



UNIVERSITAT DE
BARCELONA

MASTER FINAL PROJECT
MASTER OF ENVIRONMENTAL ENGINEERING

**Start-Up of One-Step Partial
Nitritation/Anammox Moving Bed Biofilm
Reactor to Treat Municipal-Like Wastewater**

Author

Yen Keong Cheah

June 2016

Director/s

Dr. Isaac Fernández Rodríguez
*Department of Chemical Engineering
University of Barcelona*

Title: **Start-Up of One-Step Partial Nitritation/Anammox Moving Bed Biofilm Reactor to Treat Municipal-Like Wastewater**

Student: Yen Keong Cheah

Date: June 2016

Supervisor/s: Dr. Isaac Fernández Rodríguez
Department of Chemical Engineering

ABSTRACT:

The application of partial nitritation/anammox process to remove nitrogen from wastewater is a cost effective and sustainable approach since it can save energy and resources. It was applied successfully in treating ammonium rich waste streams (Wett, 2007). This is worth to use deammonification (partial nitritation/anammox) process in sewage treatment to create an energy positive environment and therefore, this has been studied extensively for last few years to investigate its applicability in mainstream condition where both temperature (10-20 °C) and nitrogen concentration (<100 mg N/L) are very low. Systems based on Anammox can be of great help to comply with stricter wastewater discharge regulations and reduce environmental problems caused by nutrients discharges (e.g. eutrophication).

In this regard, a study of one-step Partial Nitritation/Anammox process was carried out in a moving bed biofilm reactor by using municipal-like wastewater with the aim to conduct the start-up of Partial Nitritation (PN) process in the MBBR and then stabilize it, subsequently perform the Anammox process in the same MBBR system. Finally, optimise the PN/Anammox MBBR to have higher BNR rate.

PN/Anammox process was successfully tested in a 4.5 L lab-scale MBBR for 141 days at 20 °C, with about 20% filled with Kaldnes K1 carriers. The feeding was prepared and calculated to have 50 mg NH₄⁺/L, the source of the wastewater was from the rejected water of anaerobic digester from a municipal wastewater treatment plant.

Several changes were made to achieve our objectives step by step. The analysis shows that the efficiency of NH₄⁺ removal has reached a maximum of 95% while the maximum overall percentage of nitrogen removal of 32.9% after the addition of Anammox bacteria to the reactor.

Key words: Anammox, One-Step Partial Nitritation/Anammox, Moving Bed Biofilm Reactor, Low Temperature, Municipal-Like

TABLE OF CONTENTS

ABSTRACT	i
TABLE OF CONTENTS	ii
1.0 INTRODUCTION	1
1.1 Conventional Biological Nitrogen Removal (BNR)	1
1.2 Moving Bed Biofilm Reactor	2
1.3 Kaldnes K1 Ring	3
1.4 Intermittent Aeration	4
1.5 Partial Nitrification/Anammox Process	5
1.6 Applications of PN/Anammox	8
2.0 OBJECTIVE	9
2.1 Scope of Study	9
3.0 MATERIALS AND METHODOLOGY	9
3.1 Influent	9
3.2 Operational Setup	10
3.3 Analytical Methods	11
3.3.1 Nitrogen Species	11
3.3.2 pH	12
3.3.3 Temperature and Dissolved Oxygen	12
3.3.4 Chemical Oxygen Demand	13
3.3.5 Total Suspended Solids	13
3.3.6 Conductivity	13
3.3.7 Flow rate	14
4.0 RESULTS AND DISCUSSIONS	14
5.0 CONCLUSIONS	23
6.0 NOMENCLATURE	25
7.0 BIBLIOGRAPHY	26

1.0 INTRODUCTION

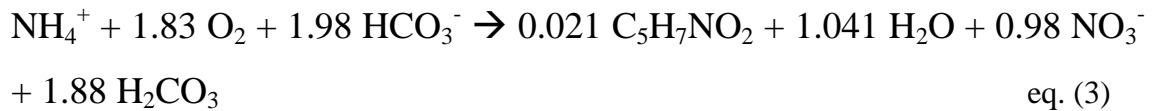
1.1 Conventional Biological Nitrogen Removal (BNR)

The removal of ammonia from wastewater has become a worldwide emerging concern because ammonia is toxic to aquatic species and causes eutrophication in natural water environments (Tchobanoglous et al. 2003). Aquatic life may suffer a loss of equilibrium, hyper excitability, increased respiratory activity and oxygen uptake, and increased heart rate. At extreme ammonia levels, aquatic life, like fish, may experience convulsions, coma, and death. Experiments have shown that the lethal concentration for a variety of fish species ranges from 0.2 to 2.0 mg/L (Oram, 2014). Nitrogen compounds in wastewater are usually removed by biological approaches mainly because of the cost and efficiency (EPA 1993; Zhu et al. 2007a, b). Based on the microbial nitrogen cycle and the metabolism of inorganic nitrogen compounds, many biological technologies and processes have been developed and implemented for nitrogen removal from wastewater, such as pre-denitrification (Anoxic/Oxic), modified Bardenpho, Bio-denitro, sequencing batch reactor (SBR), oxidation ditch (OD), step feeding, anaerobic/anoxic/aerobic (A²/O), and University of Cape Town (UCT) processes (Wentzel et al. 1992; Østgaard et al. 1997; Williams and Beresford 1998; Tchobanoglous et al. 2003; Pai et al. 2004). These processes have been widely employed in wastewater treatment plants for nitrification and denitrification (EPA 1993).

Nitrification occurs by oxidizing ammonium to nitrate. Nitrite is formed as an intermediate in this reaction. Two different groups of microorganism, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), are responsible for nitrification (Sultana 2014). AOB will convert ammonia to nitrite (eq. 1) followed by the oxidation of nitrite to nitrate by the NOB (eq. 2).

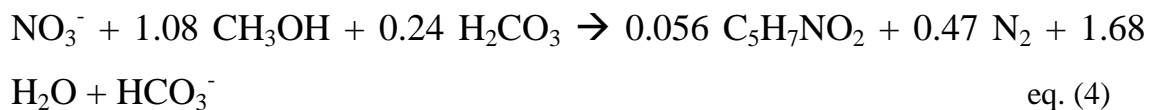


Based on the growth yields for ammonium oxidizers and nitrite oxidizers, the reaction can be written as:



The above equation shows that 4.18 g oxygen is required to oxidize per gram ammonium-nitrogen (Lin et al., 2009).

Biological conversion of nitrate to nitrite and nitrogen gas occurs in denitrification process. In this process nitrate and nitrite are electron acceptors and a biodegradable carbon source acts as electron donor. Hence, denitrification is an anaerobic reaction. The process involves the transfer of electrons from carbon substrate to nitrate and nitrite. Normally, methanol will be added as the external carbon source for this process when there is not enough biodegradable organic matter in the wastewater. The overall process can be expressed in the following equation:



1.2 Moving Bed Biofilm Reactor

For biological treatment of water, there are many different biofilm systems in use, such as trickling filters, rotating biological contactors (RBC), fixed media submerged biofilters, granular media biofilters, fluidised bed reactors, etc. After the development of moving bed biofilm reactor (MBBR) process in the late 1980s and early 1990s, it has been a commercial success. There are presently more than 400 large-scale wastewater treatment plants based on this process in operation in 22 different countries all over the world (Bjorn, 2005), including pulp and paper industry waste (Jahren et al. 2002), poultry processing wastewater (Rusten et al 1998a), cheese factory wastes (Rusten et al. 1996), refinery and slaughter house waste (Johnson et al. 2000), phenolic wastewater (Hosseini et al. 2005), dairy wastewater (Andreottola et al. 2002) and municipal wastewater (Andreottola et al. 2000a,b, 2003; Rusten et al. 1994, 1995a,b, 1997, 1998). Moreover, sequencing batch operation of MBBR has been attempted for biological phosphorus removal (Pastorelli et al. 1999; Helness et al. 1999). In addition, hundreds of small, on-site treatment units have been used based on the MBBR, in which most of

these are in Germany. There are several reasons for the fact that biofilm processes more and more often are being favoured instead of activated sludge processes, such as:

- a. The treatment plant requires less space
- b. The final treatment result is less dependent on biomass separation since the biomass concentration to be separated is at least 10 times lower
- c. The attached biomass becomes more specialised (higher concentration of relevant organisms) at a given point in the process train, because there is no sludge return

The idea of the development of the moving bed biofilm process is very clear: to adopt the best from both the activated sludge process and the biofilter processes without including the worst. Contrary to most biofilm reactors, the moving bed biofilm reactor utilises the whole tank volume for biomass growth, as does also the activated sludge reactor. Unlike the activated sludge reactor, it does not need any sludge recycle, which is the same as the case in other biofilm reactors. The biomass was allowed to grow inside the carriers which are freely to move around the whole reactor while the carriers are prevented to move out from the reactor by having a sieve in the outlet. Therefore, the MBBR is a well-combination of both activated sludge reactor and biofilm reactors and could be applied for aerobic, anoxic or anaerobic processes.

In aerobic processes, the carrier's movement is caused by the air circulation from the aeration pump; while in anoxic and anaerobic processes, a mixer/stirrer keeps the carriers moving. In the aerobic reactors, a special coarse bubble aeration system has been developed.

