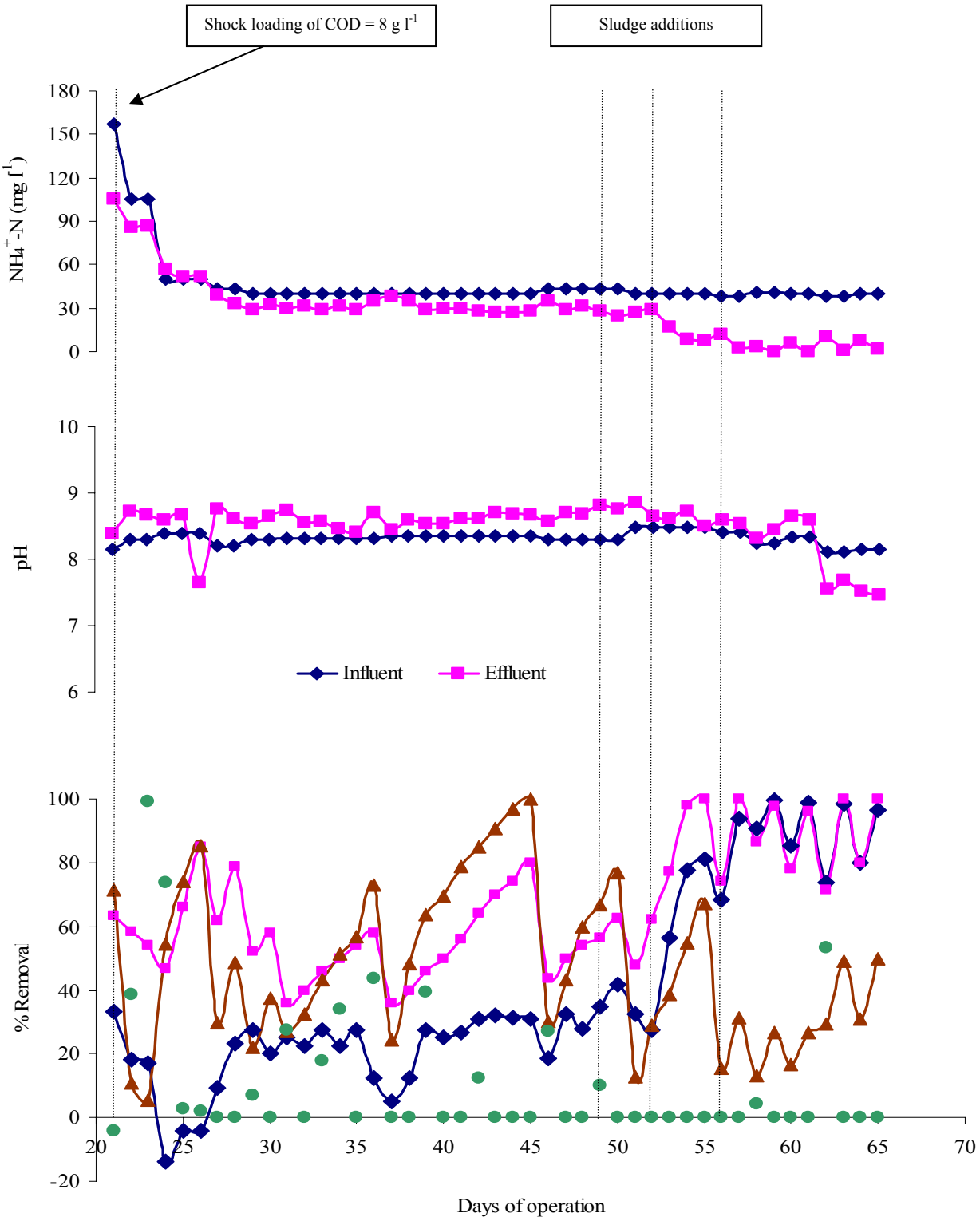


Figure 4.28 Effect of COD shock loading to the Anammox reaction; effluent concentrations and removal efficiency (cont.)

4.4.3 Co-removal of nitrogen and COD in SBR

4.4.3.1 Process operation and performance

The experiment for co-removal of nitrogen and COD was continually conducted by exploring of synthetic COD to the reactor. This experiment was started after the study of COD shock loading and when the recovery of Anammox activity in the reactor was achieved, at day 66. Synthetic COD is produced from fat milk as described in 3.4.1. The COD concentration was increased during the experiment, expressed as COD:N ratio. The initial COD to nitrogen range of 0.9-2.6 was on day 66-83 and increased to 4.0-4.1 on day 83-91. Nitrate concentration in the influent was increased to 50-70 mg N l^{-1} to reduce the competition for nitrite utilization by the co-existing bacterial group, denitrifying bacteria.

The monitoring effluent concentrations and pH condition are shown in Figure 4.29. Removal efficiency is depicted in Figure 4.30. To evaluate the unionization of ammonium in the system, especially at a relatively high temperature and pH in the experiment, the ammonium reduction was then calculated as a net reduction according to Emerson et al. (1975) using the relation between pK_a and percent of unionized ammonium at a given temperature (Equations 4.1 and 4.2).

$$pK_a = 0.0918 + 2729.92/T \quad (4.1)$$

$$\% \text{ unionized ammonium} = 100/(1+\text{antilog}\,(pK_a - pH)) \quad (4.2)$$

where T is degree Kelvin.

The applied COD to N ratios during the concurrent processes of Anammox and denitrification are indicated in Figure 4.30. The first COD to nitrogen ratio of 0.9-2.6 had no effect on the Anammox, which is shown by the net average ammonium reduction of 94% with SD of 5.8. However, the increase of COD to nitrogen ratio from 2.0 to 2.5 insulted in the increase of nitrate consumption from 44 to 80, observed during day 77-80. The inhibition effect of COD on the Anammox was observed after day 83, with COD to nitrogen ratio of 4.0-4.1, as indicated by the decreasing of net average ammonium reduction from 94% to 78% with SD 1.8. After that the reactor was operated with the normal feed without COD content. The COD remaining in the system was then diluted, resulting in the decreasing of COD to nitrogen ratio and the net average ammonium reduction was recovered to 87% (SD 4.2).

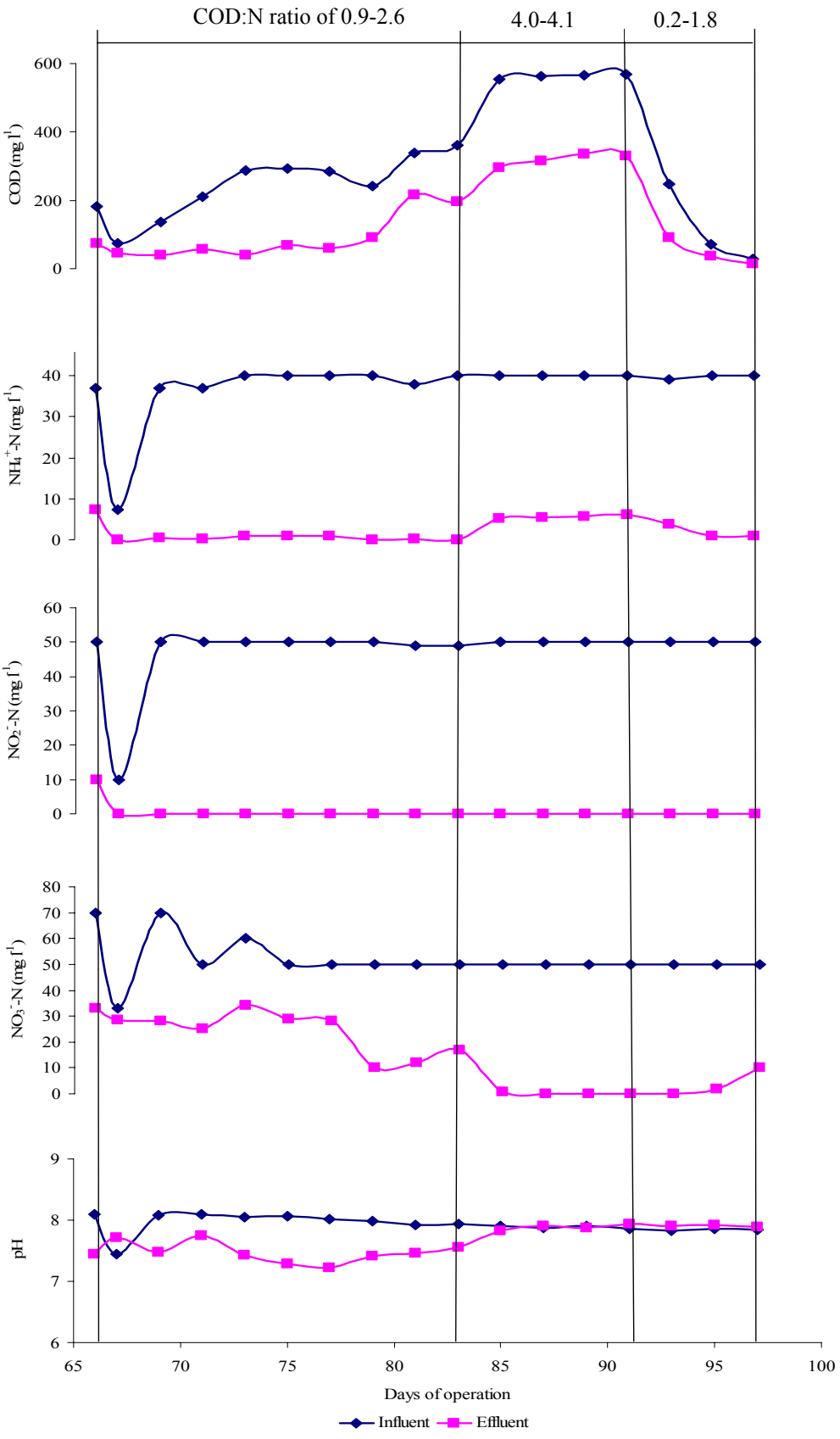


Figure 4.29 Co-removal of COD and nitrogen under the concurrent processes of Anammox and denitrification

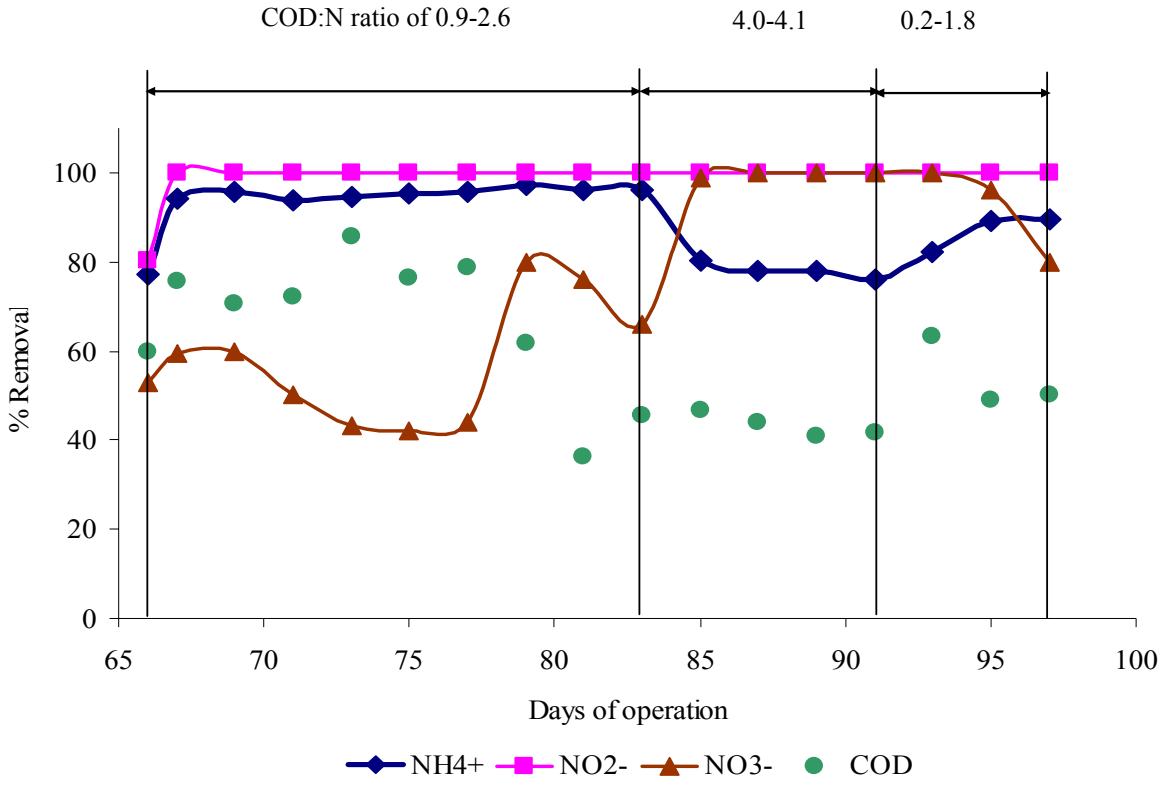


Figure 4.30 Applied COD to N ratio during the concurrent processes of Anammox and denitrification

4.4.3.2 Process evaluation

Stoichiometric parameters for Anammox during the co-process of Anamox and denitrification were calculated in each influent COD range (Table 4.5). The obtained mole ratio of nitrite to ammonium consumption of 1.28-1.37 (SD 0.03) and 1.23-1.30 (SD 0.03), for COD range of 100-300 and 300-400 mg l^{-1}, respectively, agreed with that obtained for the Anammox process, 1.32 (Strous et al., 1998). The higher mole ratio of 1.44-1.48 (SD 0.02) for COD range of 400-600 mg l^{-1} was obtained. Nevertheless, a high mole ratio of nitrate to ammonium consumption of 1.42-1.48 (SD 0.02) was recorded for the highest COD range of 400-600 mg l^{-1} with the lower mole ratio of 0.67-1.25 (SD 0.28) and 0.54-1.01 (SD 0.23) for COD range of 100-300 and 300-400 mg l^{-1}, respectively. The mole ratio of nitrate to ammonium indicated a consumption of nitrate of 0.67-1.25 (SD 0.28), 0.54-1.01 (SD 0.23), and 1.42-1.48 (SD 0.02) for COD range of 100-300, 300-400, and 400-600 mg l^{-1}, respectively. It confirmed the co-existing activity of denitrifying in the system.

For better understanding of the possible undergone processes between Anammox and denitrification in the reactor, carbon and nitrogen mass balances were performed. The possible stoichiometric reactions proposed earlier (Strous et al., 1998 and Ahn et al., 2004) were used by assuming that a concurrent process existed for autotrophic Anammox and heterotrophic denitrification and fermentation. The reactions are shown in Equation 4.3 to 4.6.

Table 4.5 Stoichiometric parameters calculated for each COD range during the concurrent processes of Anammox and denitrification

Influent COD (mg l^{-1})	NO_2^- consumed to NH_4^+ consumed, mol $(mol)^{-1}$	NO_3^- consumed to NH_4^+ consumed, mol $(mol)^{-1}$
100-300	1.28-1.37 (0.03)	0.67-1.25 (0.28)
300-400	1.23-1.30 (0.03)	0.54-1.01 (0.23)
400-600	1.44-1.48 (0.02)	1.42-1.48 (0.02)

Number in the parentheses indicates a standard deviation of each stoichiometric parameter.

Anammox

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (4.3)$$

Fermentation

$$C_6H_{12}O_6 + 0.2\ NH_4^+ + 0.2\ HCO_3^- \longrightarrow 0.2\ C_5H_7O_2N + CH_3CH_2CH_2COOH + 1.2\ CO_2 + 1.8\ H_20 \quad (4.4)$$

Denitrification

$$NO_2^- + 0.19\ CH_3CH_2CH_2COOH + H_2CO_3 \longrightarrow 0.037\ C_5H_7O_2N + HCO_3^- + 1.14\ H_2O + 0.585\ CO_2 + 0.481\ N_2 \quad (4.5)$$

$$NO_3^- + 0.29\ CH_3CH_2CH_2COOH + H_2CO_3 \longrightarrow 0.034\ C_5H_7O_2N + HCO_3^- + 1.54\ H_2O + 0.986\ CO_2 + 0.483\ N_2 \quad (4.6)$$

Through these processes, Anammox undergoes stoichiometric reaction causing COD and ammonium reductions, Equation 4.3. According to Equation 4.4, the fermentation resulted in organic acids represented by butyric acid which is used as an electron donor for denitrification as indicated in Equation 4.5 and 4.6. Thus a small fraction of COD and ammonium are consumed due to biomass synthesis.

The results of calculation based on the above hypotheses are shown in Table 4.6. At low COD concentration ranges of 100-300 and 300-400 mg l^{-1}, calculated nitrite consumption was found near to the actual consumption from the experiment, with SD of 1.9 and 0.5, respectively. It indicated well an Anammox reaction in the reactor. A higher SD of 6.2 for COD concentration range of 400 to 600 mg l^{-1} indicated an initial effect that resulted from denitrification competition. Competition between Anammox and denitrifying communities were reported earlier by a number of researchers (Dong and Tollner, 2003; Jianlong and Jing, 2005; Pathak et al., 2007). A contradictory conclusion was drawn by Ahn et al. (2004) who reported no competition between Anammox organisms and denitrifying bacteria. The reasons are unclear and need further observations. However, the

proposed stoichiometric reactions poorly predicted nitrate and COD consumptions. A similar trend was obtained for all influent COD concentration ranges. More study is needed for further clarification.

Table 4.6 Average consumptions of nitrogen and COD during the concurrent processes of Anammox and denitrification (day 66 to 97)

Influent COD ($mg\ l^{-1}$)	Consumption ($mg\ l^{-1}$)							
	NH_4^+-N		NO_2^--N		NO_3^--N		COD	
	Exp.	Cal. [a]	Exp.	Cal.	Exp.	Cal. [b]	Exp.	Cal.
100-300	35.8	34.3	48.0	45.3	34.3	43.2	148.4	85.9
300-400	39.1	38.1	49.6	50.3	30.8	40.7	177.3	80.9
400-600	34.3	31.2	50.0	41.2	49.8	57.9	243.6	115.2

[a] net ammonium consumption
[b] including nitrate produced from Anammox reaction

4.4.4 Anammox activity and confirmation

4.4.4.1 Anammox activity

Anammox activity of biomass from the Anammox-SBR was determined in batch studies, at 37 ºC incubation as described in 3.4.2. The results showed that the Anammox activity expressed as a specific nitrogen removal rate of 0.18 kg N $(kg\text{-}VSS.\ d)^{-1}$ was achieved for Anammox. The oxygen-limited autotrophic nitrification-denitrification (OLAND) sludge showed the same activity, 0.17 kg N $(kg\text{-}VSS.\ d)^{-1}$, while the specific nitrogen removal rate of 0.07 kg N $(kg\text{-}VSS.\ d)^{-1}$ was achieved for reactor operation as described in 4.4.1. The stoichiometry of the Anammox reaction obtained from batch tests was 1:1.2-1.3 (mol NH_4^+:mol NO_2^-). Regression analysis between ammonium and nitrite consumption during experimentation (Figure 4.31) showed that a similar stoichiometry of ammonium to nitrite consumption of Anammox and OLAND sludges was obtained in the range of 1:1.24-1.26 and 1:1.23-1.24 (mol NH_4^+:mol NO_2^-) with R^2 of 0.9966-0.9993 and 0.9996-0.9997, respectively.

Time course of nitrogen species and removal efficiency of Anammox sludge compared with OLAND sludge is shown in Figure 4.32a,b,c,d. Their activity in terms of ammonium and nitrite removal efficiency showed the same profiles with above 95 and 99%, respectively (Figure 4.33). Nitrate production was not detected, but its reduction was observed simultaneously with the anaerobic ammonium oxidation. This could be from the activity of co-existing heterotrophs, denitrifying bacteria, which may consume the nitrate produced by Anammox. The speculation was mentioned in the study of Schmid et al. (2003).

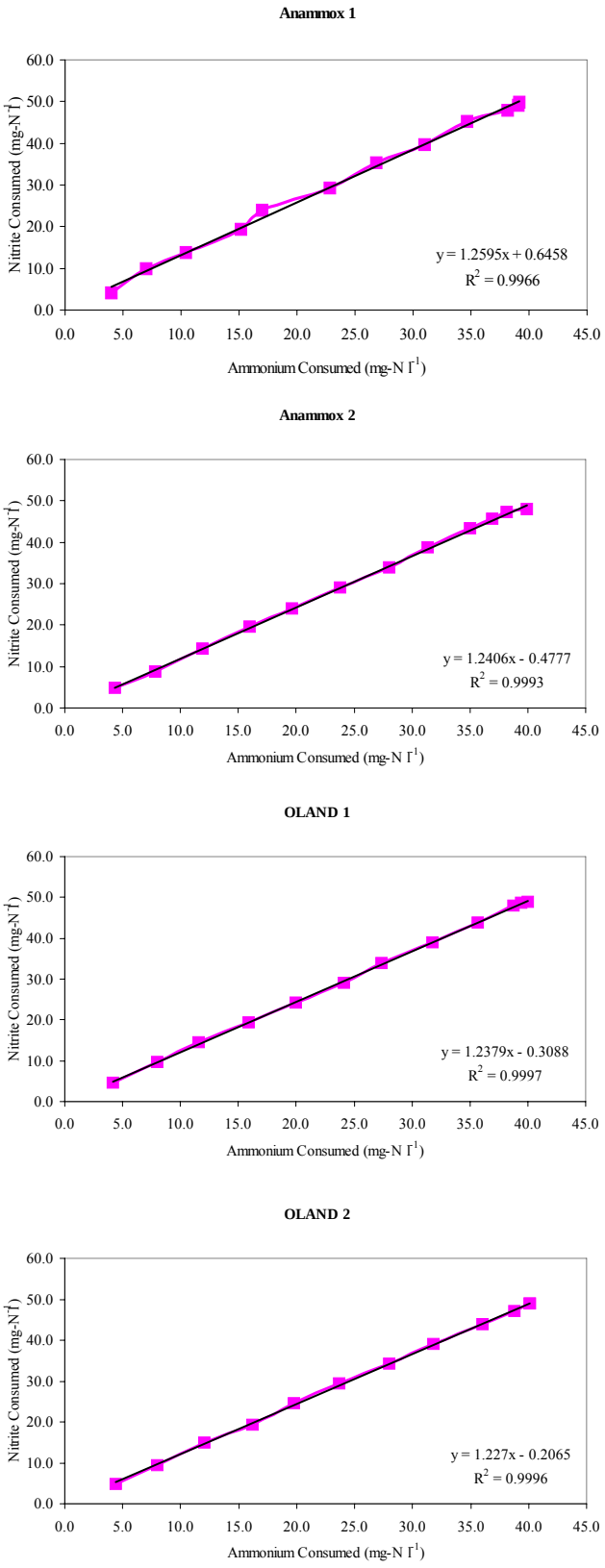


Figure 4.31 Regression analysis of ammonium and nitrite consumption from batch study of Anammox and OLAND sludges

