

**Integration of nitrification with denitrification for surface  
water and groundwater treatment**

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at Flinders University

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## **Declaration**

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for any degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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18/10/2016

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## Summary

With the growth of major cities led by the population boom over the world and poor water catchment protection, surface water and groundwater sources can be polluted by many substances, of which, ammonia is one of the primary factors. While conventional methods for ammonia treatment using chlorine present limitations during treatment processes, biological treatment solutions are highly appropriate in efficiently removing contaminants such as ammonia.

The study was conducted by an experiment at the lab-scale focused on the integration of a nitrification system with a denitrification system to remove ammonia and other nitrogen compounds in treatment surface water and groundwater. The experiment was conducted in four phases including bacteria development, primary defining capacity of nitrification and denitrification systems, maximum capacity of the nitrification system and maximum capacity of the denitrification system. The results revealed that the nitrification system with hydraulic rate of  $0.3 \text{ L min}^{-1}$  can remove  $83 \text{ mg NH}_4\text{-N day}^{-1}$  with  $4.88 \text{ m}^2$  of polypropylene media. It corresponds to  $17 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$  of ammonium nitrogen surface load. Meanwhile,  $716 \text{ mg NO}_3\text{-N day}^{-1}$  was eliminated by the denitrification system with hydraulic rate of  $15 \text{ ml min}^{-1}$  for Column 1 and 2 and  $12$  and  $15 \text{ ml min}^{-1}$  for Column 3 and 4 respectively.

A graph of the relationship among ammonium/nitrate mass, polypropylene media volume and barley straw mass was created based on the results of the experiment. The results contribute a basic foundation to select the suitable model integrating a nitrification unit and a denitrification unit in removing ammonium and nitrite in surface water and groundwater treatment.

## **1. Introduction**

### **1.1. Background of Study**

With the expanse of major cities led by the population boom over the world and poor water catchment protection, water sources including both surface water and groundwater have been affected by a deterioration of both quantity and quality. Human activities in catchment areas are believed to be a main reason for these issues (Henry & Heineke 1996). For example, discharge of poorly treated waste water or agriculture activities causes pollution for water sources which are used to supply potable water to communities (Umezawa et al. 2008). Surface water and groundwater sources can be polluted by many substances, of which ammonia is one of the primary factors. The ammonia content in these water sources varies from over  $0 \text{ mg NH}_4^+ \text{ L}^{-1}$  to  $25 \text{ mg NH}_4^+ \text{ L}^{-1}$  (Angelopoulos et al. 2009). The low concentration of ammonium nitrogen (under  $5 \text{ mg NH}_4^+ \text{ L}^{-1}$ ) has been recorded in a number of sources. For example, low concentration of ammonia from  $0.09$  to  $0.1 \text{ mg NH}_4^+ \text{ L}^{-1}$  has been found in the tap water of many households in Southeast Asian cities such as Manila and Jakarta (Umezawa et al. 2008). Ammonia seriously influences chlorine disinfection processes which is an important stage in drinking water treatment (van den Akker 2008). Inorganic chloramines will be created as a result of the reaction between chlorine and ammonia. Meanwhile, inorganic chloramines are quite stable and persist for a long time and this leads to poor water quality (Symons & Carswell 1977). In addition, a part of the amount of chlorine will be utilized for this reaction and it could lead to lack of chlorine for disinfection when the required chlorine mass only is calculated for demand disinfection. Therefore, the ammonia contamination should be considered in order to minimize its negative effects.

In the context of the water quality deterioration by nitrogen in recent decades, the demand for ammonia removal in potable water treatment plants is

increasing. Meanwhile, conventional methods for ammonia treatment using chlorine present limitations during treatment processes. This conventional approach uses pre-chlorination as an effective solution to eliminate ammonia in raw water. However, this approach has a number of disadvantages such as generating disinfection by-products and increasing chlorine consumption (van Den Akker et al. 2010). By-products such as chloramine will lead to poor treated water quality and can cause public health problems. Furthermore, in order to eliminate 1mg ammonia, an amount of chlorine 10 mg is required which could significantly increase the water treatment cost. Therefore, it is necessary to develop environment friendly approaches for ammonia removal.

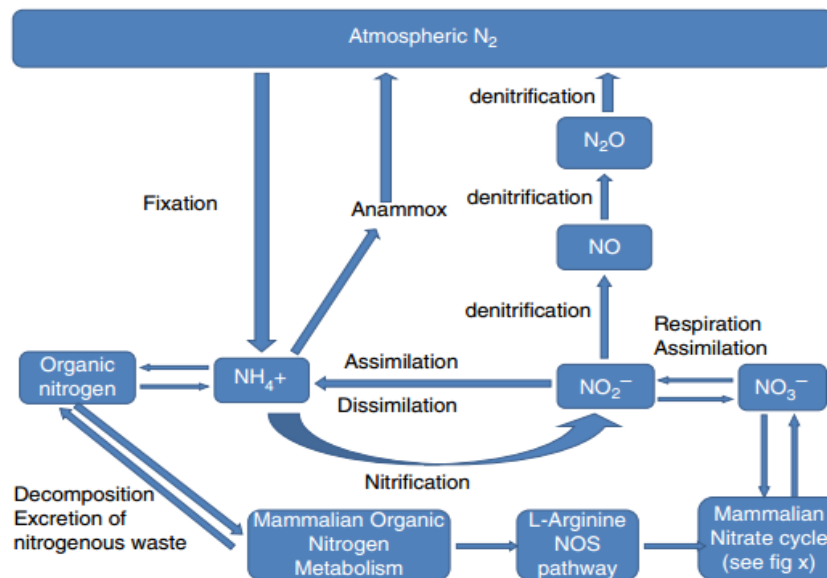
Biological treatment solutions are highly appropriate in efficiently removing contaminants such as ammonia because they can minimize chlorine consumption and disinfection by-products generated by the interference between ammonia and chlorine during the disinfection process (Rittmann, Huck & Bouwer 1989). Therefore, biological methods to remove nitrogen in water sources have been studied since the early 1990s (Pearce & Williams 1999). While nitrification is known as one effective option to biologically convert ammonia into nitrate, which does not increase chlorine demand for disinfection, denitrification is indicated as a powerful solution to transfer nitrate into nitrogen gas which is released into the atmosphere. Unfortunately, most of this research has focused on either nitrification or denitrification processes. Meanwhile, only a few researchers have investigated combining nitrification with denitrification, such as Cecen and Gönenç (1992), Kuai and Verstraete (1998), and Furukawa et al. (2006). Nevertheless, these studies were regularly conducted on wastewaters, which have extremely high nitrogen concentrations compared with those in surface water and groundwater. Hence, further study on the integration of

nitrification with denitrification is necessary at lower concentrations of ammonia contamination.

## 1.2. Nitrogen

### 1.2.1. Nitrogen compounds

Nitrogen plays a significant role in all life on Earth and is an essential element in biological processes because it is a primary factor for building blocks of proteins, amino and nucleic acids as well as other cellular constituents (Bryan 2011; Ward & Jensen 2014). The largest source of nitrogen is located in the Earth's atmosphere which accounts for about 78 percent of the atmosphere. However, the vast majority of living organisms cannot directly access atmospheric nitrogen which is an inert nitrogen form; therefore, this gaseous nitrogen must be converted to usable nitrogen compounds, such as ammonia and nitrate, by micro-organisms. Normally, these transformations of nitrogen occur in the soils and they are grouped into a system which is named as the nitrogen cycle (Figure 1.1).



**Figure 1.1.** The nitrogen cycle (Modified from Bryan 2011)



Nitrogen compounds are diverse. An overview of the nitrogen forms is provided in Table 1.1 below. However, several nitrogen compounds including nitrogen gas, ammonium, ammonia, nitrate and nitrite are seen as the most common compounds in general (Tchobanoglous et al. 2003; Wall 2013). In surface water and groundwater, ammonium, nitrite and nitrate are believed to be important compounds because they are one of the key factors leading to contamination of water sources and, therefore, have negative effects on the public health (Shrimali & Singh 2001).

**Table 1.1.** Overview of the nitrogen forms (Adapted from Tchobanoglous et al. 2003; Wall 2013)

<b>Nitrogen parameter</b>	<b>Abbreviation</b>	<b>General description</b>
Ammonia	NH <sub>3</sub>	Ammonia is low concentration in both surface and groundwater.
Ammonium	NH <sub>4</sub> <sup>+</sup>	Ammonium is regularly measured together with ammonia in a laboratory. Normally. The level of ammonium is higher than the concentration of ammonia, however it is less toxic.
Nitrate	NO <sub>3</sub> <sup>-</sup>	Nitrate is the primary form of nitrogen in water sources. It is dissolved in water and simply moves through soils.
Nitrite	NO <sub>2</sub> <sup>-</sup>	Nitrite is low level in waters and is frequently measured together with nitrate in a laboratory.

Organic nitrogen	Organic N	TKN – (NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup> )
Inorganic nitrogen	TIN	NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup> + NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup>
Total Kjeldahl nitrogen	TKN	Organic N + NH <sub>3</sub> + NH <sub>4</sub> <sup>+</sup>
Total nitrogen	TN	TKN + NO <sub>3</sub> <sup>-</sup> + NO <sub>2</sub> <sup>-</sup>

### 1.2.2. Nitrogen contamination

Surface water and groundwater are the main sources for provision of drinking water supply and other purposes throughout the world. However, a number of these water sources are seriously polluted by nitrogen compounds such as ammonia and nitrate. In a number of countries in Asia, such as Vietnam and China, the level of nitrate in both groundwater and surface water has steadily increased in recent times and exceeded the safe level which is 10 mg L<sup>-1</sup> of nitrate nitrogen based on the WHO standards (Kumazawa 2002). A survey conducted in six regions in northern Vietnam indicates that the concentrations of nitrate nitrogen of most samples are greater than 10 mg L<sup>-1</sup>; however, the nitrate in some samples reached up to 34 mg L<sup>-1</sup> (Cam et al. 2008). Meanwhile, in the European Union, Angelopoulos et al. (2009) report that the concentrations of nitrate nitrogen in over 24% of monitoring stations were above 40 mg L<sup>-1</sup> and 7% of total stations showed the level in a range of 25 – 0 mg L<sup>-1</sup> between 2000 and 2003. In the USA, up to 400 thousand sites of water sources were reported to be polluted by nitrogen (Yang & Lee 2005). In addition, the levels of nitrate nitrogen in 36% of the sample wells in the Pantanos stream of Argentina were higher than 10 mg L<sup>-1</sup>. There is also evidence of nitrogen pollution in surface water and ground water in Oceania. For example, in Australia, the concentration of nitrate nitrogen from 15 to 54

mg L<sup>-1</sup> was observed in the Northern Territory (Salvestrin & Hagare 2009). Therefore, it seems clear from these studies that nitrogen contamination in water sources has been found in many areas in the world and there are signs that its concentration has slightly increased over time.

### 1.2.3. Sources of nitrogen

Nitrogen sources leading to contamination in surface water and groundwater are divided into two main sources including artificial and natural sources. Agricultural irrigation, nitrogenous fertilizers, and human and animal waste are key artificial nitrogen sources generating pollution in water while the important natural nitrogen sources include atmospheric nitrogen deposition, contaminated land and river-aquifer interaction (Liu et al. 2013; Wall 2013).

### 1.2.4. Health effects of nitrogen

Nitrite and nitrate in drinking water are the most toxic forms of nitrogen which can cause severe health problems, even death if their concentrations are excessive. Whilst nitrite is known as the most toxic form of nitrogen to humans, nitrate also generates negative effects on public health, especially infants, because it not only is directly toxic but also can be converted to nitrite by bacteria in the human body (Wall 2013; WHO 2008). The conversion can be performed by bacteria in the stomach under particular conditions such as gastrointestinal infections or low gastric acidity. Additionally, the colon and distal small intestine are also vital sites for the conversion of nitrate into nitrite (Ward et al. 2005; WHO 2008). Humans can also encounter serious illnesses, such as methaemoglobinemia or “blue baby syndrome” in infants, hypertension and cancer, when they are using water sources accompanied by high levels of nitrate and nitrite (Knobeloch et al. 2000). Methaemoglobinemia causes shortness of breath and blueness of the skin which raises the most serious concerns, because it can directly affect infants. Methaemoglobinemia is the result of the reaction of nitrite with haemoglobin

in red blood cells, which will lead to a changed state of haemoglobin into methemoglobin. This altered form will significantly contribute to reducing the transport capacity of oxygen, even blocking these transports (El Midaoui et al. 2002; Knobloch et al. 2000; Luk & Au-Yeung 2002; WHO 2008). To limit the serious effects on public health, removing or reducing the concentration of nitrate and nitrite in drinking water is essential.

### **1.3. Nitrification**

#### **1.3.1. Nitrifying Trickling Filters (NTFs)**

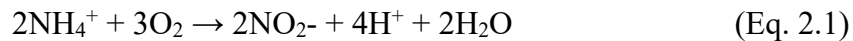
There are a number of methods to convert ammonia or ammonium into nitrate. However, application of high rate NTFs for raw water treatment is one of the most effective methods. This method is not only efficient for removing high ammonia concentration but is also a reliable solution in dealing with low ammonia concentration in surface water and groundwater (Pearce & Williams 1999; Vayenas & Lyberatos 1994).

Trickling filters have been successfully applied in the elimination of inorganic and organic substances in wastewater and potable water since the 1890s (Boller, Gujer & Tschui 1994). In general, a trickling filter system includes an influent water distribution system, trickling filter media, ventilation gates and effluent pipes or channels. Although each part of the system plays an important role in the system, trickling filter media is believed to be central in performance of this system. The media is divided into two groups based on its attributes, including rock and plastic media groups (Lewandowski & Boltz 2011). While the rock media group had been commonly applied for over 100 years, plastic media group have been developed and replaced rock media since the 1950s. The efficiency of plastic media is believed to be superior to the rock group because plastic media can assist in increasing hydraulic rates and limiting clogging (Tchobanoglous et al. 2003).

Trickling filters used to eliminate ammonia or ammonium are known as NTFs in which two microbiological processes occur including autotrophic and heterotrophic nitrification. The efficacy of NTFs in removal of ammonia and ammonium in raw water was demonstrated by a series of pilot experiments conducted by van den Akker et al. (2008) and van den Akker (2008). In these experiments, low ammonia concentrations from 0.5 to 5.0 mg NH<sub>4</sub>-N L<sup>-1</sup> were successfully removed under high hydraulic load between 72.5 and 145 m<sup>3</sup> m<sup>-2</sup> day<sup>-1</sup>.

### 1.3.2. Autotrophic nitrification

Nitrification is the process including two stages by which ammonium or ammonia is converted to nitrite followed by the transformation of nitrite to nitrate. This process cannot remove the nitrogen compound, nevertheless it assists to transform the toxic nitrogen forms ammonium or ammonia to nitrate form which can be removed by the denitrification process. In the first stage, the oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) into nitrite by ammonia oxidizing bacteria (AOB) is presented according to Equation 2.1 (Noda et al. 2004; Sharma & Ahlert 1977; Tchobanoglous et al. 2003).

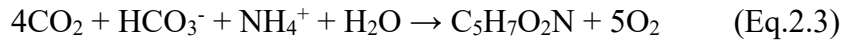


In the second stage, nitrite oxidizing bacteria (NOB) oxidize nitrite into nitrate according to Equation 2.2 (Noda et al. 2004; Tchobanoglous et al. 2003).



The two stages naturally occur in the environment with specialized bacteria such as *Nitrosomonas sp.* and *Nitrobacter sp.* in the first and second stage respectively. These two bacteria are considered as autotrophic bacteria and nitrifying bacteria. They use carbon dioxide as their carbon requirements for

the synthesis of cellular material. This reaction is indicated in Equation 2.3 (Noda et al. 2004; Watson, Valois & Waterbury 1981).



The energy generated by autotrophic bacteria is assigned to fixing carbon dioxide. This is a main reason for the reduced growth rate of nitrifiers. Wiesmann (1994) and Van Benthum, Van Loosdrecht and Heijnen (1997) indicate that the growth rate of nitrifying at 30°C is approximately 0.08 h<sup>-1</sup>, while this rate of aerobic heterotrophic organisms at the same temperature is from 0.3 to 0.5 h<sup>-1</sup>.

### 1.3.3. Heterotrophic nitrification

Heterotrophic nitrification is the process by which inorganic and organic nitrogen forms are oxidized to nitrate by heterotrophic bacteria and fungi (Watson, Valois & Waterbury 1981). In general, the mechanism of heterotrophic nitrification is similar to that in autotrophic ammonia oxidizers. Besides, this mechanism is evaluated to be linked to aerobic denitrification (Conrad 1996; Nyerges & Stein 2009; Prosser 1989).

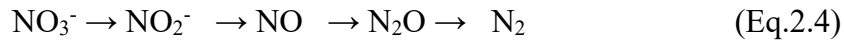
In terms of fungal nitrification, the second mechanism of heterotrophic nitrification is linked to the degradation of lignin. In addition, the mechanism can contribute to reducing organic compounds with hydroxyl radicals (Prosser 1989).

Although heterotrophic nitrification has a similar mechanism to autotrophic nitrification, the cellular rates of heterotrophic bacteria is significantly slower than that in autotrophic organisms. In drinking water systems, the number of heterotrophic nitrifiers may be negligible in comparison with autotrophic nitrifiers (Verstraete, Willy & Alexander 1973; Watson, Valois & Waterbury 1981).

## 1.4. Denitrification

### 1.4.1. Heterotrophic denitrification

Biological denitrification is the process to transform nitrate to dinitrogen gas in the anoxic or anaerobic conditions by the action of denitrifying bacteria. In the sequence of denitrification, nitrate is converted into dinitrogen gas through nitrite, nitric oxide and nitrous oxide as the Equation 2.4 (Fernández-Nava et al. 2010; Tchobanoglous et al. 2003). During the transformations in Eq. 2.4, nitrate and nitrite are used as terminal electron acceptors (Moreno et al. 2005).



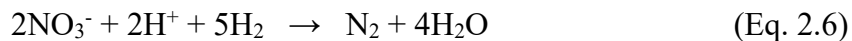
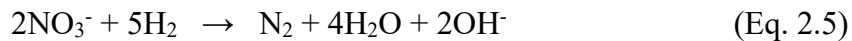
Types of bacteria in the biological denitrification process are diversified compared with the biological nitrification process. These bacteria can use both organic and inorganic carbon sources to act as a hydrogen donor and to supply the biological synthesis (Liljedahl 2014). While bacteria in the autotrophic denitrification tend to use inorganic carbon, that in the heterotrophic denitrification prefer to use organic carbon for their activations (Liu et al. 2013; Van Rijn, Tal & Schreier 2006).

Heterotrophic denitrifiers play a strong role in the success of the microbial denitrification processes. This finding has been confirmed by numerous studies and it is widely applied in the field (Soares 2000). However, intrinsic nitrate degradation cannot protect water sources because this process may occur only slowly in the limited carbon sources conditions (Devlin, Eedy & Butler 2000; Mohseni-Bandpi, Elliott & Zazouli 2013; Strauss & Lamberti 2002). Therefore, external carbon sources are necessary to support bacterial growth, respiration and enrichment. Organic carbon is a key carbon source for heterotrophic denitrifiers and is classified into liquid, solid and gas groups (Liu et al. 2013).

### 1.4.2. Autotrophic denitrification

Apart from heterotrophic denitrifiers, autotrophic denitrification is an effective way of the biological denitrification processes. In some cases, autotrophic denitrification is evaluated to be more powerful than heterotrophic denitrification. This is represented by its advantages over heterotrophic denitrification including reduction of clogging, low biomass build-up and limitation of organic carbon contamination thanks to using inorganic carbon as a carbon and energy source (Ghafari, Hasan & Aroua 2008; Pan 2007; Van Rijn, Tal & Schreier 2006). In the autotrophic growth conditions, several bacteria can gain energy sources and electron donors for microbial metabolic chain by using hydrogen and sulphur compounds such as  $S^0$ ,  $S^{2-}$ ,  $SO_3^{2-}$ , and  $H_2S$  (Matějů et al. 1992). Concerning electron donors, autotrophic denitrification can be divided into hydrogenotrophic and sulphur autotrophic denitrification (Liu et al. 2013).

Hydrogenotrophic denitrification uses hydrogen gas as a substrate in the denitrification processes. This gas is an ideal substrate because it is harmless to public health and does not required post treatments further to remove by-products (Liu et al. 2013). The pathways for hydrogenotrophic denitrification are indicated as Equation 2.5 and 2.6 (Chang, Tseng & Huang 1999; Karanasios et al. 2010).



Regarding sulphur autotrophic denitrification, this process contributes both advantages and drawbacks to autotrophic denitrification. On the one hand, elemental sulphur is not an expensive substrate in comparison with ethanol or methanol which are commonly used in a carbon sources for biological denitrification. In addition, under aerobic conditions, sulphur autotrophic denitrification also can take place, therefore deoxygenating the water sources



is not necessary (Zhang & Lampe 1999). On the other hand, sulphur autotrophic denitrification has to deal with undesirable by-product sulphates and low solubility of reduced sulphurs (Karanasios et al. 2010).

A novel heterotrophic-autotrophic denitrification approach which uses mixed bacteria, pine bark and granulated spongy iron to support its processes was proposed by Liu et al. (2013) to remove nitrogen under the aerobic conditions. Besides pine bark, a number of agricultural residues such as cotton, wood chips and wheat straw has been used as external carbon sources in heterotrophic denitrification processes (Saliling, Westerman & Losordo 2007; Soares & Abeliovich 1998; Volokita, Abeliovich & Soares 1996). However, valuable information on barley straw as a potential carbon source is limited. In heterotrophic-autotrophic denitrification, a series of processes including chemical reduction of dissolved oxygen and nitrate, biological deoxygenation, autotrophic denitrification and heterotrophic denitrification are involved in the heterotrophic-autotrophic denitrification. The results indicate that nearly 100% of nitrate nitrogen was removed after 16 days by this method, in which all above processes contributed to the effectiveness, however, the heterotrophic denitrification accounts for over 83% of the total nitrate nitrogen removal. The denitrification rate was steady at 1.23 to 1.39 mg NO<sub>3</sub>-N L<sup>-1</sup> day<sup>-1</sup> during 3.5 months (Liu et al. 2013).