1.3 Kaldnes K1 Ring

The biofilm carrier Kaldnes K1 ring is made of high density polyethylene (density $0,95 \text{ g/cm}^3$) and shaped as a small cylinder with a cross on the inside of the cylinder and "fins" on the outside (see figure 1). The cylinder has a length of 7 mm, and a diameter of 10 mm (not including fins). Lately a larger carrier (K2) was introduced with similar shape (length and diameter about 15 mm), intended to be used in plants with coarse inlet sieves.



Figure 1: Kaldnes K1 carriers

One of the important advantages of the moving bed biofilm reactor is that the filling of carrier in the reactor may be subject to preferences. The standard filling degree is 67%, resulting in a total, specific carrier area of $465 \text{ m}^2/\text{m}^3$. Since the biomass is growing primarily on the inside of the carrier, therefore the effective specific surface area of $335 \text{ m}^2/\text{m}^3$ for the K1 carrier and $235 \text{ m}^2/\text{m}^3$ for the K2 carrier, at 67% filling. In order to be able to move the carrier suspension freely, it is recommended that filling degrees should be below 70% (corresponding to $350 \text{ m}^2/\text{m}^3$ effective specific area for K1).

The rate expression normally used in biofilm processes is based on biofilm carrier area ($\text{g}/\text{m}^2\text{d}$). Because of some uncertainty with respect to how much of the available carrier area that is in fact covered by biofilm and because of easy rate comparison with other biofilters, the volumetric rates ($\text{g}/\text{m}^3_{\text{reactor volume}}\text{d}$) have been used earlier for the moving bed reactor. It has been demonstrated, however, that the biofilm area is the key parameter and therefore the design of the process is most correctly based on effective carrier area ($\text{g}/\text{m}^2_{\text{carrier area}}\text{d}$) (Ø degaard et al, 1998).

1.4 Intermittent Aeration

Intermittent aeration is one of the aeration strategies used in full-scale application of the one-stage partial nitrification/anammox process in moving bed biofilm reactors (MBBR) (Plaza et al., 2011; Rosenwinkel & Cornelius, 2005). Aeration of sequencing batch reactors (SBR) can also be considered intermittent due to the intermittent oxygen

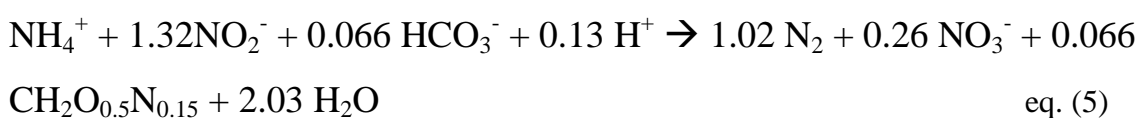
supply (Vazquez-Padin et al., 2009; Winkler et al., 2012), and can be applied to improve process efficiency and maintain anammox bacteria inside the system. In treating ammonium-rich wastewater with a low content of organic matter, anammox bacteria can avoid permanent inhibition by oxygen due to non-aerated phases offered by intermittent aeration, apart from being protected from the outer layer of the biofilm. This also provides better conditions for anammox bacteria to become one of the dominant groups of microorganisms in the system. In addition, the non-aerated phases limit nitrite oxidizers and prevent nitrite oxidising to nitrate.

In this study, since the consumption of oxygen by the bacteria is not very high and the concentration of dissolved oxygen (DO) in the reactor is maintained below 1.0 mg DO/L, therefore this aeration method is suitable to be applied in this case.

1.5 Partial Nitrification/Anammox Process

In the past decades, many researchers have put a lot of efforts in a potential engineering application, known as anaerobic ammonium oxidation (Anammox). This was the result of the intensive energy cost in the combination of nitrification/denitrification (N/DN) process, on the other hand, this autotrophic process allows over 50% of the oxygen to be saved and no organic carbon source is needed (Fux et al 2001). Theoretically, the anammox bacteria will convert nitrite to nitrogen gas directly without passing through the nitrate formation, that's why it will be able to save almost 50% of oxygen in the aeration. As a result, a new combination of oxidation of ammonium to nitrite followed by anammox process was created, it's now known as partial nitrification/anammox (PN/Anammox) process.

The anammox process bypasses the biological nitrification denitrification and converts ammonium (NH_4^+) to dinitrogen gas by using NO_2^- as electron acceptor, which has been produced previously through the partial nitrification, as mentioned in the eq. (1). The stoichiometric equation for anammox process is as follows (Strous et al., 1998):



PN/Anammox has been one of the most innovative developments in biological wastewater treatment in recent years. With its discovery in the 1990s a completely new way of ammonium removal from wastewater became available. Over the past decade many technologies have been developed and studied for their applicability to the PN/Anammox concept and several have made it into full-scale (Lackner 2014). Lackner (2014) also reported in her study about the steady growth in the number of new plants over the past years and it's estimated to have more than 100 operating installations worldwide by 2014. So far, among these 100 full-scale installations, they aim to treat the high-ammonium concentration in the side stream of municipal treatment plants or industrial effluents, but it has rarely been reported any mainstream BNR from municipal wastewater by using Anammox (Xu et al 2015). In the review of Xu (2015), they concluded there are 3 main challenges to be overcome before having any mainstream Anammox process are:

- a. High chemical oxygen demand to nitrogen (COD/N) ratio leading to denitrifiers outcompeting Anammox bacteria
- b. Numerous difficulties in selective retention of ammonia oxidizing bacteria (AOB) over nitrite-oxidizing bacteria (NOB)
- c. Insufficient accumulation of Anammox bacteria

Municipal wastewater is a potential source of chemical energy in form of organic carbon (Frijns et al. 2013). Besides, the COD/N ratio of this type of wastewater (around 10-12 (Tchobanoglous et al. 2004)) is usually significantly higher than the optimum desirable for a PN/Anammox treatment (<2-5, according to Lackner et al. (2008), or even <0.5, according to Daigger (2014)). Firstly, heterotrophs grow on biodegradable COD and compete with ammonium oxidizing bacteria (AOB) for dissolved oxygen and with Anammox for nitrite (heterotrophic denitrifiers) (Xu et al. 2015; Jenni et al. 2014). Secondly, if heterotrophs are growing, the production of sludge can increase and its physical characteristics can change, decreasing in that case the retention of biomass in the system (Jenni et al. 2014). Finally, some specific biodegradable organic compounds may be inhibitory for AOB (Gujer 2010) or Anammox biomass (Jin et al. 2012). Regarding the outcompetition phenomena, Jenni et al. (2014) have reported that the key factor for the successful operation of the process at moderate COD/N ratios (1.4 g COD/g N) is maintaining the appropriate Sludge Retention Time (SRT).

The effective selection and growth of the AOB, outcompeting the NOB, in order to obtain the oxidation to NO_2^- of about 50% of the NH_4^+ , can be much more difficult when treating these types of wastewaters (Xu et al. 2015). Two of the selection driving forces commonly used are based on high ammonium concentration (i.e. NOB selective inhibition by free ammonia (Liang et al. 2007) and on the wash-out of NOB due to the faster growth kinetics of AOB at the mesophyll range of temperature (e.g. SHARON process (Hellenga et al. 1998)). In this case, however, the wastewaters to be treated will be at ambient temperature, which, unless in hot/tropical climates, will be significantly lower than the mesophilic temperature range. In addition, the low ammonium concentration, usually around or under 50 mg/L (Gao et al. 2014), will make the inhibition by free ammonia virtually negligible (Xu et al. 2015).

In absence of inhibition factors to select AOB and wash-out NOB, the population selection in the PN step will have to rely on fine-tuning the concentrations of the involved species, i.e. oxygen and nitrogen species (Regmi et al. 2014) and, eventually, on the use of biofilms (de Clippeleir et al. 2013). The use of limiting dissolved oxygen (DO) concentrations to maintain stable conversion of ammonium to nitrite, based on oxygen affinity differences between AOB and NOB, is still a controversial matter. The main reason is that there is a wide range of oxygen affinity constants reported in the literature (Vannecke et al. 2015) due to the diversity of populations of AOB and NOB and also due to the different conditions of the experiments. Therefore, while some authors recommend the operation at limiting DO concentrations to suppress NOB (Pérez et al. 2014), others on the contrary propose the operation at non-limiting conditions (Regmi et al. 2014; de Clippeleir et al. 2013).

In addition to the challenges discussed above, retention of Anammox bacteria is another challenge. Anammox bacteria grow extremely slow with a doubling time of about 11 days in a lab-scale experiment (Strous et al. 1998) and 25 days at temperature below 20 °C (Hendrickx et al. 2012). Thus, a long SRT is essential for retention of Anammox biomass in the mainstream deammonification process, especially at temperatures below 20 °C (Hendrickx et al. 2012; Lotti et al. 2014). Granular sludge and biofilm have been suggested for effective retention of Anammox biomass (Fernández et al. 2008).

For these reasons, a MBBR with Kaldnes K1 carriers is used to overcome the problems by extending the SRT, Anammox bacteria will stay in the inner area inside the carriers to avoid being washed-out easily from the reactor. Other than that, by lowering the concentration of DO, it is expected that an aerobic condition can be created at the outer layer of bacteria on the carriers while an anoxic condition will occur at the inner layer of biofilm on the carrier, where the Anammox is located. So, several solutions are provided to overcome these limitations.

1.6 Application of PN/Anammox

The deammonification process combining partial nitrification and anaerobic ammonium oxidation has been considered as a viable option for energy-efficient used water treatment. In general, the deammonification process can be classified as sidestream for high-ammonia used water (e.g., anaerobic digester liquor) and mainstream for low-ammonia used water (e.g., municipal used water). Large-scale application of the sidestream deammonification process has been widely reported (Lackner et al. 2014), while the mainstream deammonification is being explored at its infancy stage (Regmi et al. 2014; Wett et al. 2013).