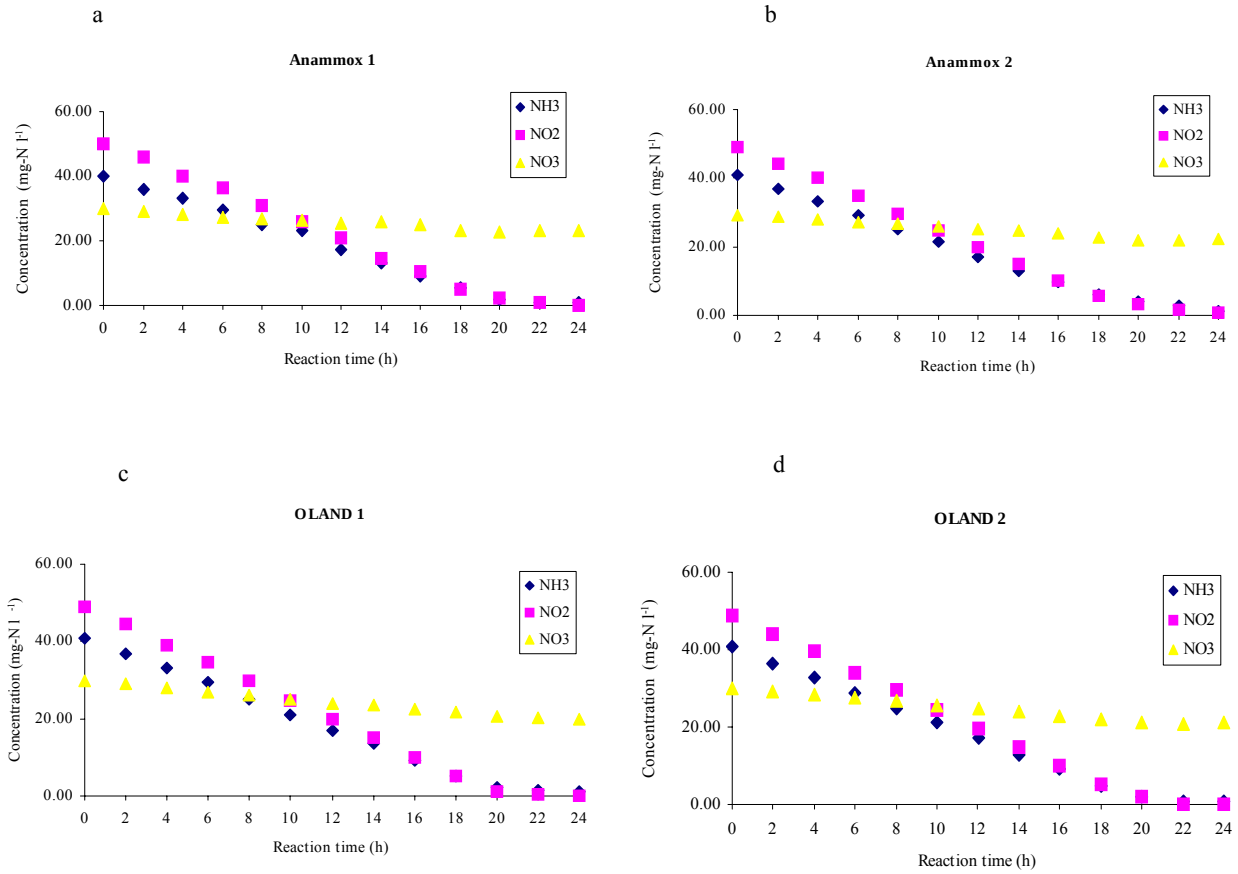


Figure 4.32 Time course of nitrogen removal (a) and (b) Anammox sludge, (c) and (d) OLAND sludge

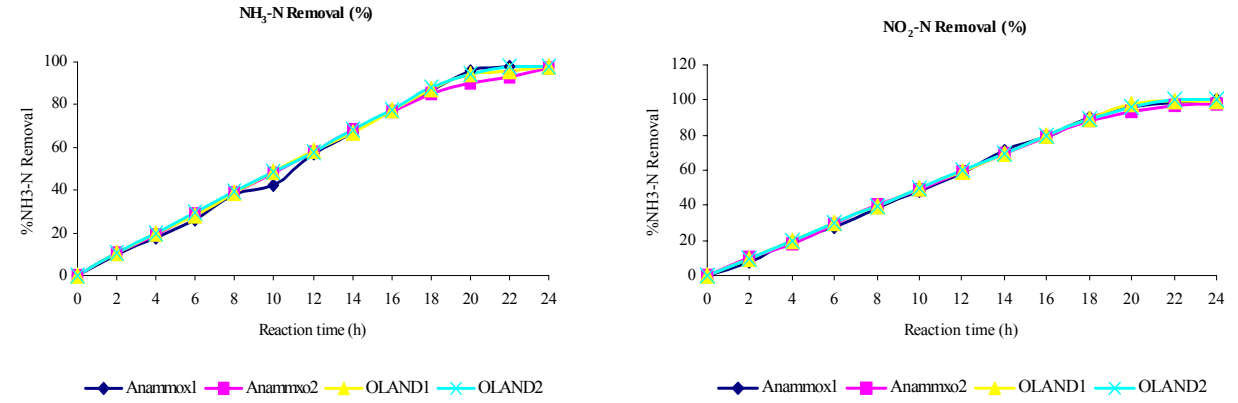


Figure 4.33 NH_4^+ and NO_2^- removal efficiency of Anammox and OLAND sludges

4.4.4.2 Hydroxylamine test and FISH analysis

Anammox biomass taken from the SBR was injected in the 50 ml vials containing 2.1 μM anaerobically prepared hydroxylamine solution. The maximum hydrazine production of 1 μM was observed at 50 min reaction time (Figure 4.34). It showed that the Anammox bacteria taken from the reactor were active and responsible for nitrogen conversion in the reactor.

At the beginning of measurement, at 10 min reaction time, hydroxylamine was rapidly converted to hydrazine by the enzyme hydrazinase as it could be detected in 0.5 μM. After that, both hydroxylamine and hydrazine concentrations were stable. Hydrazine was accumulated and started to increase after 40 min reaction time (Figure 4.34). As mentioned above, the maximum hydrazine concentration was observed at 50 min reaction time, whereas the decrease of hydroxylamine concentration did not occur but it showed almost constant value. This phenomenon might come from the supplement of hydroxylamine production according to the nitrite remaining in the liquid inside reactor, together with the produced electons from the conversion reaction of hydroxylamine, being converted to hydroxylamine by the enzyme nitrite reductase (van Dongen et al., 2001b). Considering a slower disintegration of hydrazine than the formation of hydroxylamine, then hydrazine was accumulated in the system, resulting in the detection of its increased concentration. The trend lines in the figure were created to briefly demonstrate a decreasing of hydroxylamine and a production of hydrazine concentration only.

The existence of Anammox microbes in the reactor was also observed using the FISH technique. The bacterial biomass from the reactor developed and hybridized with both probes EUB 338 mixed and Amx 820 (Figure 4.35). The hybridized results of EUB 338 mixed showed almost all bacterial sample cells, while the Amx 820 probes is specific to anaerobic ammonium-oxidizing bacteria, *C. Brocadia anammoxidans* and *C. Kuenenia stuttgartiensis*.

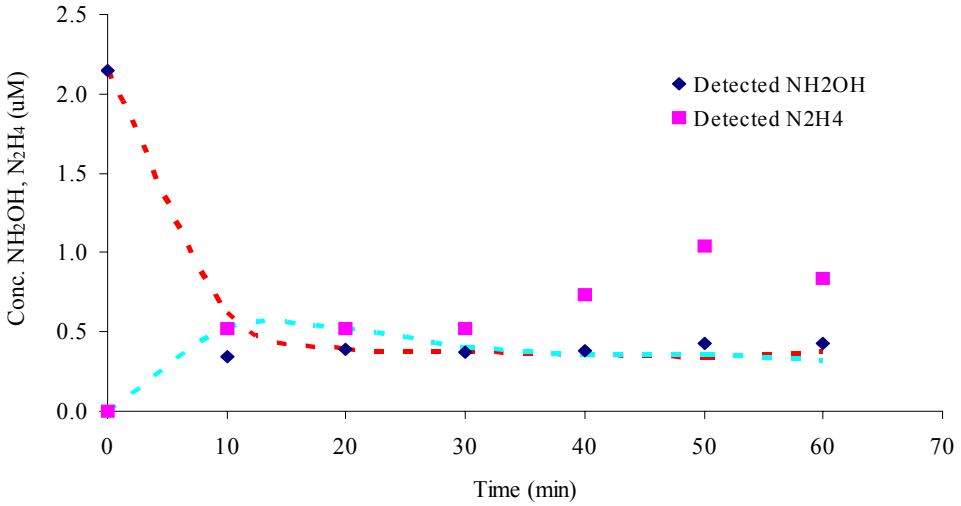


Figure 4.34 Production of hydrazine from hydroxylamine under anaerobic conditions of biomass taken from Anammox-SBR

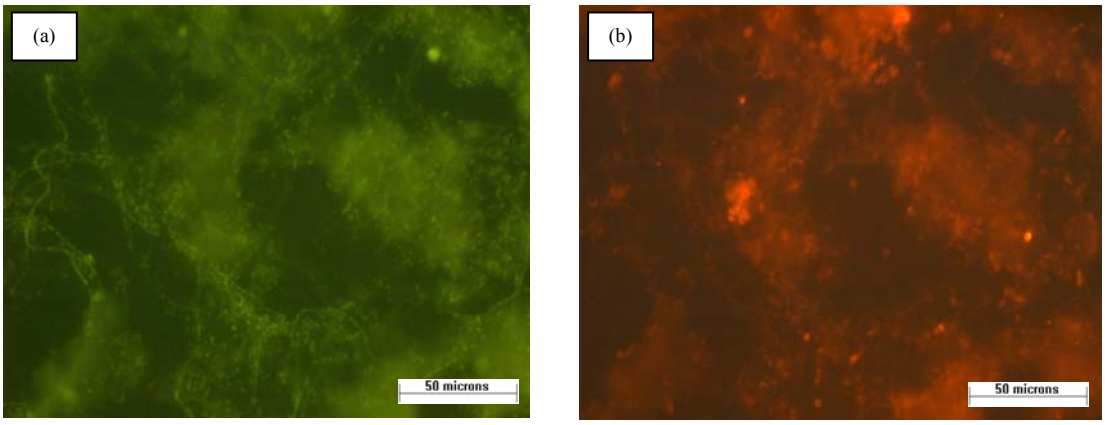


Figure 4.35 FISH images of biomass from Anammox-SBR:
Hybridized result with EUB 338 mixed probe (a) and Amx 820 probe (b)

4.5 Experimental phase IIIb : Co-removal of COD and nitrogen in Anammox-UASB

4.5.1 Process operation and performance

The three parallel 200-ml laboratory-scale UASB reactors were inoculated and operated as described in 3.5. The acclimatization of Anammox biomass in the reactors was conducted with the anaerobic granular sludge taken from the previous UASB reactor, for a half month prior monitoring. The inoculation of anaerobic granular sludge was targeted to accelerate the start-up process and the results obviously showed the stable condition for the treatment efficiency within a short period. At the beginning period of operation (18 days), ammonium, nitrite, and nitrate were fed with the same feed for all three reactors. The effluent nitrogen species, pH and COD in Anammox-UASBs during the experiment are shown in Figure 4.36. The results showed that at the beginning period of the experiment, after 18 days of operation, the Anammox occurred only in UASB 1 as evidenced by the average ammonium removal of 97% (Figure 4.37a). Nitrite and nitrate, however, were completely consumed in all reactors with the average COD removal of 55%, 85%, and 97% in UASB 1, 2, and 3, respectively (Figure 4.37a). This clearly showed the competitiveness of heterotroph, denitrifying bacteria for nitrate and nitrite consumption to remove COD in both UASB 2 and 3. Then, the influent nitrate concentration was increased in all reactors to a range of 90-100 mg N l^{-1} at the 19th day of operation. The Anammox reaction was observed consequently in UASB 2 with the average ammonium removal of 89% (Figure 4.37a). The attempts to establish the Anammox process in UASB 3 were carried out by raising the amount of nitrate to 100-120 mg N l^{-1} in all reactors at the 31st day of operation. This was done to provide a sufficient amount of nitrate for denitrification so that the nitrite would be used only by Anammox bacteria. However, significant Anammox reaction still did not occur in this reactor as the effluent ammonium concentration showed a higher value than in UASB 1 and 2 (Figure 4.36). The results indicated that the nitrate concentration of above 90 mg N l^{-1} was an excess requirement for UASB 1 and 2 as shown by the increase in nitrate concentration remaining in the effluent after the 19th day of operation (Figure 4.36).

The variation of COD concentrations ranging from 100 to 400 mg l^{-1} brought about different conversions and pH conditions as indicated in Figure 4.37. The study was carried out in three reactors having COD concentration divided into 3 classes: UASB 1 (COD 100-200 mg l^{-1}), UASB 2 (COD 200-300 mg l^{-1}), and UASB 3 (COD 300-400 mg l^{-1}). This accounted for the COD to nitrogen (COD:N) ratio of 0.9, 1.4, and 2.0, respectively.

As shown in Figure 4.36, an instant COD conversion and nitrite-nitrate reduction clearly indicated an immediate availability of denitrifying microbes in all reactors. Although over 50% of ammonium reductions were obtained for all reactors, it does not necessarily indicate the Anammox activity since there was also ionization equilibrium, especially at a relatively high temperature and pH as found in the early stage of this experiment. According to Emerson et al. (1975), the pK_a and percent unionized ammonia at a given temperature can be calculated using Equations 4.1 and 4.2, as mentioned in 4.4.3.1.

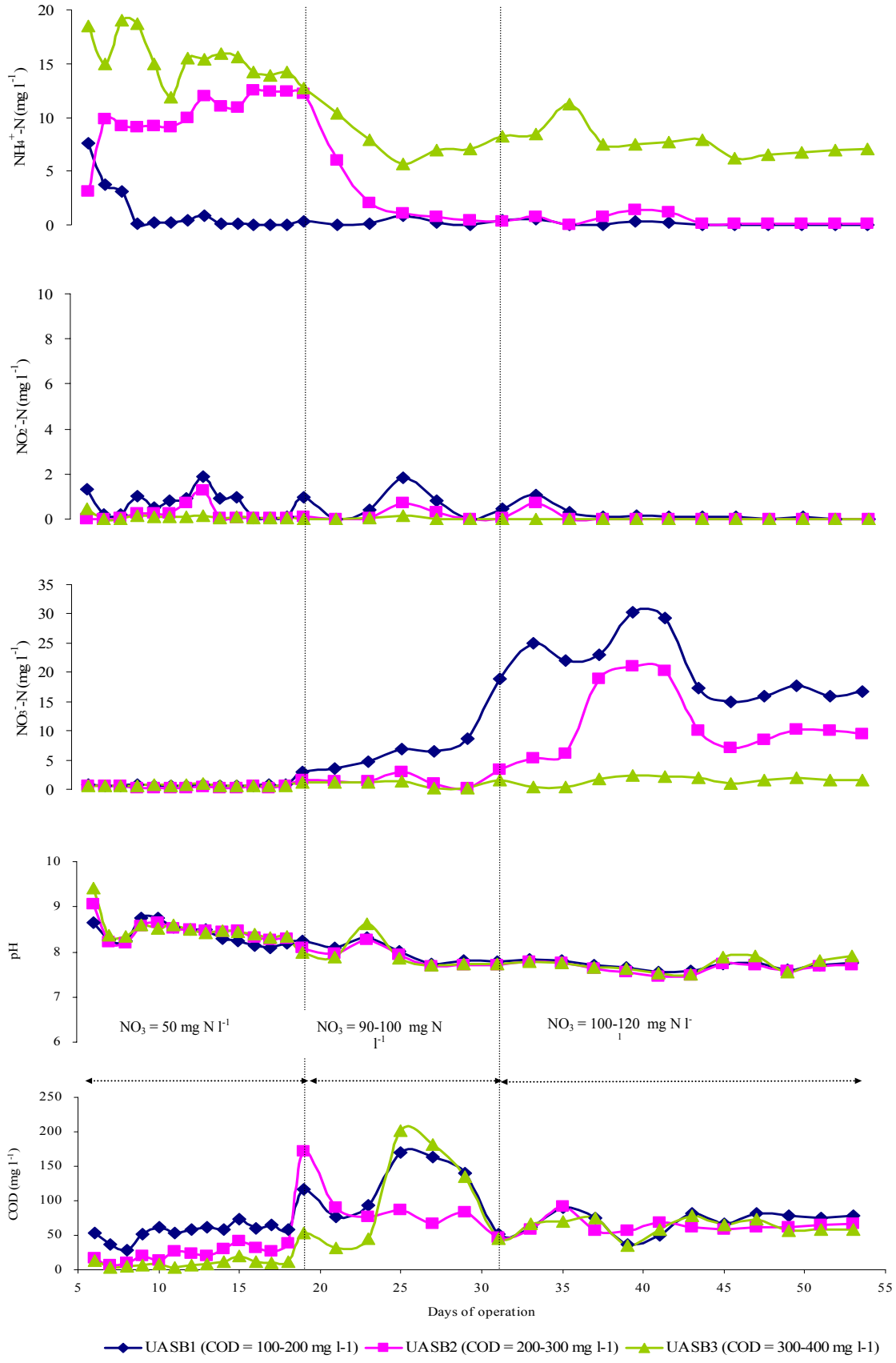
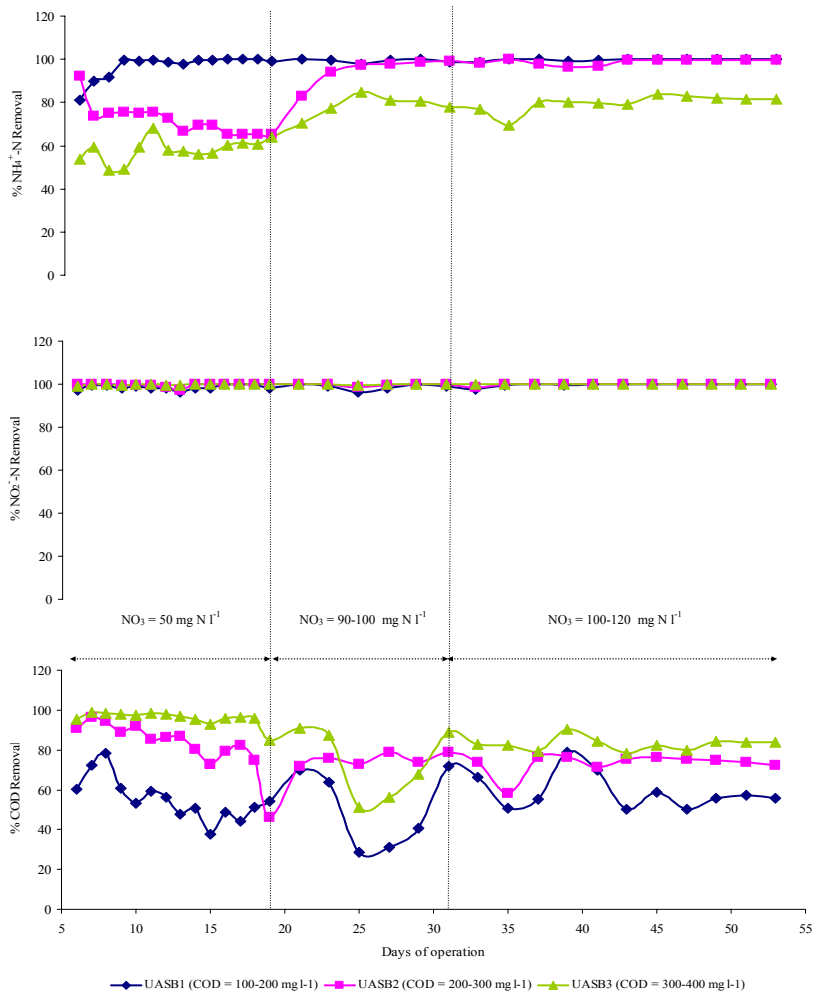


Figure 4.36 Effluent nitrogen species pH and COD in Anammox-UASBs

(a)



(b)

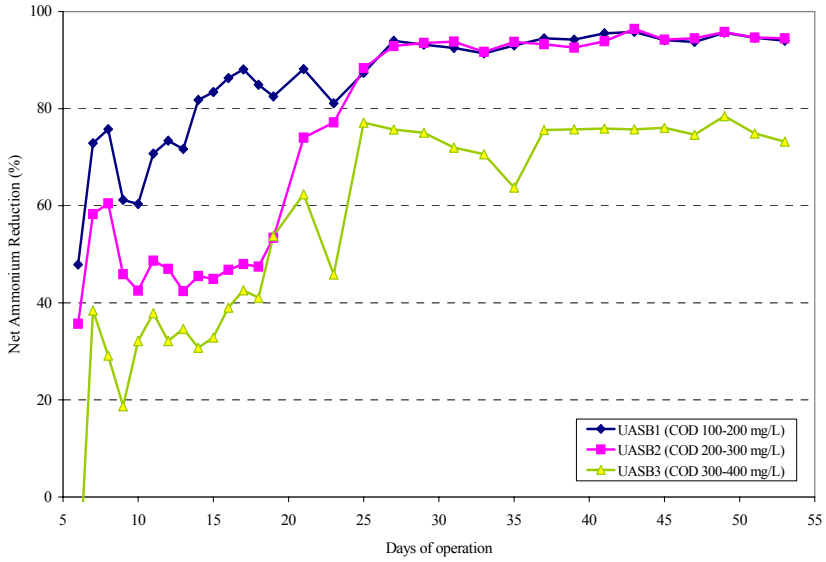


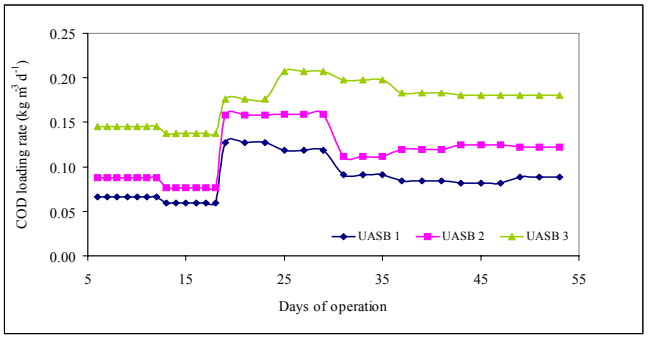
Figure 4.37 Nitrogen and COD removal efficiency (a) and the net ammonium reduction (b) in Anammox-UASBs

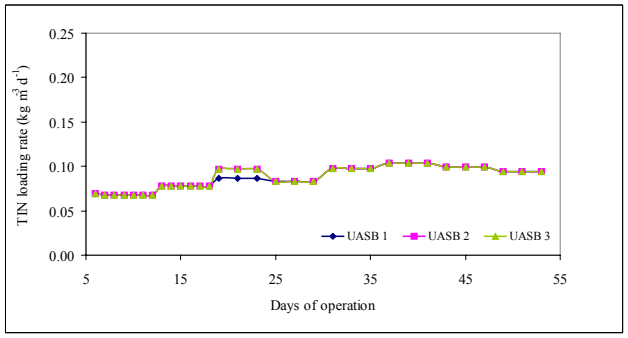
At the temperature of 35 ºC and pH of 7.5 to 9.4, as obtained in the effluent, percents of unionized ammonium were estimated to vary from 3.4 to 73.6%, respectively. It was fortunate that a high pH of the effluent prevailed only in the early stage and gradually reduced over time. The obtained ammonium conversions in all reactors ranged from 49% to a nearly complete conversion. As nitrite was completely removed in all reactors, the surplus ammonium conversion should denote the Anammox activity. To account for the ionization, net ammonium reductions were calculated and are presented in Figure 4.37b.

At low COD concentration ranges of 100 to 200 mg l^{-1}, Anammox organisms could effectively compete for nitrite, allowing efficient ammonium conversion but relatively poor COD removal as compared to a greater range of COD concentrations. Competitiveness was gradually reduced with an increase in COD concentrations. An increase in nitrate concentration on the 18^{th} day, from 50 to approximately 100 mg l^{-1}, led to a sharp increase in ammonium oxidation indicating that there was an insufficiency of nitrite-nitrate concentrations for COD ranges of over 100 mg l^{-1}. A near complete conversion of ammonium was obtained for a COD range of 200-300 mg l^{-1} after 31 days of operation. Additional nitrate concentration was then supplemented to ensure adequacy of inorganic nitrogen for denitrification at provided COD concentrations. However, it showed no further enhancement of either Anammox activity or denitrification. As a nitrite-to-ammonium ratio of the feed was maintained constantly at approximately 1.3, a greater conversion of ammonium in the feed with low COD concentration suggested a greater Anammox activity. In other words, Anammox activity was suppressed when COD concentration was over 300 mg l^{-1}. A similar conclusion can be drawn from Figure 4.39 which depicts the loading, conversion, and specific-conversion rates of nitrogen and COD at various COD ranges. The overall and specific conversion rates of COD were relatively in proportion with the applied COD concentrations while those of nitrogen were quite analogous throughout.