### **1.5. Factors influencing nitrification and denitrification**

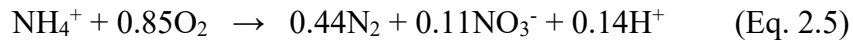
The effectiveness of a biological system heavily depends on nitrification and denitrification rates. However, it is not simple to maintain the high rate of nitrification and denitrification in the biological processes, although there are a large number of studies on their attributes (Noda et al. 2004). A series of studies demonstrate that environmental factors seriously affect the rate of nitrification and denitrification reaction including temperature, oxygen concentration, pH, hydraulic loading rate and inhibiting substances (Chen,

Ling & Blancheton 2006; Jenicek et al. 2004; Liljedahl 2014; Ling & Chen 2005; Sharma & Ahlert 1977; Zanetti et al. 2012). In these studies, deoxygenation and carbon sources are considered as essential factors for biological denitrification.

## **1.6. Integration of nitrification and denitrification**

### 1.6.1. Single stage process

A single stage process for nitrogen removal basically combines nitrification and denitrification in the same reactor to obtain carbonaceous removal, ammonia oxidation and nitrate reduction (Wang, Shammass & Hung 2010). A partial nitrification and anoxic oxidation of ammonia processes simultaneously occur under the oxygen limited environment and, as a result, ammonium is transformed into nitrogen gas. Overall reaction for nitrogen removal in the process is showed in Equation 2.7 (Sliemers et al. 2003).



The integration of nitrification and denitrification in a single stage has been studied extensively since the last decade because of its potential cost advantage and high volumetric nitrogen removal rate in comparison with the separated stage process (Abbas et al. 2014; Wyffels et al. 2003). Numerous nitrogen removal models based on the single state process were developed by different research groups around the world. In which, it is important to mention several typical models including Completely Autotrophic Nitrogen removal over Nitrite (CANON); Aerobic Deammonification (DEMON); Oxygen-Limited Autotrophic Nitrification Denitrification (OLAND); and Single-stage Nitrogen removal using Anammox and Partial nitrification (SNAP); Simultaneous Partial Nitrification, Anammox and Denitrification (SNAD).

The CANON method can achieve a very high nitrogen transformation rate under a low concentration of organic materials. It could remove up to 1.5 kg N (m<sup>3</sup> reactor)<sup>-1</sup> day<sup>-1</sup> and the rate could be higher, up to 20 times if the CANON process was maintained in a gas-lift reactor which has a high oxygen mass transfer rate (Sliemers et al. 2003).

The DEMON process was developed by Hanover University, Germany (Hippen et al. 1997). This model can convert a huge amount of the ammonium into nitrogen gas in aerobic conditions by deammonification. During the process, nitrate and nitrite are known as the intermediary substances (Hippen et al. 1997).

The OLAND system was developed by Kuai and Verstraete (1998) at Ghent University, Belgium. The system uses normal nitrifying sludge as the biocatalyst for the nitrogen removal in water sources in one step. Ammonium is oxidized and converted into nitrogen gas with nitrite as the electron acceptor. The nitrogen removal rate of the OLAND system in the lab-scale was not really high. It was only 16mg of N g of volatile suspended solids<sup>-1</sup> day<sup>-1</sup> corresponding to 50 mg of N L<sup>-1</sup> day<sup>-1</sup> (Kuai & Verstraete 1998).

SNAP was developed to effectively eliminate ammonium as an economical process. In this process, a novel biofilm reactor was applied to remove 60 to 80% of total ammonium under conditions of temperature 35°C, pH 7.5 – 7.7 and DO 2-3 mg. Both anammox bacteria and ammonium oxidizing bacteria were detected in SNAP sludge with the ratio being 15% and 8.7% respectively (Furukawa et al. 2006).

SNAD is a reliable method for nitrogen removal under limited oxygen conditions. The research results show that 19% of ammonium or 70% of total nitrogen were successfully converted into dinitrogen gas, corresponding to 0.69 kg N (m<sup>3</sup> reactor)<sup>-1</sup> day<sup>-1</sup> (Chen et al. 2009).

Although the single stage process has great advantages and can obtain ideal results in lab-scale, it should be realized that this process is dealing with several potential limitations (Wang, Shammas & Hung 2010). As the single stage process is based on anaerobic ammonium oxidation (Anammox), this is considered as the first limitation because of the extended time in the Anammox process, which needs further research to shorten this period. Additionally, the application capacity of the single stage process in the field is not really high and has only been realized at several locations (Zhang et al. 2014).

#### 1.6.2. Separated stage process

Separated stage process is known as the conventional biological process to remove ammonia or ammonium in water sources. The nitrification is the first step of the process followed by denitrification and they are accomplished in separate reactors (Wang, Shammas & Hung 2010; Windey, De Bo & Verstraete 2005). Supplemental carbon and energy sources are necessary to optimize nitrogen remove by the denitrification stage because most of degradable organics in a water source are removed in or prior to the nitrification stage (Wang, Shammas & Hung 2010).

An experiment was conducted by Cecen and Gönenç (1992) in which the integration of nitrification with denitrification occurred in two upflow submerged filters. High strength nitrogenous wastes participated in reaction in the nitrification filter to convert ammonium into nitrate. Before flow into the denitrification filters, diluted molasses was added into the water source. The results show that about 98% of ammonium was transformed in the nitrification step, however, the rate and effectiveness strongly depended on the concentration of oxygen. The finding was logical with the research results which were conducted by a number of researchers, such as Okey and Albertson (1989) and Gönenç and Harremöes (1985).

Rusten, Hem and Ødegaard (1995) developed a moving bed biofilm reactor. Small plastic elements were installed with density less than  $1.0 \text{ g/cm}^3$  in the large surface area. Two options for the integration of nitrification with denitrification were deployed in recirculated systems. In the first option, wastewater was pumped through pre-denitrification reactors before it was nitrified, and then water was returned to pre-denitrification reactors. Conversely, wastewater ran through a nitrification system with post-denitrification in the other option. The external carbon sources were fed into the second system. The results indicate that the latter system was to dominate the former one. In the similar conditions, the first system only converted from 50 to 70% of the total nitrogen, while this number was 80 to 90% in the second system.

In conclusion, nitrogen contamination in surface water and groundwater is a serious problem because it can generate negative effects on public health. Although there are a large number of studies on either nitrification or denitrification, these studies are only a part of completed stages in removing nitrogen in water sources. To absolutely remove nitrogen compounds in water, normally it requires a system which combines both nitrification and denitrification processes. Researches on a complete system to remove nitrogen have been conducted, however, they are too limited in number and they have tended to focus on wastewater which has high nitrogen concentrations greater than surface water and groundwater. Therefore, it is necessary to carry out a further study in the integration of nitrification with denitrification in treatment of nitrogen contamination in surface water and groundwater.

### **1.7. Research Question**

**What is the best method to effectively integrate nitrification and denitrification for surface water and groundwater treatment?**

The integration of a nitrification system with a denitrification system to remove ammonia and other nitrogen compounds in treatment of surface water and groundwater was studied by experimentation at lab-scale. The study was divided into several stages including installation of artificial media for nitrification and development of nitrifying bacteria, followed by supplementation of barley straw as a carbon source, deoxygenation by spongy iron and adjustment to find the optimal process rate for denitrification. It is anticipated that the findings of the study can significantly contribute to a reliable method for the treatment of nitrogen pollution in surface water and groundwater given that studies on the integration of nitrification with denitrification are limited.

This study aims to determine the most suitable combination model between nitrification and denitrification systems in treatment of nitrogen pollution in surface water and groundwater. This model will be very useful for small communities in rural areas and remote towns or villages in developing countries. In these communities, water drawn from rivers, lakes or bores can be easily contaminated by ammonia or ammonium from poor agriculture activities. A commercial water treatment plant may be beyond the economic capability of such communities, however, a low-tech practical system may offer solutions, especially when people do not have access to other potable water sources.

## 2. Material and Methods

### 2.1. Equipment and materials

#### 2.1.1. Equipment

The study was conducted at pilot-scale at the Health and Environment Group Laboratory of Flinders University. The main items of equipment used to collect and analyse data, are listed in Table 2.1 below.

**Table 2.1.** Main equipment used in the study

No.	Name of equipment	Descriptions
1	FOSS - FIAstar 5000 Analyser	Used to measure ammonium (NH <sub>4</sub> -N), nitrite (NO <sub>2</sub> -N) and nitrate (NO <sub>3</sub> -N)
2	TOC-L Shimadzu Analyser	Used to measure total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC)
3	HACH DR 2000	Used to measure total chlorine and free chlorine
4	DO meter – HANNA HI9147	Used to measure dissolved oxygen and temperature
5	pH meter - Jenway 370 pH/mV	Used to measure pH and temperature
6	Air pump	Provide oxygen for water in the reservoir
7	Peristaltic pumps – Watson Marflow 323	Pump and control flow into the pilot system

#### 2.1.2. Materials

Lake water from the main campus of Flinders University was utilized as a water source for the experiment. Ammonium chloride was used as ammonium source to supplement the water sample. Activated sludge

collected from Bolivar Waste Water Treatment Plant was used as a source from which to develop nitrifying bacteria in the Commissioning Phase. Furthermore, barley straw was collected from a hay farm in Kuitpo, South Australia. Several chemical reagents were utilized for samples analysis at the laboratory.

## **2.2. Preparation**

### **2.2.1. Water quality makeup**

In order to simulate an ammonium pollution in water sources, ammonium chloride solution was added to the water sourced from the lake at Flinders University (Figure 2.1). This addition created water samples with different concentrations of ammonium for the respective experimental phases. Ammonium chloride with the formula ( $\text{NH}_4\text{Cl}$ ) was used to adjust the quality of lake water to simulate poor quality of surface water and groundwater. An ammonium stock standard solution  $1000 \text{ mg NH}_4^+ \text{ L}^{-1}$  was prepared by dissolving a desired quantity of ammonium chloride into distilled water. This solution was stored in a refrigerator ( $5^\circ\text{C}$ ) and was stable for at least three months. Before using ammonium stock standard solution, the solution was allowed to reach room temperature. Calcium carbonate ( $70 \text{ mg L}^{-1}$ ) was added to water samples during NTF commissioning phase to supply inorganic carbon for the growth of nitrifying bacteria.

Carbon source plays a primary role in the denitrification processes. However, a low carbon content is quite common in groundwater and some surface water sources. Therefore, a supply of external carbon sources is necessary for bacterial growth. Recently, a number of studies have been conducted to determine the potential carbon sources for denitrification, such as pine bark, cotton, sawdust, wood chips, newspaper and wheat straw, and they have achieved positive results (Aslan & Türkman 2005; Kim et al. 2002; Liu et al. 2013; Robertson, Vogan & Lombardo 2008; Saliling, Westerman & Losordo

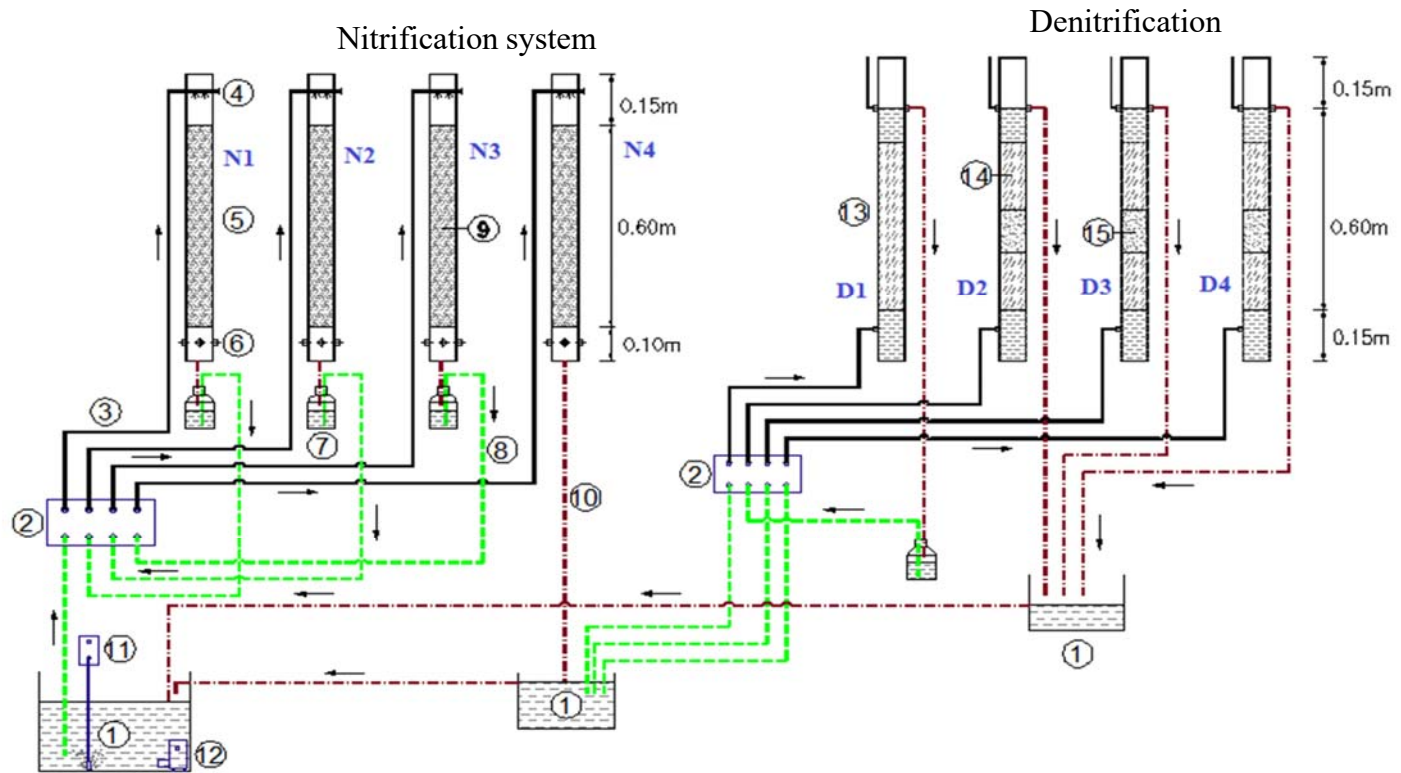


2007). However, valuable information for barley straw as an external carbon source for both nitrification and denitrification processes is still limited. Hence, the suitability of barley straw to provide inorganic and organic carbon for nitrification and denitrification systems was considered in this study.

### 2.2.2. Nitrifying trickling filters (NTFs)

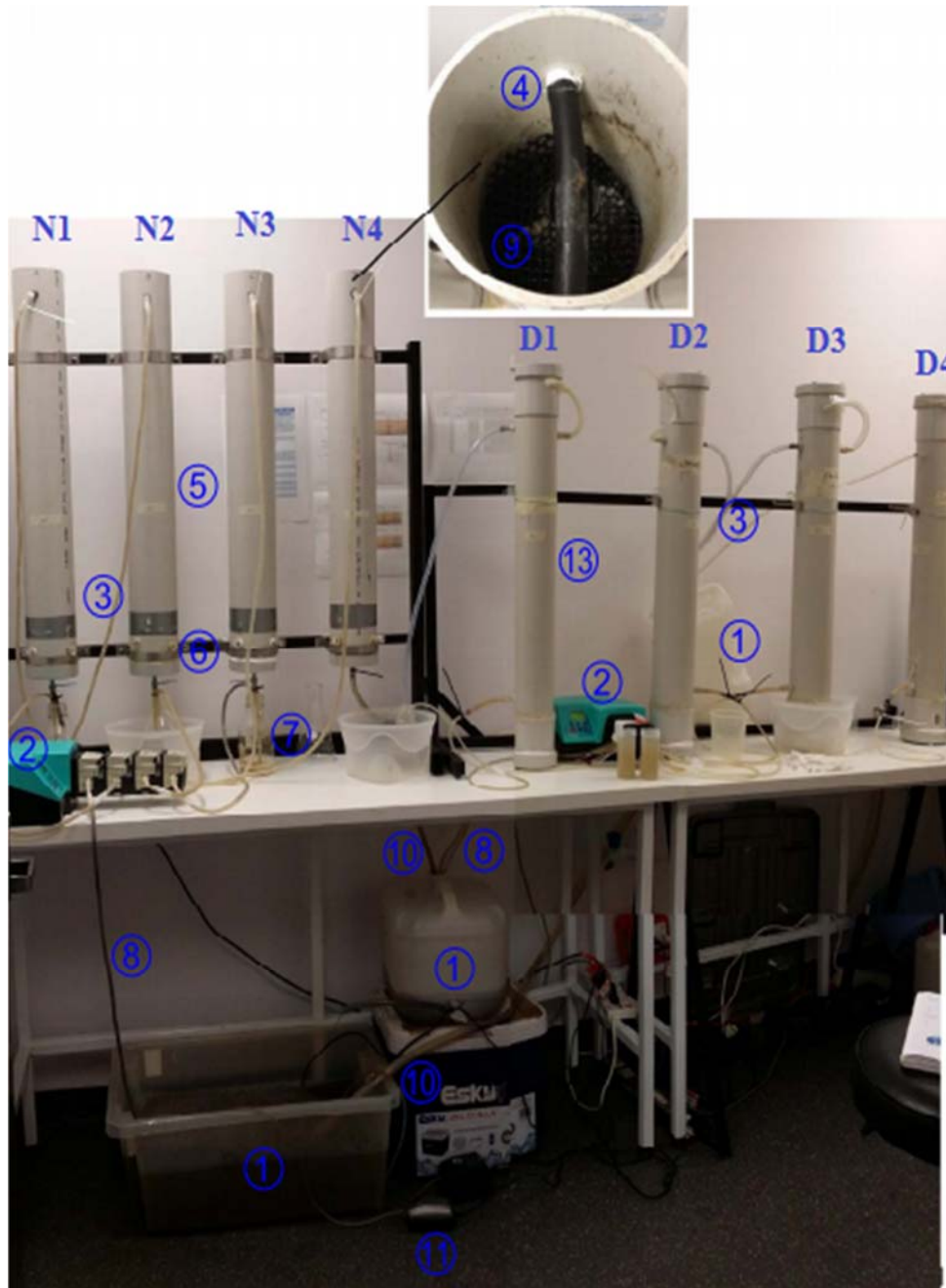
The pilot system including nitrification and denitrification systems was designed as given in Figure 2.1 and shown in Plate 2.1. The NTFs were packed in four polyvinyl chloride columns (0.104 m internal diameter, height 0.85 m). The filters used a bed (thickness, 0.6 m and volume 0.0051 m<sup>3</sup>) of TKP 312 (2H Plastic Australia) polypropylene with area to volume ratio of 240 m<sup>2</sup> m<sup>-3</sup>, void volume of 95% and average foils thickness of 0.35 mm. The effective filter surface area (1.22 m<sup>2</sup>) of each filter was similar. The 4 NTFs were operated in series, with water distribution on to the top of the NTFs by a peristaltic pump at a constant flow rate of 0.3 L min<sup>-1</sup>. The system operated under recirculated flow. The detail characteristics of TKP 312 polypropylene is indicated in Plate 2.2 and Table 2.2.

Following passage through the in series NTF, a fraction of the nitrified effluent was diverted to the denitrification system, and the remainder was returned to the NTF reservoir for recirculation through the NTFs.



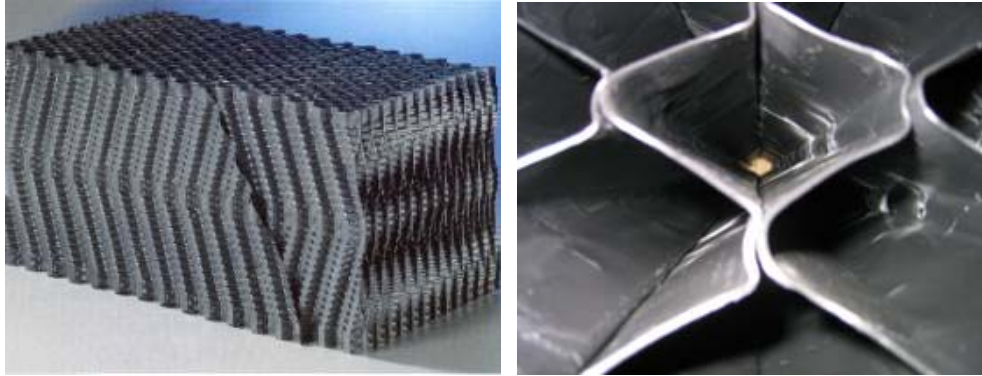
1- Plastic reservoir, 2- Peristaltic pump, 3- Discharge silicone tube, 4- Spray distribution, 5- Nitrifying column, 6- Natural ventilation ports, 7- Glass reservoir, 8- Intake silicone tube, 9- Polypropylene media, 10- Returned silicone tube, 11- Air pump, 12- Stirring pump, 13- Denitrifying column, 14- Barley straw, 15- Spongy iron

**Figure 2.1.** The schematic diagram of nitrification and denitrification systems



*1- Plastic reservoir, 2- Peristaltic pump, 3- Discharge silicone tube, 4- Spray distribution, 5- Nitrifying column, 6- Natural ventilation ports, 7- Glass reservoir, 8- Intake silicone tube, 9- Polypropylene media, 10- Returned silicone tube, 11- Air Pump, 13- Denitrifying column*

**Plate 2.1.** The nitrification and denitrification system



**Plate 2.2.** Structure of TPK 312 polypropylene (2H Plastic Australia n.d)

**Table 2.2.** Characteristics of TPK 312 polypropylene

Specification	Units	Value
Surface area	$\text{m}^2 \text{m}^{-3}$	240
Channel width	mm	12
Void volume	%	95
Average foil thickness	mm	0.35
Dry weight	$\text{kg m}^{-3}$	29
Specific weight	$\text{g cm}^{-3}$	1.03
Dimensions (Diameter x Height)	mm	104 x 300
Max service temperature	$^{\circ}\text{C}$	80
Material		Polypropylene

### 2.2.3. NTF hydraulic loading rate

Influent hydraulic flow rate to the NTFs was  $0.3 \text{ L min}^{-1}$  and constant during the experiment time. This flow rate was controlled by rotational speed of peristaltic pump. Hydraulic surface load of the nitrification system was

defined based on media surface area which was  $0.354 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1}$  as Equation 2.1 below.

$$Q_s = \frac{Q_{fr}}{A} = \frac{0.432}{1.22} = 0.354 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1} \quad (\text{Eq. 2.1})$$

Where  $Q_s$  is hydraulic surface load ( $\text{L m}^{-2} \text{ d}^{-1}$ );  $Q_{Tfr}$  is total hydraulic flow rate in a day ( $Q_{Tfr} = 0.432 \text{ m}^3 \text{ d}^{-1}$ ); and  $A$  is surface area of polypropylene media in a column ( $A = 1.22 \text{ m}^2$ ).