The traditional BNR process has several disadvantages, including intensive oxygen demand for nitrification as well as the requirement of additional organic carbon sources for denitrification. Even when there is usually enough organic matter to carry out denitrification in mainstream wastewater, it could be saved and used to produce biogas (energy/positive Waste Water Treatment Plant) or valuable products through other processes. On the other hand, Anammox process can achieve very high volumetric nitrogen removal rates up to 76 kg N/ (m³·day) (Tang et al. 2011), indicating its potential application for treating wastewater with high ammonium strength. The combined nitrification–anammox process can be achieved either in two separate reactors as the SHARON (Single reactor system for High-rate Ammonium Removal Over Nitrite)–anammox process (Hellings et al. 1998, van Dongen et al. 2001), or in a single reactor such as OLAND (Oxygen-Limited Autotrophic Nitrification– Denitrification) (Meulenberg et al. 1992), CANON process (Cho et al. 2011, Sliemers et al. 2003, Third et al. 2001), SNAP (Single-stage Nitrogen removal using Anammox and Partial

nitritation) (Furukawa et al. 2006), and DEMON (the pH-controlled DEamMONification system) (Wett 2007).

Recently, there are a lot of researchers put their effort on investigating the applicability of PN/Anammox in the mainstream and searching for the solutions to get on this sustainable track. Although there isn't any reported full-scale application on the mainstream yet, but it's clear that sooner or later the turning point can be found to increase its reliability and viability.

2.0 OBJECTIVE

2.1 Scope of Study

The PN/Anammox is the most promising biological nitrogen removal process which is extensively used for ammonium rich wastewater (Sultana, 2014). Even though the process has several challenges, but it is worth to be used in the mainstream to create an energy positive environment. Therefore, the following objectives are aimed to be achieved at the end of this research:

1. Acquisition of deep knowledge about PN/Anammox process and Moving Bed Biofilm Reactor (MBBR) to start-up the lab-scale one-step PN/Anammox MBBR.
2. For the laboratory scale experiments, a study of some literature reviews for PN/Anammox and MBBR technologies will be needed.
3. Start-up and stabilization the PN process in the MBBR first and then carry out the Anammox process in the same MBBR system. And, if possible, optimise the PN/Anammox MBBR to have higher BNR rate.

3.0 MATERIALS AND METHODOLOGY

3.1 Influent

The main source of the influent of the reactor was directly supplied with supernatant originating from dewatering of digested sludge containing high ammonium concentrations. Depending on the concentration of ammonium from this supply,

dilution was made to get around 50 mg/L of ammonium as influent to the reactor, as corresponds to a municipal-like influent. Based on the previous analysis, 0.3 g/L of NaHCO_3 was dissolved and mixed with the influent to increase the alkalinity of the system.

3.2 Operational Setup

Figure 2 indicates the overall look of the reactor and the connections of feeding tank as well as the temperature controller.

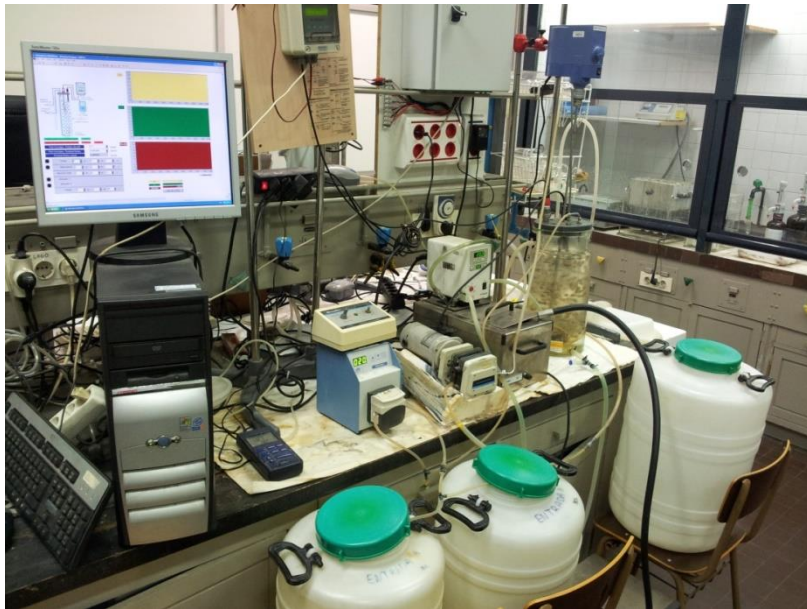


Figure 2: Reactor setup

From the figure above, we can clearly see that there are two feeding tanks (two green tops, side-by-side at the bottom of photo) where the influent was prepared by the dilution of rejected water from one Waste Water Treatment Plant (WWTP) near Barcelona mixed with a solution of sodium bicarbonate. Two pumps was used for the influent (P Selecta, Percom-I) and effluent (Cole Parmer, Masterflex), and there were connected to a timer to extend the hydraulic retention time (HRT) of reactor, automatically switching ON and OFF every 15 minutes. The feeding pump was set to its lowest value in order to get as higher as possible the HRT, currently was 0.45 day;

while the pumping rate of the output pump was always exceed that of input, so no overflow could happen. To make sure the volume of liquid inside reactor was always to be 4.5 L, the liquid was withdrawn from the surface by the output pump. A cooler was connected to the main temperature controller to maintain it at 20°C. To ensure the homogeneity in the reactor, IKA RW 16 basic WERKE stirrer was used at its lowest speed to avoid an excess of shear stress in the reactor. The aeration was set to be 5 seconds every 6 minutes and it's controlled by Advantech ADAMView program.

3.3 Analytical Methods

For the analysis, samples of effluent were taken daily while those of influent were taken every time the feeding tank was refilled. Approximately 25 mL of sample was filled in a vial (figure 3) and was stored in the fridge.

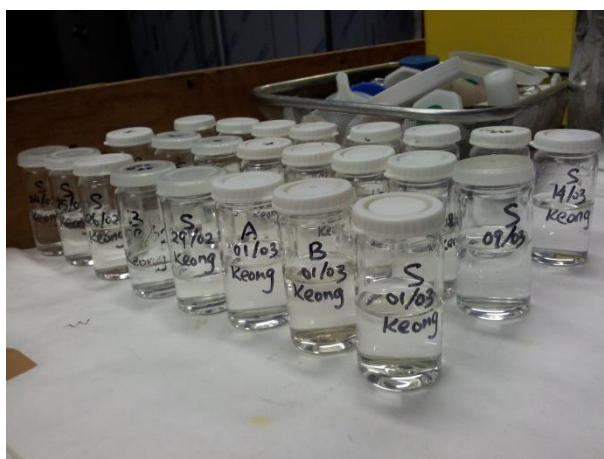


Figure 3: Samples in the vials

3.3.1 Nitrogen Species

In this research, the main concern is to remove as much as possible ammonium in the influent, by oxidizing it to nitrite and then to be removed as nitrogen. But sometimes, it could be possible to be over-oxidized to nitrate. Therefore, these three species of nitrogen are what we are interested in. With the ion-exchange chromatography, Metrohm 861 Advanced Compact IC (as indicated on the figure 4),

supported by the IC Net program, the concentration for both cation, nitrogen as ammonium (N-NH_4^+) and anions, nitrite (N-NO_2^-), nitrate (N-NO_3^-), can be detected directly in mg/L.



Figure 4: Ionic chromatograph, Metrohm 861 Advanced Compact IC and its autosampler

3.3.2 pH

The pH in the biological treatment process is crucially important, it's because the bacteria used normally can survive within a very narrow range of pH. Once the condition is out of that range, they'll simply be inactivated or even destroyed. During this research, the pH measurements were carried out continuously by Crison pH 28 probe.

3.3.3 Temperature and Dissolved Oxygen

The measurements of temperature and concentration of dissolved oxygen were measured by the same device, WTW Oxi 340i, which gave considered accurate values to them.

3.3.4 Chemical Oxygen Demand

The determination of the total oxygen requirement for both biological and non-biological oxidation of materials was carried out according to the method 5220D of the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The samples with all the required agents were digested (VELP Scientifica, Eco 26 Thermoreactor) at 150 °C for 2 hours. The absorbance of the samples was measured by a Spectrophotometer (SI Analytics, UviLine 9100) at a $\lambda=620$ nm.

3.3.5 Total Suspended Solids

According to American Public Health Association (APHA 1998), total solids can be roughly divided into 2 main groups, total suspended solid and total dissolved solids. The portion of solids that passes through a filter of 2.0 μm (or smaller) nominal pore size considered as dissolved solids, otherwise, the portion retained on the filter is suspended solids. In accordance with APHA and the laboratory guideline for Master's degree of environmental engineering, a glass microfiber filter provided by the Filter-Lab (Ref.: MFV-3) was put into 550 °C muffle furnace for 1 hour and then the weight was recorded as M_0 . A defined volume of effluent was taken (it was predicted to have very little amount of suspended solids by its clarity, so 50 mL was decided) and filtered through the filter paper. With the retained suspended solids on it, the filter paper was heated up between 103 to 105 °C for 1 hour. When it got cold, the weight was recorded again as M_1 , the difference between M_0 and M_1 was the mass of total suspended solids. To further analyse the volatile suspended solids, the same filter was put into muffle furnace for 1 hour at 550 °C. Weight was jotted down as M_2 and the difference between M_1 and M_2 was known as the mass of volatile suspended solids.