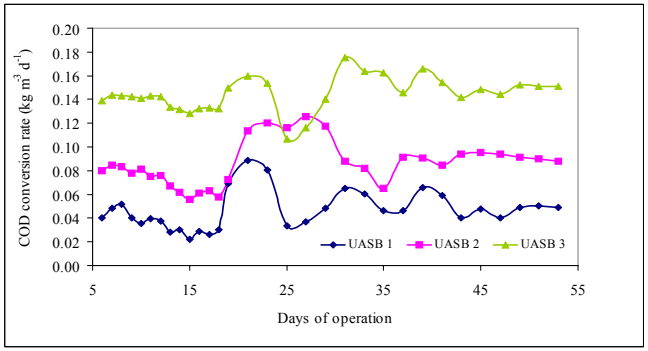
The average total nitrogen loading rate expressed as total inorganic nitrogen (TIN) of 0.08, 0.09, and 0.09 kg m^{-3} d^{-1} was explored in UASB 1, 2, and 3, respectively. The highest average specific nitrogen removal rate of 0.12 kg (kg-VSS)$^{-1}$ d^{-1} was observed in UASB 1 while the rate of 0.09 and 0.10 kg (kg-VSS)$^{-1}$ d^{-1} was recorded in UASB 2 and 3. However, the average nitrogen conversion rate of 0.08 kg m^{-3} d^{-1} was observed in all reactors. The increment of COD loading rate of 0.08, 0.11, and 0.17 kg m^{-3} d^{-1} was carried out for UASB 1, 2, and 3, respectively. In UASB 3, the denitrification showed better performance than Anammox, as shown by the higher average COD conversion and specific removal rate of 0.14 kg m^{-3} d^{-1} and 0.23 kg (kg-VSS)$^{-1}$ d^{-1}, whereas the lower rate of 0.05, 0.09 kg m^{-3} d^{-1} and 0.07, 0.13 kg (kg-VSS)$^{-1}$ d^{-1} was recorded in UASB 1 and 2, respectively. This agreed with the higher COD removal and lower ammonia removal efficiency obtained from UASB 3 than the other reactors (Figure 4.37) and the results discussed above.

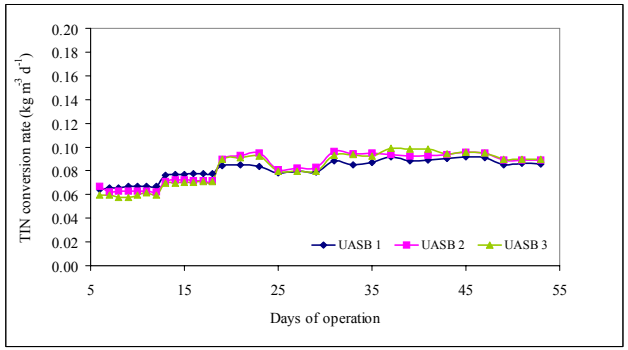
Loading rate



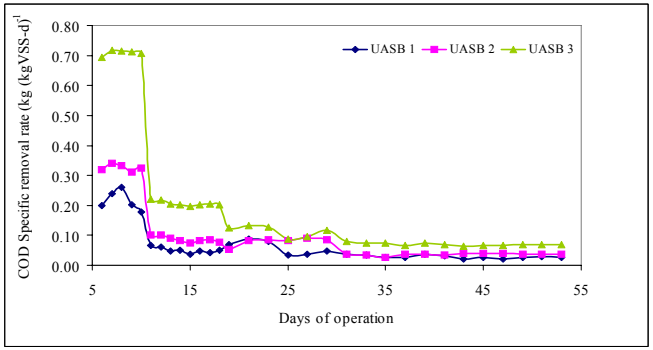


Conversion rate





Specific removal rate



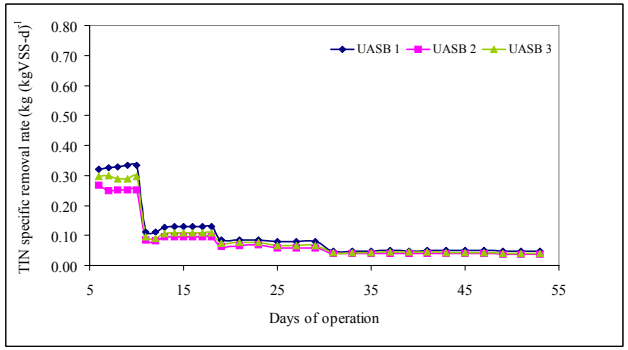


Figure 4.38 Loading, conversion, and specific removal rate of COD and nitrogen in UASB 1 (COD = 100-200 mg l^{-1}), UASB 2 (COD = 200-300 mg l^{-1}) and UASB 3 (COD = 300-400 mg l^{-1})

4.5.2 Process evaluation

Stoichiometric parameters for Anammox-UASBs were calculated during the steady state period in all UASB reactors (Table 4.7). The ratio of nitrite consumed to ammonium consumed in UASB 1, 1.34, agreed with that obtained by Strous et al. (1998) but the ratio from UASB 2, 1.59, was slightly higher. In UASB 3, the ratio 1.96, was completely different from the theoretical nitrite consumed to ammonium consumed of the Anammox process. Considered together with the effluent nitrogen species and removal profile (Figure 4.36, 4.37), it shows that the Anammox bacteria was inactive in this reactor. This assumption was confirmed by the hydroxylamine test and FISH analysis in the topic 4.5.3. Nevertheless, the nitrate production was not detected in all reactors. The nitrate consumption was observed concurrent with the ammonium oxidation, which indicated the co-processes of Anammox and denitrification, and agreed with the results from the previous study of Mulder et al. (1995), Jianlong and Jing (2005). The produced nitrate from Anammox reaction was then presumably consumed by the co-existing bacterial group, denitrifying bacteria. The nitrate to ammonium consumption was observed in a high range of 2.04-3.25 mol $(mol)^{-1}$ (Table 4.7).

Table 4.7 Stoichiometric parameters calculated in the Anammox-UASBs

	UASB 1: Low COD	UASB 2: Medium COD	UASB 3: High COD	SBR (Strous et al., 1998)
NO_2^- consumed to NH_4^+ consumed mol $(mol)^{-1}$	1.34	1.59	1.96	1.32
NO_3^- produced to NH_4^+ consumed mol $(mol)^{-1}$	-2.04[a]	-2.57[a]	-3.25[a]	0.26

[a] NO_3^- consumed to NH_4^+ consumed mol $(mol)^{-1}$

To understand the possible processes taken in the reactors, carbon and nitrogen mass balances were performed. The possible stoichiometric reactions proposed earlier by researchers (Strous et al., 1998 and Ahn et al., 2004) were used by assuming that a concurrent process existed for autotrophic Anammox and heterotrophic denitrification and fermentation. The reactions, Equations 4.3-4.6, were earlier indicated in the topic 4.4.3.2.

For clarification, the possible reactions used for calculation were again mentioned. Through these processes, Anammox undergoes stoichiometric reaction causing COD and ammonium reductions, Equation 4.3. According to Equation 4.4, the fermentation resulted in organic acids represented by butyric acid which is used as an electron donor for denitrification as indicated in Equation 4.5 and 4.6. Thus a small fraction of COD and ammonium are consumed due to biomass synthesis.

Table 4.8 shows the results of calculation based on the aforementioned hypotheses. At low COD concentration range of 100-300 mg l^{-1}, calculated nitrite consumption was found near to the actual consumption. A narrow variation of less than 5% was observed. This indicates that the reaction well represents the Anammox activities in

the reactors. A greater variation of approximately 25% for COD concentration in a range of 300 to 400 mg l^{-1} was no doubt due to denitrification competition. Competition between Anammox and denitrifying communities was reported earlier by a number of researchers (Dong and Tollner, 2003; Jianlong and Jing, 2005). Nevertheless, a contradictory conclusion was drawn by Ahn et al. (2004) who reported no competition between Anammox organisms and denitrifying bacteria. The reasons are unclear.

Although the proposed stoichiometric reactions poorly predicted nitrate and COD consumptions, a similar trend was obtained for both COD concentration ranges of 100-200 and 200-300 mg l^{-1}. More study is needed for further clarification.

Table 4.8 Average consumptions of nitrogen and COD during steady state condition (day 31 to 53)

Reactor	Consumption (mg l^{-1})							
	NH_4^+-N		NO_2^--N		NO_3^--N		COD	
	Exp.	Cal. [a]	Exp.	Cal.	Exp.	Cal. [b]	Exp.	Cal.
UASB1[c]	37.6	35.5	49.3	46.9	89.4	98.6	103.3	196.1
UASB2[d]	37.3	35.5	49.4	46.9	99.2	108.4	175.5	215.6
UASB3[e]	30.1	27.9	49.5	36.8	108.5	115.8	309.3	230.2

[a] net ammonium consumption
[b] including nitrate produced from Anammox reaction
[c] COD 100-200 mg l^{-1} [d] COD 200-300 mg l^{-1} [e] COD 300-400 mg l^{-1}

4.5.3 Anammox confirmation

Sludge samples were taken from all reactors prior to the start and at the end of experiments to observe the Anammox organisms in the system using FISH techniques. Gene probe EUB 338 mixed was used to identify most bacteria in the samples and the biomass from all reactors showed hybridized result with this probe. A specific Amx 820 gene probe was used to identify anaerobic ammonium-oxidizing bacteria, *C. Brocadia anammoxidans* and *C. Kuenenia stuttgartiensis*. Prior to operation, biomass in all reactors showed hybridization with the Amx 820 probe but remained only for UASB 1 and UASB 2 toward the end of experiments (Figure 4.39). This confirmed a poor performance of ammonium and nitrite conversions in UASB 3 as indicated earlier.

Confirmation was made through a detection of hydrazine which is a unique intermediate of Anammox activity. Figure 4.40 depicts the outcome of the study using a batch hydroxylamine test. A positive result was obtained for sludges from the UASB 1 and UASB 2 while the sludge from UASB 3 showed a negative result. As the obtained nitrogen conversions were relatively stable after 3 weeks, it was postulated that Anammox communities were suspended or eradicated due to a relatively high COD (300-400 mg l^{-1})

or COD to N ratio of 2.0 causing the out-performance of denitrifying bacteria in the systems.

The maximum hydrazine production of approximately 1 μM was detected at 20 min reaction time for the biomass taken from UASB 1 (Figure 4.40a). Since the hydroxylamine concentration was rapidly decreased after 10 min, the maximum hydrazine concentration should be detected at this time, but it was observed later, after 20 min. Considering the possible trend line in the curve, this might be caused by the slow formation of hydrazine but rapid degradation of hydroxylamine. The data of hydrazine concentration after 50 and 60 min shows higher values again. This probably comes from an error from measurement or the same earlier possible explanation of batch result of the biomass from SBR.

The result of hydroxylamine test of biomass taken from UASB 2 showed better interpretation than the other as the maximum hydrazine production was observed after 10 min, which agreed well with the rapid decreasing of hydroxylamine concentration at the same reaction time (Figure 4.40b). The result of removal efficiency of the biomass in UASB 2 showed the active cooperative function between Anammox and denitrification as in UASB 1, which is shown by the average specific nitrogen removal rate of 0.09 kg (kg-VSS)$^{-1}$ d^{-1} and the specific COD removal rate of 0.13 kg (kg-VSS)$^{-1}$ d^{-1}.

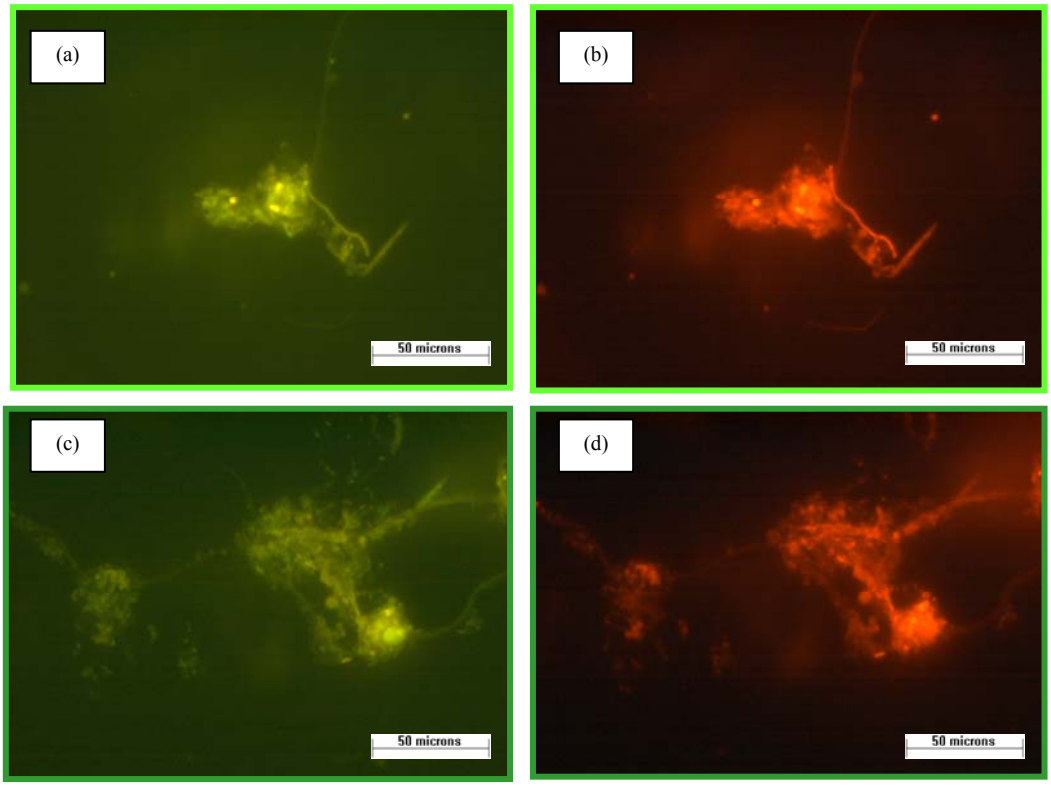
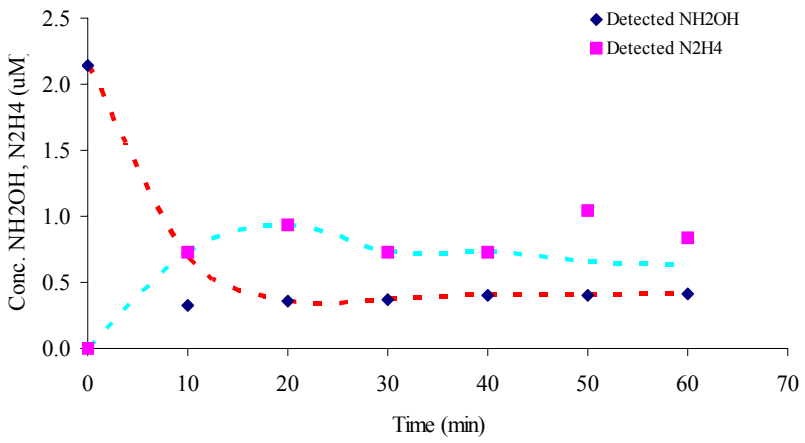


Figure 4.39 FISH images of biomass from UASB 1 (a,b) and UASB 2 (c,d) with EUB 338 mixed probe (a,c) and Amx 820 probe (b,d)

(a)



(b)

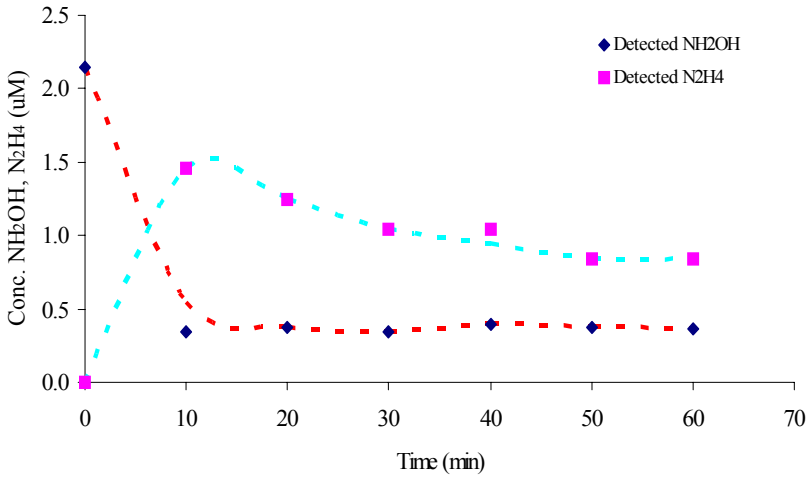


Figure 4.40 Production of hydrazine from hydroxylamine under anaerobic conditions of biomass taken from (a) UASB 1 and (b) UASB 2

4.6 Experimental phase IV : Mathematical model describing system behavior for nitrogen removal in Anammox-SBR and UASB reactor

4.6.1 Development of mathematical model describing system behavior of Anammox process

This is to develop the mathematical model for describing the system behavior of Anammox process, the important basic concepts were determined as follows:

4.6.1.1 Stoichiometry equation

The Anammox stoichiometric equation was estimated by Strous et al. (1998) as shown in the topic 4.4.3.2, Equation 4.3. The equation is depicted here again for clarification.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \longrightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (4.3)$$

This equation was used to develop the model for describing the system behavior of the Anammox process in SBR in this study. From the equation, it is shown that ammonium and nitrite are the main substrates in Anammox process. NH_4^+ acts as an electron donor and is oxidized to dinitrogen gas with NO_2^- as electron acceptor under anaerobic conditions. The considered products from this process were N_2, NO_3^-, and biomass ($CH_2O_{0.5}N_{0.15}$). The model was developed by considering only the change of concentration of NH_4^+, NO_2^-, NO_3^-, N_2, and biomass.

4.6.1.2 Reaction rate of the process

The reaction rate of the process with unequal stoichiometric coefficient could be expressed in the following form (Metcalf and Eddy, 2003).

$$a\,A + b\,B \longrightarrow c\,C + d\,D \quad (4.7)$$

The concentration or reaction change could be expressed by:

$$r = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = \frac{1}{c}\frac{d[C]}{dt} = \frac{1}{d}\frac{d[D]}{dt} \quad (4.8)$$

From Equation 4.3, the reaction of Anammox process could be expressed as.

$$r_{AN} = -\frac{d[NH_4^+]}{dt} = -\frac{1}{1.32}\frac{d[NO_2^-]}{dt} = \frac{1}{1.02}\frac{d[N_2]}{dt} = \frac{1}{0.26}\frac{d[NO_3^-]}{dt}$$

$$= \frac{1}{0.066}\frac{d[CH_2O_{0.5}N_{0.15}]}{dt} \quad (4.9)$$

when r_{AN} = reaction rate of Anammox process
$[NH_4^+]$ = concentration of NH_4^+-N, mol l^{-1}
$[NO_2^-]$ = concentration of NO_2^--N, mol l^{-1}
$[N_2]$ = concentration of N_2, mol l^{-1}
$[NO_3^-]$ = concentration of NO_3^--N, mol l^{-1}

The rate expression of saturation or mixed-order (Metcalf & Eddy, 2003) was selected as the main rate expression in model development. This rate expression is shown as follows:

$$r = \pm \frac{kC}{K + C} \tag{4.10}$$

k = maximum reaction rate, mg $l^{-1}min^{-1}$
K = Constant, mg l^{-1}
C = Substrate concentration, mg l^{-1}

The Monod equation based on the saturation or mixed-order (Orhon and Artan, 1994) was considered together (Equation 4.11).

$$\mu = \mu_m \frac{S}{K_s + S} \tag{4.11}$$

μ = specific growth rate, T^{-1}
μ_m = maximum specific growth rate, T^{-1}
S = growth limiting substrate, mg l^{-1}
K_s = half velocity constant, mg l^{-1}

An additional Monod term was used to describe an eventual inhibition of the Anammox organisms by COD (Equation 4.12). This inhibition term was considered and applied based on the previous report of Dapena-Mora et al. (2004b). The details for an additional inhibition COD term are explained in Appendix D.

$$\mu = \mu_m \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \tag{4.12}$$

K_{sCOD} = half velocity constant for COD under the concurrent operation of Anammox and denitrification, mg l^{-1}
S_{COD} = Influent COD concentration, mg l^{-1}

The common microbial growth was defined by means of differential equation (Equation 4.13).

$$\frac{dX}{dt} = \mu X \tag{4.13}$$

Substitute μ from Equation 4.11 to Equation 4.13, the new equation including the decay coefficient became.

$$\frac{dX}{dt} = \mu_m \frac{S}{K_s + S} . X - K_d X \quad (4.14)$$

Modified Equation 4.14 to the Anammox process, it gave

$$\frac{d X_{AN}}{dt} = \mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} . \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} . \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} . \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} . X_{AN} - (K_{dAN} . X_{AN}) \quad (4.15)$$

X_{AN}	=	Anammox biomass, mg l^{-1}
K_{dAN}	=	decay coefficient for Anammox, d^{-1}
S_{NH}	=	influent ammonium, mg l^{-1}
S_{NO2}	=	influent nitrite, mg l^{-1}
S_{NO3}	=	influent nitrate, mg l^{-1}
K_{sNH}	=	half velocity constant for ammonium in Anammox, mg l^{-1}
K_{sNO2}	=	half velocity constant for nitrite in Anammox, mg l^{-1}
K_{sNO3}	=	half velocity constant for nitrate in denitrification, mg l^{-1}

The Equation 4.14 was the main typical equation used for developing the model.

4.6.1.2.1 Reaction rate of the process obtained from the experiment

To find out the reaction rate of Anammox process for ammonium, nitrite, and nitrate utilizations in the reactor operation, the change in ammonium, nitrite, and nitrate concentrations over time was plotted in various reaction orders; zero, first, and second-order (Figure 4.41-4.43). Theoretically, for each characteristic kinetic plot, a specific rate law shows a straight line. In the substrate concentration, [S] vs time, *t* plot, only the zero-order reaction produces a straight line. In the ln [S] vs *t* plot, only the first-order reaction produces a straight line, and in the 1/[S] vs *t* plot, only the second-order reaction produces a straight line. The obtained plots showed that the reaction rate for the reactor operation was close to both zero-order and first-order reactions based on no significant difference between the R^2 values of 0.9116 and 0.9662, which were obtained for zero-order and first-order plots. The slope or rate constant considered as first-order reaction was 0.5241, 0.4679, and 0.0687 h^{-1} with R^2 value of 0.9662, 0.9626, and 0.8294 for ammonium, nitrite, and nitrate utilizations, respectively. While, the rate constant for ammonium, nitrite, and nitrate utilizations of 8.511, 11.835, and 0.6924 h^{-1} with R^2 value of 0.9116, 0.923, and 0.8242 was calculated based on zero-order reaction.