Meanwhile the irrigation rate was  $50.82 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1}$ . This value was calculated from Equation 2.2 and it was based on the total hydraulic flow rate per unit of cross sectional area of a nitrification column per day.

$$Q_I = \frac{Q_{Tfr}}{A_s} = \frac{0.432}{0.0085} = 50.82 \text{ m}^3 \text{ m}^{-2} \text{ d}^{-1} \quad (\text{Eq. 2.2})$$

Where  $Q_I$  is irrigation rate ( $\text{m}^3 \text{ m}^{-2} \text{ d}^{-1}$ ); and  $A_s$  is cross sectional area of a nitrification column ( $A_s = 0.0085 \text{ m}^2$ ).

The corresponding irrigation velocity was  $2.1 \text{ m h}^{-1}$  and it was defined as Equation 2.3 below.

$$V_I = \frac{Q_{fr}}{A_s} = \frac{0.018}{0.0085} = 2.12 \text{ m h}^{-1} \quad (\text{Eq. 2.3})$$

Where  $V_I$  is irrigation velocity ( $\text{m h}^{-1}$ ); and  $Q_{fr}$  is hydraulic flow rate per hour ( $Q_{fr} = 0.018 \text{ m}^3 \text{ h}^{-1}$ ).

The flow rates in the denitrification system were  $1.5$ ,  $1.2$  and  $1.5 \text{ ml min}^{-1}$  for Group 1, 2 and 3 respectively in all phases except Experimental Phase 4. In the Phase 4, the flows rates in each group of the denitrification system were increased three fold. They were  $4.5 \text{ ml min}^{-1}$  for Groups 1 as well as 3, and  $3.6 \text{ ml min}^{-1}$  for Group 2.

#### 2.2.4. Denitrifying design and operation

Denitrification system was divided into three groups: Group 1 comprised the first two columns in the series, Group 2 was the third column and Group 3 with the last column. The descriptions of each Group are showed in Table 2.3 below. The nitrified effluent from the in series NTF was delivered by peristaltic pump to the base of each enclosed, denitrifying filter to ensure that barley straw and spongy iron in each column were submerged. Following passage through the denitrifying filters, the water was returned to the reservoir of the nitrification system for subsequent recirculation through the NTFs and the denitrifying filters. Barley straw (200g/filter) provided organic carbon for the denitrifying processes, while spongy iron was included to assist in elimination of dissolved oxygen in the water.

**Table 2.3.** The descriptions of groups in the denitrification system

Groups	Columns	Inside diameter (m)	Total height (m)	Effective filter height (m)	Materials	
					Barley straw (g)	Spongy iron (g)
1	1	0.104	0.9	0.75	200	-
	2	0.104	0.9	0.75	200	20
2	3	0.104	0.9	0.75	200	20
3	4	0.104	0.9	0.75	200	20

### 2.3. Methods

In the study, the experiment was divided into five main phases including Commissioning phase, Experimental phase 1, Experimental phase 2, Experimental phase 3, and Experimental phase 4. In the commissioning

phase, nitrifying bacteria were developed in NTFs in the presence of ammonium spiked lake water. After that, all water in the system was replaced by new lake water spiked with ammonium in the Experimental phase 1. This Phase assisted to basically define the capacity of nitrification and denitrification systems. Thereafter, capacity of the nitrification and denitrification systems were measured in the Experimental phase 2 and 3 respectively. A further explanation of these phases is provided below.

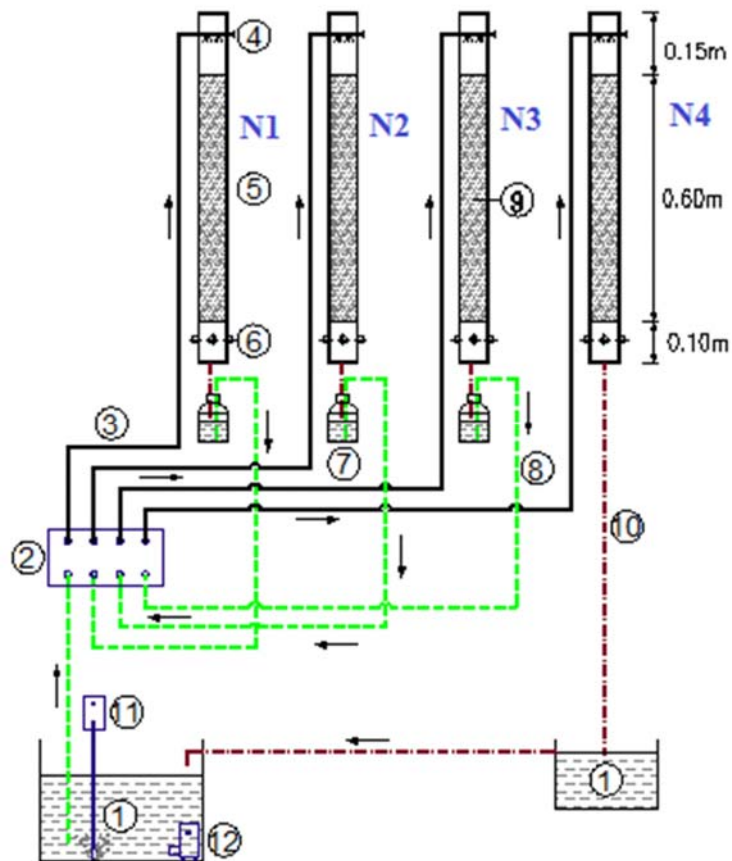
### 2.3.1. Commissioning phase: Growing bacteria

The Commissioning phase was conducted over 20 days in three stage. In the first two stages of the Commission phase, only nitrification system was operated. The first stage (7 days) polypropylene media was immersed in diluted activated sludge (Plate 2.3), which was collected from Bolivar Waste Water Treatment Plant. Enrichment of nitrifying bacteria was stimulated by the addition of 10 mg  $\text{NH}_4\text{-N L}^{-1}$ . *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria in the nitrification system use carbon dioxide for syntheses and growth. To alleviate potential inorganic carbon limitation issues, an external carbon source, being calcium carbonate ( $70 \text{ mg L}^{-1}$ ), was added to the activated sludge. Activated sludge was circulated by a pump and aerated.



**Plate 2.3.** Immersing polypropylene media in diluted activated sludge

In stage 2 of the commissioning the media was packed into the nitrifying columns and the activated sludge was recirculated through the filter media over 3 days at a flow rate of  $0.3 \text{ L min}^{-1}$  (Figure 2.2). The activated sludge was supplemented with  $5 \text{ mg NH}_4\text{-N L}^{-1}$  and  $35 \text{ mg}$  of carbonate. The return activated sludge resident in the reservoir was continuously mixed and aerated before recirculation through the NTFs.



- 1- Plastic reservoir, 2- Peristaltic pump, 3- Discharge silicone tube,  
 4- Spray distribution, 5- Nitrifying column, 6- Natural ventilation ports,  
 7- Glass reservoir, 8- Intake silicone tube, 9- Polypropylene media,  
 10- Returned silicone tube, 11- Air pump, 12 – Stirring pump

**Figure 2.2.** The schematic diagram of diluted activated sludge circulation in the nitrification system



In Stage 3, the last 10 days of the commissioning, the activated sludge was replaced by 60 L water collected from the lake at the main campus of Flinders University. Then, 2.5 mg NH<sub>4</sub>-N L<sup>-1</sup> was added to this water to simulate the water quality of a polluted surface or groundwater. The water was pumped to the nitrification system at a flow rate of 0.3 L min<sup>-1</sup>. Most effluent water was circulated to the nitrifying reservoir, from which 4.2 ml min<sup>-1</sup> was pumped to the feed reservoir for the denitrification system with the denitrified effluent returned to the nitrifying systems feed reservoir. The nitrifying reservoir was kept aerated and stirred. The schematic diagram of this stage is shown as Figure 2.1.

### 2.3.2. Experimental phase 1: Defining the capacity of nitrification and denitrification systems

Experimental phase 1 was conducted over 14 days to determine the efficiency of both the nitrification and denitrification systems. The results of this Phase play a significant role in determining influent ammonium and nitrate concentration in the following Phases. In the first 7 days, 60 L of fresh lake water was prepared to replace the water used in the final stage of commissioning. In addition, 150 mg NH<sub>4</sub>-N (4.3 mg NH<sub>4</sub>-N L<sup>-1</sup>) was added into the nitrifying reservoir containing 35 L of lake water. The rest of new lake water (25 L) was added into denitrifying columns. The flow in the nitrification system was still circulated at a constant hydraulic rate at 0.3 L min<sup>-1</sup>, while the flow rates through the denitrification system were 1.5, 1.2 and 1.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively. In the following 7 days, 0.6 mg NH<sub>4</sub><sup>+</sup> L<sup>-1</sup> was daily loaded into the nitrification system. Nitrate converted from ammonium by nitrifying columns was pumped to the denitrifying columns to primarily evaluate the effectiveness of the denitrification system. All other conditions such as stirring and aeration remained the same as in the previous Phase. The schematic diagram of the systems is showed as Figure 2.1.

### 2.3.3. Experimental phase 2: Measuring maximum capacity of the nitrification system

The whole volume of water in the both nitrification and denitrification systems was replaced by 50L of new lake water. In the first 7 days of the Phase, 150 mg NH<sub>4</sub>-N day<sup>-1</sup> was daily loaded to the system and following six days, this figure was reduced to 100 mg NH<sub>4</sub>-N day<sup>-1</sup>. All conditions were similar to the previous Phase. The schematic diagram of the systems is showed as Figure 2.1.

### 2.3.4. Experimental phase 3: Measuring maximum capacity of the denitrification system

The last experimental phase was conducted for 5 days including two sub stages. The first stage was operated in the first four days. A constant concentration of ammonium (100 mg NH<sub>4</sub>-N L<sup>-1</sup>) was continuously supplied to the nitrification system. The water in the NTF feed reservoir of stirred and aerated. However, the flow rates to the denitrification system were increased three times compared with those in Phases 1 and 2. They were 4.5, 3.6 and 4.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively.

Follow that, the operation of the NTFs was paused and whole water in denitrification columns was pumped out. Fresh lake water, 35L with 22.86 mg NO<sub>3</sub>-N L<sup>-1</sup> was supplied to the denitrification system. The operation of air pump and stirring pump were remained. The system was operated over a day and sampling was conducted hourly.

## 2.4. Sampling and data analysis

During the experiment, lake water that was prepared for the experiment was sampled. Additionally, influent and effluent samples of each column of both nitrification and denitrification systems was collected daily. About 50 ml of water was collected for each sample at 11am ( $\pm$  1h). At the same time of

sampling, the dissolved oxygen (DO), the temperature and the potential of hydrogen (pH) was measured by a DO meter and a pH meter. The samples were filtered through glass microfiber filters (exclusion size, 4  $\mu\text{m}$ ) before analysis. This filtration can assist to eliminate negative effects of sediment on the analysis results of ammonium, nitrite and nitrate concentrations.

All lake water samples were measured free and total chlorine by HACH DR 2000. These measurements are necessary because chlorine in lake water can interact with ammonium to reduce the concentration of ammonium in water samples. Water samples were analysed for total organic carbon (TOC), inorganic carbon (IC) were analysed by TOC-L Shimadzu Analyzer. Ammonium ( $\text{NH}_4\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ) and nitrate ( $\text{NO}_3\text{-N}$ ) were analysed as described in Standard Methods for the Examination of Water and Wastewater (Greenberg et al., 1992) using a FOSS - FIAstar 5000 Analyzer.

### **3. Results**

#### **3.1. Quality of lake water**

All lake water samples used in the experiment were analysed for free chlorine ( $\text{Cl}_2$  free), total chlorine ( $\text{Cl}_2$  total) – since Flinders Lake is supplied with potable water, DO, ammonium, nitrate, nitrite, total organic carbon and inorganic carbon. The results are shown in Table 3.1. The results indicate that virtually all inorganic nitrogen compounds including ammonium and nitrate were not present in the lake water samples. Only very small amounts of nitrite nitrogen, which is not stable, were found in two of four lake water samples. In addition, the concentration of free and total chlorine also was low  $0.01 \text{ mg L}^{-1}$  to  $0.09 \text{ mg L}^{-1}$  and  $0 \text{ mg L}^{-1}$  to  $0.06 \text{ mg L}^{-1}$  respectively. These concentrations of both free and total chlorine could not create negative effects on the performance of the nitrification and denitrification systems.

**Table 3.1.** The quality of lake water before being used for the experiment

<b>Date</b>	<b>DO mgL<sup>-1</sup></b>	<b>pH</b>	<b>Cl<sub>2</sub> free mgL<sup>-1</sup></b>	<b>Cl<sub>2</sub> total mgL<sup>-1</sup></b>	<b>NH<sub>4</sub>-N mgL<sup>-1</sup></b>	<b>NO<sub>2</sub>-N mgL<sup>-1</sup></b>	<b>NO<sub>3</sub>-N mgL<sup>-1</sup></b>	<b>TOC mgL<sup>-1</sup></b>	<b>IC mgL<sup>-1</sup></b>
04/6/2016	6.0	7.65	0.09	0.01	0	0.05	0	4.49	5.31
11/6/2016	6.3	7.43	0.01	0	0	0.04	0	4.62	5.84
18/6/2016	5.8	7.35	0.03	0.06	0	0	0	6.32	8.24
21/7/2016	5.83	7.46	0.06	0.05	0	0	0	1.81	7.57

### **3.2. Commissioning phase: Growing bacteria**

The Commissioning phase was conducted over 20 days (25/05/2016 to 13/06/2016). Firstly, polypropylene media was immersed in diluted activated sludge collected from Bolivar Waste Water Treatment Plant for 7 days (25/05/2016 to 31/05/2016). During this time, nitrifiers were fed with 10 mg  $\text{NH}_4\text{-N L}^{-1}$  from ammonium chloride and 70 mg  $\text{L}^{-1}$  of carbonate from calcium carbonate. Activated sludge was stirred and provided oxygen by a stirring pump and an air pump respectively. pH, DO and temperature were measured daily at 11.00 am ( $\pm 0.5\text{h}$ ). The value of pH was in the range of 7.5 to 8, while DO ranged from 6.0 to 7.6 mg  $\text{L}^{-1}$  at 19°C.

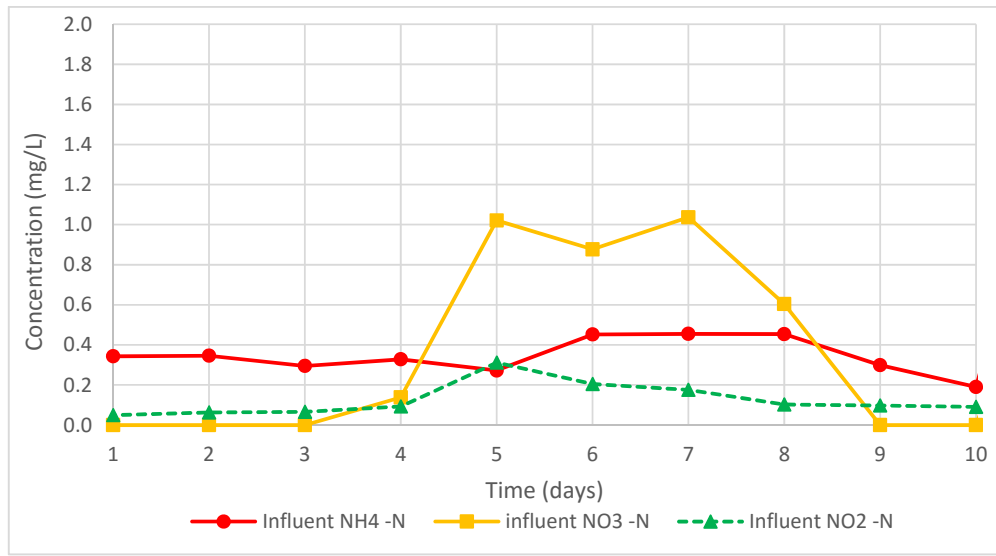
After that, the development of biofilm was continued by packing polypropylene media to nitrifying columns. The sludge was recirculated to the media during the following three days (01/06/2016 to 03/06/2016) at a flow rate of 0.3 L  $\text{min}^{-1}$ . Ammonium (5 mg  $\text{NH}_4\text{-N L}^{-1}$ ) and carbonate (35 mg  $\text{L}^{-1}$ ) were added to the system in this stage. Moreover, the operation of stirring pump and air pump were continued. pH, DO and temperature were continually measured. The pH values were between 7.6 and 8.0. Meanwhile the DO value was around 6.4 mg  $\text{L}^{-1}$  at room temperature (20°C).

Finally, the activated sludge solution was replaced by 60 L water collected from the lake at the main campus of Flinders University on 4th June 2016. This stage was maintained over 10 days (04/06/2016 to 13/06/2016). In order to make up water quality of polluted surface water and groundwater, 2.5 mg  $\text{NH}_4\text{-N L}^{-1}$  was added to the prepared water. The water was loaded onto the NTFs in re-circulation mode at a flow rate of 0.3 L  $\text{min}^{-1}$ . Most effluent water was returned to the nitrifying reservoir, while only 4.2 ml  $\text{min}^{-1}$  was loaded to the denitrification system. The denitrification flows also returned to the nitrifying reservoir. The reservoir was kept aerated and stirred as described above. The changes of ammonium, nitrate and nitrite were monitored at the

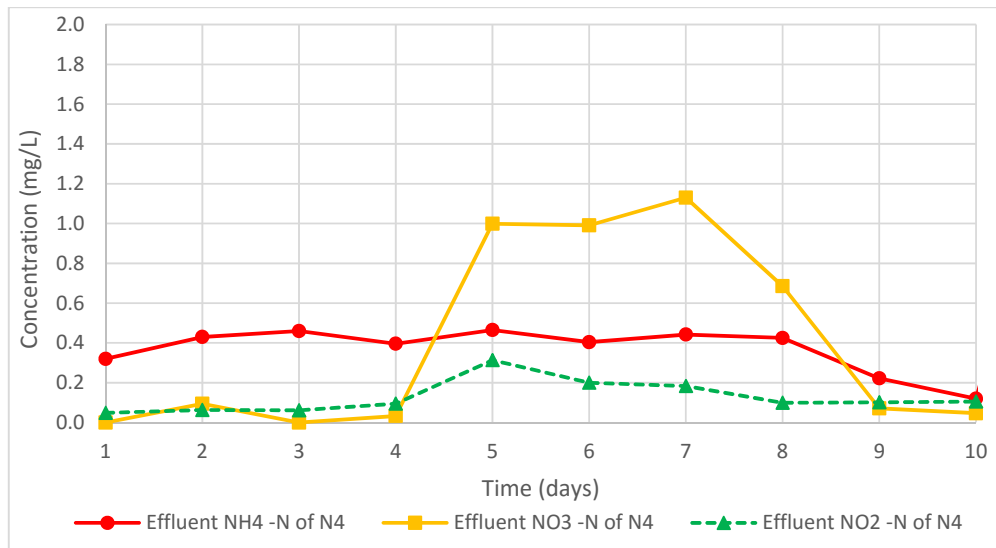
nitrifying reservoir and effluent flow of the nitrification system. The results are shown as Figure 3.1, Figure 3.2, and Figure 3.3.

As presented in Figure 3.1 and Figure 3.2, the pattern of nitrate and nitrite in influent and effluent points of the nitrification system was quite similar. In the first 4 days, the effectiveness of the system was limited. The concentration of nitrate and nitrite was constant and under  $0.15 \text{ mg L}^{-1}$ . However, the nitrate mass formed significantly increased to  $1.0 \text{ mg L}^{-1}$  in the following day, while this mass of nitrite also reached to over  $0.3 \text{ mg L}^{-1}$ . After that the nitrate production remained constant, reducing after day 7. The comparison of change in ammonium, nitrate and nitrite in the influent and effluent are shown in Figure 3.3.

Furthermore, the change in ammonium, nitrate and nitrite also were measured at influent and effluent points of all denitrification columns. Figure 3.4 presents results of the changes in ammonium in the denitrification system during the 10 days of the Commissioning phase. It is obvious that the influent ammonium concentration to the denitrification system was smaller than that in the effluent of each denitrification columns. The ammonium concentrations in denitrifying columns were quite high in the first days. They were between over  $1.0$  and  $5.5 \text{ mg NH}_4\text{-N L}^{-1}$ . While the influent ammonium concentration was only below  $0.5 \text{ mg NH}_4\text{-N L}^{-1}$ . Even in the last days of Commissioning Phase, effluent ammonium concentrations were still double influent ammonium concentration. It is unusual because normally, effluent concentration is equal or lower influent concentration. The high effluent ammonium concentrations was due to ammonification of the barley straw. The comparison of influent and effluent concentration of nitrate and nitrite of the denitrification system is shown in Figure 3.5, Figure 3.6, Figure 3.7 and Figure 3.8 respectively. The results indicate that while the influent concentration of nitrate was quite high between day 5 and 8, effluent concentration was zero.