3.3.6 Conductivity

The Crison CM 35, with the accuracy of 0.01 μS , was used to obtain the conductivity of the influent and effluent of the reactor.

3.3.7 Flow rate

At the beginning of the experiment, the flow rate was calculated by collecting the total volume of effluent liquid within certain period of time, normally 5 minutes, and up to 3 sets of data were taken to reduce any possible error. After the increment of hydraulic retention time, every set of data was changed to 15 minutes in favour of the timer used. Also, 3 sets of data were taken. Since the effluent was taken out by suction from the top of the reactor (as shown in figure 5), the effluent wasn't flowing out constantly. It means sometimes, there would have output flowing out continuously and several minutes without any drop of effluent. That's why the effluent of a complete cycle of 15 minutes was taken as better measurement.

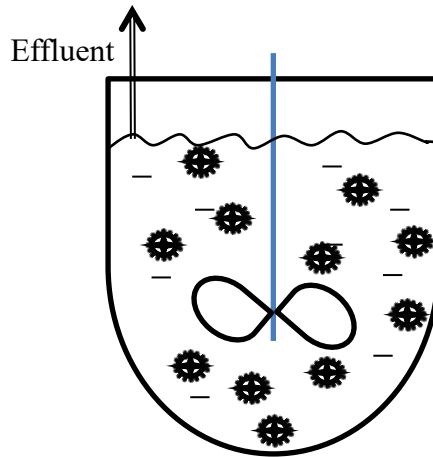


Figure 5: Flow rate withdraw by suction

4.0 Results and Discussion

The experiment was started on 25th of January 2016. Before that, the reactor had been preserved by putting the temperature down to 5 °C, so that the previously grown nitrifying bacteria could be inactivated temporally. Due to this, the start-up became easier. During the first 2 weeks, there was no data taken since a huge variation and unstable data would be obtained. From the day 16, data recording was started. This experiment was divided into 6 stages as there were 6 main changes applied to the system. The first stage was the start-up of reactor, which ranged from 25 of January (1st day) to 18 of February (25th day). Second stage began when the timer was used to

change the HRT. And then, 3rd stage was due to the intention of reducing the DO by sealing the top of reactor on 26 of April (day 93). Next, on 12 of May (day 109), Anammox bacteria was added to the reactor a first time. 1.5 L of granular Anammox sludge was put into the reactor. The Anammox inoculum contained 1.48 g VSS/L, so this was the beginning of stage 4. At stage 5, the aeration rate was changed from 4 s/6 min. to 4 s/10 min. Lastly, the sixth stage indicated the addition of Anammox for the second time, 200 mL of liquid containing similar concentration of Anammox granules was added. In all the figures of this section, those numbers 1 to 6 indicate the number of stage of experiment.

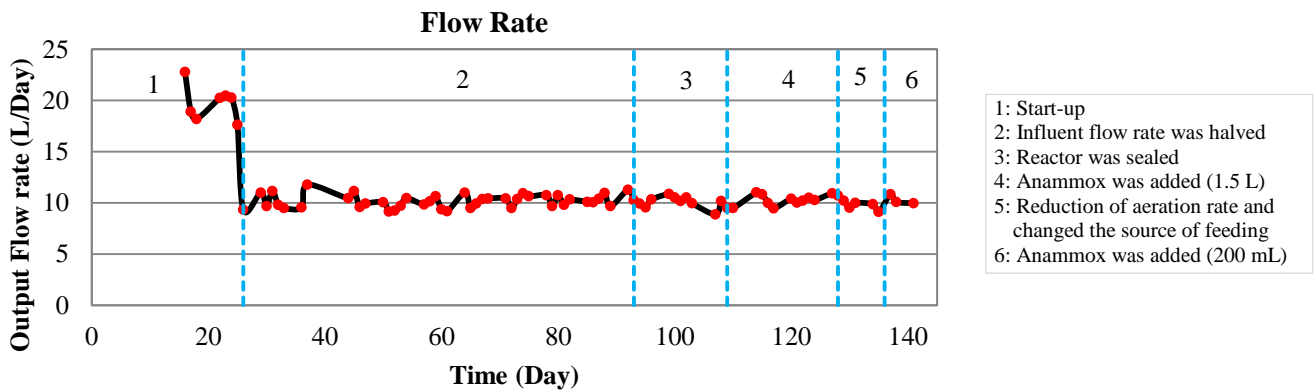


Figure 6: Effluent flow rate

At the beginning of the experiment, the feeding pump was set to its lowest value, but still, for this experiment with a 4.5 L reactor, it was considered very high its flow rate, which gave an average of 19.76 L/d, equivalent to 0.23 day of HRT. After a deep consideration, at day 25, a timer connected to the pumps had been used to reduce this value. This explained the sharp drop between 25th and 26th day which can be clearly seen in the figure 6. Afterwards, the flow rate yielded an average of 10.13 L/d, representing a HRT of 0.44 day. Basically, the flow rate changed very slightly.

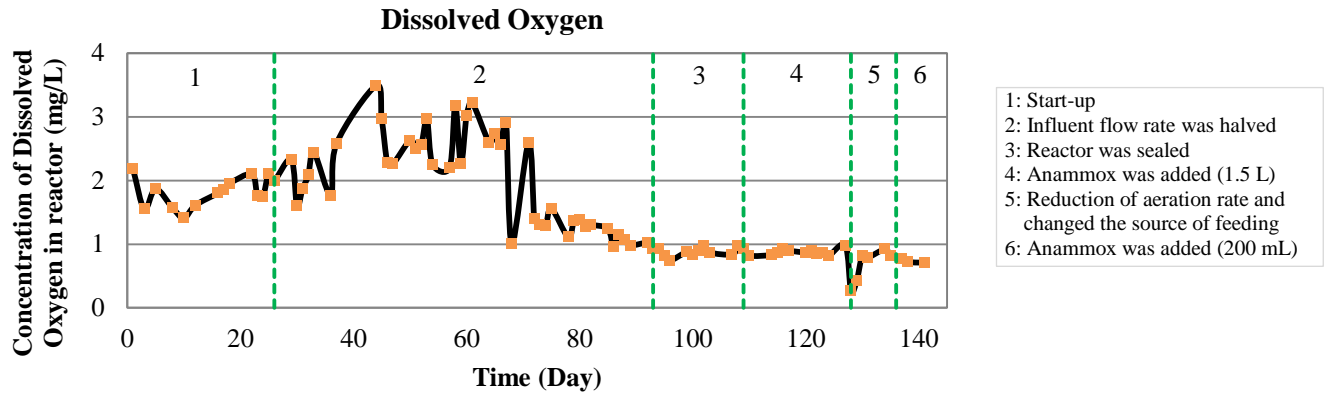


Figure 7: Concentration of dissolved oxygen in the reactor

From figure 7, we can clearly see that there is a huge change of the DO concentration between 1 and 3 mg/L in stage 1 and 2. Since at these stages, the focus was to increase the overall consumption of ammonium by the bacteria, therefore this variation wouldn't give much negative impact to the system. Basically, the variation was due to the environmental condition in the laboratory, since the reactor wasn't totally sealed and the mechanical stirring applied was quite strong to keep the support rings well mixed and in suspension, therefore, it is possible that the oxygen transference in the liquid surface was important. Besides, the feeding itself was saturated of DO, due to the fact of being kept in open tanks. In addition, it was found that the oximeter used during the first days in the continuous measurement wasn't functioning well. After the day 68 it was replaced by a new oximeter.

For the next stage, the holes on the top of reactor had been closed to avoid any oxygen from the air dissolving into the system. After the day 75, there is a decreasing trend of DO until the end of experiment. During the last 4 stages, it's found that the DO was so difficult to keep below 0.5 mg/L. In short, the DO concentration was finally kept below 1.0 mg/L to promote the anoxic condition in inner part the biofilm for the reactions to take place.

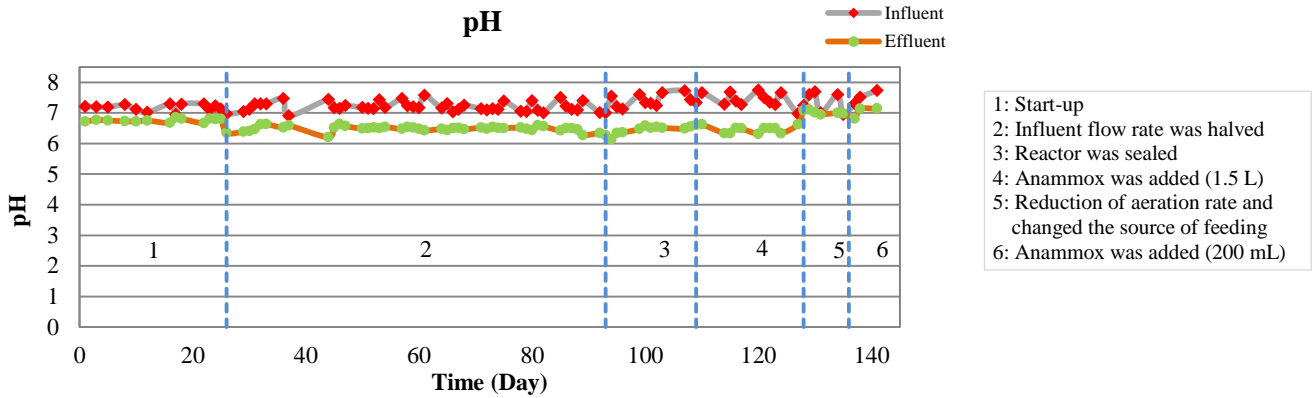


Figure 8: pH of reactor

Graph of pH of reactor versus time has been plotted as shown in figure 8. From the overall view, the pH didn't have any big change. It was reported that the average pH of feed and reactor were 7.20 and 6.59, respectively. In the reactor, nitrification took place at which hydrogen ions were produced and this would consume the alkalinity. So, the pH of effluent was always lower than that of influent. In case of having too low pH, the bacteria would be negatively affected and it could cause the reaction to be broken down. But during the experiment, the pH was well controlled at which it's within the acceptable range with a maximum of 7.14 and a minimum of 6.13.