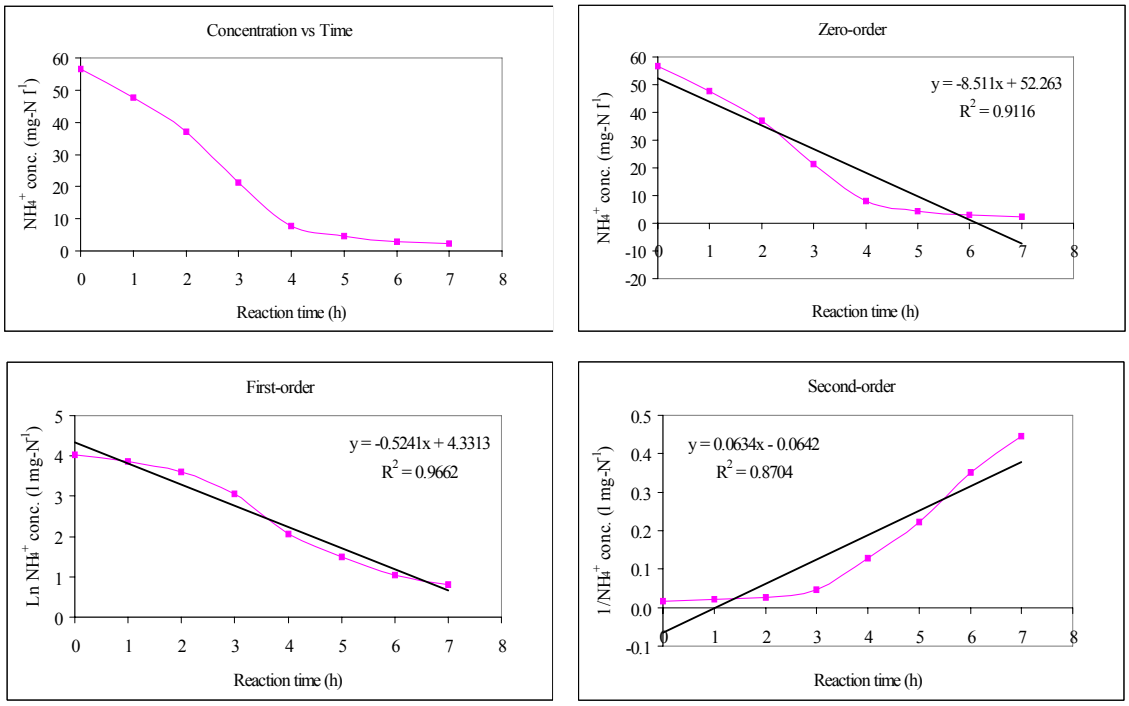


Figure 4.41 Experimental reaction rate of ammonium utilization in Anammox process

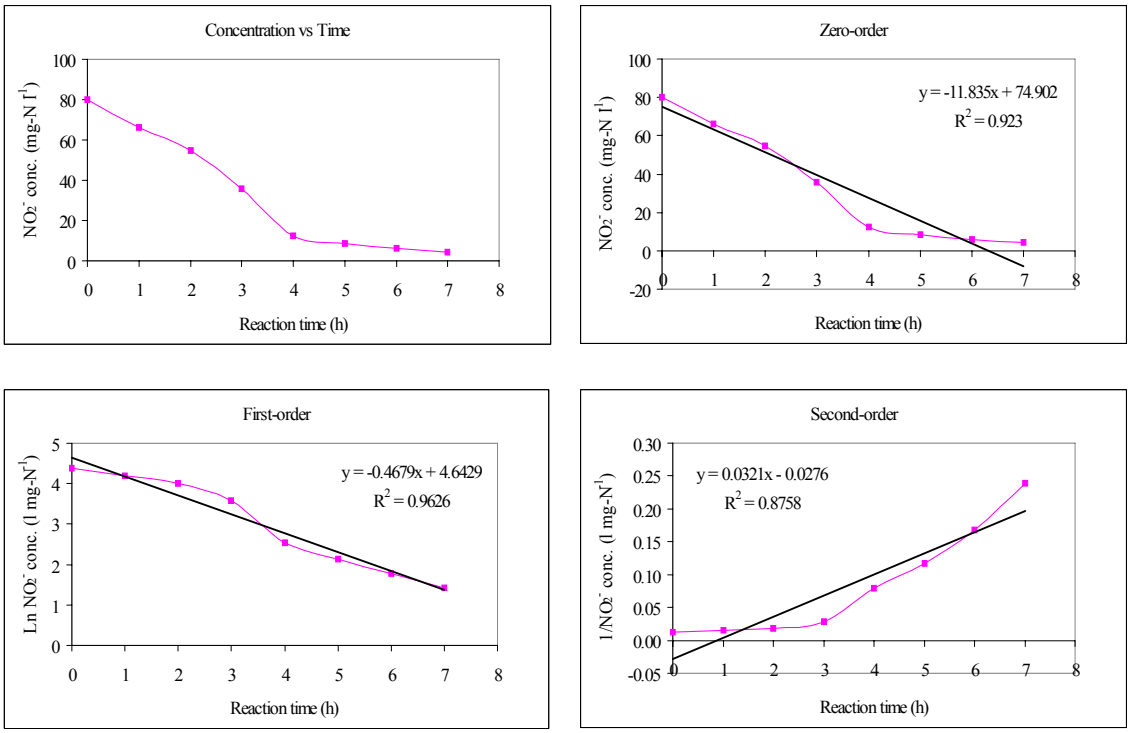


Figure 4.42 Experimental reaction rate of nitrite utilization in Anammox process

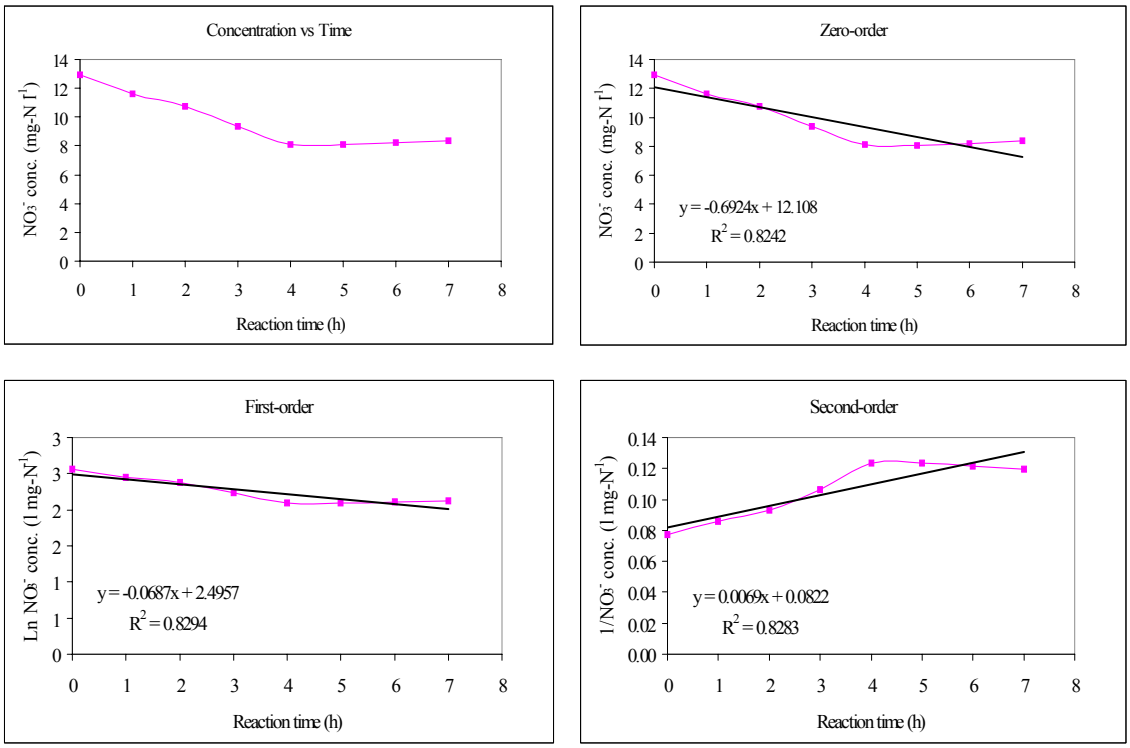


Figure 4.43 Experimental reaction rate of nitrate utilization in Anammox process

4.6.1.3 Matrix format

The Peterson matrix format was selected to present the stoichiometry of the Anammox process. The concept of stoichiometry and process rate was determined following the method in Activated sludge model 1, ASM 1 (Henze et al., 2000). The obtained matrix format for Anammox process in this study was shown in Table 4.9.

1) Determination of stoichiometric coefficient

The stoichiometric coefficient in matrix format (Table 4.9) was computed from the stoichiometric equation 4.3 and rate expression in equation 4.9 as follows.

Process rate of growth process (dX_{AN}/dt) was the main process for constructing the other processes (dS_{NH}/dt, dS_{NO2}/dt, dS_{NO3}/dt).

To apply the model for describing the concurrent ammonium oxidation and nitrate reduction as found in the experiment, more than one substrate was considered as a potential growth rate limitation in this study, which includes ammonium, nitrite, and nitrate.

1.1) Stoichiometric coefficient for X_{AN}

$$r_{AN} = \frac{d\,(CH_2O_{0.5}N_{0.15})}{dt} = \frac{d(X_{AN})}{dt}$$

$$= (1)\ \mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN}$$

The stoichiometric coefficient of growth process for X_{AN} is equal to 1 as indicated in Table 4.9.

1.2) Stoichiometric coefficient for $S_{NH\text{-}N}$

$$r_{S\,NH\text{-}N} = -\frac{d\,[NH_4^+ - N]}{dt} = \frac{1}{0.066}\frac{d\,[CH_2O_{0.5}N_{0.15}]}{dt}$$

$$= -\frac{1}{14}\frac{d\,(NH_4^+ - N)}{dt} = \frac{1}{(0.066)(24.1)}\frac{d\,(CH_2O_{0.5}N_{0.15})}{dt}$$

$$= \frac{d\,(NH_4^+ - N)}{dt} = \frac{-14}{(0.066)(24.1)}\frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(NH_4^+ - N)}{dt} = -8.80\,\frac{d\,(X_{AN})}{dt}$$

where [ ] is the concentration in mol l^{-1}
( ) is the concentration in mg l^{-1}

1.3) Stoichiometric coefficient for $S_{NO2\text{-}N}$

$$r_{S\,NO2\text{-}N} = -\frac{1}{1.32}\frac{d\,[NO_2^- - N]}{dt} = \frac{1}{(0.066)(24.1)}\frac{d\,(X_{AN})}{dt}$$

$$= -\frac{1}{(1.32)(14)}\frac{d\,(NO_2^- - N)}{dt} = \frac{1}{(0.066)(24.1)}\frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(NO_2^- - N)}{dt} = \frac{-(1.32)(14)}{(0.066)(24.1)}\frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(NO_2^- - N)}{dt} = -11.62\,\frac{d\,(X_{AN})}{dt}$$

Table 4.9 Stoichiometric and process rate matrix format for Anammox process

Component i j process	1 S_{NH-N}	2 S_{NO2-N}	3 S_{NO3-N}	4 S_{N2}	5 X_{AN}	6 X_t	7 X_f	*Process rate, ρ_j (mg l^{-1} h^{-1})
1. Growth	-8.80	-11.62	2.29	17.96	1			$\mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{s\,COD}}{K_{sCOD} + S_{COD}} \cdot X_{AN}$
2. Decay	Ammonium, mg l^{-1}	Nitrite, mg l^{-1}	Nitrate, mg l^{-1}	N gas, mg l^{-1}	-1 Biomass, mg l^{-1}	f_t Inert, mg l^{-1}	1- f_t Slowly degradable substrate, mg l^{-1}	$K_{d\,AN} \cdot X_{AN}$

* N_2 was not attained in the process rate in this study.

1.4) Stoichiometric coefficient for S_{NO3-N}

$$r_{S\,NO3-N} = \frac{1}{(0.26)(14)} \frac{d\,(NO_3^- - N)}{dt} = \frac{1}{(0.066)(24.1)} \frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(NO_3^- - N)}{dt} = \frac{(0.26)(14)}{(0.066)(24.1)} \frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(NO_3^- - N)}{dt} = 2.29 \frac{d\,(X_{AN})}{dt}$$

1.5) Stoichiometric coefficient for S_{N2}

$$r_{S\,N2} = \frac{1}{(1.02)(28)} \frac{d\,(N_2)}{dt} = \frac{1}{(0.066)(24.1)} \frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(N_2)}{dt} = \frac{(1.02)(28)}{(0.066)(24.1)} \frac{d\,(X_{AN})}{dt}$$

$$= \frac{d\,(N_2)}{dt} = 17.96 \frac{d\,(X_{AN})}{dt}$$

The stoichiometric coefficient in this study was directly computed on concentration (mg l^{-1}). It was not converted on COD basis as in the earlier ASM1 modeling, which all organic constituents have been expressed as equivalent amounts of chemical oxygen demand. The advantage of this method is that there is no need of a conversion factor for converting the laboratory result in mg l^{-1} to mg COD l^{-1}. Then, the laboratory result could be fitted directly with the model result as discussed in the later part.

2) Process in the model

Two main processes were involved in the model: the growth rate and the decay rate of Anammox biomass. The growth rate of Anammox was composed of the two limiting substrates of ammonia and nitrite. According to the study of co-processes between Anammox and denitrification in this study, another limiting substrate, nitrate was then computed in the model simulation. The inhibition term of COD was also incorporated to the process growth rate. The decay rate depended on the concentration of Anammox biomass in the reactor.

4.6.1.4 Mass balance

Each component in the matrix format (Table 4.9) was introduced into the sequencing batch reactor mass balance.

The mass balance equation for batch reactor (Metcalf and Eddy, 2003) was:

$$\text{Accumulation} = \text{inflow} - \text{outflow} \pm \text{conversion} \quad (4.16)$$

$$V\frac{dC}{dt} = QC_0 - QC \pm rV \quad (4.17)$$

The model was considered only during the reaction phase. Thus, the inflow and outflow were neglected. The typical equation during reaction phase was:

$$\frac{dC}{dt} = r$$

when C = concentration of limiting substrate, mg l^{-1}

r = rate of reaction, mg l^{-1}. time $^{-1}$

The balance equation for each component in the model could be expressed as:

Balance on NH_4^+-N

$$\frac{d\,S_{NH}}{dt} = -8.80\,\mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN} \quad (4.18)$$

Balance on NO_2^--N

$$\frac{d\,S_{NO2}}{dt} = -11.62\,\mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN} \quad (4.19)$$

Balance on NO_3^--N

$$\frac{d\,S_{NO3}}{dt} = 2.29\,\mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN} \quad (4.20)$$

Balance on N_2

$$\frac{d\,N_2}{dt} = 17.96\,\mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN} \quad (4.21)$$

Balance on X_{AN}

$$\frac{d\,X_{AN}}{dt} = \mu_m \frac{S_{NH}}{K_{sNH} + S_{NH}} \cdot \frac{S_{NO2}}{K_{sNO2} + S_{NO2}} \cdot \frac{S_{NO3}}{K_{sNO3} + S_{NO3}} \cdot \frac{K_{sCOD}}{K_{sCOD} + S_{COD}} \cdot X_{AN} - (K_{dAN} \cdot X_{AN}) \quad (4.22)$$

4.6.1.5 Kinetic coefficient

The kinetic coefficient for autotroph Anammox used in the model comprised maximum specific growth rate of Anammox (μ_m), decay coefficient (K_{dAN}), affinity constants for ammonium and nitrite (K_{sNH} and K_{sNO2}). The values of each constant according to Dapena-Mora et al. (2004b) are as follows:

μ_m	=	0.08	d^{-1}
K_{dAN}	=	0.0011	d^{-1}
K_{sNH}	=	0.3	mg N l^{-1}
K_{sNO2}	=	0.3	mg N l^{-3}

The kinetic coefficient for heterotrophic denitrifying including, the affinity constants for COD and nitrate, was manually fitted to the available data. The affinity constants for COD was set to 0.15-0.18 g m^{-3} based on the simulation results of a concurrent operation of Anammox and denitrification operated in SBR and UASB, while the affinity constant for nitrate was set to 0.3 g N m^{-3}. The values of each constant are as follows:

K_{sCOD}	=	0.15-0.18	mg N l^{-3}
K_{sNO3}	=	0.3	mg N l^{-3}

4.6.2 Model simulation for Anammox-SBR and Anammox-UASB

The obtained models for describing system behavior of the Anammox process for nitrogen removal (Equation 4.18-4.22) in this study emphasized the solids production and substrate removal. All modelling was performed in MATLAB 6.5 using function ODE 45 as a tool for solving a differential equation in the obtained model and then plotting the predicted model curves.

MATLAB's standard solver for ordinary differential equations (ODEs) is the function ode45. This function implements a Runge-Kutta method with a variable time step for efficient computation. Ode45 id designed to handle the following general problem.

$$\frac{dy}{dt} = f(t, y) \qquad y(t_0) = y_0$$

where t is the independent variable (time, position, volume) and y is a vector of dependent variables (temperature, position, concentrations) to be found. The mathematical problem is specified when the vector of functions on the right-hand side of equation, f(t,y), is set and the initial conditions, y = yo at time to, are specified.

The obtained model curves in comparison of predicted and experimental curves on the Anammox biomass cultivated in SBR both in the first and second enrichment are shown in Figure 4.44. The model curve of Anammox biomass (X_{AN}) was simulated using the initial concentrations at time prior to the exponential growth of biomass as observed through the experiments. The obtained values were found slightly different for each experiment ranging from 500 to 700 mg MLSS/L. The comparison of predicted and experimental curves on the effluent ammonium, nitrite, and nitrate concentrations from

SBR during the first and second enrichment are shown in Figures 4.45-4.50. Simulation results from the co-removal of nitrogen and COD in SBR and UASBs are described and shown in Figure 4.51-4.54. Comparison of predicted and experimental curves on the ammonium and nitrite removal in SBR operating at different initial ammonium and nitrite concentrations are shown in Figure 4.55-4.56.

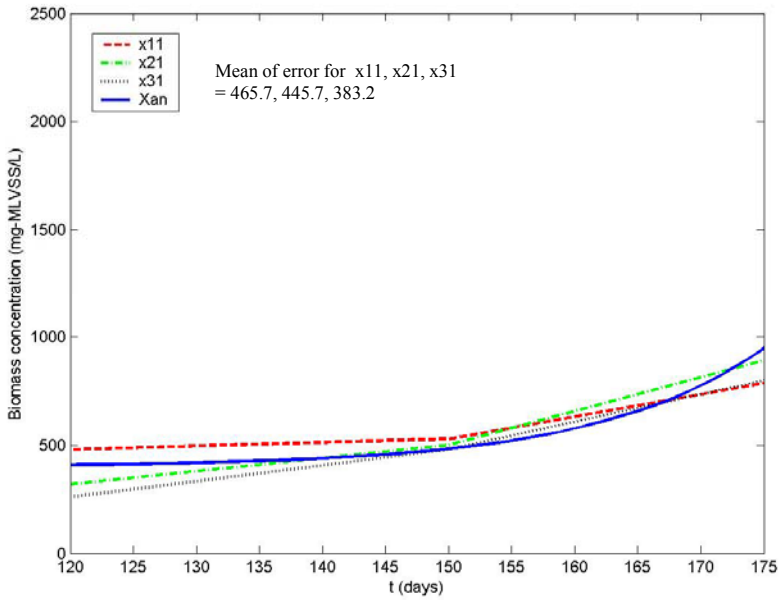
4.6.2.1 Model curves in comparison with experimental data of Anammox biomass (X_{AN}) during experimental phase I

1) Experimental phase Ia

The time evolution of the Anammox biomass concentration obtained from the model curve (X_{AN}), as well as the concentration of MLVSS from experimental data in all 3 SBRs (X11, X21, and X31) is depicted in Figure 4.44a. The experimental curve of Anammox biomass concentration in all reactors showed well fitted with a calculated curve after day 120, while the obtained results of nitrogen removal efficiency in all reactors showed a significant removal of nitrogen after 3 months of operation (Figure 4.10). Both experimental and calculated curves shows that Anammox organisms became dominant after day 120^{th}. It was quite close to the experimental results that showed an achievement of steady state conditions for nitrogen removal after day 120, also. The color change of seed sludge from black in SBR 1 (X11) and SBR 3 (X31), brownish in SBR 2 (X21), to an orangish color after day 120 indicated the dominance of Anammox organisms in the system. However, the color was not reddish as previously reported by van de Graaf et al. (1996) or pinkish as reported by Toh et al. (2002). The experimental data of cultivated Anammox biomass in all reactors showed a tendency of an exponential curve after 120 days of operation. According to the proposed steps for Anammox enrichment from conventional sludges stated in 4.1.2, the turnover of bacteria during the initial phase of enrichment resulted in the decrease of MLVSS in all reactors (X11, X21, and X31). The propagation phase lasted approximately 10 to 12 weeks then the experimental biomass curve showed an exponential way after 4 months of enrichment. This also might be due to a very slow growing of Anammox which needed a longer acclimatization period than a typical growth of heterotrophic bacteria. The error between the experimental data and the calculated data obtained from the equation was presented in terms of mean of error, which was 465.7, 445.7, and 383.2 for X11, X21, and X31, respectively.

At the beginning of the enrichment period, most of the seed sludge was heterotrophic including some autotrophic such as Anammox organisms. These heterotrophic bacteria were supposed to decrease because they did not have suitable substrates. Then, the MLVSS concentration of mixed sludge in all reactors (X11, X21, and X31) was decreased at the beginning of enrichment period. Anammox organisms, which are autotrophs, could survive and start to increase their population, which obviously seen after day 120. However, the results from SEM of SBR 1 (X11) (Figure 4.11) revealed that some heterotrophs still remained in the system. This was probably because of heterotrophic biomass was able to live on cell lysis products and from the biodegradable substrate in the influent even in the absence of oxygen, since these organisms could use both nitrate and nitrite as electron acceptors. For instance, from leaks as mentioned earlier, the aerobic bacteria that was still remaining in the system would consume this oxygen for growth and allow the Anammox bacteria to grow in an anoxic environment accordingly. This illustrates the benefit of using mixed sludge culture for Anammox enrichment.

(a)



(b)

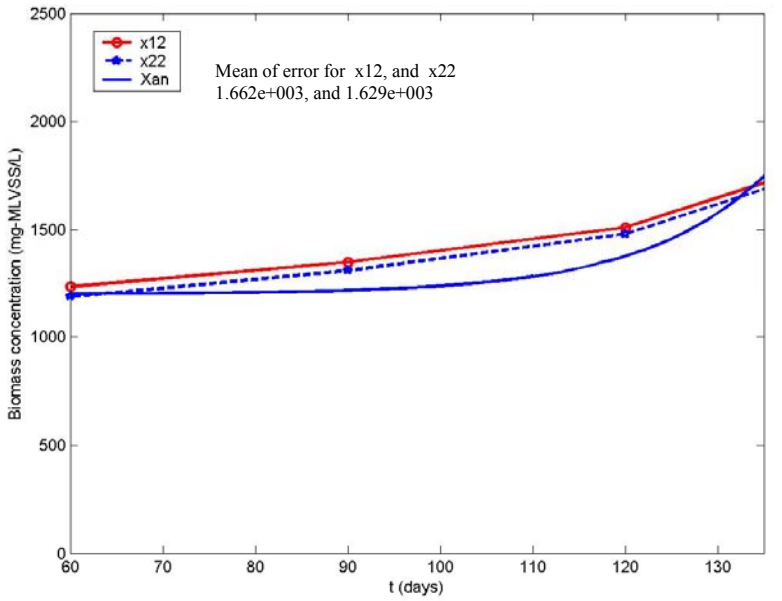


Figure 4.44 Comparison of calculated and experimental curves on the Anammox biomass cultivated in SBR versus time in experimental phase Ia (a) and Ib (b)

2) Experimental phase Ib

The calculated model curve compared with the experimental data of Anammox biomass concentration (X_{AN}) in terms of MLVSS in experimental phase Ib is shown in Figure 4.44b. The mean of error between experimental data and predicted model curve was 1,662 and 1,629 for SBR 1 (X12) and SBR 2 (X22).