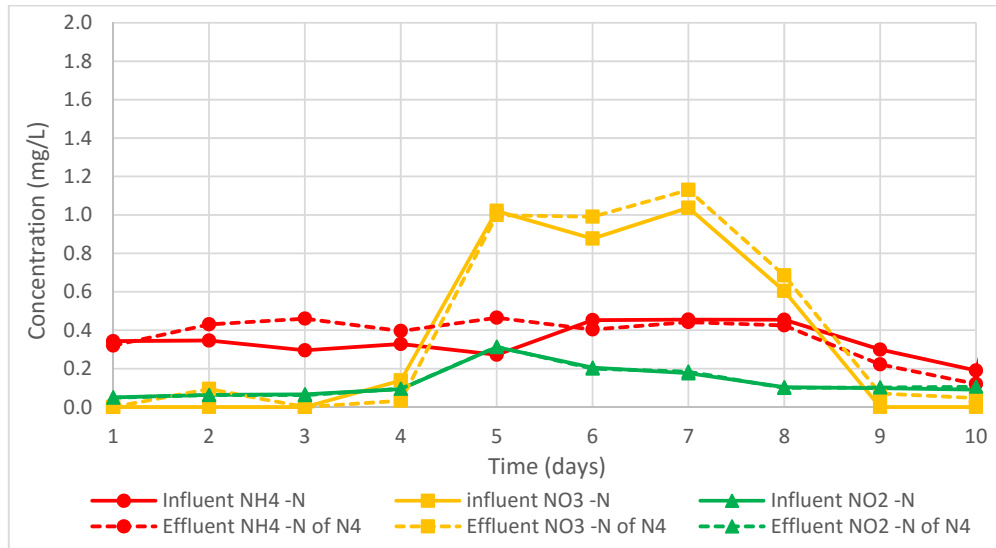


**Figure 3.1.** Influent ammonium, nitrate and nitrite nitrogen of the nitrification system during Commissioning Phase under following conditions: Hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $2.5 \text{ mg NH}_4\text{-N L}^{-1}$

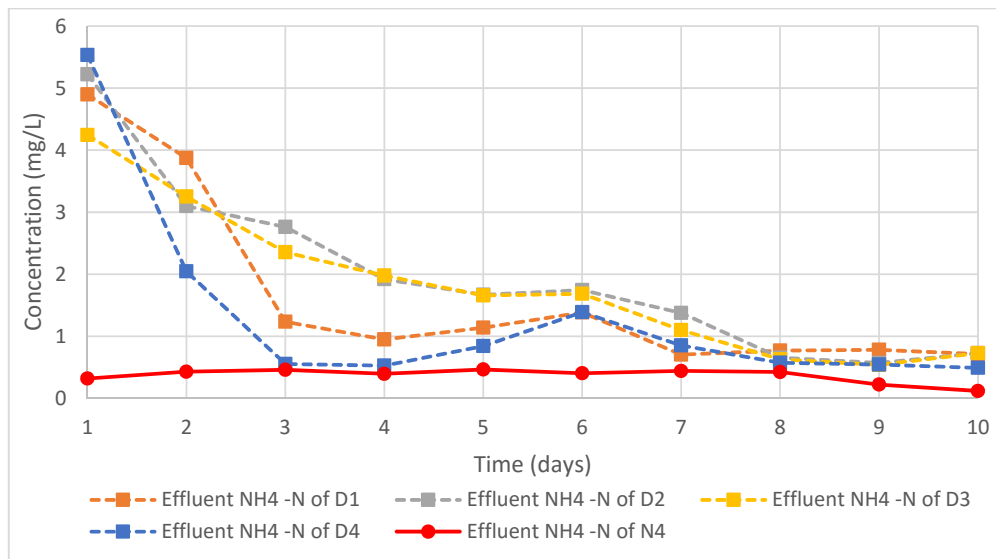


**Figure 3.2.** Effluent ammonium, nitrate and nitrite of nitrogen the nitrification system during Commissioning Phase under following conditions: Hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $2.5 \text{ mg NH}_4\text{-N L}^{-1}$

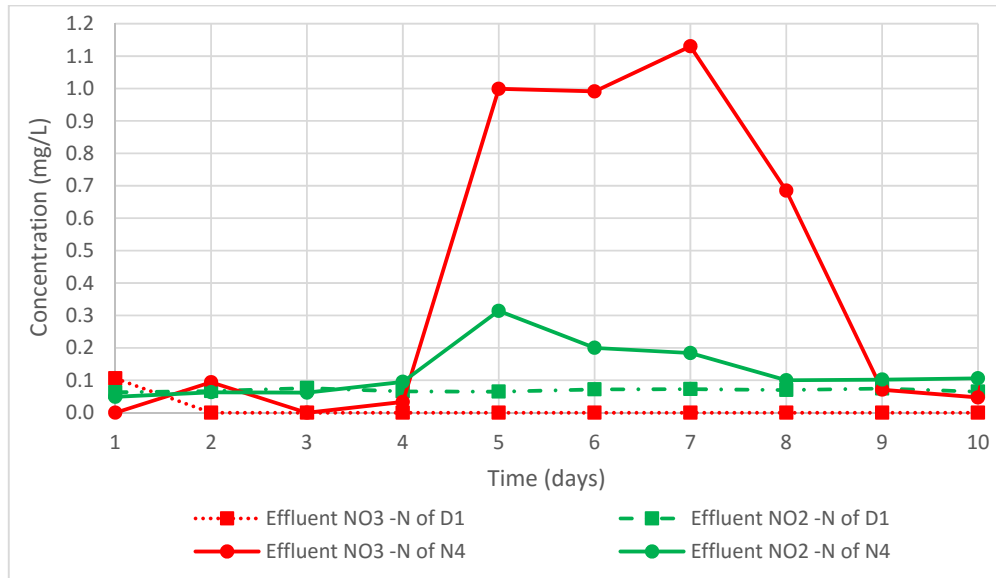




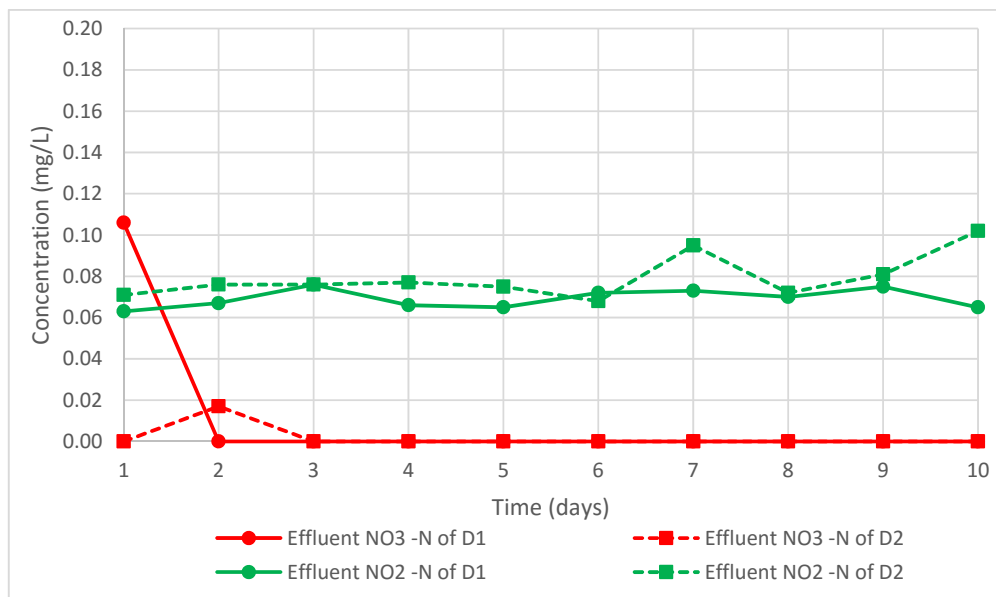
**Figure 3.3.** Comparison between influent and effluent ammonium, nitrate and nitrite nitrogen of the nitrification system during Commissioning Phase under following conditions: Hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $2.5 \text{ mg NH}_4\text{-N L}^{-1}$



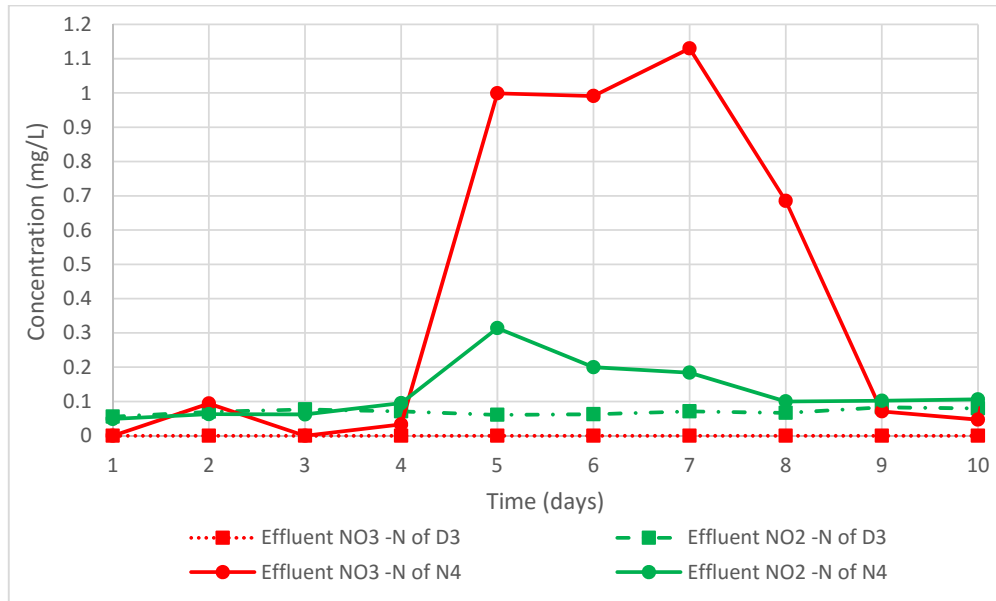
**Figure 3.4.** Influent and effluent ammonium of the nitrification and denitrification systems during Commissioning Phase under hydraulic rate of  $1.5 \text{ ml min}^{-1}$  for Column 1 and 2, and  $1.2, 1.5 \text{ ml min}^{-1}$  for Column 3 and 4 respectively



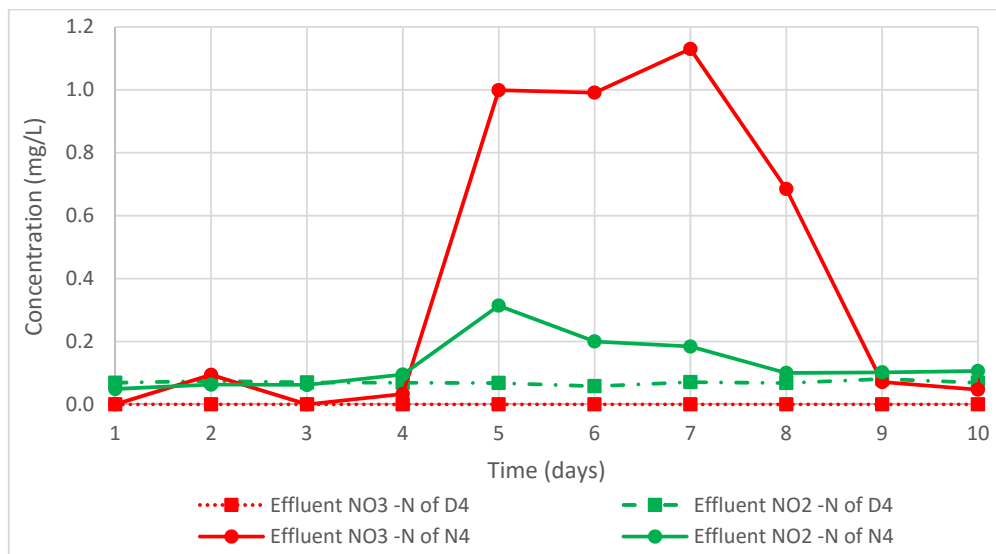
**Figure 3.5.** Comparison between influent and effluent nitrate and nitrite of Column 1 of the denitrification system during Commissioning Phase under hydraulic rate of  $1.5 \text{ ml min}^{-1}$



**Figure 3.6.** Comparison between influent and effluent nitrate and nitrite of Column 2 of the denitrification system during Commissioning Phase under hydraulic rate of  $1.5 \text{ ml min}^{-1}$



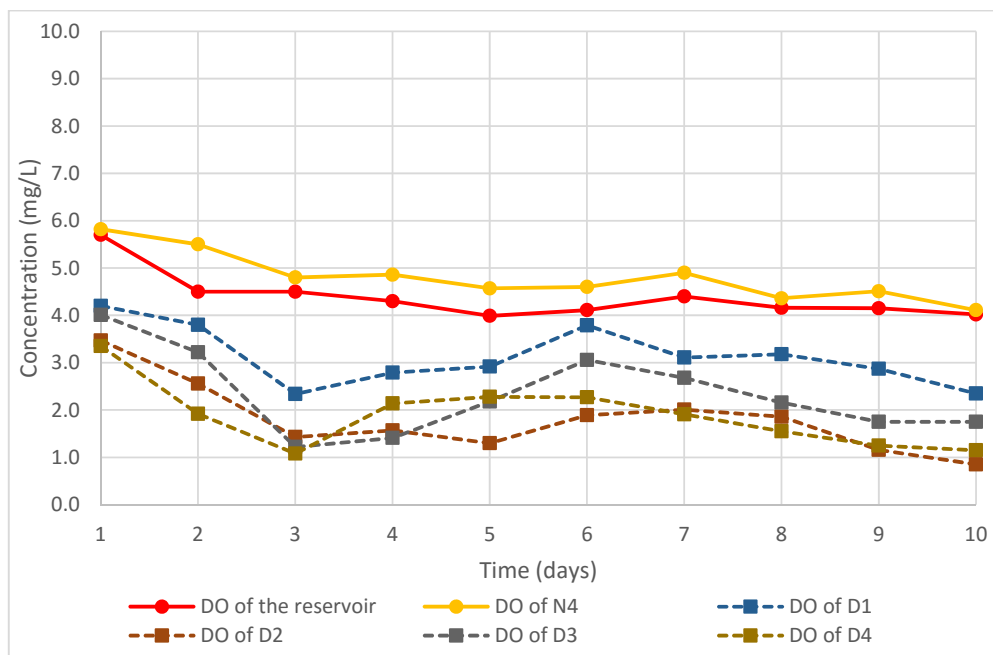
**Figure 3.7.** Comparison between influent and effluent nitrate and nitrite of Column 3 of the denitrification system during Commissioning Phase under hydraulic rate of  $1.2 \text{ ml min}^{-1}$



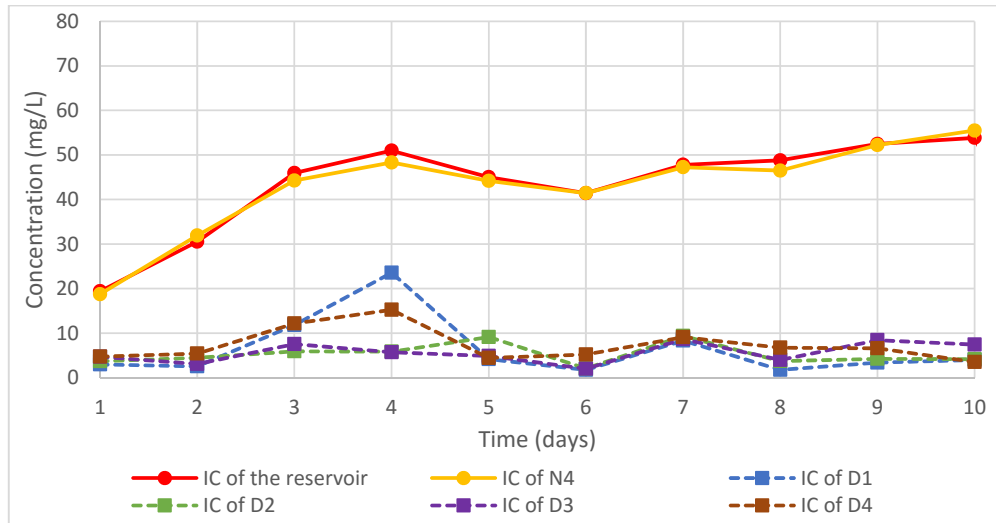
**Figure 3.8.** Comparison between influent and effluent nitrate and nitrite of column 4 of the denitrification system during Commissioning Phase under hydraulic rate of  $1.5 \text{ ml min}^{-1}$

Furthermore, pH, DO and temperature were monitored daily. The pH values in the nitrification and denitrification system were from 7.7 to 8.5 and 4.0 to 5.9 respectively. Meanwhile the room temperature was around 21°C. The DO concentrations of both systems is shown in Figure 3.9. A trend of reducing DO concentrations was noted during the Commissioning Phase in both nitrification and denitrification systems.

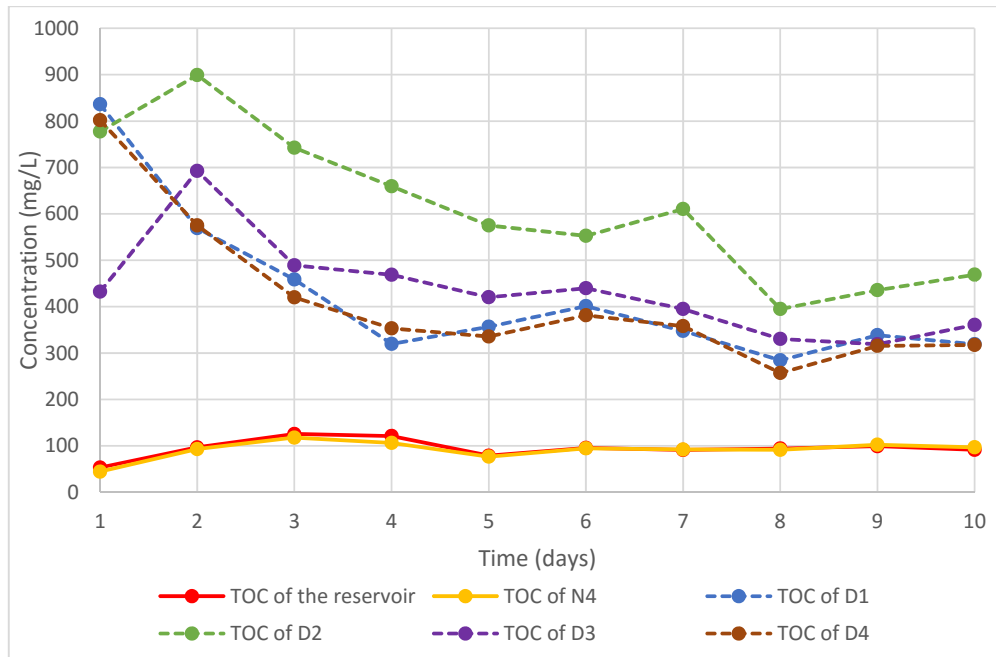
The change in inorganic carbon and total organic carbon is shown in Figure 3.10 and Figure 3.11 respectively. Inorganic carbon values in the nitrification system were much higher than those in the denitrification system. Conversely, total organic carbon in the nitrification system was smaller than that in the denitrification system (Figure. 3.11).



**Figure 3.9.** Change in dissolved oxygen in the nitrification and denitrification systems during the Commissioning Phase under following conditions: Nitrification hydraulic rate of 0.3 L min<sup>-1</sup>, denitrification hydraulic rate of 4.5 ml min<sup>-1</sup>, recirculation flow, and initial ammonium nitrogen of 2.5 mg NH<sub>4</sub>-N L<sup>-1</sup>



**Figure 3.10.** Change in inorganic carbon in the nitrification and denitrification systems during the Commissioning Phase



**Figure 3.11.** Change in total organic carbon in the nitrification and denitrification systems during the Commissioning Phase under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $2.5 \text{ mg NH}_4\text{-N L}^{-1}$

### **3.3. Experimental phase 1: Defining the capacity of nitrification and denitrification systems**

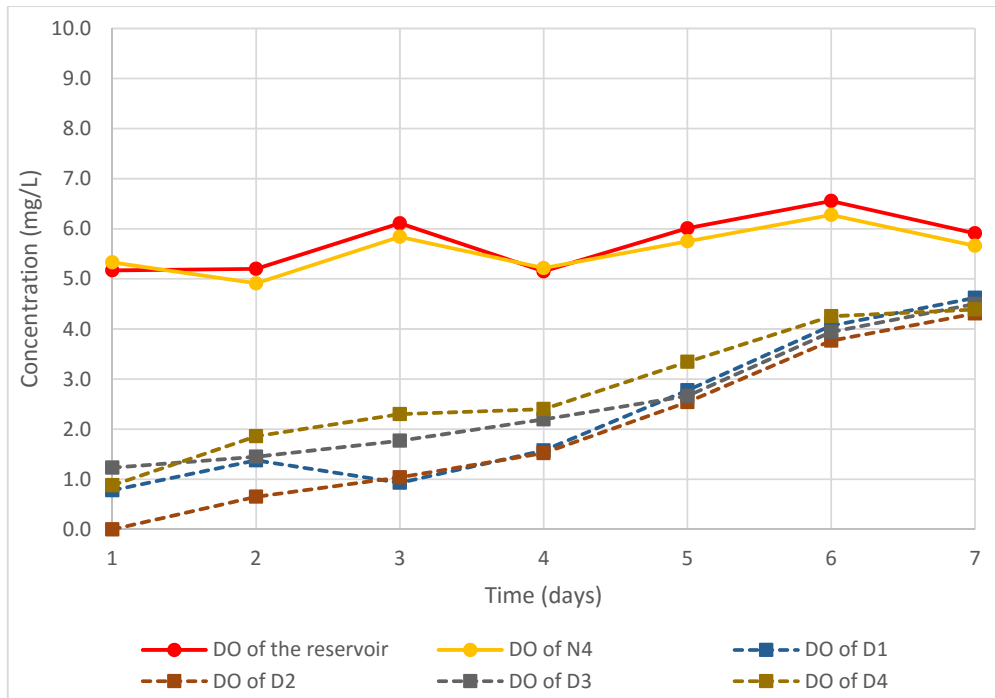
The Experimental phase 1 includes two stages and was conducted over 14 days (14/06/2016 to 27/06/2016) to determine the efficiency of both nitrification and denitrification systems.