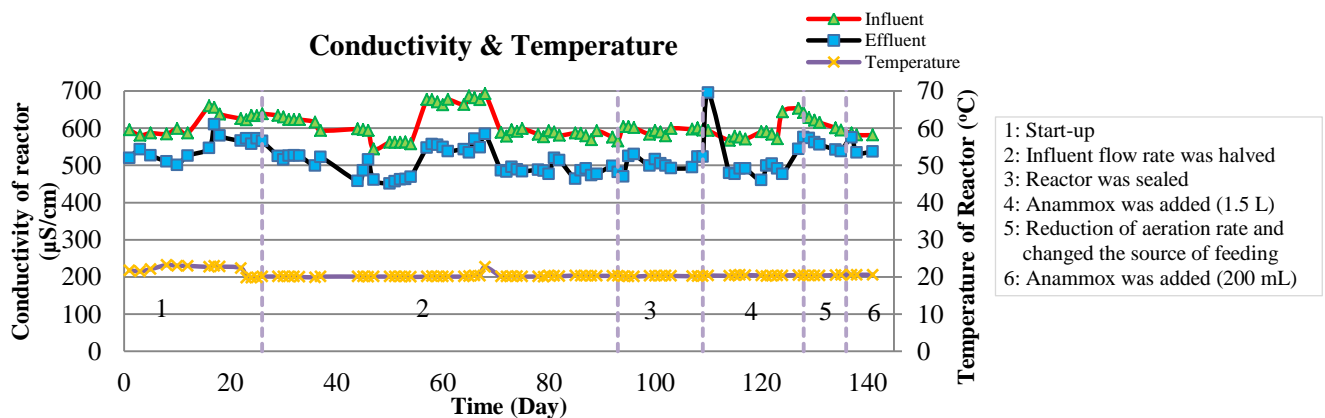


Figure 9: Temperature profile and conductivity of influent and effluent

Figure 9 represents the temperature profile of reactor and also the conductivity of both influent and effluent. The temperature was practically a straight line since there wasn't any big change of ambient temperature which could affect the control system.

The heat exchanger was functioning very well during the process; this ensured that the temperature was around the pre-set value: 20 °C.

For the graphs of conductivity, there can be said to have an estimated value for the influent and effluent close to 600 and 500 $\mu\text{S}/\text{cm}$ respectively. In the only case at which the conductivity of effluent increased suddenly was just after the addition of Anammox bacteria. After putting them into the reactor, to encourage the Anammox retention in the Kaldnes carriers, feeding was paused for 12 hours approximately. Hence, the excess accumulation of ions nitrite and nitrate, when the next day the effluent was taken and analysed, it showed an unusual peak of conductivity. Meanwhile, it can be observed that there is always an increment after every feeding preparation and slow decrement in between one new feeding and other.

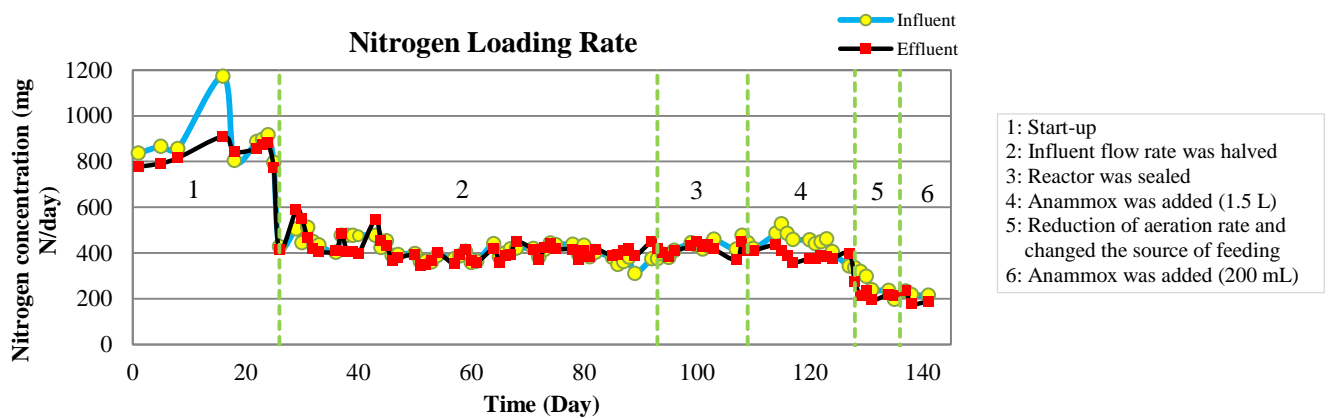


Figure 10: Nitrogen loading rate

Figure 10 gives an overview of nitrogen loading rate in and out of the reactor. From stage 1 until end of stage 3, almost all pairs of data for influent and effluent were virtually equal except one, so the nitrogen removal in these stages was not very significant to be observed yet. At day 16, a 25% extra of rejected water has been taken to prepare the feeding. So, it can be noticed that a small rise and fall occurred in stage 1. Also, after day 26, nitrogen loading rate was stepped down one level which was affected by the timer. It was observed a nearly 50% diminution in this rate, resulting to a mean value of 417.9 and 407.0 mg N/day, for influent and effluent respectively, during the second to fourth stage. It can be seen that starting from the fourth stage, there is actually

a small net removal of nitrogen, which could indicate that Anammox bacteria was performing its job. Since at the fifth stage the source of feeding has been changed, the concentration of nitrogen as ammonium was unexpectedly lower than before and caused a continuously decline in the total nitrogen feeding. If the nitrogen feeding could be the same as before, the gap between the lines of influent and effluent would be more evident.

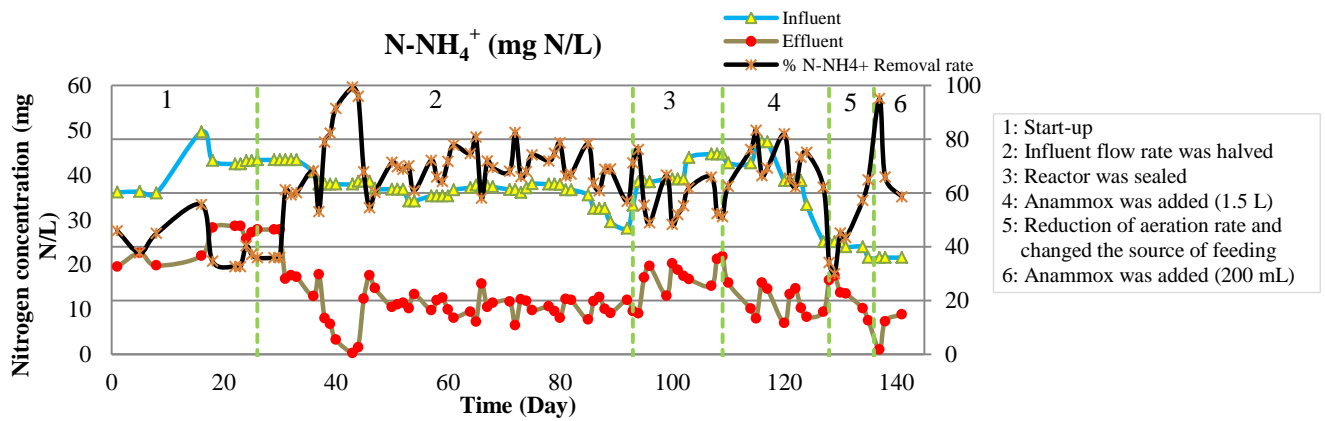


Figure 11: Concentration of nitrogen as ammonium

In this research, the removal of ammonium is also one of the objectives. It's better to get a larger gap between the concentration of ammonium in the influent and effluent, which would indicate a remarkable milestone of the investigation. As reveals in Figure 11, the graph of ammonium removal and its concentration at the effluent was mirror between each other. Consistent changes up and down can be found during the whole process. The influent was calculated to make sure that the ammonium concentration could be kept constantly, but unfortunately, it wasn't succeed especially at the last 2 stages of the experiment where it was reducing persistently. A simple conclusion can be made, there was a rapid oxidation activities in the storage tank where the feeding sourced was, so the amount of ammonium was losing without any control taken.