In the second enrichment, the Anammox process was accelerated using the combination of excess sludge from the activated sludge process and the existing Anammox biomass from a previous experiment, which resulted in a short enrichment period of two months. This agreed with the initial increasing time of biomass concentration after 60 days of operation found in both reactors, X12 and X22 (Figure 4.44b), together with the 96 % ammonium removal efficiency obtained (Figure 4.16).

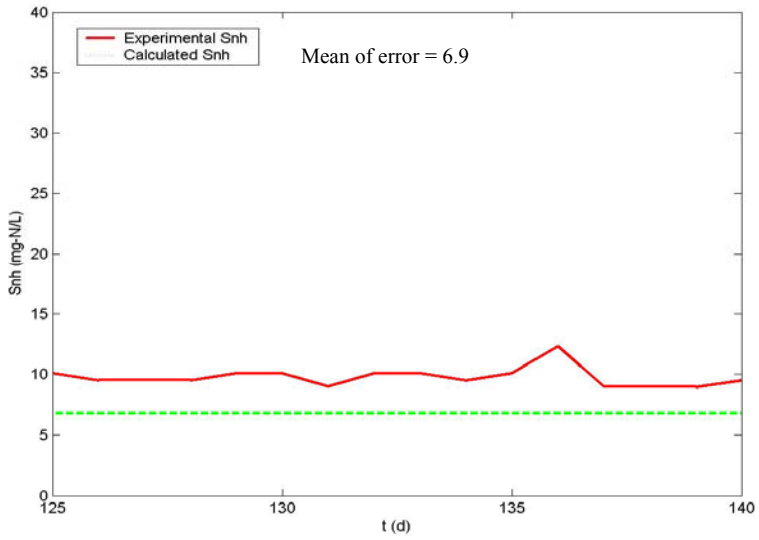
The experimental data of cultivated Anammox biomass (X12 and X22) showed a tendency of exponentiation faster than in the first enrichment (X11, X21, and X31) and agreed with the model curve (X_{AN}). The observed Anammox concentration in this enrichment period (X12 and X22) was close to the calculated one (X_{AN}) after 60 days of operation (Figure 4.44b), while the lower concentration was recorded in the first enrichment (X11, X21, and X31) during this time (Figure 4.44a).

4.6.2.2 Model curves in comparison with experimental data of effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) in Anammox-SBR during experimental phase Ia

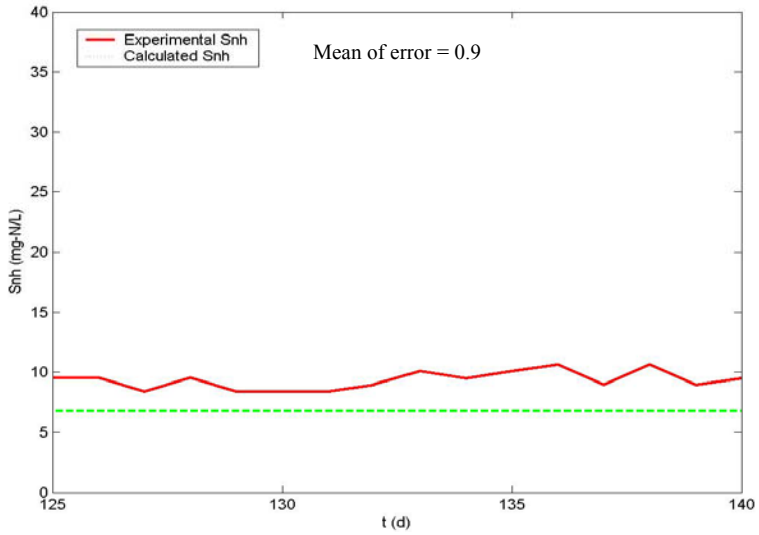
The calculated values of the effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) concentrations were compared with experimental data in Figures 4.45-4.47. The error between the experimental and calculated data based on the obtained equations was expressed as the mean of error. The mean of error between experimental data of effluent ammonium (1.0, 6.3, 4.7), nitrite (4.2, 6.4, 7.1), and nitrate (0.5, 0.5, 3.9) was obtained for SBR1, 2, and 3, respectively. From these data, the calculated values of ammonium and nitrite after 120 days of operation, when steady state condition was achieved, agreed well with the experimental data. Similarly, the measured values of nitrate were also fitted with the calculated values.

Simulation results after an achievement of steady state conditions for nitrogen removal showed an agreement between an experimental effluent ammonium, nitrite, and nitrate with a model curve (Figure 4.45-4.47). It indicated the dominance of Anammox in the system, which also was clearly confirmed by the hybridised results from FISH analysis (Figure 4.12-4.14). However, the experimental effluent nitrite and nitrate showed lower amounts than these obtained from the model calculation curve (Figure 4.46 and 4.47). The small amount of consumption of nitrate in all reactors was still observed. It indicated an occurrence of a concurrent process of ammonium oxidation by Anammox and nitrate reduction by denitrifying, using organics from cell lysis. Anammox would be a major process and denitrification was the minor process. Therefore, the produced nitrate by Anammox might be partially consumed by denitrifying bacteria, resulting in the detection of continuously decreasing effluent nitrate concentrations after 120 days of operation in all SBRs (Figure 4.47).

(a)



(b)



(c)

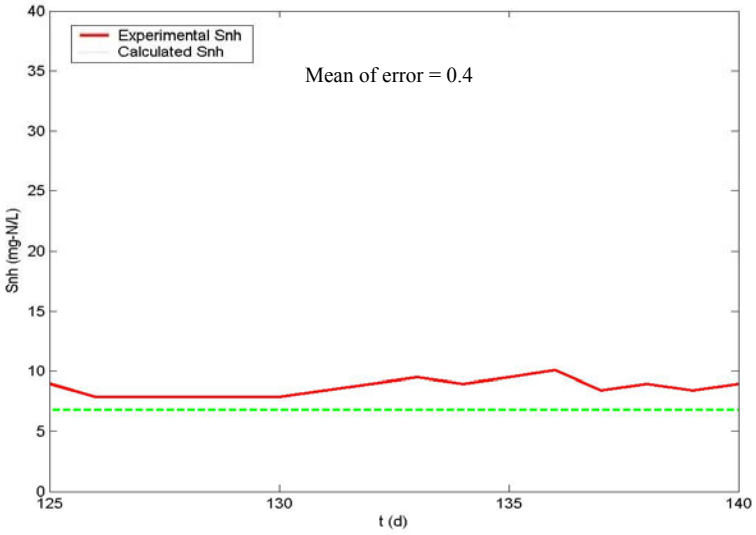
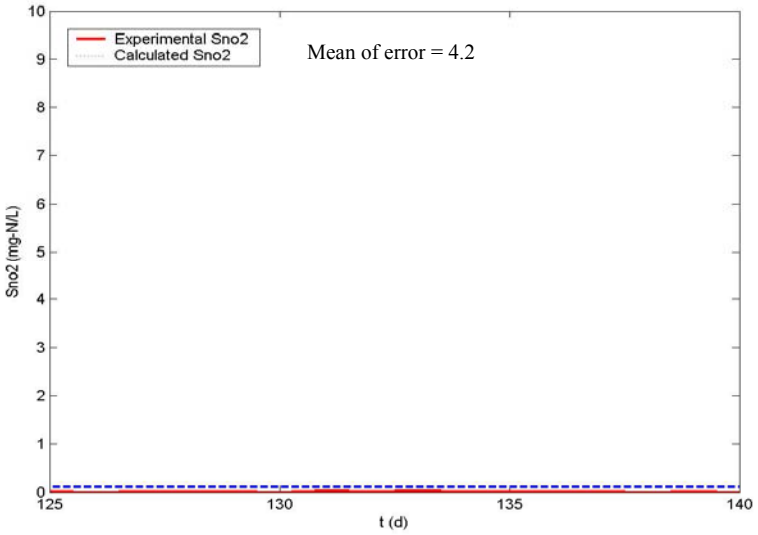
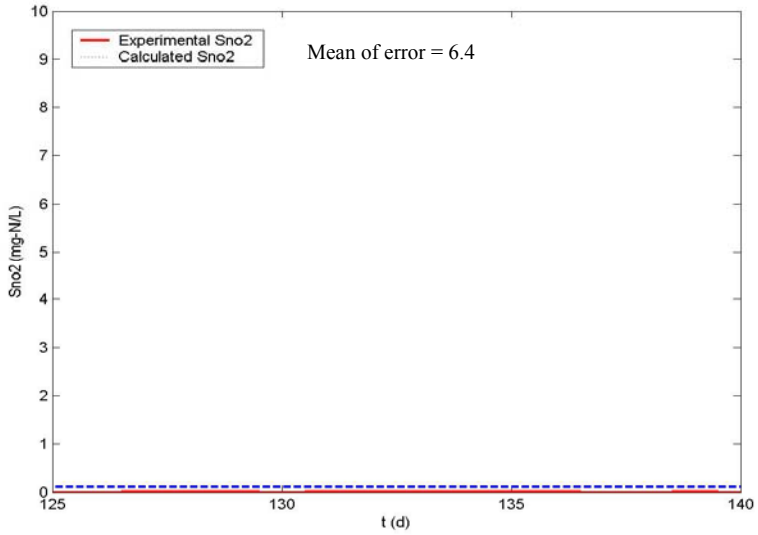


Figure 4.45 Comparison of calculated and experimental curves on the effluent ammonium in Anammox-SBR during experimental phase Ia (a) SBR 1, (b) SBR 2, and (c) SBR 3

(a)



(b)



(c)

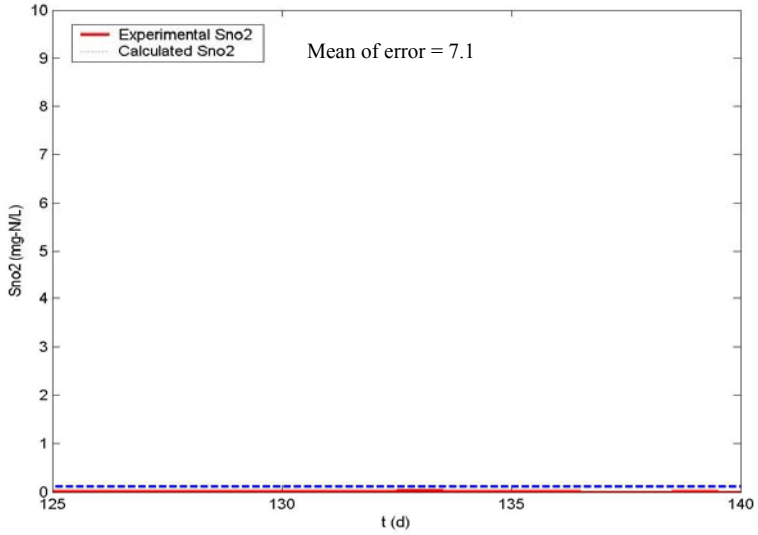
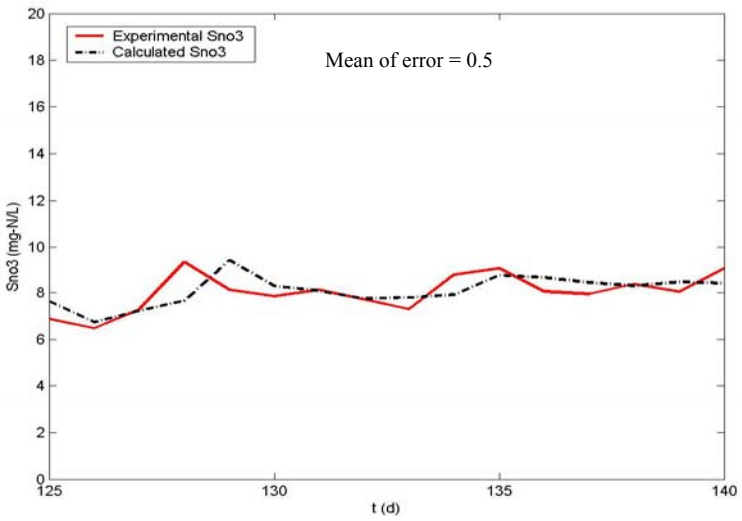
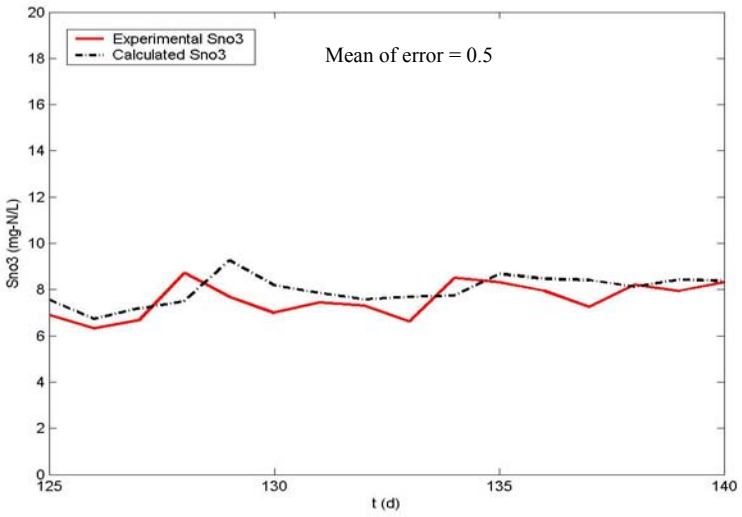


Figure 4.46 Comparison of calculated and experimental curves on the effluent nitrite in Anammox-SBR during experimental phase Ia (a) SBR 1, (b) SBR 2, and (c) SBR 3

(a)



(b)



(c)

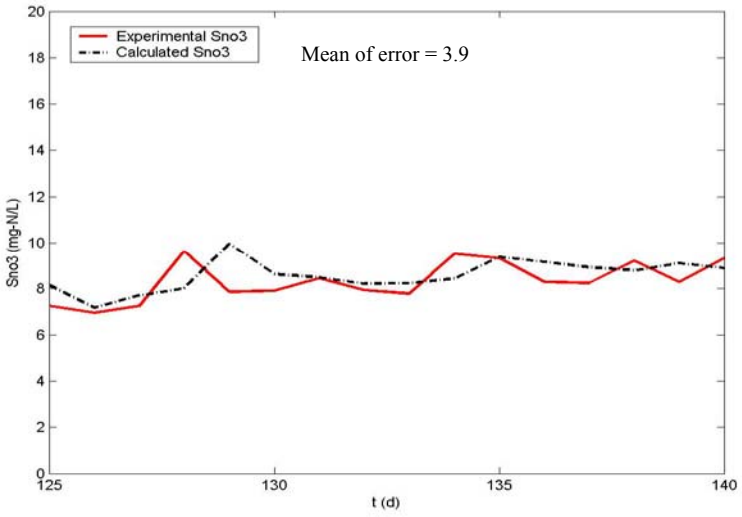


Figure 4.47 Comparison of calculated and experimental curves on the effluent nitrate in Anammox-SBR during experimental phase Ia (a) SBR 1, (b) SBR 2, and (c) SBR 3

4.6.2.3 Model curves in comparison with experimental data of effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) in Anammox-SBR during experimental phase Ib

The calculated values of the effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) concentrations during experimental phase Ib were compared with experimental data in Figures 4.48-4.50. The mean of error between experimental data and the calculated one of effluent ammonium (5.2, 6.8), nitrite (6.5, 8.6), and nitrate (0.1, 0.3) was obtained for SBR 1 and SBR 2, respectively.

The calculated values of effluent ammonium rapidly decreased and showed relatively constant value within one week, whereas the experimental data showed a constant value after 25 days for both SBR 1 and SBR 2 (Figure 4.48). The experimental effluent nitrite curve agreed with the calculated curve (Figure 4.49). However, the experimental effluent nitrite and nitrate observed in both SBR 1 and SBR 2 showed a lower amount than in the calculated data (Figure 4.49 and 4.50). This shows the possibility of a co-existing bacterial group, denitrifying, to play a concurrent function for denitrification using organics from lysis of non-preferable organisms in the system. This co-process was also detected during the first enrichment without Anammox seed. The obtained comparison curves indicated the possibility of achieving Anammox enrichment in SBR within a short period of two months when Anammox seed was provided. The confirmation of Anammox domination in the system was shown from the hybridised results by FISH analysis (Figure 4.18).

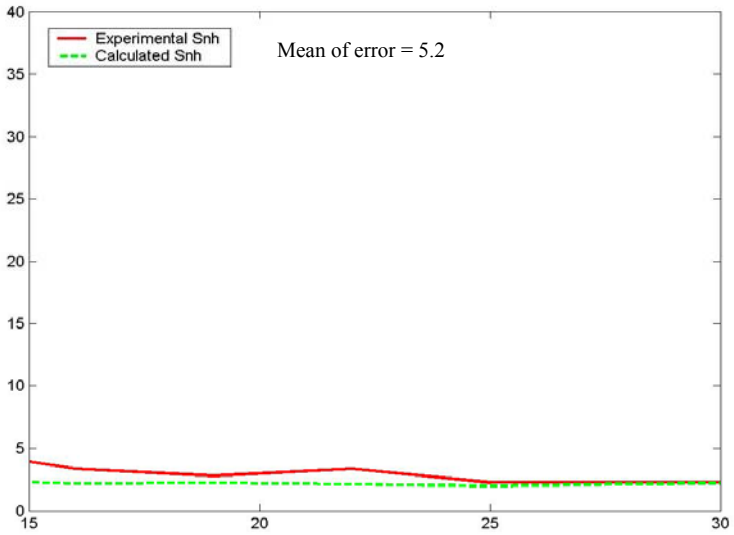
4.6.2.4 Model curves in comparison with experimental data of effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) in Anammox-SBR during the co-removal of nitrogen and COD study

The calculated values of the effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) concentrations during the study of co-removal of nitrogen and COD in SBR were compared with the experimental data in Figure 4.51. The mean of error between experimental data and the calculated one of effluent value was 6.3, 6.9, and 0.3 for ammonium, nitrite, and nitrate, respectively.

Synthetic COD produced from fat milk was introduced to the system for observing the co-removal of nitrogen and COD in the reactor after the study of shock loading of COD, when a preferable ammonium removal was achieved again, at the 66th day. The experimental effluent nitrite curve showed an agreement with the model curve after day 70 (Figure 4.51b). The decreasing of experimental effluent nitrate curve after day 77, which obviously different from the model curve, it indicated the high performance of denitrification to remove COD.

The ratio of COD to N in the feed was gradually increased within the range of 0.9-2.6, resulting in the higher effluent ammonium observed during the day 83-93 (Figure 4.51a). The higher consumption of nitrate for COD removal was also observed, which was shown by a significantly lower amount of experimental effluent nitrate than the calculated curve (Figure 4.51c). After 93 days of operation, the experimental effluent ammonium was fitted to the model curve again (Figure 4.51a) due to the COD to N ratio in the feed being reduced to approximately 1.8 as shown in Figure 4.30.

(a)



(b)

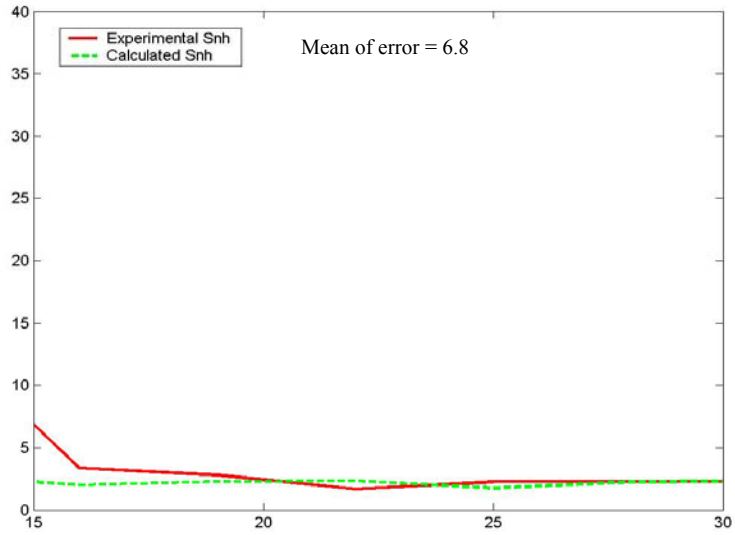
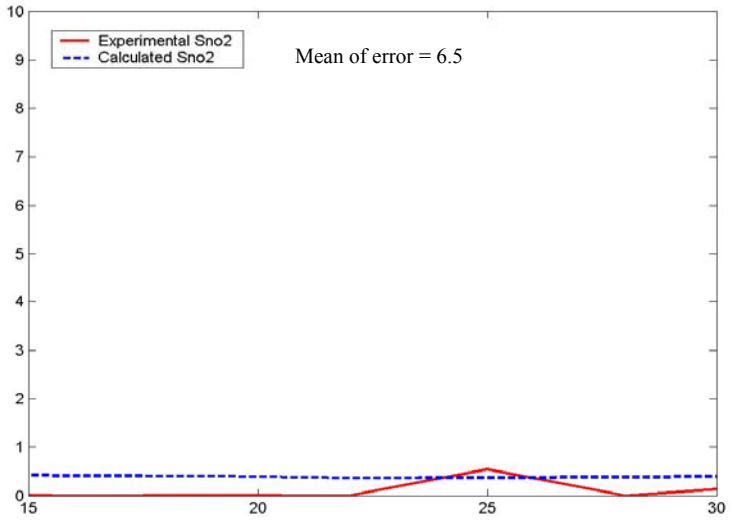


Figure 4.48 Comparison of calculated and experimental curves on the effluent ammonium in Anammox-SBR during experimental phase Ib (a) SBR 1, (b) SBR 2

(a)



(b)

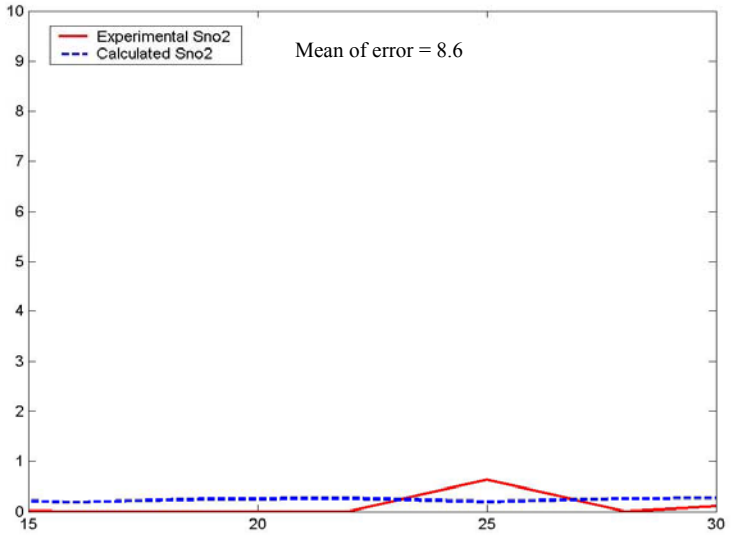
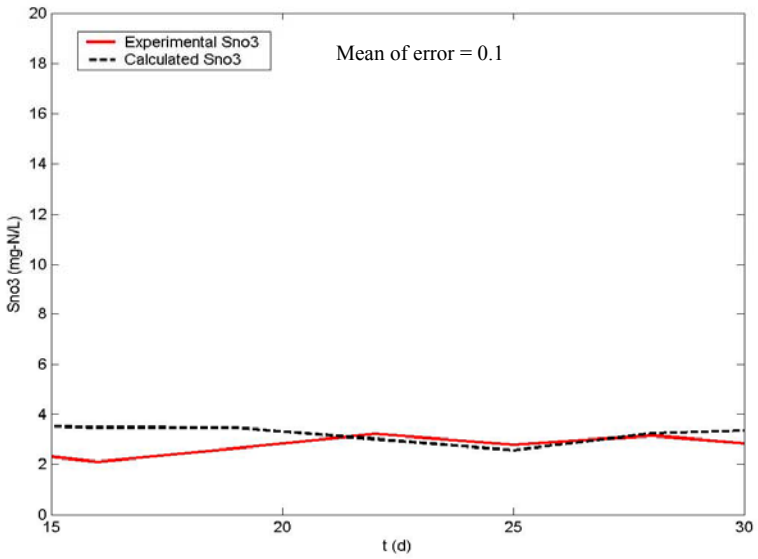