#### **3.3.1. Capacity of the nitrification system**

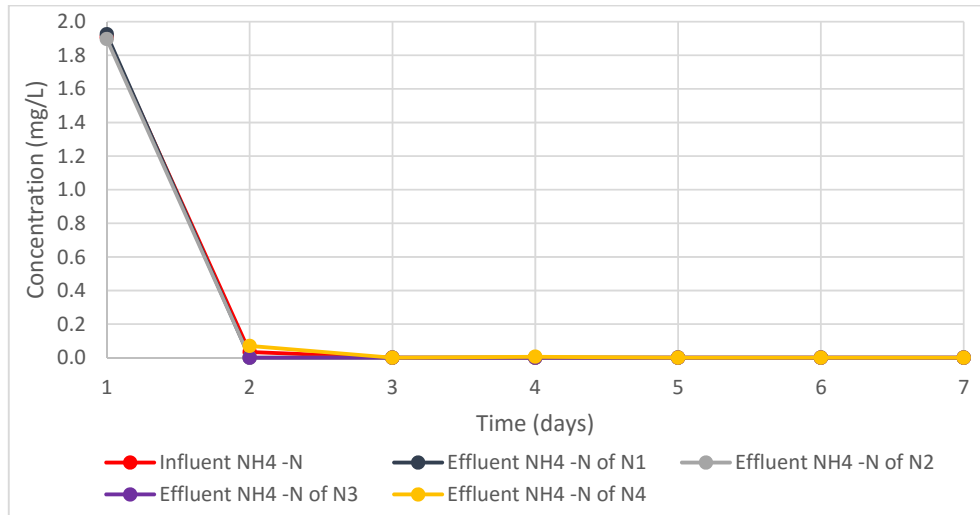
The first stage of this Phase was conducted in 7 days (14/06/2016 to 20/06/2016). The main task of this stage was to measure the capacity of the nitrification system. Accordingly, 35 L of fresh lake water was amended to yield 4.3 mg NH<sub>4</sub>-N L<sup>-1</sup> for the nitrification system. Another 25 L of fresh lake water, containing only native ammonium, was used to replace the water in the denitrification system used in the Commissioning Phase. The hydraulic rate loading of the NTF systems was 0.3 L min<sup>-1</sup> and it was still a recirculation flow configuration. While the flow rates in the denitrification system were 1.5, 1.2 and 1.5 ml min<sup>-1</sup> for columns in Group 1, 2 and 3 respectively. The temperature was measured during this stage and ranged from 20.8 to 21.4°C. The pH values were from 7.1 to 8.1 for the nitrification system and from 4.7 to 6.2 for the denitrification system. The change in DO is shown in Figure 3.12. The DO values in the nitrification system increased slightly over the 7 days from over 5.0 mg L<sup>-1</sup> to nearly 6.0 mg L<sup>-1</sup>, the DO values in the denitrification increased over 4 fold from around 1 mg L<sup>-1</sup> to over 4 mg L<sup>-1</sup>.

The changes in ammonium in the nitrification columns are shown in Figure 3.13. The initial concentration of ammonium was 4.3 mg NH<sub>4</sub>-N L<sup>-1</sup> as mentioned above. This concentration in the nitrifying reservoir reduced to 1.9 mg NH<sub>4</sub>-N L<sup>-1</sup> after 24 hours and 0.034 mg NH<sub>4</sub>-N L<sup>-1</sup> after 48 hours. Then ammonium was not detected in the nitrifying reservoir and effluent point of the system during following days of the Phase. The average daily

rate of nitrification achieved to be over  $2.1 \text{ mg NH}_4\text{-N L}^{-1}$  ( $75 \text{ mg NH}_4\text{-N day}^{-1}$ ).



**Figure 3.12.** Change in DO in the nitrification and denitrification systems during the first stage of Phase 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$

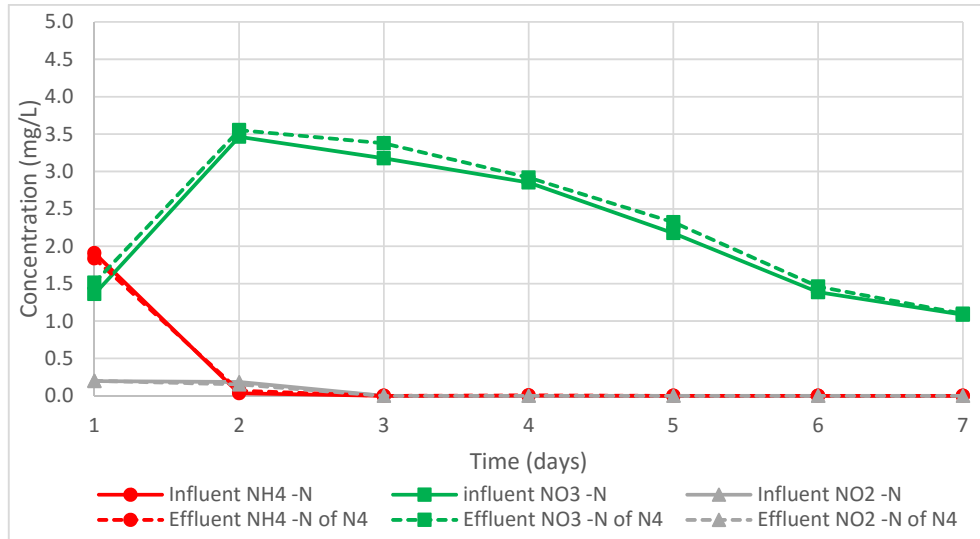


**Figure 3.13.** Change in ammonium in the nitrification system during the first stage of Phase 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$

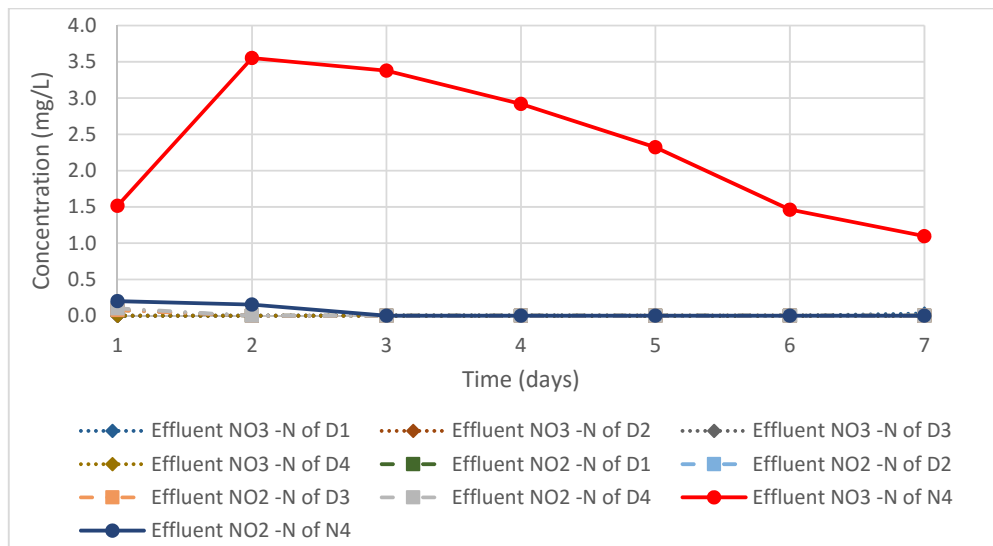
Figure 3.14 below presents the relationship among influent and effluent ammonium, nitrate and nitrite of the nitrification system. While the concentration of nitrate was quite high, the values of nitrite were limited, suggesting almost complete nitrification. They only reached to  $0.2 \text{ mg NO}_2\text{-N L}^{-1}$  in the first two days, then no nitrite was detected. The nitrate mass reached a maximum value around  $3.5 \text{ mg NO}_3\text{-N L}^{-1}$  at day 2 before steadily reducing. From the analysis of these measurement, it was determined that about  $0.5 \text{ mg NO}_3\text{-N L}^{-1}$  on average was converted daily to nitrogen gas. This number was utilized for calculation influent ammonium mass in the nitrification system in the next Phase. Although the concentration of nitrate in the nitrification was quite high, there was no nitrate detected at effluent points of the denitrification system (Figure 3.15).

The analysis results of IC and TOC during the stage are shown in Figure 3.16 and Figure 3.17 respectively. While IC values in the nitrification system tended to increase, the values in the denitrification system halved in 7 days.

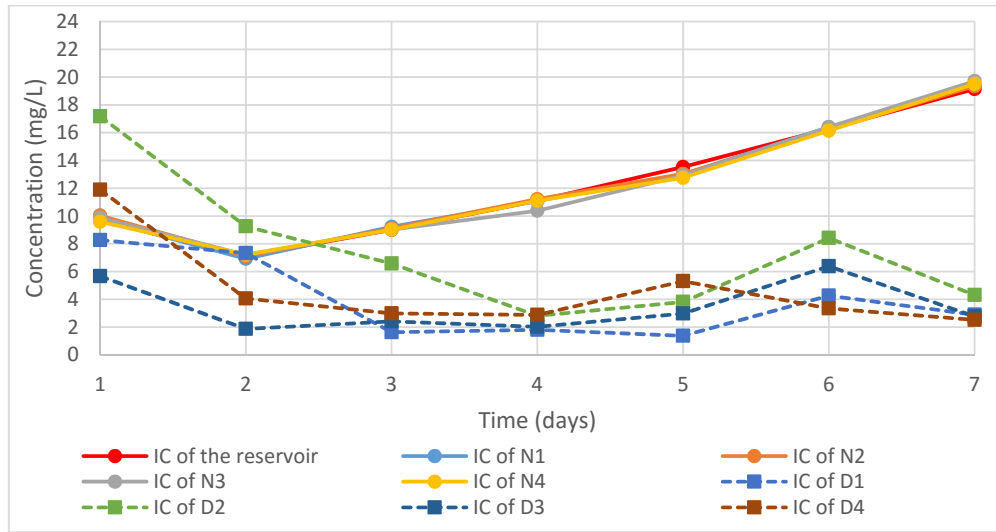




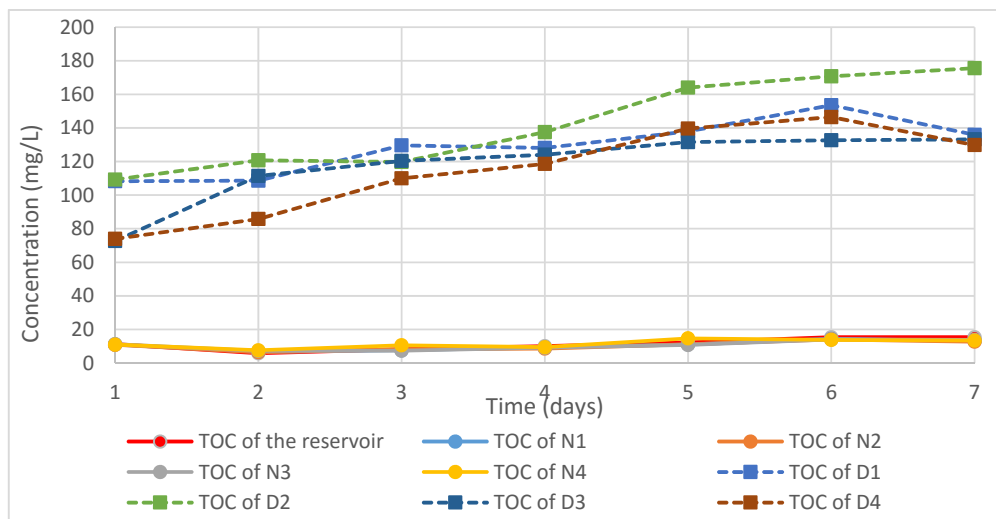
**Figure 3.14.** Influent and effluent ammonium, nitrate and nitrite of the nitrification system during the first stage of Phase 1 under following conditions: hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$



**Figure 3.15.** Influent and effluent nitrate and nitrite of the denitrification system during the first stage of Phase 1 under following conditions: hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$



**Figure 3.16.** Change in inorganic carbon of the nitrification and denitrification systems during the first stage of Phase 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$



**Figure 3.17.** Change in TOC of the nitrification and denitrification systems during the first stage of Phase 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$

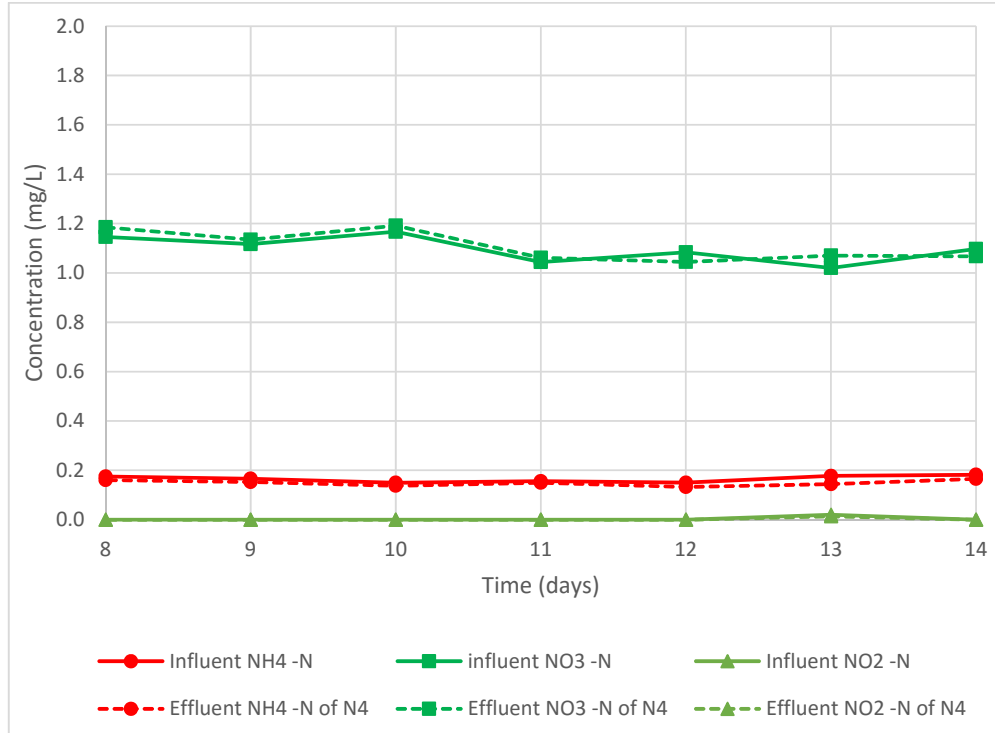
### 3.3.2. Capacity of the denitrification system

The data shown in Figure 3.14 in the first 7 days of the Phase 1 indicated the denitrification system could remove  $0.5 \text{ mg NO}_3\text{-N L}^{-1} \text{ d}^{-1}$  at a corresponding hydraulic flow rate  $4.2 \text{ ml min}^{-1}$ . Meanwhile, the theory of nitrogen mass balance contends that total influent nitrogen including ammonia, nitrate and nitrite equals total effluent nitrogen. Consequently, in order to maintain the nitrate concentration of  $0.5 \text{ mg NO}_3\text{-N L}^{-1}$  to sustain denitrification, it requires  $0.5 \text{ mg NH}_4\text{-N L}^{-1}$  to be added to the nitrifying reservoir.

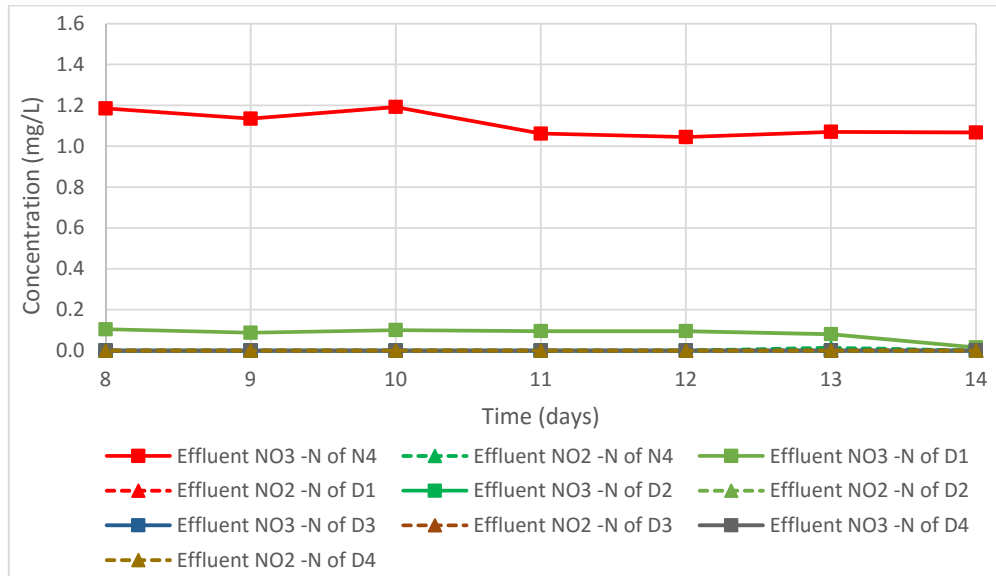
In the following 7 days (21/06/2016 to 27/06/2016), a constant concentration of ammonia  $0.5 \text{ mg NH}_4\text{-N L}^{-1}$  ( $30 \text{ mg NH}_4\text{-N day}^{-1}$ ) was daily loaded to evaluate the effectiveness of the denitrification system. All other conditions such as stirring and aeration remained as for the previous Phase. The pH values of the nitrification system were in the range 8.0 to 8.3 and they were between 4.4 and 4.8 for the denitrification system. The temperature was about  $21^\circ\text{C}$ . Meanwhile, DO values in the nitrification were still higher than that in the denitrification system. DO concentrations were from  $4.0 \text{ mg L}^{-1}$  to  $6.0 \text{ mg L}^{-1}$  in nitrifying columns, while these figures were between  $2.9 \text{ mg L}^{-1}$  and  $5.2 \text{ mg L}^{-1}$  for denitrifying columns.

The results for nitrate concentrations in effluent flow of the nitrification system (Figure 3.18) shows that the concentrations were stable throughout this Phase ( $1.0 \text{ mg NO}_3\text{-N L}^{-1}$  to  $1.2 \text{ mg NO}_3\text{-N L}^{-1}$ ). Figure 3.19 shows the concentrations of influent and effluent nitrate and nitrite in denitrifying columns. While no nitrate and nitrite were detected at effluent flows of Columns 2, 3 and 4, a small concentration of nitrate  $< 0.1 \text{ mg NO}_3\text{-N L}^{-1}$  was detected in Column 1 (Figure 3.20). Effluent flow from denitrifying Column 1 was considered as influent flow to denitrifying Column 2. The comparison between influent and effluent factors in Column 1 and 2 was as shown in Figure 3.21.

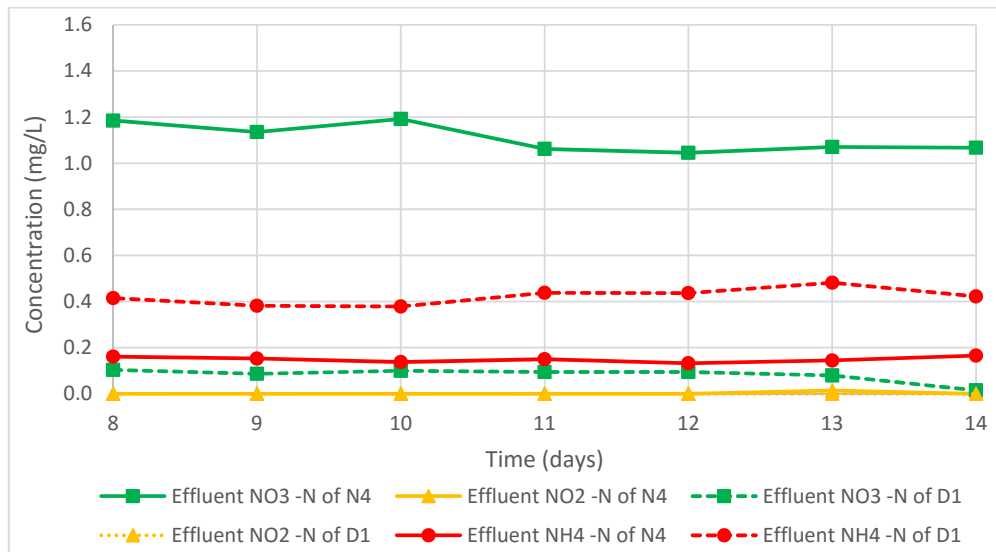
After finishing Phase 1, the system was continually maintained in all conditions without sampling during 23 days (28/06/2016 to 20/07/2016).