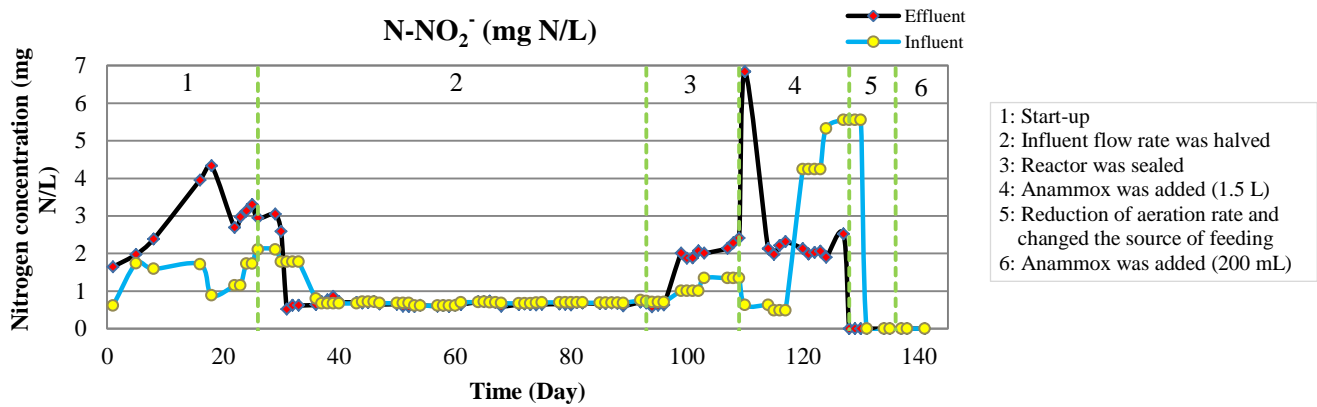


Figure 12: Concentration of nitrogen as nitrite

The concentration of nitrogen in term of nitrite for both influent and effluent was summarised in Figure 12. With the same effect of the use of timer, the conversion of ammonium to nitrite was extremely low. Before that, there was still a boost in the production of nitrite biological reaction and had reached as high as 4.33 mg N-NO₂⁻/L at day 18. But then it went downwards. Excess oxygen and sufficient reaction time had led to further oxidation of nitrite to nitrate. As it was mentioned in the introduction, the effective suppression of the nitrite oxidation is one of the biggest challenges of this process. At day 109, when the anammox was added for the first time, a record was obtained, the accumulation of ion nitrite was the highest, 6.84 mg N/L was reported. But then, within the last 2 weeks, all the inlet and outlet yielded zero nitrite. Some technical problems have been found later on the ionic chromatograph, which might give results with error, generating questions on the reliability of these results.

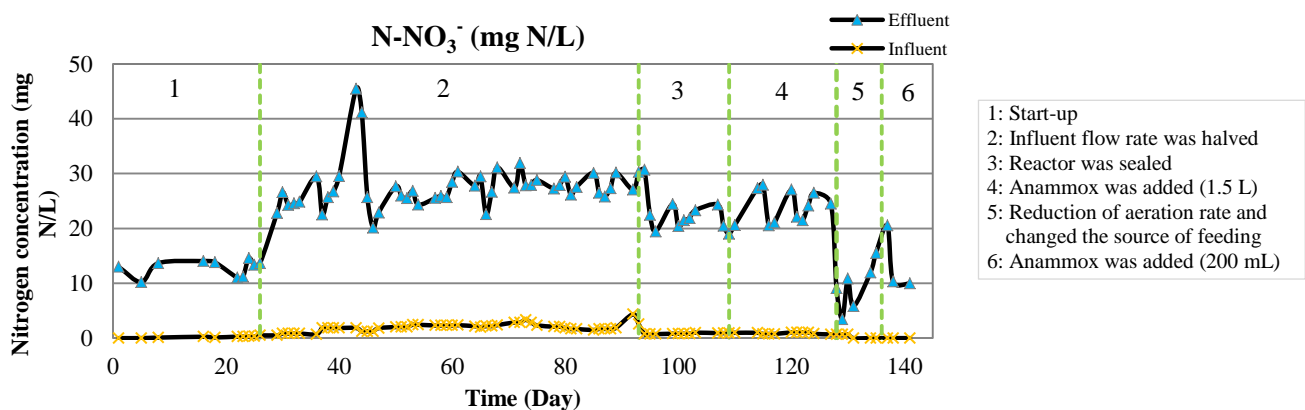


Figure 13: Concentration of nitrogen as nitrate

Figure 13 compares the concentration of nitrate nitrogen (N-NO_3^-) in the effluent and the influent. Both graphs are having, in general, an increasing trend at which the effluent is seemed to have it more significantly. Different from the real WWTP, the feed was prepared manually by diluting the supernatant effluent from the anaerobic digester of WWTP. During the transportation and storage, oxygen could easily dissolve into it and partially oxidize the ammonium, producing ions nitrite and nitrate. A great increment of ion nitrate in the output after day 26 was observed which was caused by the reduction of influent flow rate by the use of the timer; a better yield was obtained after that. From day 40 to 45, the feeding was run out, the HRT at that time was predicted to be longer than usual; hence, higher nitrate conversion was achieved unexpectedly.

After went through the start-up step, one of the objectives could be said to be partially achieved which was to have high consumption rate of ammonium by the bacteria. Therefore, the next immediate step of work was the reduction of the concentration of dissolved oxygen (3rd and 5th stages), so that the oxidation of ammonium could stop at nitrite instead of nitrate. Since nitrite and ammonium are the substrates for Anammox process, concentration of nitrate was then lowered down as what had been anticipated to occur, and the results proved it right.

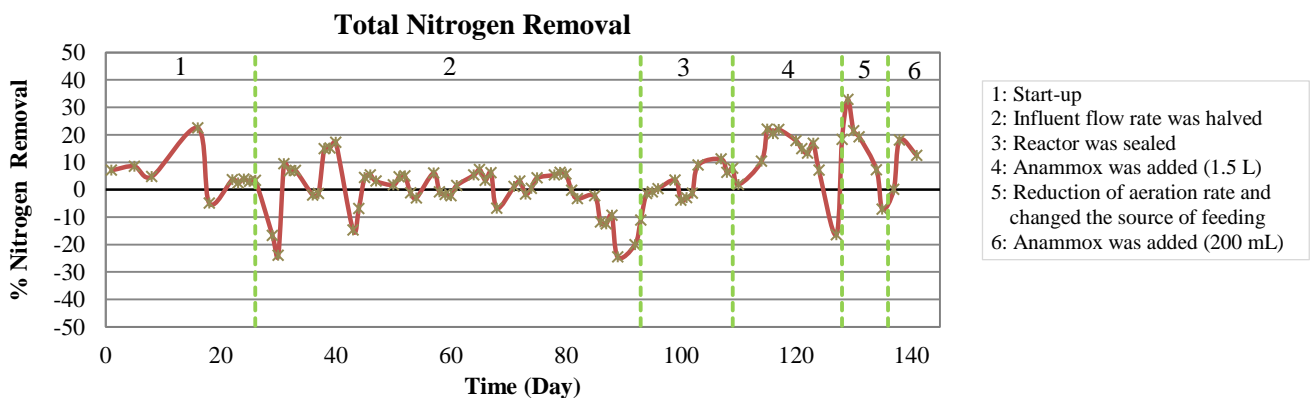


Figure 14: Total nitrogen removal

As mentioned before, this project was intended to remove as much as nitrogen (N-NH_4^+ , N-NO_2^- , N-NO_3^-) as possible; therefore, the most interesting parameter should

be the percentage of total nitrogen removal. After a series of calculations, its daily percentage has been drawn in figure 14 at which both positive and negative values were obtained, but in general, mostly were positive. Theoretically, there couldn't have any negative value because it stands for producing more nitrogen instead of removal, but it wouldn't have possibility to get it happened. For those small negative values, they could be led by the changes of either flow rate or nitrogen concentration or even both, being that the reactor wasn't large enough to overcome the effect of these changes. Another possibility would be the lysis of biomass or some degradation of solids entering the system. In any case, from stage 4, the trend was towards the net removal of nitrogen, possibly due to incipient Anammox activity and, maybe, some denitrification (see COD consumption in Figure 15).

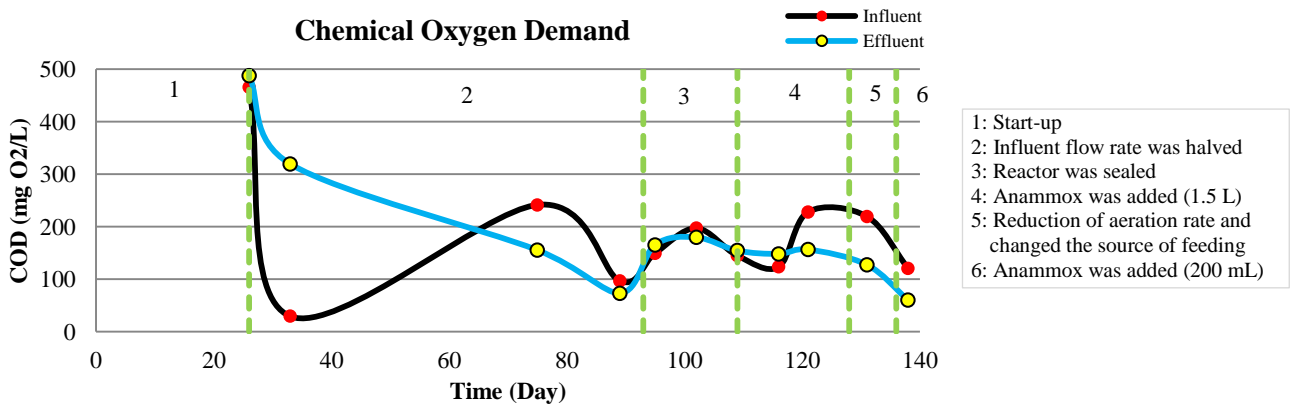


Figure 15: Chemical oxygen demand

After analysing chemical oxygen demand of both influent and effluent, it's difficult to rely only on the COD due to the reasons below:

- The source of wastewater wasn't fresh and was stored in fridge for a long period of time
- The wastewater had contact with the air which might reduce the COD of influent
- Since the feeding is diluted real wastewater, large amount of water was added to get the desired concentration of ammonium while diluting the COD as well.