Figure 4.49 Comparison of calculated and experimental curves on the effluent nitrite in Anammox-SBR during experimental phase Ib (a) SBR 1, (b) SBR 2

(a)



(b)

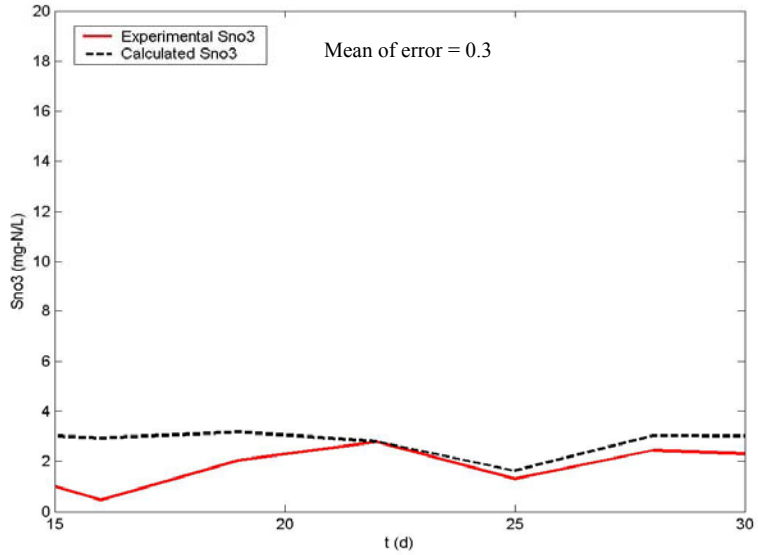
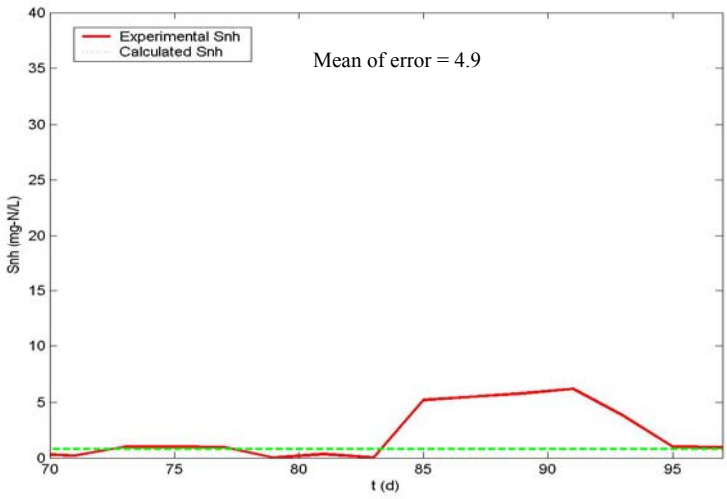
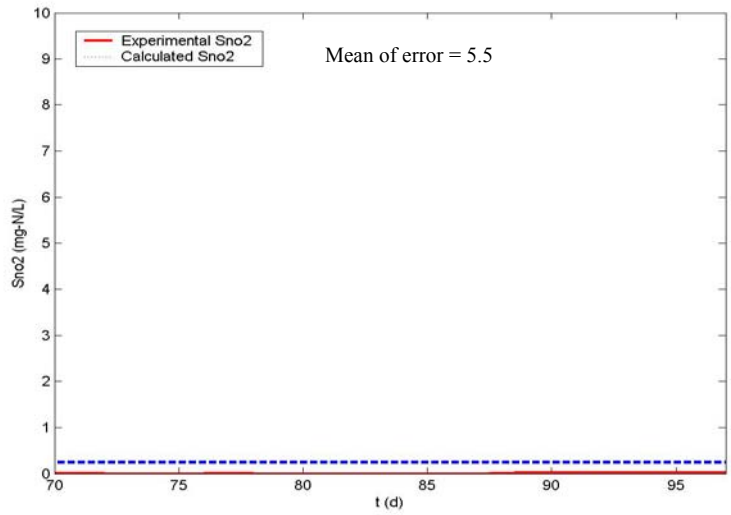


Figure 4.50 Comparison of calculated and experimental curves on the effluent nitrate in Anammox-SBR during experimental phase Ib (a) SBR 1, (b) SBR 2

(a)



(b)



(c)

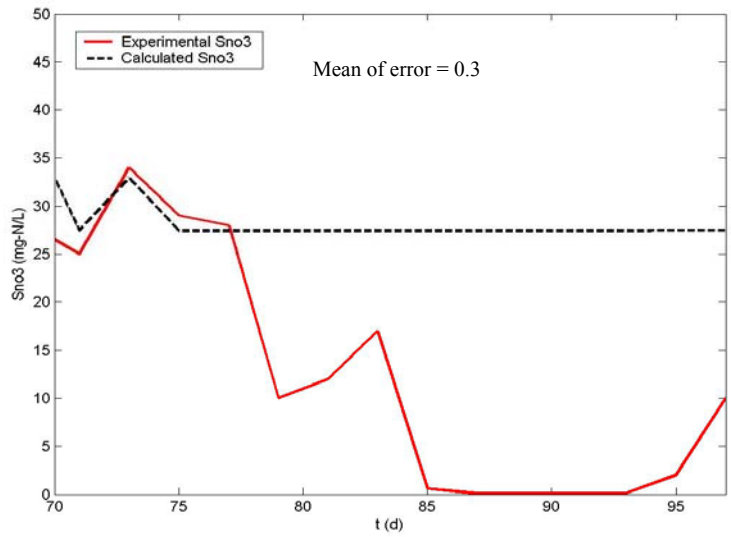


Figure 4.51 Comparison of calculated and experimental curves on the effluent ammonium (a), nitrite (b) and nitrate (c) in Anammox-SBR during the co-removal of nitrogen and COD study

4.6.2.5 Model curves in comparison with experimental data of effluent ammonium (S_{NH}), nitrite (S_{NO2}), and nitrate (S_{NO3}) in Anammox-UASB during the co-removal of nitrogen and COD study

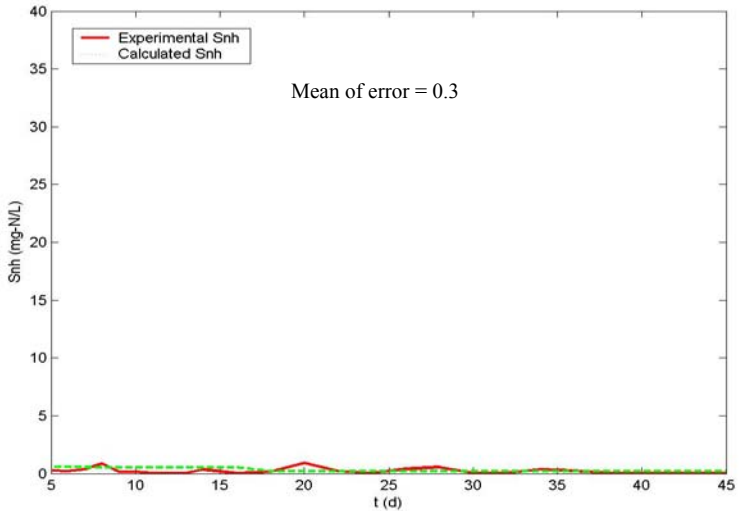
As shown in Figure 4.52a, the measured effluent ammonium (S_{NH}) from UASB 1 fed with low COD content of 100-200 mg l^{-1} agreed with the calculated curve within one week. The observed ammonium removal of 97% (Figure 4.35a) achieved in this reactor after 8 days of operation supports the output from this model simulation. The experimental effluent ammonium curve from this reactor agreed with the calculated curve through the experiment with mean of error of 0.3. The experimental curve of ammonium from UASB 2 and 3 fed with higher COD content of 200-300 and 300-400 mg l^{-1} showed higher amount than in the calculated curve during the beginning operation period to the 18th day. As a complete consumption of nitrite and nitrate with a high COD removal of 85% and 97% in UASB 2 and 3 were obtained, it clearly showed the competitiveness of heterotroph, denitrifying bacteria, for nitrate and nitrite consumption to remove COD in both UASB 2 and 3. It also agreed with the comparison curves in Figure 4.53 that showed a lower amount of experimental effluent nitrite than a calculated curve of S_{NO2} found in UASB 2 and 3.

The attempts to promote Anammox process as a main process in UASB 2 and 3 were carried out by increasing the amount of nitrate to the range of 90-100 mg N l^{-1} on the 19th day of operation. The Anammox activity was observed consequently in UASB 2 with the average ammonium removal of 89% (Figure 4.35a). The experimental effluent ammonium curve of UASB 2 after 20 days of operation agreed well with the output from model simulation (Figure 4.52b). However, Anammox activity still did not occur in UASB 3 fed with high COD content of 300-400 mg l^{-1} as the preferable ammonium removal could not be achieved (Figure 4.35a). The measured effluent of nitrate showed the excess amount after the 19th day and 31st day for UASB 1 and 2 (Figure 4.54a, b). A difference between experimental effluent nitrate and calculated curve was observed in UASB 3 through the entire period of study (Figure 4.54c).

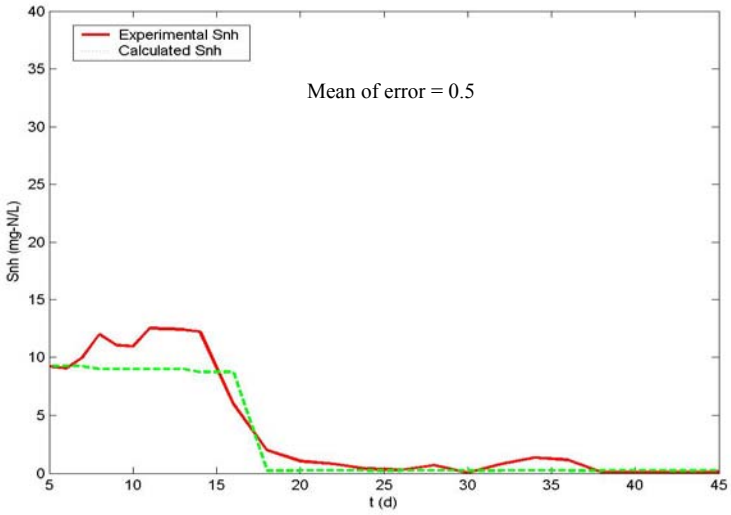
An attempt to boost the Anammox process in UASB 3 was pursued by increasing the amount of NO_3^- to 100-120 mg N l^{-1} on the 31st day of operation as explained in the previous section 4.5.1. The Anammox reaction still did not occur in this reactor, based on the comparison with the result from model simulation (Figure 4.52c). It agreed with the recorded effluent NH_4^+ concentration during this period that showed higher value than in UASB 1 and 2 (Figure 4.34). The mean of error between measured data of effluent ammonium (0.3, 0.5, 5.5), nitrite (0.1, 4.1, 9.6), and nitrate (4.9, 0.3, 6.7) during a concurrent operation of Anammox and denitrification was obtained for UASB 1, 2, and 3, respectively.

The obtained comparison curves between experimental and simulation indicated the possibility of a co-process between Anammox and denitrification in the UASB reactor by providing the optimum COD to N ratio to the system. As explained above, Anammox was achieved as a main process in both UASB 1 and 2 within the COD range of 100-300 mg l^{-1}, which was equivalent to the COD to N ratio of 0.9-1.4. It was shown by the good fit of experimental curve to the calculated curve of effluent ammonium in Figure 4.52a, b and the disagreement of the experimental curve with the calculated curve of effluent ammonium from UASB 3 (Figure 4.52c) fed with excess range of the optimum COD to N ratio as suggested above.

(a)



(b)



(c)

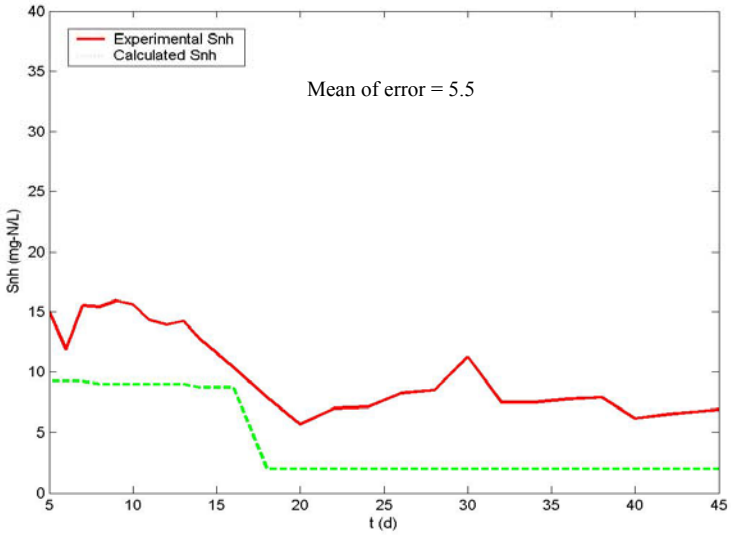
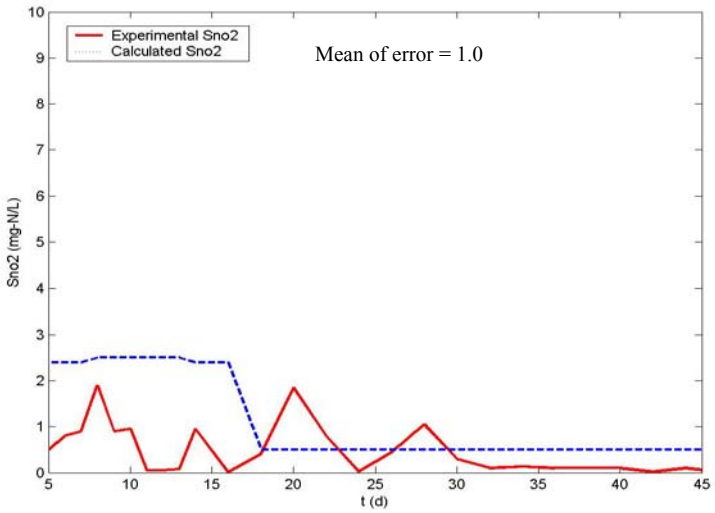
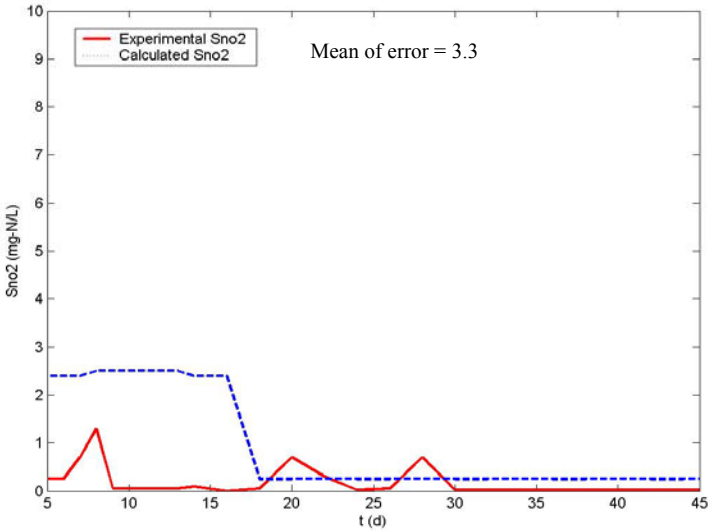


Figure 4.52 Comparison of calculated and experimental curves on the effluent ammonium in Anammox-UASB during the co-removal of nitrogen and COD study (a) UASB 1, (b) UASB 2, and (c) UASB 3

(a)



(b)



(c)

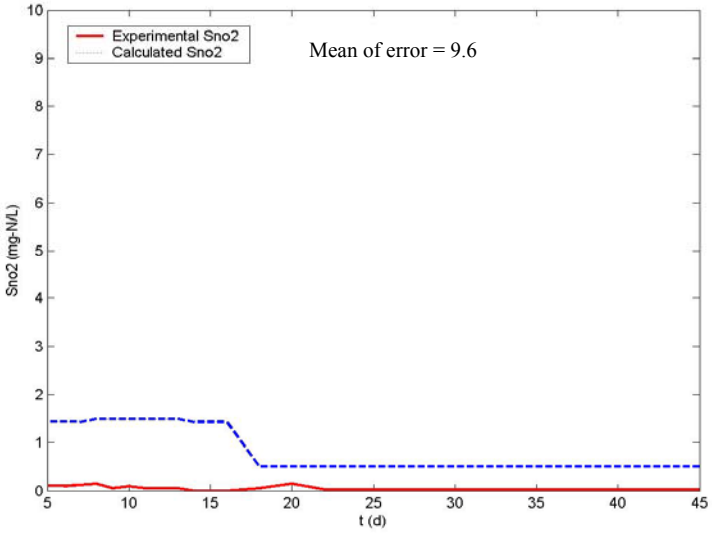
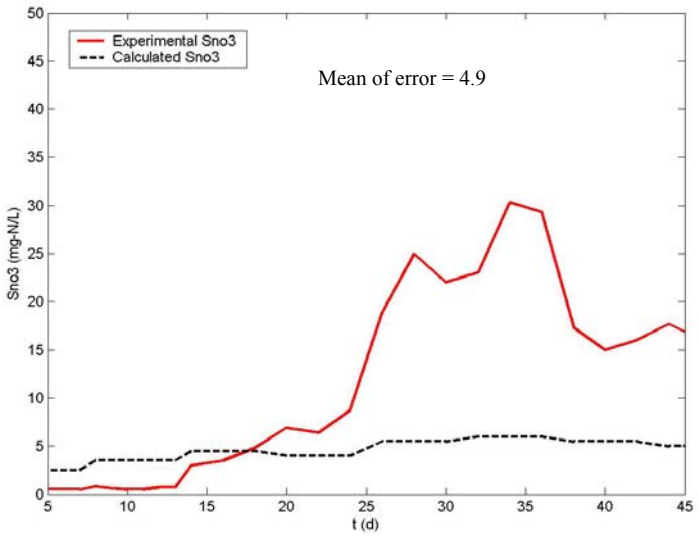
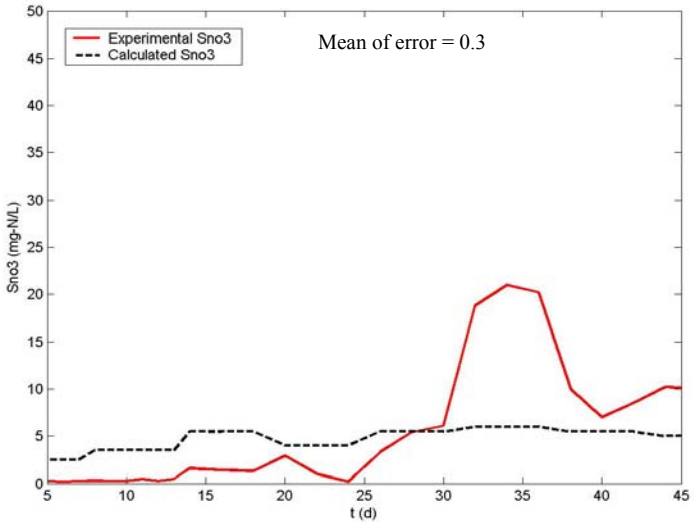


Figure 4.53 Comparison of calculated and experimental curves on the effluent nitrite in Anammox-UASB during the co-removal of nitrogen and COD study (a) UASB 1, (b) UASB 2, and (c) UASB 3

(a)



(b)



(c)

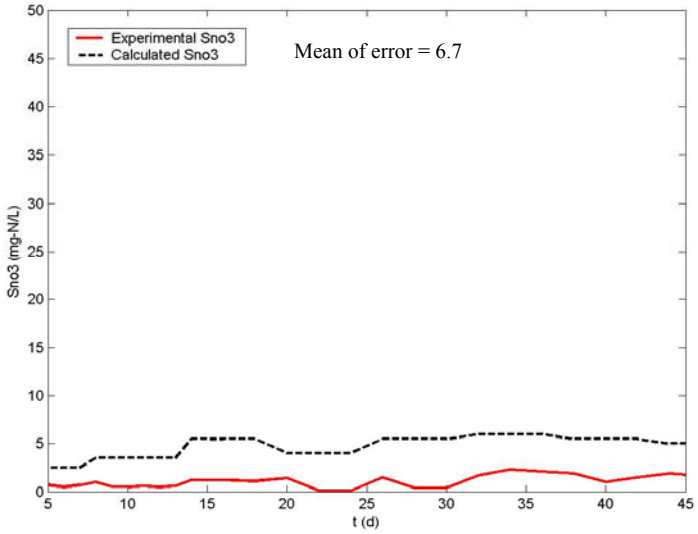


Figure 4.54 Comparison of calculated and experimental curves on the effluent nitrate in Anammox-UASB during the co-removal of nitrogen and COD study (a) UASB 1, (b) UASB 2, and (c) UASB 3

4.6.2.6 Simulation results on ammonium (S_{NH}) inhibition study

An ammonium inhibition study for Anammox-SBR was conducted by adding initial ammonium concentrations of approximately 45, 65, 75, and 115 mg N l^{-1} (experimental run NH1, NH2, NH3, and NH4, respectively) to the reactor. The concentration of effluent ammonium in each experimental run was monitored every hour for 7 h. The simulated effluent ammonium curve (Snh) was obtained from MATLAB program using equation 4.18. The equation was used for predicting the effluent ammonium from the Anammox-SBR under provided conditions and then compared with the experimental effluent data in each run. Nitrite and nitrate concentrations used in the equation were set as a constant value while COD was set as zero. Anammox biomass was manually fitted to the available data.

The experimental data of ammonium concentration decreasing during 7 h reaction time in the run NH1, with mean of error of 11.7, agreed with the model curve (Snh). The ratio of ammonium to nitrite of the run NH1 was 1:1.5. This ratio was reported as the appropriate ratio in this study. As the ammonium concentration was incremented in the run NH2 and NH3, with higher value of mean of error, 30.0 and 45.3, the results showed disagreement with the model curve. However, there was no report about the inhibition effect on the Anammox activity at these concentrations. A complete inhibition was obviously shown at approximately 115 mg NH_4^+-N l^{-1} in the experimental run NH4, with a mean of error of above one hundred (Figure 4.55).

4.6.2.7 Simulation results on nitrite (S_{NO2}) inhibition study

Nitrite inhibition study for Anammox-SBR was conducted by adding initial nitrite concentrations of approximately 60, 60, 70, 80, 80, 90, and 120 mg N l^{-1} (experimental run NO1, NO2, NO3, NO4, NO5, NO6, and NO7, respectively) to the reactor. The concentration of effluent nitrite in each experimental run was monitored every hour for 7 h. The simulated effluent nitrite curve (Sno2) was obtained from MATLAB program using equation 4.19. The equation was used for predicting the effluent nitrite from the Anammox-SBR under provided conditions and then compared with the experimental effluent data in each run. Ammonium and nitrate concentrations used for simulation were set as a constant value, while COD was set as zero. Anammox biomass was manually fitted to the available data.