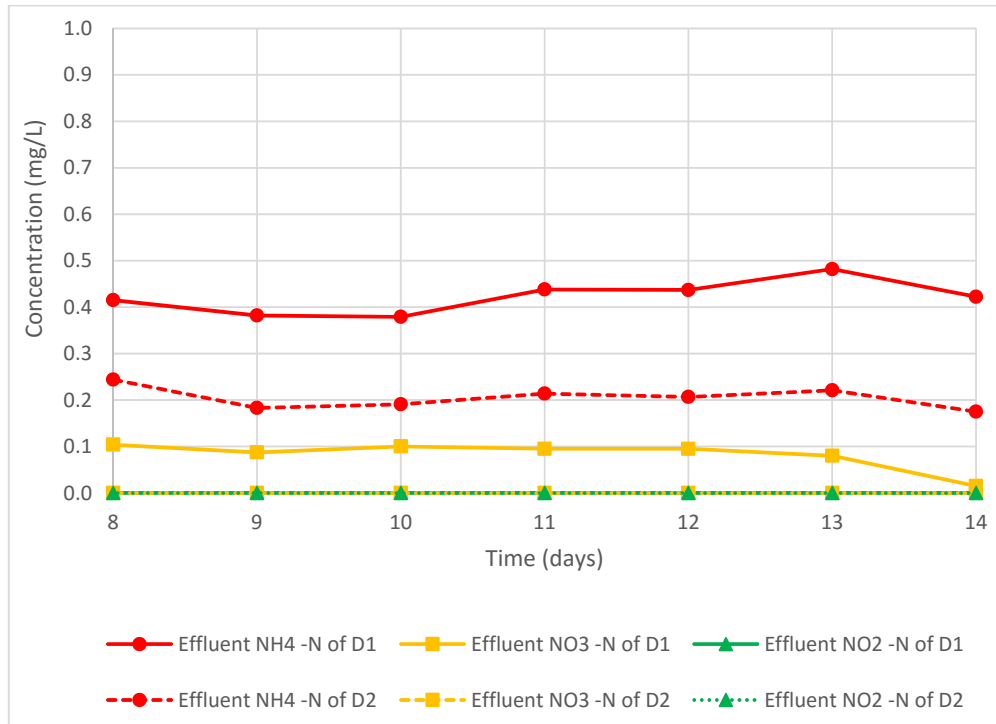
**Figure 3.18.** Influent and effluent ammonium, nitrate and nitrite of the nitrification system during the second stage of Phase 1 under following conditions: hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $0.5 \text{ mg NH}_4\text{-N L}^{-1} \text{ day}^{-1}$



**Figure 3.19.** Influent and effluent ammonium, nitrate and nitrite of the denitrification system the second stage of Phase 1 under following conditions: hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $0.5 \text{ mg NH}_4\text{-N L}^{-1} \text{ day}^{-1}$



**Figure 3.20.** Influent and effluent ammonium, nitrate and nitrite of denitrification Column 1 during the second stage of Phase 1 under following conditions: hydraulic rate of  $1.5 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $0.5 \text{ mg NH}_4\text{-N L}^{-1} \text{ day}^{-1}$



**Figure 3.21.** Influent and effluent ammonium, nitrate and nitrite of denitrification Column 2 during the second stage of Phase 1 under following conditions: hydraulic rate of  $1.2 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $0.5 \text{ mg NH}_4\text{-N L}^{-1} \text{ day}^{-1}$

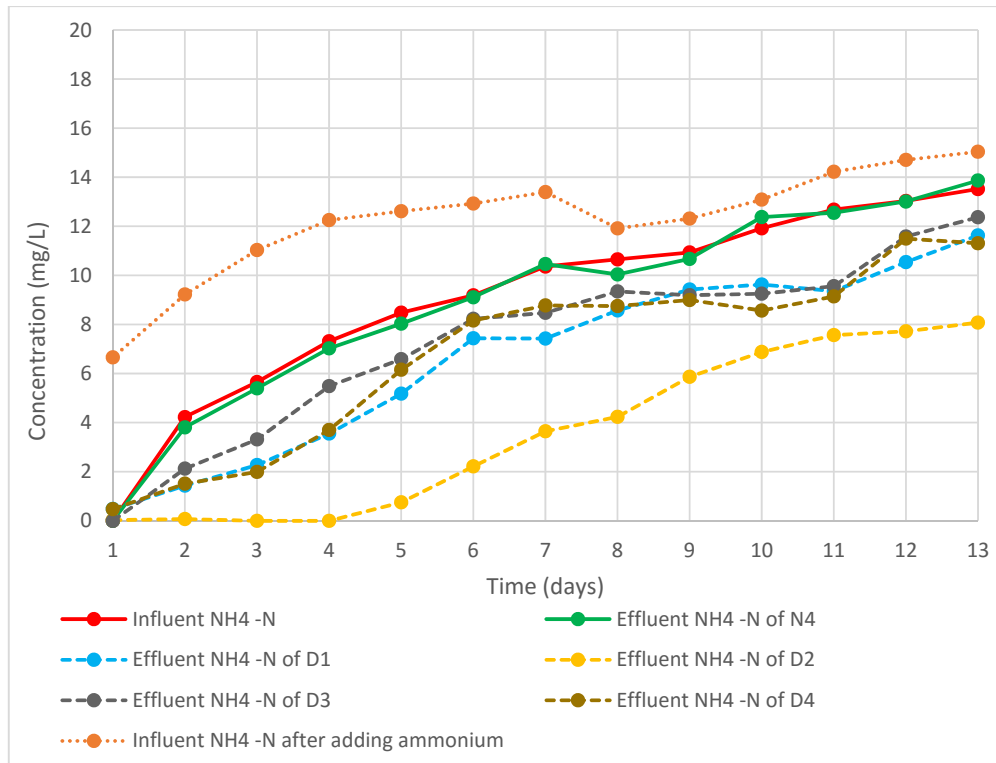
### **3.4. Experimental phase 2: Measuring maximum capacity of the nitrification system**

Experimental Phase 2 was conducted during 13 days (21/07/2016 to 02/08/2016). In order to commence the Phase 2, 50 L of lake water was prepared to replace the whole volume of water in both the nitrification and denitrification system. In this procedure, 25 L of fresh lake water was added 150 mg NH<sub>4</sub>-N day<sup>-1</sup> to maintain a concentration of 6.0 mg NH<sub>4</sub>-N L<sup>-1</sup> in the influent feed to the nitrification system. 150 mg NH<sub>4</sub>-N was daily loaded to the system in the first 7 days. Then this figure was adjusted to 100 mg NH<sub>4</sub>-N per day in following six days. Another 25 L of fresh lake water, containing only native ammonium, was used to replace the whole former water in the denitrifying columns. All other conditions were similar to the previous Phase.

The daily (room) temperature during the Phase 2 was about 21°C. The pH value of nitrification was between 6.0 and 7.0. The pH values in the Group 1 of the denitrification system including Column 1 and 2 were in range 4.0 to 5.0. These values were lower than pH in Group 2 and 3 which were from 5.1 to 6.4. DO in the nitrifying columns changed between 4.2 mg L<sup>-1</sup> and 6.3 mg L<sup>-1</sup> in comparison the DO in the denitrifying columns ranged between 2.6 mg DO L<sup>-1</sup> to 5.5 mg DO L<sup>-1</sup>.

In this experimental phase, besides defining influent and effluent ammonium concentrations of both nitrification and denitrification systems, ammonium concentration in the nitrifying reservoir was measured after adding the ammonium chloride mass. Its results were as shown in Figure 3.22. Based on the daily initial ammonium concentrations and effluent ammonium concentrations of the whole system, ammonium mass which was daily converted was defined. Figure 3.22 indicates that all ammonium concentration values in the nitrifying and denitrifying systems steadily increased during this experimental period. The effluent ammonium

concentration in Column 2 (D2) of the denitrification system was lowest in comparison with other denitrifying columns. It is in agreement when column D2 was in series with denitrifying Column 1. The influent flow of Column D2 is the discharge flow of the denitrifying Column 1 which has much lower ammonium concentration than that in the influent flows of other denitrifying columns.

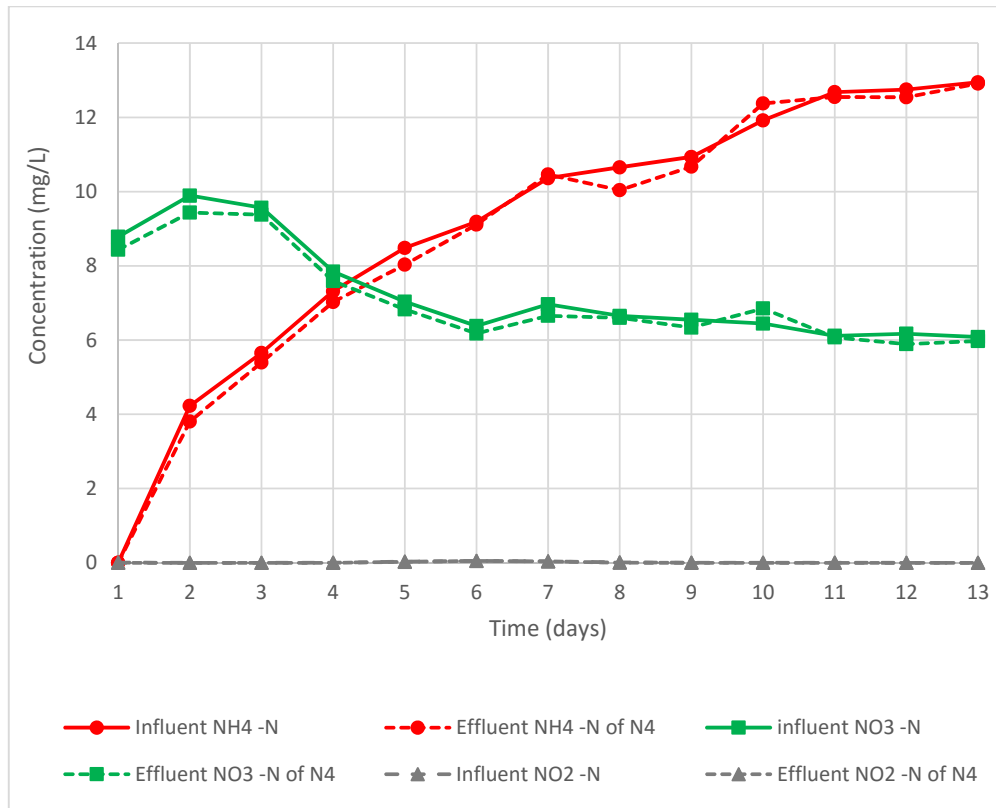


**Figure 3.22.** Comparison between influent and effluent ammonium during Experimental Phase 2 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $6 \text{ mg NH}_4\text{-N L}^{-1}$  in the first 7 days and adjust to  $4 \text{ mg NH}_4\text{-N L}^{-1}$  in following six days

Nitrate and nitrite production during the Experimental Phase 2 are shown in Figure 3.23. While only very low nitrite concentrations under  $0.06 \text{ mg NO}_2\text{-N L}^{-1}$  were found at both influent and effluent points, nitrate

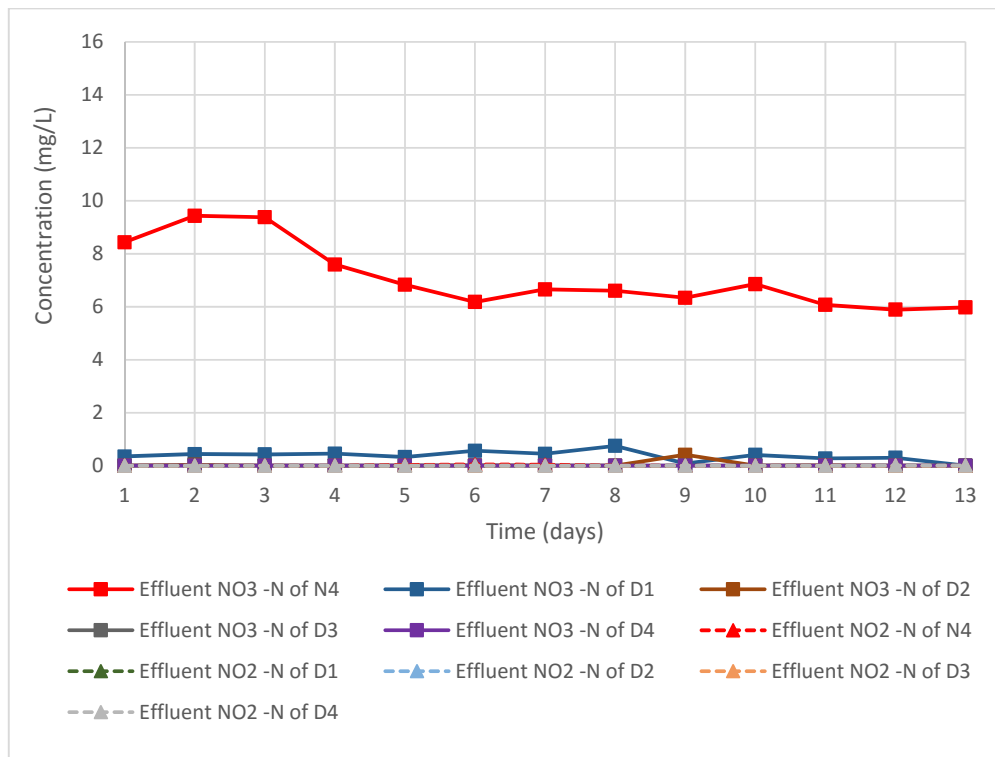


concentrations were quite high. In the first day of the Phase, over 8.4 mg  $\text{NO}_3\text{-N L}^{-1}$  was detected in the system and it reached a peak at around 9.5 mg  $\text{NO}_3\text{-N L}^{-1}$  in the following day. However, nitrate concentration in the nitrification system began declining from day 3 to day 6 before stabilising around 6.2 mg  $\text{NO}_3\text{-N L}^{-1}$  during the rest of Phase 2. It was notable that effluent nitrate masses were lower than that at the influent point during most of this period.



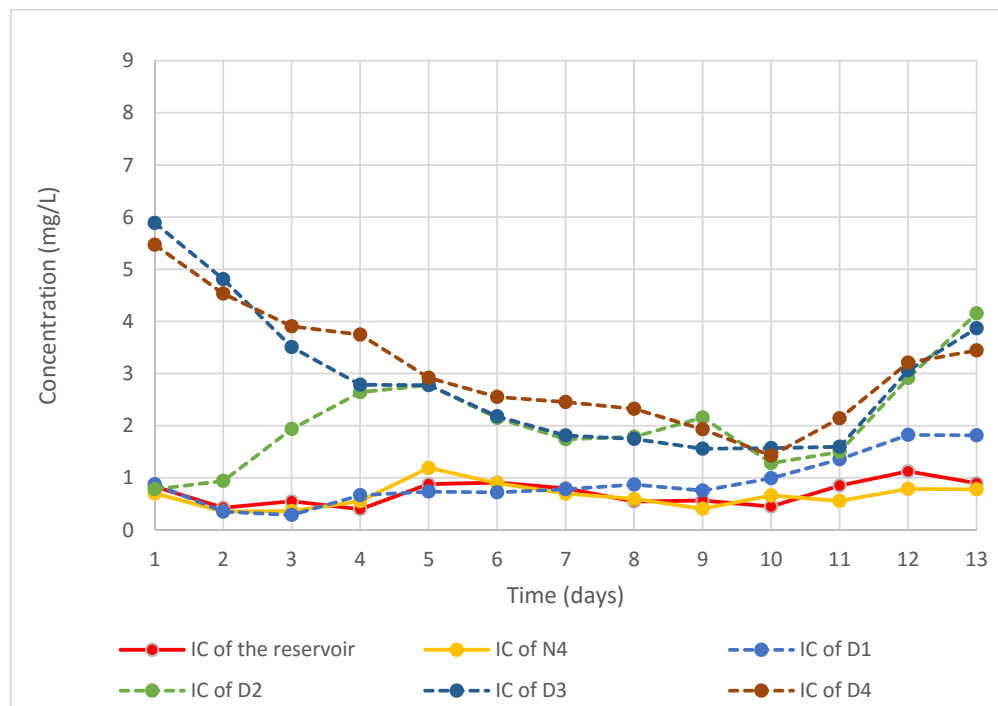
**Figure 3.23.** Influent and effluent ammonium, nitrate and nitrite of the nitrification system during Experimental Phase 2 under following conditions: hydraulic rate of  $0.3 \text{ L min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $6 \text{ mg NH}_4\text{-N L}^{-1}$  in the first 7 days and adjust to  $4 \text{ mg NH}_4\text{-N L}^{-1}$  in following six day

Although effluent nitrate concentration of the nitrification system, which supplied the nitrate concentration to the denitrifying columns, was quite high, most nitrate was transferred to nitrogen gas by denitrification process. The results are presented in Figure 3.24 and show that the effluent nitrate concentrations in Group 2 and 3 of the denitrification system were zero during all of the Experimental phase. A limited amount of nitrate between 0.28 mg NO<sub>3</sub>-N L<sup>-1</sup> and 0.75 mg NO<sub>3</sub>-N L<sup>-1</sup> was found daily in denitrifying Column 1. Similarly, low effluent nitrate concentration under 0.4 mg NO<sub>3</sub>-N L<sup>-1</sup> was detected in several days during this period.



**Figure 3.24.** Influent and effluent nitrate and nitrite of the denitrification system during Experimental Phase 2 under following conditions: hydraulic rate of 4.5 ml min<sup>-1</sup>, recirculation flow, and ammonium nitrogen of 6 mg NH<sub>4</sub>-N L<sup>-1</sup> in the first 7 days and adjust to 4 mg NH<sub>4</sub>-N L<sup>-1</sup> in following six days

Change in inorganic carbon of nitrifying columns in this Phase was definitely different in comparison with all previous Phases. The concentrations of inorganic carbon in nitrifying columns were much higher than that in the denitrifying columns in the previous Phases. However, in this Phase, inorganic carbon concentration in the nitrification system was lower than that in the denitrification system (Figure 3.25). Most of the inorganic carbon values in the nitrification system were under  $1 \text{ mg L}^{-1}$  and they were quite stable rather than increasing as in previous experimental Phases.



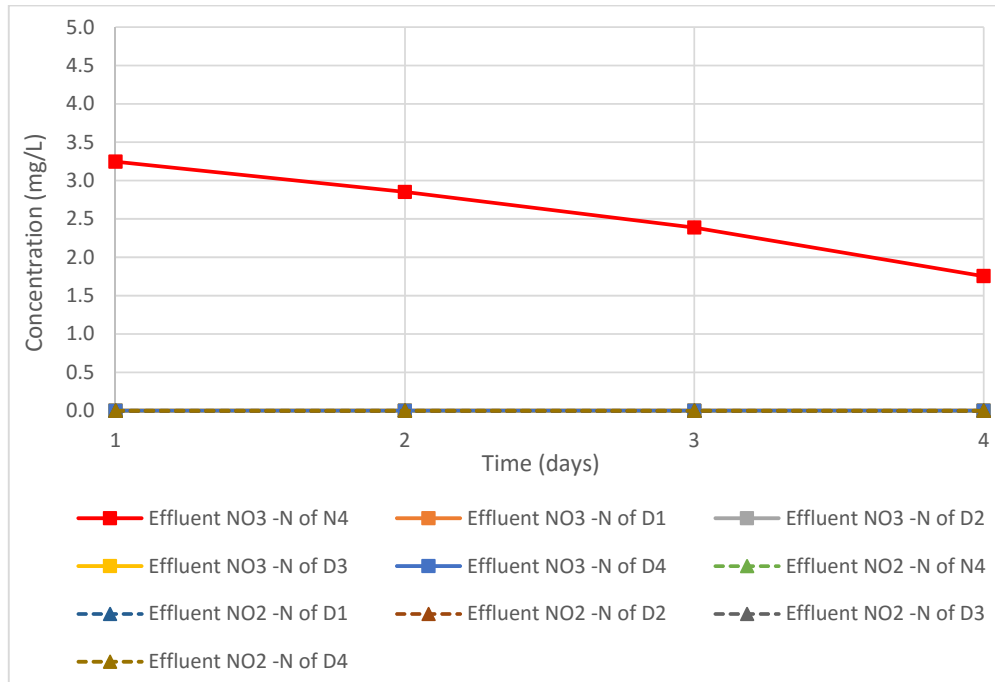
**Figure 3.25.** Change in IC of the both nitrification and denitrification systems Experimental Phase 2 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $6 \text{ mg NH}_4\text{-N L}^{-1}$  in the first 7 days and adjust to  $4 \text{ mg NH}_4\text{-N L}^{-1}$  in following six days

### **3.5. Experimental phase 3: Measuring maximum capacity of the denitrification system**

The last experimental phase was conducted over five days (03/08/2016 to 07/08/2016). In the first four days, all conditions remained as at the end of Experimental Phase 2. The constant concentration of ammonia, 100 mg NH<sub>4</sub>-N L<sup>-1</sup>, was continued to supply to the nitrification system. The operation condition of stirring and aeration had not changed. However, flow rates of the denitrification system were increased by three times. They were 4.5, 3.6 and 4.5 ml min<sup>-1</sup> for columns in Group 1, 2 and 3 respectively.

The temperature, pH and DO were continually measured during this Phase. The water temperature in the system was constant around 21°C. Meanwhile DO values of nitrification and denitrification system were in range 6.6 mg L<sup>-1</sup> to 7.3 mg L<sup>-1</sup> and 4.8 mg L<sup>-1</sup> to 5.6 mg L<sup>-1</sup> respectively. In nitrifying columns, pH values changed from 6.5 to 6.9, while these values in denitrifying columns were between 4.5 and 5.9.

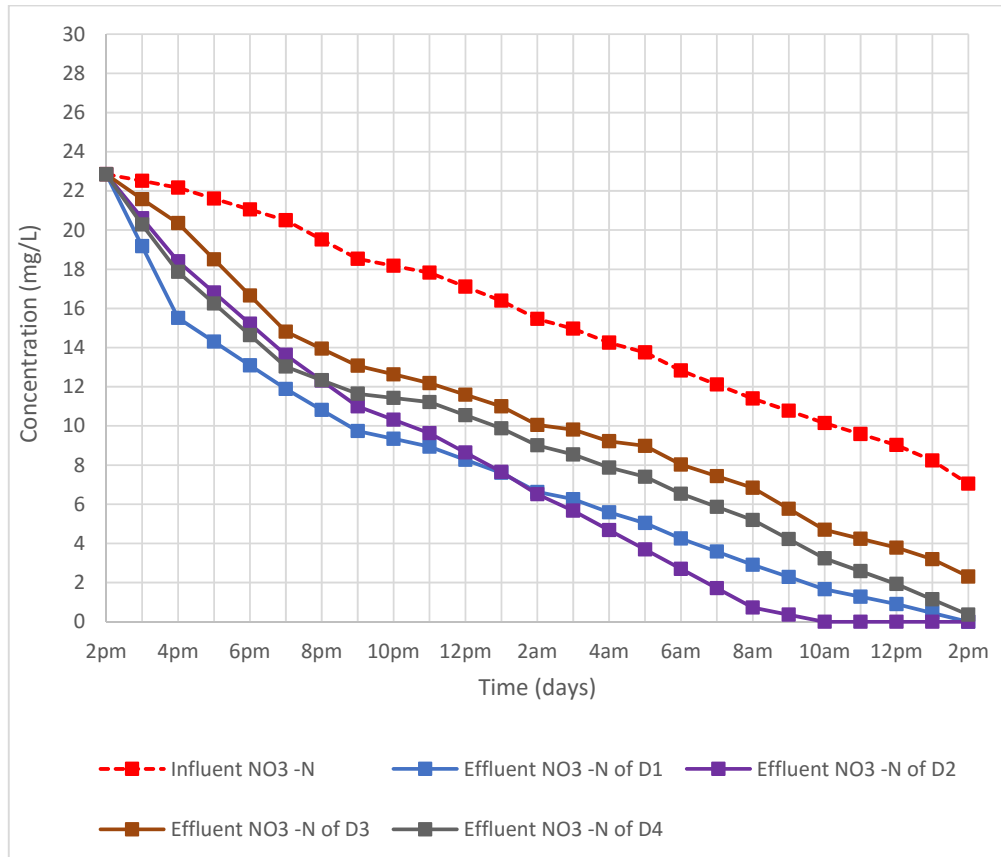
The influent nitrate and nitrite concentrations supplied to the denitrification system were the effluent nitrate and nitrite of Column 4 of the nitrification system. As shown in Figure 3.26, influent nitrate concentration significantly decreased from over 3.2 mg NO<sub>3</sub>-N L<sup>-1</sup> to around 1.7 mg NO<sub>3</sub>-N L<sup>-1</sup> in four days. Although flow rates of the denitrification system were increased to establish nitrate and nitrite at effluents points, there were no nitrate and nitrite detected at the effluent points of the denitrifying columns (Figure 3.26). This indicated that the capacity of denitrifying columns could be increased further. However, based on Experimental Phase 2, the nitrification system reached its maximum capacity in production of nitrate and nitrite. Therefore, it was impossible to increase nitrate in the nitrifying reservoir or flow rates of the denitrification system.