Due to those factors stated above, the results of COD can be classified as secondary parameter for the overall review of the performance of this experiment. Two

lines of COD of influent and effluent were plotted in the figure 15, both lines are crossing each other from the first stage to fourth stage indicating the inaccuracy of those values at most of the time of the experiment. But, in the end, it's interesting that a clear difference between the COD of influent and effluent can be observed, these should be the results based on the characteristic of biological reactor which removes the COD from the wastewater.

5.0 CONCLUSIONS

This investigation was carried out to study and evaluate the partial nitrification/Anammox technology in moving bed biofilm reactors (one-reactor system). Literature review and experimental work carried out in this thesis confirmed the sustainability and the potential advantages of the partial nitrification/Anammox as a viable option for the treatment of low strength ammonium wastewaters.

A laboratory-scale MBBR was assembled to allow understanding of the parameters involved in the process and directly examine its influence on the process performance. Results and findings concerning the laboratory-scale reactor are given in chapter 4. The following conclusions can be stated:

- a. By varying and adjusting carefully operational parameters such as DO concentration, temperature and HRT (i.e. inflow rate) is possible to obtain high and stable efficiency of the whole process.
- b. As high as 95% efficiency of the removal of N-NH_4^+ has been achieved after adding Anammox bacteria to the reactor while the maximum removal of nitrogen can be reached was 33% approximately. It's likely that more operation time was needed to obtain better performances.
- c. Without lowering the DO concentration, the percentage of nitrogen removal was very low, on average of 0.13% even though the percentage of ammonium removal was pretty high, 63.07% because of the complete nitrification of ammonium to nitrate.
- d. The conductivity was reported as a good parameter to monitor the performance of the process and the ammonium removal (Bertina, 2010), however, in this study, the

conductivity was performing very constantly compared to the ammonium at the effluent.

- e. Ratios such as COD/N and Alkalinity/N in the wastewater prior to treatment are extremely important for the stability of the process. A too high COD might enhance denitrifiers' growth, which could outcompete Anammox bacteria on a long-term scale. A too low alkalinity may not be sufficient to cope with the general decrease in pH of the partial nitrification/Anammox process. In this case, the addition of sodium bicarbonate helped to increase the alkalinity to avoid persistent large falling of the pH.
- f. Nitrite was the limiting factor for the Anammox bacteria in the one-stage partial nitrification/Anammox reactor and its concentration inside the reactor was 11.96% of the concentration of ammonium.
- g. An increase of the density of Kaldnes K1 with biomass was found one month after the starting-up of the MBBR.
- h. Although the small variation of temperature didn't affect the performance in this study, but the time frame where the sample was taken should be kept as consistent as possible.
- i. A sufficiently high nitrogen loading rate is required for a stable partial nitrification/Anammox process in order to not limit the slow growth rate of Anammox bacteria. If the load is too low the decay rate might exceed the Anammox bacteria growth rate.
- j. The changes of overall nitrogen removal at the last 3 weeks might due to the frequent changes made to the system. Especially for the Anammox bacteria to get used to a new condition, it would take longer time than others.
- k. It has been found that the percentage of ammonium removal reached its high peak every 3 to 5 days at which were those days after a new feeding tank was prepared. An assumption was made based on this: the ammonium was oxidized from day to day inside the feeding tank, hence, the concentration of ammonium wouldn't be the same daily. It's recommended to take a sample of influent every day and calculate a mean value of it, so more precise feeding concentration of ammonium could be obtained.

6.0 Nomenclature

Anammox - Anaerobic Ammonium Oxidation

APHA - American Public Health Association

BNR - Biological Nitrogen Removal

COD - Chemical Oxygen Demand

HRT - Hydraulic Retention Time

MBBR - Moving Bed Biofilm Reactor

N - Nitrogen

N/DN - Nitrification and Denitrification

N-NH₄⁺ - Nitrogen species as ammonium

N-NO₂⁻ - Nitrogen species as nitrite

N-NO₃⁻ - Nitrogen species as nitrate

PN - Partial Nitritation

SRT - Sludge Retention Time

VSS - Volatile Suspended Solids

WWTP - Waste Water Treatment Plant

7.0 Bibliography

- [1]. Al-Omari A., Wett B., Nopens I., de Clippeleir H., Han M., Regmi P., Bott C., Murthy S., Model-based evaluation of mechanisms and benefits of mainstream shortcut nitrogen removal processes, *Water Sci. Technol.*, 71(6) (2015), 840-847.
- [2]. Andreottola, G., P. Foladori, M. Ragazzi and R. Villa, 2002. Dairy wastewater treatment in a moving bed biofilm reactor. *Water Sci. Technol.*, 45: 321-328. <http://www.iwaponline.com/wst/04512/wst045120321.htm>.
- [3]. Andreottola, G., P. Foladori, G. Gatti, P. Nardelli, M. Pettena and M. Ragazzi, 2003. Upgrading of a Small Overloaded Activated Sludge Plant Using a MBBR System. *J. Environ. Sci. Health, Part A*, 38: 2317-2328. <http://www.informaworld.com/smpp/content~db=all?content=10.1081/ESE-120023388>.
- [4]. Andreottola, G., P. Foladori, M. Ragazzi and F. Tatano, 2000a. Experimental comparison between MBBR and activated sludge system for the treatment of municipal wastewater. *Water Sci. Technol.*, 41: 375-382. <http://www.iwaponline.com/wst/04104/wst041040375.htm>.
- [5]. Andreottola, G., P. Foladori and M. Ragazzi, 2000b. Upgrading of a small wastewater treatment plant in a cold climate region using a moving bed biofilm reactor (MBBR) system. *Water Sci. Technol.*, 41: 177-185. <http://www.iwaponline.com/wst/04101/wst041010177.htm>.
- [6]. APHA (1998). *Standard methods for the examination of water and wastewater*, 20th Washington DC:APHA.
- [7]. Bertina (2010). *Study on One-Stage Partial Nitritation/Anammox Process in Moving Bed Biofilm Reactors: A Sustainable Nitrogen Removal*. Politecnico Di Torino, Italy: Turin.
- [8]. Daigger G.T., Oxygen and carbon requirements for biological nitrogen removal processes accomplishing nitrification, nitritation, and anammox, *Water Environ. Res.*, 86(3) (2014), 204-209.
- [9]. de Clippeleir H., Vlaeminck S.E., de Wilde F., Daeninck K., Mosquera M., Boeckx P., Verstraete W., Boon N., N. One-stage partial nitritation/anammox at 15 °C on pretreated sewage: feasibility demonstration at lab-scale, *Appl. Microbiol. Biot.*, 97(23) (2013), 10199-10210.
- [10]. EPA (1993) *Nitrogen control*. USEPA, Washington, DC.

- [11]. Fernández I., Vázquez-Padín J.R., Mosquera-Corral A., Campos J.L., Méndez R., Biofilm and granular systems to improve Anammox biomass retention, *Biochem. Eng. J.*, 42(3) (2008), 308-313.
- [12]. Frijns J., Hofman J. and Nederlof M., The potential of (waste) water as energy carrier, *Energ. Convers. Manage.*, 65 (2013), 357-363.
- [13]. Gao D.W., Lu J.C., Liang H., Simultaneous energy recovery and autotrophic nitrogen removal from sewage at moderately low temperatures, *Appl. Microbiol. Biot.*, 98(6) (2014), 2637-2645.
- [14]. Gujer W., Nitrification and me – A subjective review, *Water Res.*, 44 (2010), 1-19.
- [15]. Hellinga C., Schellen A.A.J.C., Mulder J.W., van Loosdrecht M.C.M, Heijnen J.J., The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water, *Water Sci. Technol.*, 37(9) (1998), 135-142.
- [16]. Helness, H. and H. Ødegaard, 1999. Biological Phosphorus Removal in a Sequencing Batch Moving Bed Biofilm Reactor. *Water Sci. Technol.*, 40: 161-168. <http://www.iwaponline.com/wst/04004/wst040040161.htm>.
- [17]. Hendrickx, T.L.G., Wang, Y., Kampman, C., Zeeman, G., Temmink, H., Buisman, C.J.N., 2012. Autotrophic nitrogen removal from low strength wastewater at low temperature. *Water Res.* 46(7), 2187-2193.
- [18]. Hosseini, S.H. and S.M. Borghei, 2005. The treatment of phenolic wastewater using a moving bed bio-reactor. *Process Biochem.*, 40: 1027-1031. DOI: 10.1016/j.procbio.2004.05.002.
- [19]. Jahren, S.J., J.A. Rintala and H. Ødegaard, 2002. Aerobic moving bed biofilm reactor treating thermomechanical pulping whitewater under thermophilic conditions. *Water Res.*, 36: 1067-1075. DOI: 10.1016/S0043-1354(01)00311-6.
- [20]. Jenni S., Vlaeminck S.E., Morgenroth E., Udert K.M., Successful application of nitrification/anammox to wastewater with elevated organic carbon to ammonia ratios, *Water Res.*, 49 (2014), 316-326.
- [21]. Jin R.C., Yang G.F., Yu J.J. and Zheng P., The inhibition of the Anammox process: a review, *Chem. Eng. J.* 197 (2012), 67-79.
- [22]. Johnson, C.H., M.W. Page and L. Blaha, 2000. Full scale moving bed biofilm reactor results from refinery and slaughter house treatment facilities. *Water Sci. Technol.*, 41: 401-407. <http://www.iwaponline.com/wst/04104/wst041040401.htm>.