The experimental run of different initial nitrite concentration (NO1-NO7) was compared with the calculated model curve (Sno2) (Figure 4.56). It revealed that the experimental data of effluent nitrite in the run NO5 and NO6, with a mean of error of 29.0 and 38.0, was close to the effluent model curve (Sno2). The ratio of ammonium to nitrite consumed of 1:1.5 and 1:1.6 in the run NO5 and NO6 agreed with the results from the ammonium inhibition study in 4.6.2.6. The results of the run NO1 to NO4, with a mean of error of 10.8, 30.4, 20.8, 21.1, respectively, still showed the possibility of the process to remove nitrogen, but its capability can be increased up to the model curve (Sno2). The curve of the run NO7, with a mean of error of 103.0, showed the inhibited nitrite concentration to the Anammox in this study, which was approximately 120 mg NO_2-N l^{-1}.

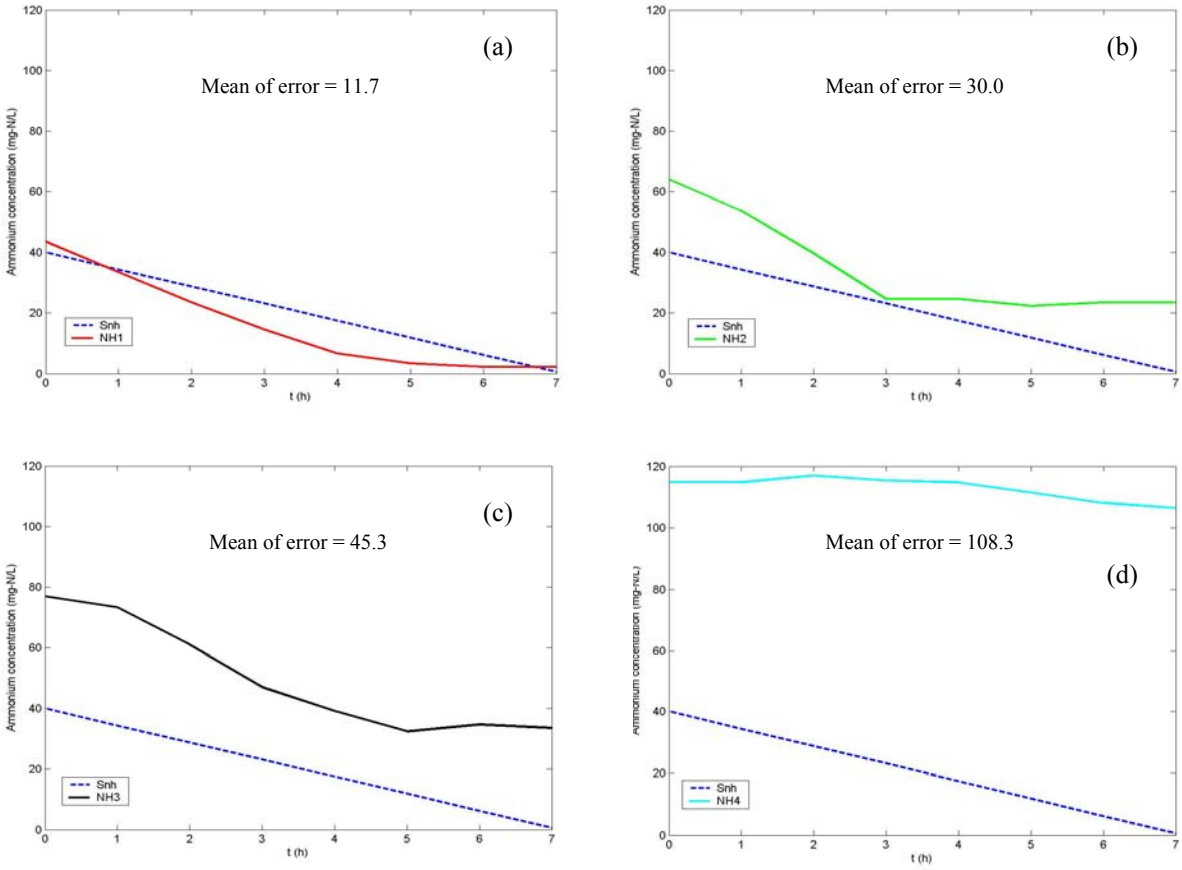


Figure 4.55 Comparison of calculated and experimental curves on the effluent ammonium in Anammox-SBR for different initial ammonium concentrations (a) 45 mg N l^{-1}, (b) 65 mg N l^{-1}, (c) 75 mg N l^{-1}, and (d) 115 mg N l^{-1}

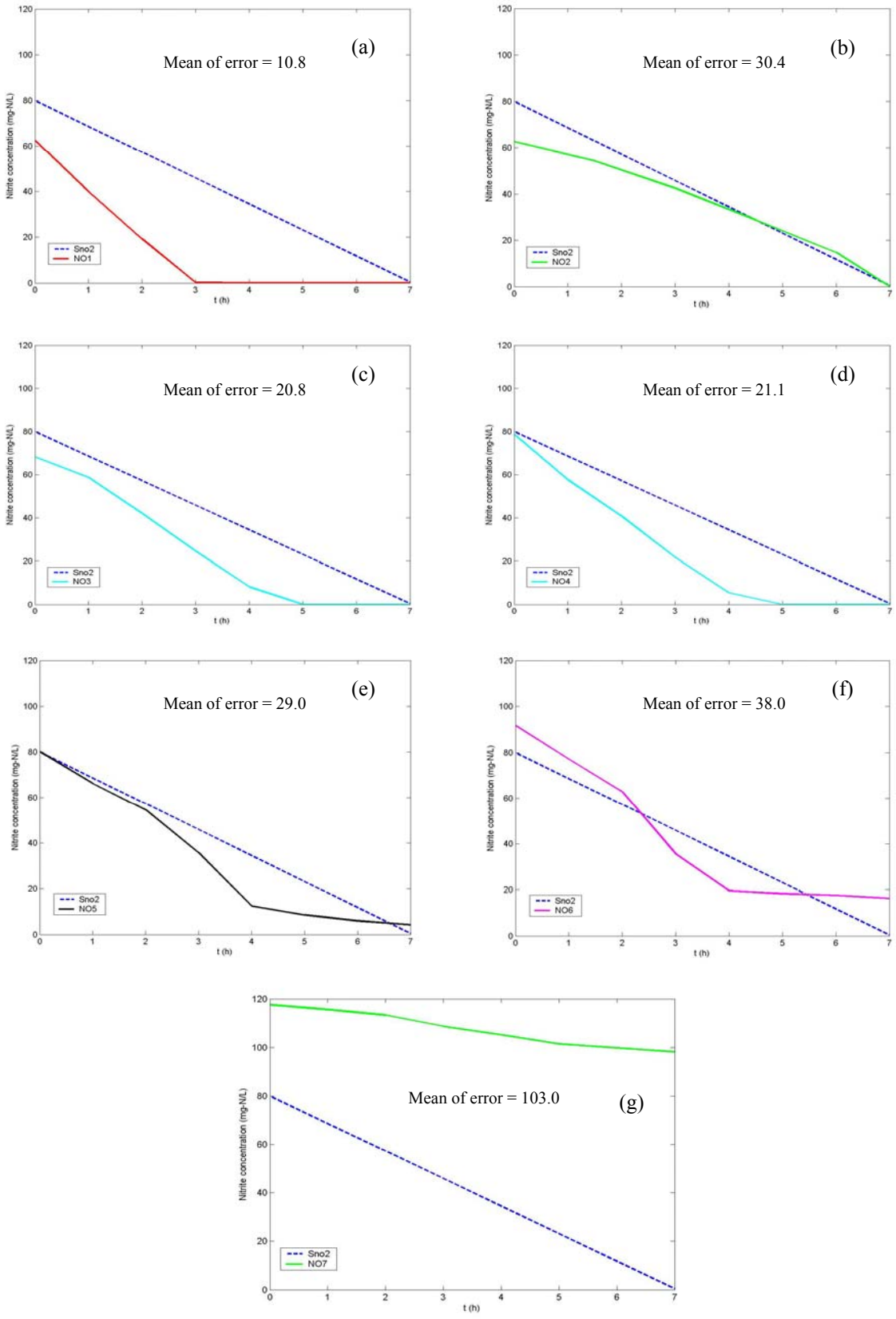


Figure 4.56 Comparison of calculated and experimental curves on the effluent nitrite in Anammox-SBR for different initial nitrite concentrations (a) and (b) 60 mg N l^{-1}, (c) 70 mg N l^{-1}, (d) and (e) 80 mg N l^{-1}, (f) 90 mg N l^{-1}, and (g) 120 mg N l^{-1}

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Anammox could be enriched using conventional sludges including UASB sludge, activated sludge, and anaerobic digestion sludge. The successful cultivation was achieved in the SBR operated with a time sequence of 5-7 h reaction time, 30 min of settle period, and 15 min discharge period. The cultivation process could be divided into three phases: initial phase (approx. 5-7 weeks), propagation phase (approx. 10-12 weeks) and stationary phase. Near perfect removal of nitrite was obtained while ammonium removal was close to 80% based on the NH_4^+ to NO_2^- ratio of 1:1.5 and NH_4^+ concentration of 45-47 mg N l^{-1}. Acceleration of the Anammox process was achieved within a period of 2 months when Anammox seed was provided.

The lower ratios of NH_4^+:NO_2^- under 1:1.2, could be used to acclimatize the Anammox biomass without adverse effect to the Anammox reaction, but it might extend the enrichment period of Anammox biomass. The NH_4^+ and NO_2^- concentrations of greater than 120 mg N l^{-1} and phosphate concentration of more than 170 mg P l^{-1} were found to inhibit the Anammox process. Both sludge concentration and reaction time affected the nitrogen removal rate. The optimum specific removal rate was obtained at sludge concentration of 1,000 mg MLSS l^{-1}.

The concurrent operation of Anammox and denitrification processes was achieved in both the SBR and the UASB reactor. The results showed that the Anammox process was suspended with an increase in COD concentration or COD to N ratio. The suppression of Anammox activity occurred at COD concentration over 400 mg l^{-1} and COD to N ratio of over 4.0. This may be slightly different based on the reactor used. High concentration of COD strongly inhibited the Anammox reaction and possibly eradicate the Anammox community.

The developed models were well described the system behavior of the Anammox process in SBR and UASB reactors for nitrogen removal and biomass production especially during steady state conditions. Poor simulation results in the early stage of operation might be due to the turnover of bacteria during the initial and propagation phase of enrichment, which lasted approximately 15 to 19 weeks prior to an exponential growth. The model also incorporated the COD inhibition due to concurrent denitrification activity and be able to predict the performance satisfactory.

5.2 Recommendations

- UASB sludge was recommended as seed sludge for Anammox enrichment as it showed faster acclimatization period.

- Application of the Anammox process treating real wastewater still needs more information and further study. The suggested COD:N ratio should be not more than 4.0 and 2.0 for the SBR and the UASB reactor, respectively.

- Study of co-existing of Anammox and other kinds of bacterial group besides denitrifying will be beneficial for practical application.

- Further study on factors affecting Anmamox process including inhibitors is needed since they can cause failure to the system.

- Further work on model simulation and verification is needed for more accurate interpretation and practical use.

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Appendix A

Stoichiometry of sludge S1-S3

Table A1 Stoichiometry of sludge S1 experiment during steady state condition

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 :	NO_2 :	NO_3
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	-1	-1.32	0.26
28	1	43.28	10.08	-33.20	49.32	0.00	-49.32	8.69	6.87	-1.82	8.11	7.33	-1	-1.49	-0.05
29	1	42.08	9.52	-32.56	49.29	0.00	-49.29	7.63	6.48	-1.15	7.85	7.52	-1	-1.51	-0.04
30	1	40.32	9.52	-30.80	49.53	0.01	-49.52	8.20	7.29	-0.91	7.91	7.67	-1	-1.61	-0.03
31	1	44.72	9.52	-35.20	54.05	0.00	-54.05	8.70	9.33	0.63	8.12	7.91	-1	-1.54	0.02
32	1	42.32	10.08	-32.24	52.39	0.01	-52.37	10.73	8.13	-2.60	8.15	8.01	-1	-1.62	-0.08
33	1	43.28	10.08	-33.20	52.75	0.00	-52.75	9.43	7.87	-1.56	8.15	8.03	-1	-1.59	-0.05
34	1	45.28	8.96	-36.32	52.62	0.01	-52.61	9.19	8.13	-1.06	8.27	8.09	-1	-1.45	-0.03
35	1	42.96	10.08	-32.88	52.63	0.00	-52.62	8.82	7.72	-1.10	8.12	7.99	-1	-1.60	-0.03
36	1	45.68	10.08	-35.60	53.58	0.02	-53.56	8.86	7.29	-1.57	8.11	7.99	-1	-1.50	-0.04
37	1	46.88	9.52	-37.36	56.44	0.01	-56.43	9.00	8.78	-0.22	8.10	8.08	-1	-1.51	-0.01
38	1	47.52	10.08	-37.44	55.48	0.01	-55.47	9.97	9.05	-0.92	8.19	8.03	-1	-1.48	-0.02
39	1	47.68	12.32	-35.36	56.20	0.00	-56.19	9.85	8.05	-1.80	8.15	8.03	-1	-1.59	-0.05
40	1	49.12	8.96	-40.16	56.19	0.00	-56.19	9.61	7.95	-1.66	8.14	8.04	-1	-1.40	-0.04
41	1	47.76	8.96	-38.80	56.19	0.00	-56.19	9.45	8.38	-1.07	8.08	7.94	-1	-1.45	-0.03
42	1	48.16	8.96	-39.20	55.24	0.00	-55.24	9.63	8.05	-1.58	8.14	7.95	-1	-1.41	-0.04
43	1	48.16	9.52	-38.64	55.24	0.00	-55.24	9.56	9.05	-0.51	8.12	7.97	-1	-1.43	-0.01
44	1	48.32	9.52	-38.80	55.24	0.00	-55.24	9.76	8.93	-0.83	8.13	8.06	-1	-1.42	-0.02
45	1	46.32	8.40	-37.92	55.01	0.00	-55.01	10.63	7.95	-2.68	8.43	8.32	-1	-1.45	-0.07
46	1	47.60	8.96	-38.64	60.24	0.00	-60.24	10.78	7.66	-3.12	8.28	7.99	-1	-1.56	-0.08
47	1	49.76	8.96	-40.80	59.05	0.00	-59.05	10.17	8.46	-1.71	8.17	7.99	-1	-1.45	-0.04
48	1	50.56	7.84	-42.72	59.53	0.00	-59.53	9.72	9.33	-0.39	8.08	8.01	-1	-1.39	-0.01
49	1	51.84	10.08	-41.76	63.58	0.00	-63.58	12.60	8.52	-4.08	8.10	7.97	-1	-1.52	-0.10
50	1	53.68	11.76	-41.92	61.67	0.00	-61.67	9.99	8.21	-1.78	8.12	7.91	-1	-1.47	-0.04

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 :	NO_2 :	NO_3
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	-1	-1.32	0.26
51	1	56.16	12.32	-43.84	64.05	0.07	-63.98	11.10	8.97	-2.13	8.02	7.78	-1	-1.46	-0.05
52	1	55.12	12.88	-42.24	62.17	0.00	-62.17	10.92	9.07	-1.85	8.04	7.88	-1	-1.47	-0.04
Average													-1	- 1.50	-0.04

Table A2 Stoichiometry of sludge S2 experiment during steady state condition

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 :	NO_2 :	NO_3
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	-1	-1.32	0.26
21	2	40.48	10.64	-29.84	46.91	0.00	-46.907	7.25	4.74	-2.51	8.05	7.52	-1	-1.57	-0.08
22	2	38.64	11.76	-26.88	47.74	0.00	-47.74	6.95	5.80	-1.15	8.03	7.58	-1	-1.78	-0.04
23	2	38.56	8.96	-29.60	47.98	0.00	-47.979	7.34	6.21	-1.13	8.02	7.50	-1	-1.62	-0.04
24	2	36.16	8.96	-27.20	46.91	0.00	-46.907	8.02	6.25	-1.77	7.97	7.47	-1	-1.72	-0.06
25	2	44.96	10.64	-34.32	54.89	0.00	-54.885	8.91	6.99	-1.92	7.89	7.50	-1	-1.60	-0.06
26	2	41.84	8.96	-32.88	50.01	0.00	-50.007	7.38	6.44	-0.94	8.09	7.68	-1	-1.52	-0.03
27	1	40.16	10.64	-29.52	49.17	0.00	-49.171	8.13	8.11	-0.02	7.96	7.76	-1	-1.67	-0.00
28	1	41.04	9.52	-31.52	48.22	0.00	-48.222	8.60	6.89	-1.71	8.11	7.62	-1	-1.53	-0.05
29	1	41.92	9.52	-32.40	49.29	0.00	-49.293	7.63	6.33	-1.30	7.93	7.54	-1	-1.52	-0.04
30	1	40.32	8.40	-31.92	49.53	0.00	-49.525	8.16	6.68	-1.48	7.92	7.66	-1	-1.55	-0.05
31	1	44.40	9.52	-34.88	54.05	0.00	-54.051	8.53	8.72	0.19	8.12	7.99	-1	-1.55	0.01
32	1	42.32	8.40	-33.92	52.39	0.00	-52.381	10.56	7.68	-2.88	8.17	7.89	-1	-1.54	-0.08
33	1	42.80	8.40	-34.40	52.74	0.00	-52.744	9.30	6.99	-2.31	8.12	7.83	-1	-1.53	-0.07
34	1	44.80	8.40	-36.40	52.62	0.00	-52.621	8.94	7.44	-1.50	8.22	7.82	-1	-1.45	-0.04
35	1	42.80	8.96	-33.84	52.62	0.00	-52.62	8.62	7.31	-1.31	8.04	7.92	-1	-1.55	-0.04
36	1	45.36	10.08	-35.28	53.58	0.01	-53.567	8.74	6.62	-2.12	8.09	7.97	-1	-1.52	-0.06

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 : -1	NO_2 : -1.32	NO_3 0.26
		influent	effluent		influent	effluent		influent	effluent		influent	effluent			
37	1	46.88	9.52	-37.36	56.44	0.01	-56.43	8.81	8.50	-0.31	8.09	8.05	-1	-1.51	-0.01
38	1	47.52	10.08	-37.44	55.48	0.01	-55.476	9.89	8.31	-1.58	8.19	7.95	-1	-1.48	-0.04
39	1	47.68	10.64	-37.04	56.19	0.00	-56.194	9.64	7.95	-1.69	8.13	7.99	-1	-1.52	-0.05
40	1	48.64	8.96	-39.68	56.19	0.00	-56.193	9.58	7.25	-2.33	8.13	7.95	-1	-1.42	-0.06
41	1	47.76	10.64	-37.12	56.19	0.00	-56.193	9.25	8.21	-1.04	8.05	7.83	-1	-1.51	-0.03
42	1	48.64	8.96	-39.68	55.24	0.00	-55.242	9.58	7.95	-1.63	8.11	7.80	-1	-1.39	-0.04
43	1	48.16	9.52	-38.64	55.24	0.00	-55.243	9.54	8.31	-1.23	8.08	7.85	-1	-1.43	-0.03
44	1	48.32	9.52	-38.80	55.24	0.00	-55.243	9.55	8.87	-0.68	8.10	7.98	-1	-1.42	-0.02
45	1	46.32	9.52	-36.80	55.01	0.00	-55.006	10.61	8.29	-2.32	8.41	8.01	-1	-1.49	-0.06
46	1	47.92	10.64	-37.28	60.24	0.00	-60.243	10.88	8.07	-2.81	8.20	7.90	-1	-1.62	-0.08
47	1	50.24	10.08	-40.16	59.05	0.00	-59.05	10.28	8.23	-2.05	8.14	7.90	-1	-1.47	-0.05
48	1	50.88	11.20	-39.68	59.53	0.00	-59.529	9.66	9.50	-0.16	8.05	7.95	-1	-1.50	-0.00
49	1	52.80	11.76	-41.04	63.58	0.00	-63.579	12.65	7.54	-5.11	8.09	7.88	-1	-1.55	-0.12
50	1	54.16	11.76	-42.40	61.67	0.00	-61.671	9.71	8.29	-1.42	8.09	7.92	-1	-1.45	-0.03
51	1	56.16	11.76	-44.40	64.05	0.00	-64.05	11.12	8.33	-2.79	8.02	7.70	-1	-1.44	-0.06
52	1	54.96	10.64	-44.32	62.15	0.00	-62.15	10.74	7.91	-2.83	8.02	7.90	-1	-1.40	-0.06
Average													-1	-1.53	-0.05

Table A3 Stoichiometry of sludge S3 experiment during steady state condition

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 : -1	NO_2 : -1.32	NO_3 0.26
		influent	effluent		influent	effluent		influent	effluent		influent	effluent			
27	3	44.72	10.08	-34.64	57.72	0.00	-57.72	7.58	9.56	1.98	7.93	7.50	-1	-1.67	0.06
28	3	38.08	7.28	-30.80	47.62	0.00	-47.62	8.86	8.74	-0.12	8.00	7.41	-1	-1.55	-0.00

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 :	NO_2 :	NO_3
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	-1	-1.32	0.26
29	3	38.48	7.28	-31.20	46.08	0.00	-46.08	8.98	7.80	-1.18	7.85	7.52	-1	-1.48	-0.04
30	2	37.68	6.72	-30.96	47.51	0.00	-47.51	8.69	9.17	0.48	7.99	7.56	-1	-1.53	0.02
31	2	37.92	5.04	-32.88	46.91	0.00	-46.91	8.16	7.38	-0.78	8.02	7.48	-1	-1.43	-0.02
32	2	37.04	6.16	-30.88	47.74	0.00	-47.74	7.71	7.33	-0.38	8.02	7.50	-1	-1.55	-0.01
33	2	36.96	6.16	-30.80	47.98	0.00	-47.98	7.77	7.82	0.05	7.99	7.40	-1	-1.56	0.00
34	2	35.36	6.16	-29.20	46.91	0.00	-46.91	8.48	7.42	-1.06	7.94	7.36	-1	-1.61	-0.04
35	2	44.16	8.40	-35.76	54.89	0.00	-54.88	9.24	7.50	-1.74	7.86	7.48	-1	-1.53	-0.05
36	2	41.20	7.84	-33.36	50.01	0.00	-50.01	7.53	7.60	0.07	8.09	7.54	-1	-1.50	0.00
37	1	39.84	8.40	-31.44	49.17	0.00	-49.17	8.46	8.84	0.38	7.92	7.69	-1	-1.56	0.01
38	1	40.40	8.96	-31.44	48.22	0.00	-48.22	8.81	7.25	-1.56	8.09	7.61	-1	-1.53	-0.05
39	1	41.76	7.84	-33.92	49.29	0.00	-49.29	7.74	6.95	-0.79	7.93	7.52	-1	-1.45	-0.02
40	1	39.84	7.84	-32.00	49.53	0.00	-49.52	8.34	7.25	-1.09	7.91	7.73	-1	-1.55	-0.03
41	1	44.24	7.84	-36.40	54.05	0.00	-54.05	8.69	9.64	0.95	8.14	7.87	-1	-1.48	0.03
42	1	41.84	7.84	-34.00	52.39	0.01	-52.37	10.82	7.87	-2.95	8.13	7.80	-1	-1.54	-0.09
43	1	42.64	7.84	-34.80	52.75	0.00	-52.75	9.36	7.91	-1.45	8.09	7.79	-1	-1.52	-0.04
44	1	44.64	8.40	-36.24	52.62	0.00	-52.62	9.20	8.44	-0.76	8.20	7.80	-1	-1.45	-0.02
45	1	42.80	8.96	-33.84	52.62	0.01	-52.62	8.90	7.93	-0.97	8.04	7.89	-1	-1.55	-0.03
46	1	45.36	9.52	-35.84	53.58	0.01	-53.57	8.92	7.78	-1.14	8.08	7.91	-1	-1.49	-0.03
47	1	46.72	8.96	-37.76	56.44	0.01	-56.43	9.14	9.52	0.38	8.07	7.91	-1	- 1.49	0.01
48	1	47.36	9.52	-37.84	55.48	0.01	-55.47	10.18	9.33	-0.85	8.15	7.90	-1	-1.47	-0.02
49	1	47.52	10.08	-37.44	56.20	0.00	-56.19	9.93	8.29	-1.64	8.11	8.00	-1	-1.50	-0.04
50	1	48.48	8.40	-40.08	56.19	0.00	-56.19	9.68	8.25	-1.43	8.14	7.97	-1	-1.40	-0.04
51	1	47.60	8.96	-38.64	56.19	0.00	-56.19	9.54	9.21	-0.33	8.06	7.89	-1	-1.45	-0.01
52	1	48.16	8.40	-39.76	55.24	0.00	-55.24	9.87	8.29	-1.58	8.13	7.85	-1	-1.39	-0.04
53	1	48.00	8.96	-39.04	55.24	0.00	-55.24	9.63	9.33	-0.30	8.09	7.86	-1	-1.42	-0.01