**Figure 3.26.** Change in influent and effluent nitrate and nitrite of the denitrification system in Experimental Phase 3 under following conditions: hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $12.6 \text{ ml min}^{-1}$ , recirculation flow, and ammonium nitrogen of  $4 \text{ mg NH}_4\text{-N L}^{-1}$

In order to evaluate capacity of the denitrification system, another approach was applied. The nitrification was paused and all of the water in denitrification columns was pumped out and replaced by 35L of new lake water with  $22.86 \text{ mg NO}_3\text{-N L}^{-1}$ . The operation of the air pump and stirring pump was continued. The system was operated over one day and sampling was conducted hourly.

The results of converted nitrate in a day are as shown in Figure 3.27. Initial nitrate concentration in denitrifying columns were  $22.86 \text{ mg NO}_3\text{-N L}^{-1}$ . After 24 hours, nitrate concentration in the reservoir remained over  $7 \text{ NO}_3\text{-N L}^{-1}$ , while these figures in Column 3 and 4 were  $2.3 \text{ NO}_3\text{-N L}^{-1}$  and  $0.36 \text{ NO}_3\text{-N L}^{-1}$  respectively. In Column 1 and 2 of Group 1, nitrate was not detected at the effluent discharge points.



**Figure 3.27.** Change in influent and effluent nitrate of the denitrification system in a last day of Experimental Phase 3 under following conditions: hydraulic rate of  $42 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $22.86 \text{ mg NO}_3\text{-N L}^{-1}$

## 4. Discussion

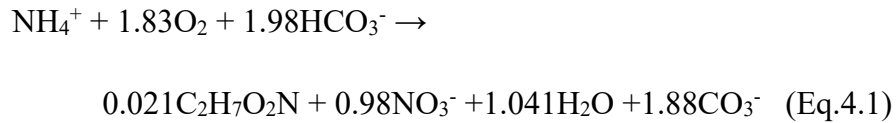
### 4.1. Nitrifiers and denitrifiers development

#### 4.1.1. Substrate

In order to achieve bacterial growth in the Commissioning Phase of the study, calcium carbonate was added to dilute activated sludge as a necessary substrate for bacteria growth. As mentioned, nitrification includes a two-step process of ammonia oxidizing bacteria and nitrite oxidizing bacteria with *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria respectively. Both bacteria are autotrophic and their carbon source for the synthesis of cellular material is inorganic carbon. Absence of an external inorganic carbon source during this period could result in low bacteria growth. Therefore, 70 mg L<sup>-1</sup> of calcium carbonate was supplied when media was immersed in activated sludge solution and other 35 mg L<sup>-1</sup> of calcium carbonate was added when recirculation flows was initiated. These calcium carbonate masses played a role as a food source to enhance nitrifiers growth in the nitrification system. However, ammonium is not consumed by nitrifiers because bacteria only use ammonium as their energy transfer source (Villaverde, Garcia-Encina & Fdz-Polanco 1997).

A number of studies have reported the importance of inorganic carbon in nitrification processes. These studies have confirmed that low inorganic carbon concentration in water sources limits nitrification activity (Furukawa, Kenji et al. 1993; Guisasola et al. 2007; Kimura, Isaka & Kazama 2011; Wett & Rauch 2003). Furthermore, Guisasola et al. (2007) indicated that the minimum inorganic carbon concentration needed to maintain nitrification rate is 3 mmol C L<sup>-1</sup> in a water source. If its concentration was lower, nitrification rate was limited, especially for ammonia oxidizing process. For the oxidation of 1 mg ammonium to nitrate, it regularly consumes around 7.0 mg alkalinity (as calcium carbonate). This theory has been confirmed by a

range of studies (Chen, Ling & Blancheton 2006; Liptak & Liu 1997; van den Akker 2008). The complete nitrification process is described as Equation 4.1 as follows (Zhang & Bishop 1996).



Calcium carbonate added to dilute activated sludge is not only the substrate for autotrophic biomass but also increases the pH in solution to buffer acidification. It is recognized that the nitrification process strip carbon dioxide and hydrogen is released. As a result, pH significantly reduces and an acid environment in the water source can be established (Villaverde, Garcia-Encina & Fdz-Polanco 1997; Wett & Rauch 2003). Meanwhile, an optimum pH for ammonia oxidizing bacteria and nitrite oxidizing bacteria is in the range of 7.0 to 9.0 (Zhang & Bishop 1996). Hence alkalinity such as calcium carbonate is useful to maintain and improve pH in a water source.

#### 4.1.2. pH, DO and temperature

In a range of factors affecting nitrification and denitrification processes, pH, DO and temperature are considered as primary parameters. Any change in these factors could cause a low growth rate of bacteria. The nitrifiers growth rate is limited when pH is lower than 6.5 or higher than 9.0. With lower pH, development of nitrifiers might be prevented because acid formation occurs and it is toxic to bacteria. In contrast, with higher pH values, the oxidation process might be inhibited or nitrite is accumulated in the system (Wu, Zheng & Xing 2014). In the Commissioning Phase, pH was maintained at optimal values between 7.3 and 8.6 in the nitrification system. In this condition, nitrifiers could achieve a high rate of growth (Lájer 2012). Meanwhile, pH values in denitrifying columns were in a range of 5.0 to 6.6 which were lower than optimum pH value between 7.0 and 7.5 (Thomas, Lloyd & Boddy 1994). The pH of the denitrification system was lower than that within the



nitrification system. This led to decomposition of barley straw in the denitrifying columns (Holmes, Plant & Water 2010).

Dissolved oxygen and nitrifiers growth is believed to have a close relationship. The maximum growth rate of nitrifiers has been reported to be affected by dissolved oxygen concentration over 4.0 mg L<sup>-1</sup> (Poquillon & Petit 1989). In order to complete nitrification, a relatively large amount of oxygen is required. In reality, approximately 4.6 mg of oxygen is necessary to oxidize 1.0 mg ammonium to nitrate, Table 4.1 by Gerardi (2003).

**Table 4.1.** Oxygen consumption for complete nitrification (modified from Gerardi 2003)

Biochemical reaction	Oxygen consumption (mg)
1 mg NH <sub>4</sub> -N to 1 mg NO <sub>2</sub> -N	3.43
1 mg NO <sub>2</sub> -N to 1 mg NO <sub>3</sub> -N	1.14
1 mg NH <sub>4</sub> -N to 1 mg NO <sub>3</sub> -N	4.57

In the Commissioning Phase, dissolved oxygen concentration in the NTFs was in range of 6.0 mg L<sup>-1</sup> to 7.6 mg L<sup>-1</sup> in the first 10 days and 4.0 mg L<sup>-1</sup> to 5.9 mg L<sup>-1</sup> in the last 10 days. The concentration was not optimum for nitrification in the first 10 days of the Commissioning Phase because in those days, ammonium concentration was quite high. However, in the last 10 days of this Phase when ammonium concentration declined to 2.5 mg NH<sub>4</sub>-N L<sup>-1</sup>, the oxygen demand also significantly reduced. Dissolved oxygen concentration in the denitrification system declined from around 4.0 mg L<sup>-1</sup> to over 1.0 mg L<sup>-1</sup>, however, it still did not reach to the optimum value of 0.15 mg DO L<sup>-1</sup> to 0.35 mg DO L<sup>-1</sup> (Hocaoglu et al. 2011) for denitrifying process. As a result, denitrification process occurred but it might not obtain maximum rate. Although, effluent dissolved oxygen concentrations of denitrifying columns were higher than the optimum value, dissolved oxygen

concentrations inside denitrifying columns might reach the optimum value because environments in the denitrifying columns are not homogeneous. Hence, dissolved oxygen concentrations at different points are may not be similar.

In terms of temperature, although the Commissioning phase was operated in cold weather conditions (May and June 2016), temperature in the water source was regularly between 19°C and 21°C because the experiment was conducted in a room of the Laboratory. The temperature during the experiment period was not an optimum temperature for both nitrifiers and denitrifiers growth, however, these temperatures did not inhibit bacteria activity. Indeed, optimum temperature for biological processes is believed to be between 25°C and 35°C. In the range of this temperature, the nitrifiers growth might attain a maximum rates (Cruikshank & Gilles 2007; Tchobanoglous et al. 2003). Conversely, higher or lower temperatures can limit nitrifying and denitrifying bacteria activity. Tchobanoglous et al. (2003) indicated that nitrification and denitrification ceased, when the temperature rises to 50°C. In addition, when temperature is under 15°C, bacteria become quite inactive and autotrophic nitrifying bacteria cease functioning at around 5°C (Henze 2008).

#### 4.1.3. Nitrifiers development

As shown in Figure 3.3, ammonia oxidizing bacteria had developed since day 1 of the last stage of the Commissioning Phase and it was maintained during the period, whereas, nitrite oxidizing bacteria only appeared and developed after 3 days of this stage. There are several explanations for this situation including the time required for bacteria growth, flow rates and total organic carbon. Firstly, it is not simple to start-up a nitrifying system in a short time. It regularly requires up to 30 days to grow bacteria (Jubany et al. 2008; Sudarno et al. 2010) and up to 100 days to reach a nitrifying stability (Vallés-

Morales et al. 2004; Verstraete, Vanstaen & Voets 1977). In comparison, the Commissioning Phase of this study was conducted over 20 days, therefore, the presentation of oxidizing bacteria understandably might be slow.

Secondly, a part of ammonium mass was pumped and kept to the denitrifying columns in several days before returning the nitrifying reservoir. This might lead to reduce ammonium concentration in the nitrification system. As a result, the efficiency of nitrifying columns was affected. The total denitrification flow rate of  $4.5 \text{ ml min}^{-1}$  was very low. Furthermore, the volume of a denitrifying column was 6.37 L, while flow rates were 1.5, 1.2 and  $1.5 \text{ ml min}^{-1}$  for Group 1, 2 and 3 respectively. As a result, it required 3 days to replace all the water in Group 2 and 3, meanwhile 6 days was required for Group 1.

Finally, total organic carbon was considered as a main factor affecting growth of nitrifiers. A large amount of total organic carbon was washed out from barley straw in the denitrification system and it was returned to the nitrification system (Figure 3.11). This carbon source may have suppressed the nitrification process. van Den Akker et al. (2010) demonstrated the impact of high concentrations of organic carbon on the performance of the nitrifying trickling filter. They found that if organic carbon was of a greater load than  $5.5 \text{ mg sBOD}_5 \text{ L}^{-1}$ , the entire filter bed of the nitrification system was severely inhibited.

#### 4.1.4. Denitrifiers development

The results shown from Figure 3.5 to Figure 3.8 reveal that bacteria in denitrifying columns developed well. Influent nitrate in each column was transferred to nitrogen gas and effluent nitrate could not be found in effluent points. However, nitrite was still found at effluent points of all columns. This finding suggests that it might require more time to develop bacteria in the denitrification system to completely remove nitrate and nitrite.

## 4.2. Nitrification performances

The performances of the nitrification system was evaluated in two steps. In the first step, the nitrification was primarily defined as its capacity to converting ammonium to nitrate and nitrite. This step was conducted in the first stage of Experimental Phase 1. Based on this primary calculation, the average maximum capacity of the nitrification system was determined in the next step which was presented as Experimental Phase 2.

As mentioned above, in order to basically define nitrifying capacity, 150 mg  $\text{NH}_4\text{-N}$  was added to 35 L of water in the nitrifying reservoir. The initial ammonium concentration was 4.3 mg  $\text{NH}_4\text{-N L}^{-1}$ . The results showed in Figure 3.12 indicated that after one day, ammonium concentration in the reservoir significantly reduced to 1.9 mg  $\text{NH}_4\text{-N L}^{-1}$ . In the next day, this value was 0.034 mg  $\text{NH}_4\text{-N L}^{-1}$  and no ammonium was found in the following days. These ammonium concentrations were not original values because water in the nitrifying reservoir was diluted by return flows 4.2 ml  $\text{min}^{-1}$  (6.05 L  $\text{day}^{-1}$ ) from the denitrification system. The original ammonium concentration values at the nitrifying reservoir were 2.3 mg  $\text{NH}_4\text{-N L}^{-1}$  and 0.04 mg  $\text{NH}_4\text{-N L}^{-1}$  for the first and second day respectively. They were defined as Equation 4.1 below (Doucette 1997).

$$C_1 = \frac{C_2 \times V_2}{V_1} \quad (\text{Eq. 4.1})$$

$$V_1 = V - V_d \quad (\text{Eq. 4.2})$$

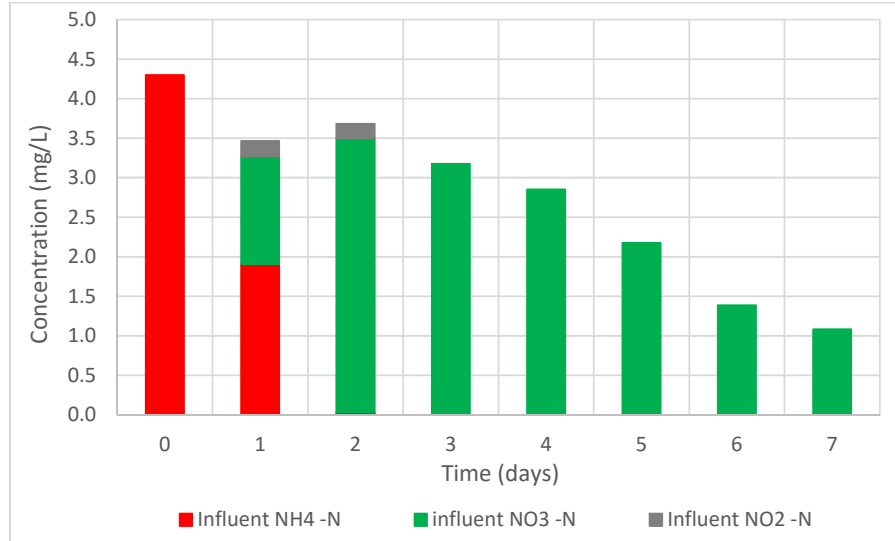
Where  $C_1$  is original ammonium concentration at the measure time (mg  $\text{NH}_4\text{-N L}^{-1}$ );  $C_2$  is diluted ammonium concentration at the measure time (mg  $\text{NH}_4\text{-N L}^{-1}$ );  $V_1$  is original water volume in the nitrifying reservoir ( $V_1 = 28.95$  L) and is defined as Equation 4.2;  $V_2$  is diluted volume of water in the nitrifying reservoir ( $V_2 = 35$  L);  $V$  is initial volume of water in the nitrifying reservoir

( $V = V_2 = 35\text{L}$ );  $V_d$  is volume of water pumped to the denitrification per day  
 $V_d = 6.05\text{ L}$ .

The converted rate of ammonium in the first day was  $2.0\text{ mg NH}_4\text{-N L}^{-1}$  and this figure was  $2.26\text{ mg NH}_4\text{-N L}^{-1}$  in the second day. Basically, the average converted rate of ammonium in the nitrification system was  $2.13\text{ mg NH}_4\text{-N L}^{-1}\text{ day}^{-1}$ . It was equal to a mass of  $75\text{ mg NH}_4\text{-N}$  converted per day by the system as a whole. The data analysis of ammonium showed that no ammonium was detected in denitrification columns during the stage.

The data in Figure 4.1 indicated that most of ammonium mass was converted to nitrate and nitrite in the first two days. In the first day,  $1.36\text{ mg NO}_3\text{-N L}^{-1}$  was formed in comparison with  $0.2\text{ mg NO}_2\text{-N L}^{-1}$ . The remaining ammonium concentration was  $1.9\text{ mg NH}_4\text{-N L}^{-1}$ . The total values of ammonium, nitrate and nitrite concentration was  $3.46\text{ mg L}^{-1}$ . This concentration was diluted by return flow from the denitrification system, therefore it is necessary to convert to original value by Equation 4.1 and 4.2. As a result the total original concentration of ammonium, nitrate and nitrite was  $4.18\text{ mg L}^{-1}$  compared to  $4.3\text{ mg L}^{-1}$  in the influent. It is obvious to recognize that  $2.8\%$  ( $0.12\text{ mg L}^{-1}$ ) of influent mass was unaccounted for after one day. The deficient mass could be explained by a small amount of nitrate and nitrite were pumped to the denitrification system and were converted to nitrogen gas. Similarly, the decline of total ammonium, nitrate and nitrite in following days can also be explained in the same way.

However, in day 2, the original total values of ammonium, nitrate and nitrite concentration was nearly  $4.45\text{ mg L}^{-1}$  (diluted concentration value was  $3.68\text{ mg L}^{-1}$ ). This number was  $3.5\%$  higher than the initial value  $4.3\text{ mg L}^{-1}$ . In reality, it is not simple to obtain  $100\%$  mass balance in complex experimental systems, various factors could affect experimental results.



**Figure 4.1.** Ammonium, nitrate and nitrite in the nitrifying reservoir during the first stage of Experimental Phase 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$

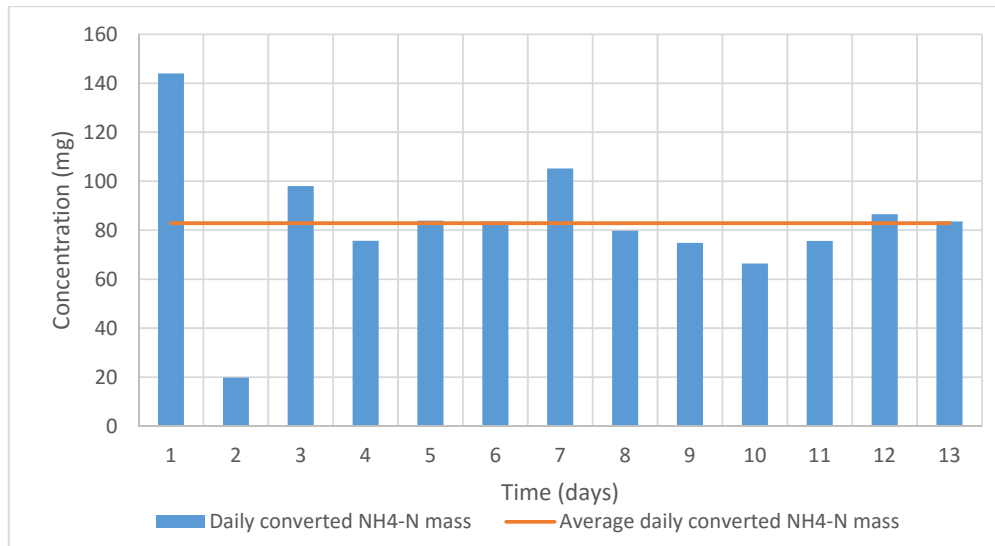
Based on the primary capacity of the nitrification system, the next step was conducted as Experimental Phase 2. The selected ammonium mass was  $150 \text{ mg NH}_4\text{-N day}^{-1}$  which was twice that compared to the initial value of  $75 \text{ mg NH}_4\text{-N day}^{-1}$ . It was selected because the loss of ammonium mass could be higher when a part of the ammonium was pumped to denitrifying columns.

The converted ammonium masses were calculated based on Equation 4.3 below and their results were presented in Figure 4.2. The results indicated that in the first two days, converted ammonium masses were unusual. While  $144 \text{ mg NH}_4\text{-N}$  was converted in the first day, this figure was only  $20 \text{ mg NH}_4\text{-N}$  in the second day. In the following days converted ammonium masses fluctuated around the average value which was nearly  $83 \text{ mg NH}_4\text{-N per day}$ .

$$M_c = M_{in} - M_{out} \quad (\text{Eq. 4.3})$$

$$M_{\text{out}} = \sum C_i \cdot V_i \quad (\text{Eq. 4.4})$$

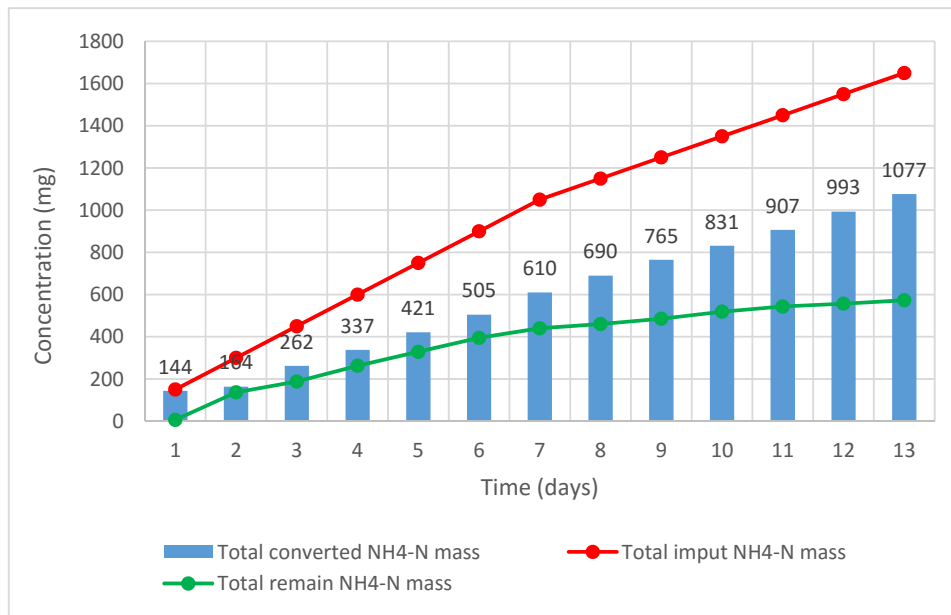
Where  $M_c$  is daily mass of ammonium was converted (mg);  $M_{\text{in}}$  is total daily input ammonium mass (mg);  $M_{\text{out}}$  is total daily output ammonium mass (mg) and  $M_{\text{out}}$  is measured as Equation 3.7;  $C_i$  and  $V_i$  are daily ammonium concentrations and volumes of each nitrifying and denitrifying columns, which were shown as Figure 3.22.