- [23]. Joss A., Salzgeber D., Eugster J., König R., Rottermann K., Burger S., Fabijan P., Leumann S., Mohn J. and Siegrist H. (2009). Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. *Environmental Science and Technology* **43**(14), 5301-5306.
- [24]. Kartal B., van Niftrik L., Rattray J., van de Vossenberg J.L.C.M., Schmid M.C., Damsté J. S., Jetten M.S.M., Strous M., *Candidatus* 'Brocadia fulgida': an autofluorescent anaerobic ammonium oxidizing bacterium, *FEMS Microbiol. Ecol.*, 63 (2008), 46-55.
- [25]. Lackner S., Terada A., Smets B.F., Heterotrophic activity compromises autotrophic nitrogen removal in membrane-aerated biofilms: Results of a modeling study, *Water Res.*, 42(4) (2008), 1102-1112.
- [26]. Lackner, S.; Gilbert, E.M.; Vlaeminck, S.E.; Joss, A.; Horn, H.; Van Loosdrecht, M.C.M. (2014) Full-scale partial nitritation/anammox experiences - An application survey. *Water Research*, 55, pp. 292-303.
- [27]. Liang Z., Liu J.X., Control factors of partial nitritation for landfill leachate treatment, *J. Environ. Sci.*, 19(5) (2007), 523-529.
- [28]. Lin Y.-M., Tay J.-H., Liu Y. and Hung Y.-T. (2009). Biological Nitrification and Denitrification Processes. In: *Handbook of Environmental Engineering*, Humana Press, Totowa NJ, pp. 539-588.
- [29]. Lotti T., Kleerebezem R., van Erp Taalman Kip C., Hendrickx T.L., Kruit J., Hoekstra M., van Loosdrecht M.C., Anammox growth on pretreated municipal wastewater, *Environ. Sci. Technol.*, 48(14) (2014), 7874-7880.
- [30]. Oram, B. (2014). Ammonia in Groundwater, Runoff, and Streams. Water Research Centre. B.F. Environmental Consultants Inc. 10 April 2016. <http://www.water-research.net/index.php/ammonia-in-groundwater-runoff-and-streams>.
- [31]. Østgaard K, Christensson M, Lie E, Jönsson K, Welander T (1997) Anoxic biological phosphorus removal in a full-scale UCT process. *Water Res* 31(11):2719–2726.
- [32]. Pai TY, Tsai YP, Chou YJ, Chang HY, Leu HG, Ouyang CF (2004) Microbial kinetic analysis of three different types of EBNR process. *Chemosphere* 55: 109–118.
- [33]. Pastorelli, G., R. Canziani, L. Pedrazzi and A. Rozzi, 1999. Phosphorus and nitrogen removal in moving-bed sequencing batch biofilm reactors. *Water Sci.*

- Technol., 40: 169-176. <http://lequia.udg.es/lequianet/WatSciTech/04004/0169/040040169.pdf>.
- [34]. Pérez J., Lotti T., Kleerebezem R., Picioreanu C., van Loosdrecht M.C.M., Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study, *Water Res.*, 66 (2014), 205-218.
- [35]. Plaza, E., Stridh, S., Örnmark, J., Kanders, L. & Trela, J. 2011. Swedish Experience of the Deammonification Process in a Biofilm System. Proc. of the IWA Specialized conference "Nutrient recovery and management 2011" 9-12 January, 2011, Miami, USA, 1333-1345.
- [36]. Regmi P., Miller M.W., Holgate B., Bunce R., Park H., Chandran K., Wett B., Murthy S., Bott C.B., Control of aeration, aerobic SRT and COD input for mainstream nitrification/denitrification, *Water Res.*, 57 (2014), 162-171.
- [37]. Rosenwinkel, K. H. & Cornelius, A. 2005. Deammonification in the Moving-Bed Process for the Treatment of Wastewater with High Ammonia Content. *Chemical Engineering & Technology*, 28, 49-52.
- [38]. Rusten, B., J.G. Siljudalen, A. Wien and D. Eidem, 1998a. Biological pretreatment of poultry processing wastewater. *Water Sci. Technol.*, 38: 19-28. DOI: 10.1016/S0273-1223(98)00493-4.
- [39]. Rusten, B., J.G. Siljudalen and H. Strand, 1996. Upgrading of a biological-chemical treatment plant for cheese factory wastewater. *Water Sci. Technol.*, 34: 41-49. DOI: 10.1016/S0273-1223(96)00819-0.
- [40]. Rusten, B., M. McCoy, R. Proctor and J.G. Siljudalen, 1998b. The innovative moving bed biofilm reactor/solids contact reaeration process for secondary treatment of municipal wastewater. *Water Environ. Res.*, 70: 1083-1089. <http://www.ingentaconnect.com/content/wef/wer/1998/00000070/00000005/art00015>.
- [41]. Rusten, B., O. Kolkin and H. Ødegaard, 1997. Moving bed biofilm reactors and chemical precipitation for high efficiency treatment of wastewater from small communities. *Water Sci. Technol.*, 35: 71-79. DOI: 10.1016/S0273-1223(97)00097-8.
- [42]. Rusten, B., L. Hem and H. Ødegaard, 1995a. Nitrification of municipal wastewater in novel moving bed biofilm reactors. *Water Environ. Res.*, 67: 75-86. <http://www.ingentaconnect.com/content/wef/wer/1995/00000067/00000001/art00009>.

- [43]. Rusten, B., L. Hem and H. Ødegaard, 1995b. Nitrogen removal from dilute wastewater in cold climate using moving bed biofilm reactors. *Water Environ. Res.*, 67: 65-74. <http://www.ingentaconnect.com/content/wef/wer/1995/00000067/00000001/art00008>.
- [44]. Rusten, B., J.G. Siljudalen and B. Nordeidet, 1994. Upgrading to nitrogen removal with the KMT moving bed biofilm process. *Water Sci. Technol.*, 29: 185-195. <http://www.iwaponline.com/wst/02912/wst029120185.htm>.
- [45]. Sultana R (2014) Partial nitrification/anammox process in a moving bed biofilm reactor operated at low temperatures, Lic. 2014, Royal Institute of Technology: Stockholm. pp. 2-3.
- [46]. Strous M., Heijnen J. J., Kuenen J. G. and Jetten M. S. M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* 50(5), 589-596.
- [47]. Tang, C.J., Zheng, P., Wang, C.H., Mahmood, Q., Zhang, J.Q., Chen, X.G., Zhang, L., Chen, J.W., 2011. Performance of high-loaded anammox UASB reactors containing granular sludge. *Water Res.* 45, 135–144.
- [48]. Tchobanoglous G, Burton FL, Stensel DH (2003) *Wastewater Engineering: Treatment Disposal and Reuse*, 4th Ed. McGraw-Hill, New York.
- [49]. Tchobanoglous G., Burton F.L., Stensel H.D., Metcalf Eds.& Eddy: *Wastewater Engineering: Treatment and Reuse*, McGraw-Hill, New York, 2004.
- [50]. Vannecke T.P.W., Volcke E.I.P., Modelling microbial competition in nitrifying biofilm reactors, *Biotechnol. Bioeng.*, 112(12) (2015), 2550-2561.
- [51]. Vazquez-Padin, J., Fernandez, I., Figueroa, M., Mosquera-Corral, A., Campos, J.L. and Mendez, R., 2009. Applications of Anammox based processes to treat anaerobic digester supernatant at room temperature. *Bioresour. Technol.* 100 (12), 2988-2994.
- [52]. Wentzel MC, Ekama GA, Marais GVR (1992) Process and modelling of nitrification denitrification biological excess phosphorus removal systems: a review. *Water Sci Technol* 25(6):59–82.
- [53]. Williams SC, Beresford J (1998) The effect of anaerobic zone mixing on the performance of a three-stage Bardenpho plant. *Water Sci Technol* 38(1):55–62.
- [54]. Winkler, M.K.H., Yang, J.J., Kleerebezem, R., Plaza, E., Trela, J., Hultman, B. and van Loosdrecht, M.C.M., 2012. Nitrate reduction by organotrophic Anammox

bacteria in a nitrification/anammox granular sludge and a moving bed biofilm reactor. *Bioresour. Technol.* 114, 217-223.

- [55]. Xu G, Zhou Y, Yang Q, Lee ZM, Gu J, Lay W, Cao Y and Liu Y. The challenges of mainstream deammonification process for municipal used water treatment. *Appl Microbiol Biotechnol* 99, 2485–2490 (2015).
- [56]. Zhu GB, Peng YZ, Wu SY, Wang SY (2007a) Effect of influent flow rate distribution on the performance of step-feed biological nitrogen removal process. *Chem Eng J* 131:319–328.
- [57]. Zhu GB, Peng YZ, Wang SY, Zuo JL, Wang YY, Guo JH (2007b) Development and experimental evaluation of a steady-state model for the step-feed biological nitrogen removal process. *Chinese Journal of Chemical Engineering* 15(3):411–417.