Cycle	Reaction Time (days)	NH_3-N (mg l^{-1})		ΔNH_3	NO_2-N (mg l^{-1})		ΔNO_2	NO_3-N (mg l^{-1})		ΔNO_3	pH		NH_3 :	NO_2 :	NO_3
		influent	effluent		influent	effluent		influent	effluent		influent	effluent	-1	-1.32	0.26
54	1	48.16	7.84	-40.32	55.24	0.00	-55.24	9.84	9.27	-0.57	8.10	8.00	-1	-1.37	-0.01
55	1	45.84	7.84	-38.00	55.01	0.00	-55.01	10.73	8.62	-2.11	8.41	7.93	-1	-1.45	-0.06
56	1	47.44	8.40	-39.04	60.24	0.00	-60.24	10.97	7.33	-3.64	8.17	7.82	-1	-1.54	-0.09
57	1	49.60	10.08	-39.52	59.05	0.00	-59.05	10.07	7.21	-2.86	8.12	7.79	-1	-1.49	-0.07
58	1	50.88	11.76	-39.12	59.53	0.00	-59.53	9.37	9.93	0.56	8.02	7.86	-1	-1.52	0.01
59	1	52.96	11.76	-41.20	63.58	0.00	-63.58	12.77	8.82	-3.95	8.06	7.82	-1	-1.54	-0.10
60	1	54.16	12.88	-41.28	61.67	0.00	-61.67	10.08	9.91	-0.17	8.08	7.79	-1	-1.49	-0.00
61	1	56.48	11.76	-44.72	64.05	0.00	-64.05	11.58	9.09	-2.49	7.98	7.68	-1	-1.43	-0.06
62	1	54.96	10.64	-44.32	62.15	0.00	-62.15	10.95	8.56	-2.39	8.02	7.80	-1	-1.40	-0.05
Average													-1	-1.50	-0.03

Appendix B

Nitrogen removal efficiency of sludge S1-S3

Table B1 Nitrogen removal efficiency of sludge S1

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
1	32.16	75.04	-133	50.80	120.00	-136	1.00	6.40	84
4	32.16	75.60	-135	50.80	126.00	-148	1.30	8.00	84
10	32.16	87.36	-172	50.40	124.00	-146	1.30	8.70	85
14	32.16	88.48	-175	51.00	115.00	-125	1.00	6.00	83
17	32.16	79.52	-147	51.03	110.00	-116	1.60	8.40	81
23	71.84	71.68	0	51.00	114.00	-124	1.30	9.20	86
26	60.16	54.88	9	50.80	108.00	-113	1.50	4.80	69
29	51.52	54.88	-7	51.03	119.06	-133	0.94	2.20	57
33	50.56	56.00	-11	51.03	144.77	-184	0.94	2.70	65
37	50.56	58.80	-16	51.03	98.77	-94	0.94	2.61	64
43	50.56	55.40	-10	51.03	77.06	-51	0.94	12.59	93
46	50.56	59.92	-19	51.03	72.39	-42	0.94	8.59	89
49	50.56	62.16	-23	51.03	67.24	-32	0.94	7.04	87
52	70.32	56.00	20	96.55	91.10	6	6.54	11.85	45
55	70.32	62.72	11	96.55	84.24	13	6.54	12.63	48
58	70.32	63.28	10	96.55	89.67	7	6.54	10.10	35
61	70.32	63.84	9	96.55	74.53	23	6.54	9.36	30

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
64	79.20	72.80	8	93.61	110.96	-19	7.34	0.16	-4488
67	79.20	71.12	10	93.61	81.24	13	7.34	0.08	-9075
70	79.20	75.60	5	93.61	52.96	43	7.34	1.10	-567
73	82.56	84.56	-2	91.94	67.96	26	2.45	1.10	-123
76	82.56	87.36	-6	91.94	44.10	52	2.45	0.29	-745
79	93.36	94.08	-1	77.82	67.39	13	2.86	5.80	51
82	96.96	80.64	17	90.02	66.96	26	8.33	5.39	-55
85	94.24	98.56	-5	106.21	69.39	35	3.05	3.76	19
88	67.36	57.68	14	82.83	58.90	29	5.80	8.64	33
91	54.08	52.64	3	61.19	53.94	12	7.91	5.54	-43
94	49.84	51.52	-3	64.10	51.01	20	7.15	6.33	-13
97	48.32	51.52	-7	63.27	51.94	18	8.71	6.13	-42
100	49.92	51.52	-3	62.58	51.01	18	7.35	5.87	-25
103	51.12	52.08	-2	63.62	52.54	17	6.61	7.31	10
106	50.08	52.64	-5	62.63	53.61	14	8.22	8.48	3
109	51.44	49.84	3	61.40	53.74	12	8.90	8.13	-9
111	49.84	50.96	-2	62.86	56.67	10	8.79	8.25	-7
113	50.56	49.28	3	63.10	56.21	11	7.90	7.89	0

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
115	49.68	48.72	2	63.80	57.61	10	7.85	8.25	5
117	49.12	43.68	11	64.44	53.01	18	8.04	8.89	10
119	46.08	41.44	10	62.05	44.47	28	8.78	8.70	-1
121	54.24	39.20	28	67.59	39.47	42	9.61	10.07	5
123	50.00	12.32	75	61.28	0.01	100	8.26	7.52	-10
124	41.12	18.48	55	49.17	3.85	92	8.43	8.40	0
125	43.28	10.08	77	49.32	0.00	100	8.69	6.87	-26
126	42.08	9.52	77	49.29	0.00	100	7.63	6.48	-18
127	40.32	9.52	76	49.53	0.01	100	8.20	7.29	-13
128	44.72	9.52	79	54.05	0.00	100	8.70	9.33	7
129	42.32	10.08	76	52.39	0.01	100	10.73	8.13	-32
130	43.28	10.08	77	52.75	0.00	100	9.43	7.87	-20
131	45.28	8.96	80	52.62	0.01	100	9.19	8.13	-13
132	42.96	10.08	77	52.63	0.00	100	8.82	7.72	-14
133	45.68	10.08	78	53.58	0.02	100	8.86	7.29	-21
134	46.88	9.52	80	56.44	0.01	100	9.00	8.78	-2
135	47.52	10.08	79	55.48	0.01	100	9.97	9.05	-10
136	47.68	12.32	74	56.20	0.00	100	9.85	8.05	-22

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
137	49.12	8.96	82	56.19	0.00	100	9.61	7.95	-21
138	47.76	8.96	81	56.19	0.00	100	9.45	8.38	-13
139	48.16	8.96	81	55.24	0.00	100	9.63	8.05	-20
140	48.16	9.52	80	55.24	0.00	100	9.56	9.05	-6
141	48.32	9.52	80	55.24	0.00	100	9.76	8.93	-9
142	46.32	8.40	82	55.01	0.00	100	10.63	7.95	-34
143	47.60	8.96	81	60.24	0.00	100	10.78	7.66	-41
144	49.76	8.96	82	59.05	0.00	100	10.17	8.46	-20
145	50.56	7.84	84	59.53	0.00	100	9.72	9.33	-4
146	51.84	10.08	81	63.58	0.00	100	12.60	8.52	-48
147	53.68	11.76	78	61.67	0.00	100	9.99	8.21	-22
148	56.16	12.32	78	64.05	0.07	100	11.10	8.97	-24
149	55.12	12.88	77	62.17	0.00	100	10.92	9.07	-20

Table B2 Nitrogen removal efficiency of sludge S2

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
1	32.16	75.04	-133	51.03	121.00	-137	2.60	18.00	86
4	32.16	66.08	-105	50.80	120.00	-136	2.00	17.00	88

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
10	32.16	69.44	-116	50.80	129.00	-154	1.80	17.90	90
14	32.16	87.36	-172	51.00	117.00	-129	2.40	20.40	88
17	32.16	72.80	-126	51.03	112.00	-119	2.10	21.00	90
23	68.00	68.32	0	51.03	115.00	-125	3.00	18.50	84
26	58.24	45.92	21	51.90	108.00	-108	2.80	23.00	88
29	46.40	44.80	3	51.03	119.06	-133	0.94	2.20	57
33	44.80	51.52	-15	51.03	99.91	-96	0.94	14.04	93
37	44.80	49.84	-11	51.03	44.77	12	0.94	31.59	97
43	44.80	48.72	-9	51.03	14.77	71	0.94	34.86	97
46	44.80	48.16	-8	51.03	6.67	87	0.94	74.04	99
49	44.80	50.40	-13	51.03	2.10	96	0.94	64.24	99
52	63.60	53.76	15	59.33	47.96	19	39.23	27.51	-43
55	63.60	53.20	16	59.33	29.53	50	39.23	59.35	34
58	63.60	53.20	16	59.33	13.81	77	39.23	46.29	15
61	63.60	53.20	16	59.33	1.53	97	39.23	60.57	35
64	73.12	64.40	12	51.90	56.24	-8	17.71	38.53	54
67	73.12	62.72	14	51.90	39.10	25	17.71	39.35	55
70	73.12	61.60	16	51.90	24.10	54	17.71	49.14	64

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
73	74.56	59.92	20	75.45	65.67	13	29.90	34.45	13
76	74.56	75.60	-1	75.45	51.67	32	29.90	31.18	4
79	86.64	84.00	3	82.14	77.67	5	20.51	32.94	38
82	91.20	77.28	15	95.90	65.67	32	23.84	21.10	-13
85	93.28	59.36	36	105.84	47.96	55	7.54	1.31	-476
88	56.16	50.96	9	76.70	37.11	52	5.10	5.58	9
91	52.16	52.08	0	54.96	30.94	44	7.04	4.31	-63
94	49.68	50.96	-3	57.53	27.61	52	6.80	5.52	-23
97	48.16	50.40	-5	56.58	26.11	54	8.48	6.40	-32
100	49.60	47.04	5	55.20	20.21	63	7.43	5.33	-39
103	49.84	45.36	9	54.82	15.77	71	6.46	5.80	-11
106	48.16	31.36	35	52.13	3.17	94	7.79	5.99	-30
109	45.36	20.16	56	46.99	0.00	100	8.19	5.46	-50
111	41.36	15.68	62	47.51	0.00	100	8.02	5.99	-34
113	40.48	10.64	74	46.91	0.00	100	7.25	4.74	-53
115	38.64	11.76	70	47.74	0.00	100	6.95	5.80	-20
117	38.56	8.96	77	47.98	0.00	100	7.34	6.21	-18
119	36.16	8.96	75	46.91	0.00	100	8.02	6.25	-28

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
121	44.96	10.64	76	54.89	0.00	100	8.91	6.99	-27
123	41.84	8.96	79	50.01	0.00	100	7.38	6.44	-15
124	40.16	10.64	74	49.17	0.00	100	8.13	8.11	0
125	41.04	9.52	77	48.22	0.00	100	8.60	6.89	-25
126	41.92	9.52	77	49.29	0.00	100	7.63	6.33	-21
127	40.32	8.40	79	49.53	0.00	100	8.16	6.68	-22
128	44.40	9.52	79	54.05	0.00	100	8.53	8.72	2
129	42.32	8.40	80	52.39	0.00	100	10.56	7.68	-37
130	42.80	8.40	80	52.74	0.00	100	9.30	6.99	-33
131	44.80	8.40	81	52.62	0.00	100	8.94	7.44	-20
132	42.80	8.96	79	52.62	0.00	100	8.62	7.31	-18
133	45.36	10.08	78	53.58	0.01	100	8.74	6.62	-32
134	46.88	9.52	80	56.44	0.01	100	8.81	8.50	-4
135	47.52	10.08	79	55.48	0.01	100	9.89	8.31	-19
136	47.68	10.64	78	56.19	0.00	100	9.64	7.95	-21
137	48.64	8.96	82	56.19	0.00	100	9.58	7.25	-32
138	47.76	10.64	78	56.19	0.00	100	9.25	8.21	-13
139	48.64	8.96	82	55.24	0.00	100	9.58	7.95	-21

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
140	48.16	9.52	80	55.24	0.00	100	9.54	8.31	-15
141	48.32	9.52	80	55.24	0.00	100	9.55	8.87	-8
142	46.32	9.52	79	55.01	0.00	100	10.61	8.29	-28
143	47.92	10.64	78	60.24	0.00	100	10.88	8.07	-35
144	50.24	10.08	80	59.05	0.00	100	10.28	8.23	-25
145	50.88	11.20	78	59.53	0.00	100	9.66	9.50	-2
146	52.80	11.76	78	63.58	0.00	100	12.65	7.54	-68
147	54.16	11.76	78	61.67	0.00	100	9.71	8.29	-17
148	56.16	11.76	79	64.05	0.00	100	11.12	8.33	-33
149	54.96	10.64	81	62.15	0.00	100	10.74	7.91	-36

Table B3 Nitrogen removal efficiency of sludge S3

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
1	32.16	75.04	-133	54.00	118.00	-119	1.50	4.80	69
4	32.16	66.64	-107	53.50	125.00	-134	1.30	7.20	82
10	32.16	73.92	-130	57.28	122.00	-113	1.20	8.00	85
14	32.16	86.24	-168	57.28	115.00	-101	1.50	6.50	77
17	32.16	1.12	97	51.03	115.00	-125	1.20	7.80	85

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
23	27.04	6.72	75	51.03	112.00	-119	1.30	8.00	84
26	23.04	3.36	85	57.28	110.00	-92	1.50	3.20	53
29	22.08	12.32	44	51.03	119.06	-133	0.94	2.20	57
33	26.24	20.16	23	51.03	0.02	100	0.94	79.76	99
37	37.92	3.92	90	57.28	0.00	100	47.75	112.00	57
43	37.76	2.80	93	52.37	0.00	100	66.52	89.14	25
46	24.16	5.04	79	66.94	0.22	100	53.11	116.49	54
49	29.28	2.24	92	51.15	0.01	100	66.70	105.88	37
52	36.08	24.64	32	58.13	67.81	-17	63.02	45.47	-39
55	41.92	26.32	37	96.02	56.10	42	28.68	66.29	57
58	37.60	34.72	8	103.49	92.39	11	43.20	31.18	-39
61	46.24	30.80	33	109.94	129.10	-17	22.78	29.14	22
64	60.32	48.72	19	124.80	125.24	0	18.64	20.16	8
67	70.56	60.48	14	122.47	121.81	1	12.64	3.43	-269
70	75.36	58.80	22	122.59	116.10	5	2.90	20.57	86
73	72.96	71.12	3	128.02	116.10	9	13.57	21.80	38
76	78.56	65.52	17	135.73	120.53	11	19.52	18.50	-6
79	80.88	75.04	7	121.49	112.39	7	13.27	23.14	43

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
82	86.08	69.44	19	115.74	96.67	16	18.24	16.61	-10
85	91.04	81.76	10	114.70	91.24	20	6.26	16.82	63
88	62.56	50.96	19	89.07	65.53	26	9.53	16.97	44
91	52.16	46.48	11	63.08	56.01	11	10.29	10.87	5
94	48.08	43.68	9	64.70	50.81	21	8.68	11.19	22
97	46.08	40.32	13	63.21	45.61	28	10.10	11.05	9
100	46.72	29.12	38	60.77	30.34	50	8.76	9.25	5
103	44.72	10.08	77	57.72	0.00	100	7.58	9.56	21
106	38.08	7.28	81	47.62	0.00	100	8.86	8.74	-1
109	38.48	7.28	81	46.08	0.00	100	8.98	7.80	-15
111	37.68	6.72	82	47.51	0.00	100	8.69	9.17	5
113	37.92	5.04	87	46.91	0.00	100	8.16	7.38	-11
115	37.04	6.16	83	47.74	0.00	100	7.71	7.33	-5
117	36.96	6.16	83	47.98	0.00	100	7.77	7.82	1
119	35.36	6.16	83	46.91	0.00	100	8.48	7.42	-14
121	44.16	8.40	81	54.89	0.00	100	9.24	7.50	-23
123	41.20	7.84	81	50.01	0.00	100	7.53	7.60	1
124	39.84	8.40	79	49.17	0.00	100	8.46	8.84	4

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
125	40.40	8.96	78	48.22	0.00	100	8.81	7.25	-22
126	41.76	7.84	81	49.29	0.00	100	7.74	6.95	-11
127	39.84	7.84	80	49.53	0.00	100	8.34	7.25	-15
128	44.24	7.84	82	54.05	0.00	100	8.69	9.64	10
129	41.84	7.84	81	52.39	0.01	100	10.82	7.87	-37
130	42.64	7.84	82	52.75	0.00	100	9.36	7.91	-18
131	44.64	8.40	81	52.62	0.00	100	9.20	8.44	-9
132	42.80	8.96	79	52.62	0.01	100	8.90	7.93	-12
133	45.36	9.52	79	53.58	0.01	100	8.92	7.78	-15
134	46.72	8.96	81	56.44	0.01	100	9.14	9.52	4
135	47.36	9.52	80	55.48	0.01	100	10.18	9.33	-9
136	47.52	10.08	79	56.20	0.00	100	9.93	8.29	-20
137	48.48	8.40	83	56.19	0.00	100	9.68	8.25	-17
138	47.60	8.96	81	56.19	0.00	100	9.54	9.21	-4
139	48.16	8.40	83	55.24	0.00	100	9.87	8.29	-19
140	48.00	8.96	81	55.24	0.00	100	9.63	9.33	-3
141	48.16	7.84	84	55.24	0.00	100	9.84	9.27	-6
142	45.84	7.84	83	55.01	0.00	100	10.73	8.62	-24

Date	Influent NH_4^+	Effluent NH_4^+	% Rem	Influent NO_2^-	Effluent NO_2^-	% Rem	Influent NO_3^-	Effluent NO_3^-	% Produce
143	47.44	8.40	82	60.24	0.00	100	10.97	7.33	-50
144	49.60	10.08	80	59.05	0.00	100	10.07	7.21	-40
145	50.88	11.76	77	59.53	0.00	100	9.37	9.93	6
146	52.96	11.76	78	63.58	0.00	100	12.77	8.82	-45
147	54.16	12.88	76	61.67	0.00	100	10.08	9.91	-2
148	56.48	11.76	79	64.05	0.00	100	11.58	9.09	-27
149	54.96	10.64	81	62.15	0.00	100	10.95	8.56	-28

Appendix C

NH_4^+-N and NO_2^--N consumption

Table C1 NH_4^+-N and NO_2^--N consumed at different sludge quantity

	NH_4^+ removal rate				
Reaction time	500	1000	1500	2000	2500
7	0.04	0.05	0.06	0.05	0.07
24	0.02	0.04	0.03	0.03	0.02
48	0.02	0.02	0.02	0.01	0.01
72	0.03	0.02	0.01	0.01	0.01
	NO_2^- removal rate				
Reaction time	500	1000	1500	2000	2500
7	0.05	0.06	0.08	0.08	0.10
24	0.02	0.05	0.05	0.04	0.03
48	0.02	0.03	0.02	0.02	0.01
72	0.03	0.02	0.02	0.01	0.01

Removal rate = Substrate consumed . (sludge conc. Time)$^{-1}$

Appendix D

Inhibition term of COD

Inhibition term of COD for a concurrent processes of Anammox and denitrification

I. The Analogy of enzyme kinetics with Monod equation (Orhorn et al., 1994)

$$E + S \underset{k2}{\overset{k1}{\rightleftharpoons}} ES \xrightarrow{k3} E + P \qquad (1)$$

where S = concentration of substrate
E = concentration of free enzyme
ES = concentration of enzyme – substrate complex
P = concentration of products

The rate of formation of the enzyme – substrate complex is expressed as:

$$\frac{d}{dt} ES = k_1 E.S - (k_2 + k_3) ES \qquad (2)$$

The equilibrium condition is the simplify assumption,

thus the term $\frac{d}{dt} ES$ is zero. The above equation becomes:

$$E = \frac{k_2 + k_3}{k_1} . \frac{ES}{S} \qquad (3)$$

The mass balance of enzyme species yield:

$$E = E_0 - ES \qquad (4)$$

where; E_0 = initial free enzyme concentration

Substitute E in equation (3) to equation (4) it yields

$$ES = E_0 \frac{S}{K_s + S} \qquad (5)$$

where; $K_s = \frac{k_2 + k_3}{k_1}$

The Monod equation is expressed as:

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (6)$$

II. Expression for non competition inhibition

In non competition inhibition, the magnitude of inhibition is not depending on the concentration of S or ES. The mass balance equation for the non competition inhibition is expressed as:

$$E_0' = E_0 - EI \quad (7)$$

where E_0' = initial free enzyme concentration that is available for substrate S

EI = enzyme – inhibition complex

The reaction of inhibition of inhibitor (I) is expressed as:

$$E_0' + I = EI \quad (8)$$

E_0' is available enzyme for substrate, the inhibitor will combine with E_0' to form inhibitor enzyme complex.

The constant of equation (8) is expressed as:

$$K_I = \frac{E_0' . I}{EI} \quad (9)$$

Combine equation (7) and (8), then:

$$EI = \frac{E_0 . I}{K_I + I} \quad (10)$$

In the case of non competitive inhibition, equation (4) can be expressed as:

$$E = E_0' - ES \quad (11)$$

Equation (7) and (1) can be expressed as:

$$E_0' = E_0 - EI = E + ES \quad (12)$$

From equation (10), (3), and (12);

$$EI = \frac{E_0 . I}{K_I + I}$$

$$E = K_s . \frac{ES}{S} \quad \text{where } K_s = \frac{k_2 + k_3}{k_1} .$$

$$E_0' = E_0 - EI = E + ES$$

$$ES = E_0 - EI - E$$

$$ES = E_0 - \left\{ \frac{E_0 . I}{K_I + I} \right\} - \frac{K_s \ ES}{S}$$

$$ES + \frac{K_s \ ES}{S} = E_0 - \left\{ \frac{E_0 . I}{K_I + I} \right\}$$

$$ES \left(1 + \frac{K_s}{S}\right) = E_0 \left\{ 1 - \frac{I}{K_I + I} \right\}$$

$$ES \frac{(S + K_s)}{S} = E_0 \left\{ \frac{K_I + I - I}{K_I + I} \right\}$$

$$ES = E_0 \left\{ \frac{K_I}{K_I + I} \right\} \left\{ \frac{S}{Ks + S} \right\} \qquad (13)$$

The system will behave as if it contained less enzyme in the presence of a non competitive inhibitor, thus the equation will apparently reduced to a lower level;

$$ES = E_0 \left\{ \frac{K_I}{K_I + I} \right\} \qquad (14)$$

or $$\mu = \mu_m \left\{ \frac{Ks}{Ks + S} \right\}$$

where $K_s = K_I$

$S = I$