**Figure 4.2.** Daily average converted ammonium mass 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$

Figure 4.3 shows the total converted ammonium mass during 13 days of the Experimental Phase 2. During this phase,  $1650 \text{ mg NH}_4\text{-N}$  was added to the system, of which nearly  $1077 \text{ mg NH}_4\text{-N}$  was converted to nitrate and nitrite, with only  $573 \text{ mg NH}_4\text{-N}$  remaining. The average ammonium removal rate was  $83 \text{ mg NH}_4\text{-N day}^{-1}$ . It corresponds to  $0.2 \text{ mg NH}_4\text{-N L}^{-1} \text{ h}^{-1}$ . Although this rate was higher than that in the Experimental Phase 1, it was not considered overly high. In a study, Mai et al. (2016) achieved ammonia

removal rate of  $0.44 \text{ mg NH}_4\text{-N L}^{-1} \text{ h}^{-1}$ . The pilot in their study was quite similar to the nitrification system in this study, however, installation of nitrifying columns was parallel rather than in series as in this study. The ammonium conversion rate in the study could not achieve a higher level because the external energy source for nitrifying bacteria was limited. Figure 3.25 indicates that inorganic carbon concentration during Phase 2 was lower than  $1.1 \text{ mg L}^{-1}$ . This concentration was very low and, as a result, it could inhibit the nitrification process (Guisasola et al. 2007; Kimura, Isaka & Kazama 2011). Therefore, the average maximum ammonium conversion rate achieved  $83 \text{ mg NH}_4\text{-N per day}$ .



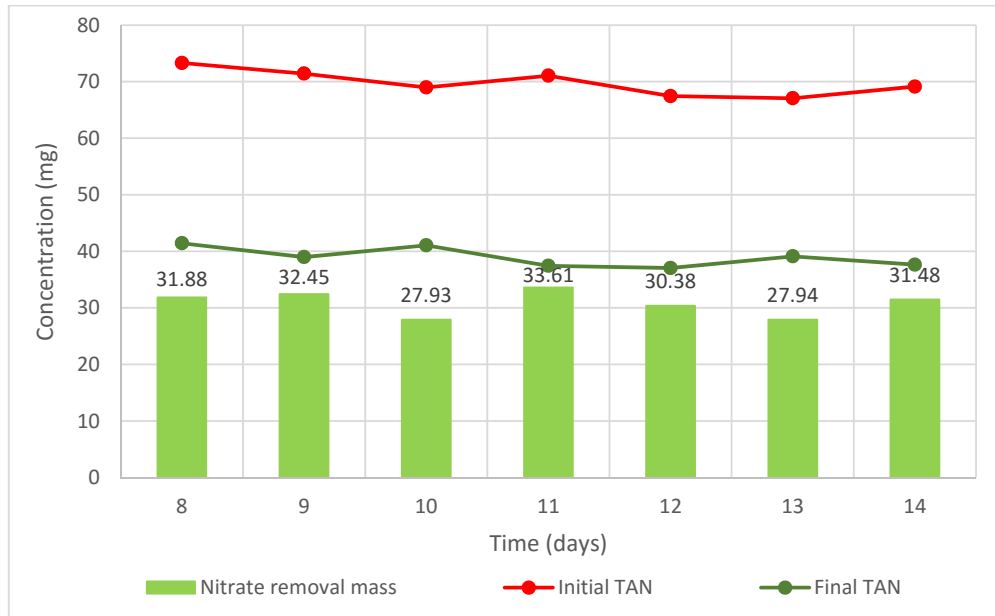
**Figure 4.3.** Defining total converted ammonium mass 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $4.3 \text{ mg NH}_4\text{-N L}^{-1}$



### 4.3. Denitrification performances

#### 4.3.1. Denitrification capacity

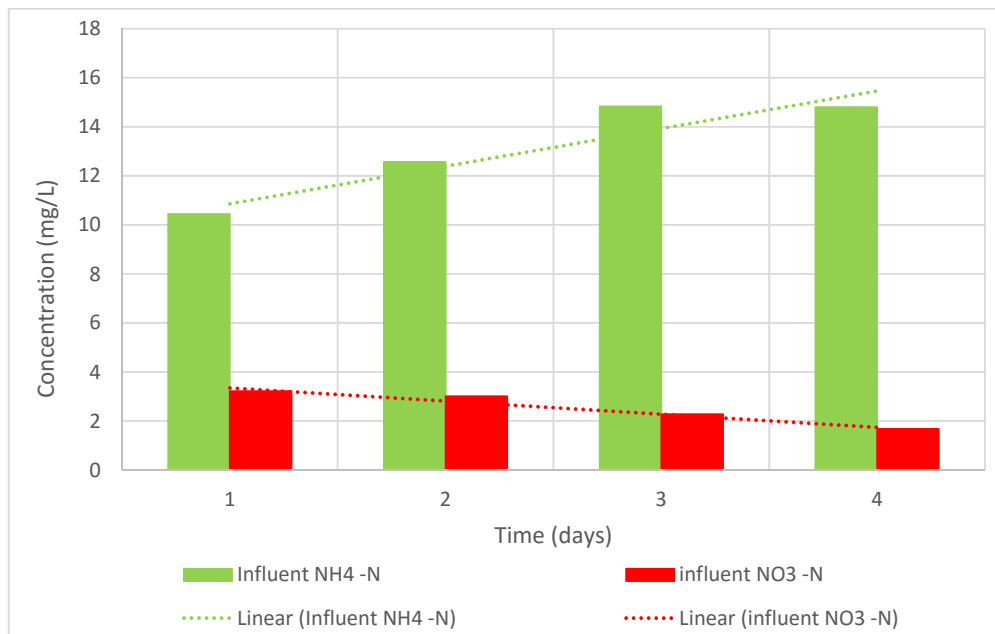
The primary average capacity of the denitrification system was defined to be approximately  $0.62 \text{ mg NO}_3\text{-N L}^{-1} \text{ day}^{-1}$  in the Experimental Phase 1. The result was calculated based on the second stage of the Experimental Phase 1 (21/06/2016 to 27/06/2016). As shown in Figure 4.4, initial total ammonia nitrogen including ammonium, nitrate and nitrite during this period fluctuated around  $70 \text{ mg day}^{-1}$ . After a day, this number significantly reduced to around  $40 \text{ mg day}^{-1}$ . This reduction was caused by the removal of nitrate from the denitrification system. This system removed daily over  $30 \text{ mg NO}_3\text{-N}$ . The nitrate removal rate was only a primary value because it was calculated based on the denitrification flow rate  $4.2 \text{ ml min}^{-1}$ . The rate could change if nitrate concentration or flow rate was not stable (Canto et al. 2008; Karanasios et al. 2010; Liu et al. 2013; Wang et al. 2013). This theory is in line with previous studies. For example, Park, Choi and Pak (2005) conducted a denitrification study with an initial nitrate concentration in the range of  $20 \text{ mg NO}_3\text{-N L}^{-1}$  to  $150 \text{ mg NO}_3\text{-N L}^{-1}$ . Their results revealed that the nitrate removal rate depended on initial nitrate concentration. The removal rate increased when nitrate loading increased. Similarly, the change in nitrate removal rate was observed in a study by Park et al. (2005) where the initial nitrate concentration changed from  $20 \text{ mg NO}_3\text{-N L}^{-1}$  to over  $490 \text{ mg NO}_3\text{-N L}^{-1}$ .



**Figure 4.4.** Total ammonia nitrogen (TAN) and nitrate removal mass 1 under following conditions: Nitrification hydraulic rate of  $0.3 \text{ L min}^{-1}$ , denitrification hydraulic rate of  $4.5 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $0.5 \text{ mg NH}_4\text{-N L}^{-1} \text{ day}^{-1}$

Although influent nitrate concentration at the end of Phase 2 was quite high an approximately  $6.0 \text{ mg NO}_3\text{-N L}^{-1}$ , in an attempt to achieve nitrate at effluent points, indicative of the maximum denitrification rate, of denitrifying columns, denitrification flow rates increased three times from  $1.5$ ,  $1.2$  and  $1.5 \text{ ml min}^{-1}$  to  $4.5$ ,  $3.6$  and  $4.5 \text{ ml min}^{-1}$  for group 1, 2 and 3 respectively. However after four days of the Experimental Phase 3, no nitrate or nitrite were detected at effluent points of the denitrifying columns. This result demonstrated that the capacity of the denitrification was much higher, and the finding is in agreement with previous studies which revealed that the efficiency of denitrification systems was high. These studies found that large amount of nitrate (approximately  $13 \text{ mg L}^{-1}$ ) could be converted to nitrogen gas in a short time (Kaplan et al. 1987; Kim et al. 2010; Schmidt & Clark 2012).

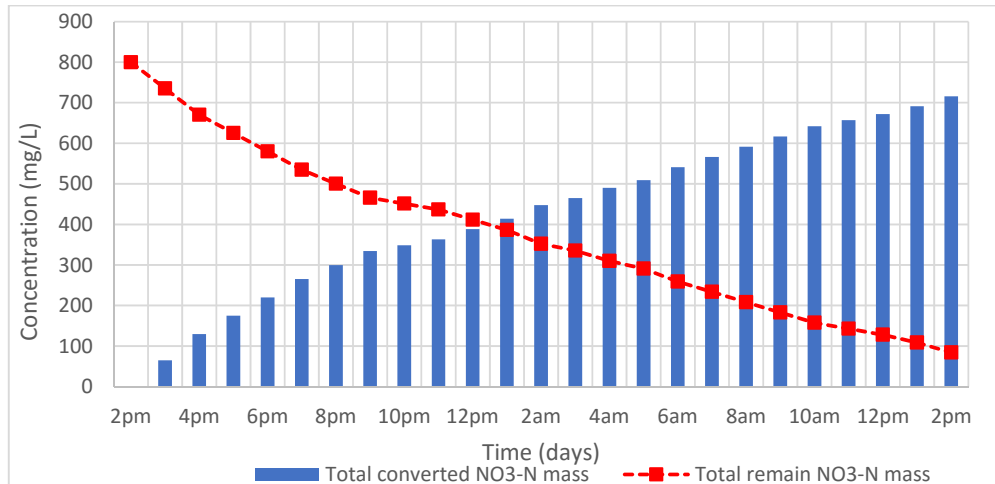
Figure 4.5 below presents trends in concentration of influent ammonium and nitrate at the nitrifying reservoir. It is clear that while ammonium concentration had an increasing trend during the time, nitrate concentration which was produced from influent ammonium was on a continual decline. Ammonium concentration increased from over 10 mg NH<sub>4</sub>-N L<sup>-1</sup> to nearly 15 mg NH<sub>4</sub>-N L<sup>-1</sup> in four days. Conversely, nitrate concentration decreased by approximately 1.6 mg NO<sub>3</sub>-N L<sup>-1</sup> from 3.2 mg NO<sub>3</sub>-N L<sup>-1</sup> to 1.6 mg NO<sub>3</sub>-N L<sup>-1</sup>. From the data, it could be inferred that the ammonium removal rate reached maximum capacity and, therefore, could not produce more nitrate. Meanwhile, nitrate mass could disappear in next several days. Therefore, a decision was made to stop the system and change the method for defining the maximum capacity of the denitrification system.



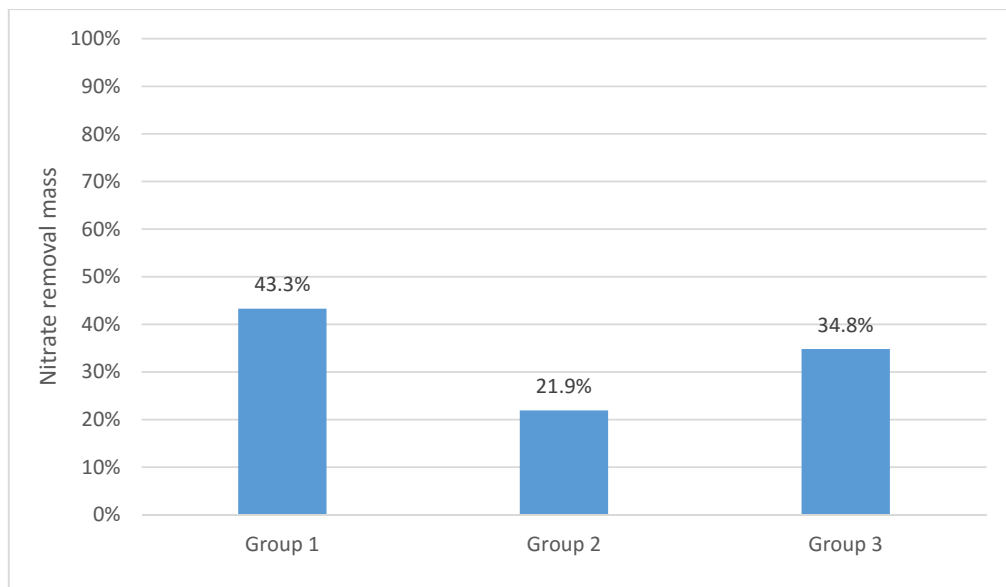
**Figure 4.5.** Ammonium and nitrate in the nitrifying reservoir under following conditions: hydraulic rate of 0.3 L min<sup>-1</sup>, denitrification hydraulic rate of 12.6 ml min<sup>-1</sup>, recirculation flow, and ammonium nitrogen of 4 mg NH<sub>4</sub>-N L<sup>-1</sup>

Accordingly, the nitrification system which was applied to provide nitrate to the denitrification system by converting ammonium to nitrate was stopped. An external nitrate source was utilized as influent nitrate to the denitrifying columns. In addition, the denitrification flow rates were increased 10 times and the sampling time was changed from daily to hourly. All changes were made to ensure that nitrate could be found at effluent points of the denitrification system and, as a result, effluent nitrate was detected from the denitrification columns, indicating saturation of the denitrification capacity of the columns (Figure 3.27).

Total nitrate removal mass was calculated based on Equation 4.3 and 4.4 in which ammonium mass was replaced by nitrate mass. The results are shown in Figure 4.4 below. Input nitrate mass was 800 mg NO<sub>3</sub>-N, and after 24 hours this mass remained 84 mg NO<sub>3</sub>-N. Consequently, 716 mg NO<sub>3</sub>-N was converted to nitrogen gas in a day at a total flow rate was 42 ml min<sup>-1</sup>. It equates to a nitrate removal rate of approximately 11 mg NO<sub>3</sub>-N L<sup>-1</sup> h<sup>-1</sup>. The efficiency of the denitrification system was contributed to by the capacity of three Groups of denitrifying columns. Group 1 with denitrifying column 1 and 2 accounted for over 43% of the total nitrate removal mass. Group 2 and 3 contributed nearly 22 and 35% respectively (Figure 4.5). The efficiency of Group 1 was highest because it combined two denitrifying columns while Group 2 and 3 only had one column.



**Figure 4.4.** Total converted and remaining nitrate in a day of the denitrification system in Experimental Phase 3 under following conditions: hydraulic rate of  $42 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $22.86 \text{ mg NO}_3\text{-N L}^{-1}$



**Figure 4.5.** Nitrate removal mass of groups in the denitrification system in Experimental Phase 3 under following conditions: hydraulic rate of  $42 \text{ ml min}^{-1}$ , recirculation flow, and initial ammonium nitrogen of  $22.86 \text{ mg NO}_3\text{-N L}^{-1}$

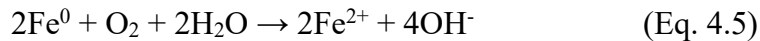
#### 4.3.2. Organic carbon and spongy iron

Organic carbon is considered to be one of the most important factors controlling the denitrification rate. It directly influences the heterotrophic process which accounts for the majority of denitrifiers in the experiment (Knowles 1982). In order to serve organic carbon for denitrifiers bacteria, barley straw was utilized in the experiment. The changes in total organic carbon during the whole time of the experiment is shown in Figure 4.6. It should be noted that all of the water in the nitrification and denitrification systems was replaced at the end of Commissioning Phase and Experimental Phase 1. During the Commissioning phase the concentrations of total organic carbon in each denitrifying column were very high and these concentrations significantly reduced after 10 days of the Phase. In general, it reduced around 2.5 times from around 800 mg L<sup>-1</sup> to over 300 mg L<sup>-1</sup>. The high total organic carbon concentrations in denitrifying columns could be caused by wash out of the soluble components of barley straw. The components were washed out when barley straw in the columns was first immersed in water (Holmes, Plant & Water 2010).

Total organic carbon concentrations in Column 1 and 4 immediately reached the highest value at the first day, while Column 2 and 3 achieved the highest concentration in the following day. Column 2 was installed in series with Column 1, effluent flow of Column 1 containing high total organic carbon concentration was the influent flow to Column 2. As a result, the concentration of total organic carbon in Column 2 increased and took time to reach the highest value. In addition, the total organic carbon concentration in this column was maintained higher than that in other columns during the Experiment. In terms of total organic carbon in Column 3, its flow rate was lower than other columns and this could be a reason for taking longer to reach the highest TOC concentration.

In Phase 1, the concentrations of total organic carbon in columns changed in range from 75 mg L<sup>-1</sup> to over 200 mg L<sup>-1</sup>. These values were higher than those in Phase 2 and 3 which were between approximately 20 mg L<sup>-1</sup> and nearly 140 mg L<sup>-1</sup>. This difference could be explained by the changes of influent ammonium concentration. While total ammonium mass added to the system in Phase 1 was only 360 mg NH<sub>4</sub>-N, this number in Phase 2 and 3 was 2050 mg NH<sub>4</sub>-N. The increase of ammonium concentration led to an increased nitrate concentration. As a result, more organic carbon was utilized to convert nitrate to nitrogen gas in the denitrification system. Based on the performance of the denitrification system, it could be seen that barley straw was a good external carbon source for denitrifiers. This result is in line with other studies of Holmes, Plant and Water (2010), Hashemi et al. (2010) and Zhou (2010).

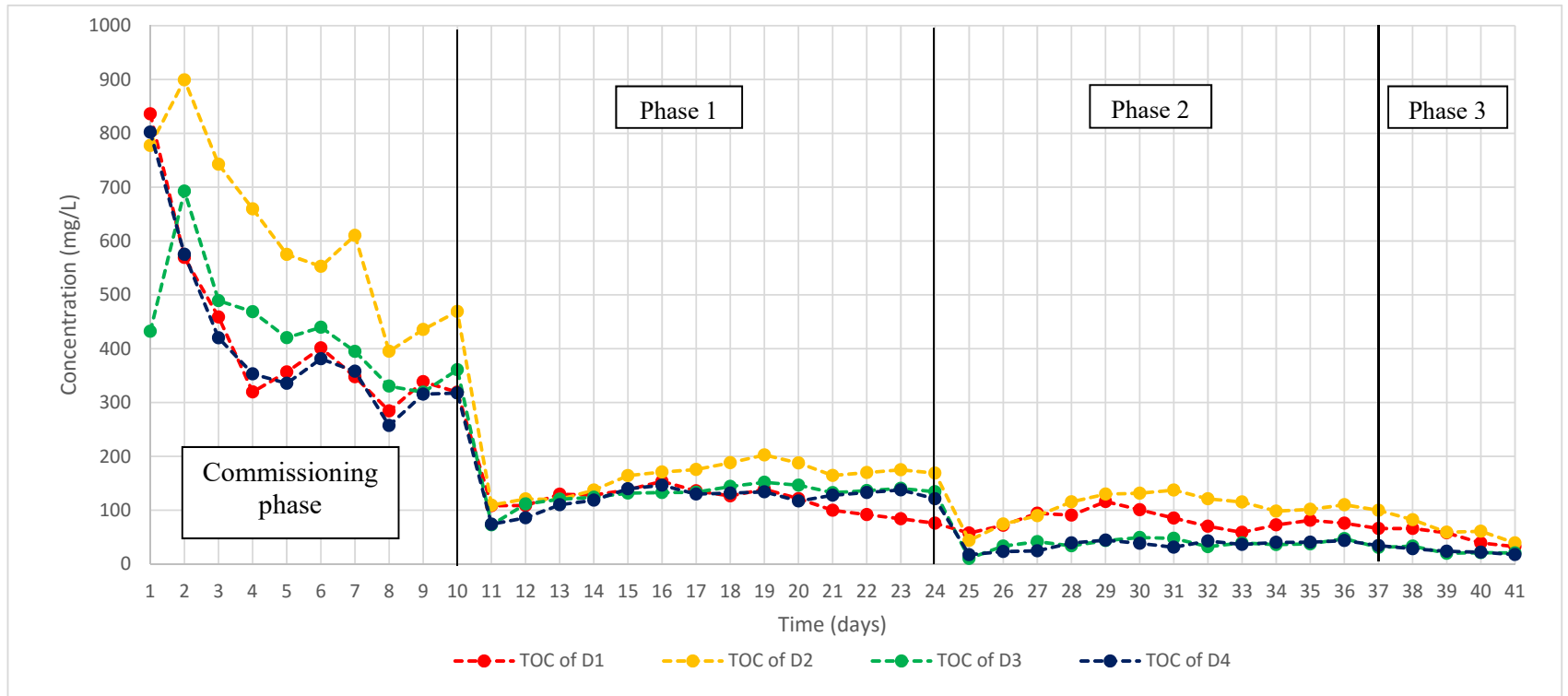
In the previous studies, spongy iron was evaluated as a suitable material for deoxygenation, which can assist in enhancing the denitrification rate (Huang et al. 2015; Liu et al. 2013). Dissolved oxygen is removed by spongy iron via chemical reduction process as Equation 4.5 (Della Rocca, Belgiorno & Meriç 2007).



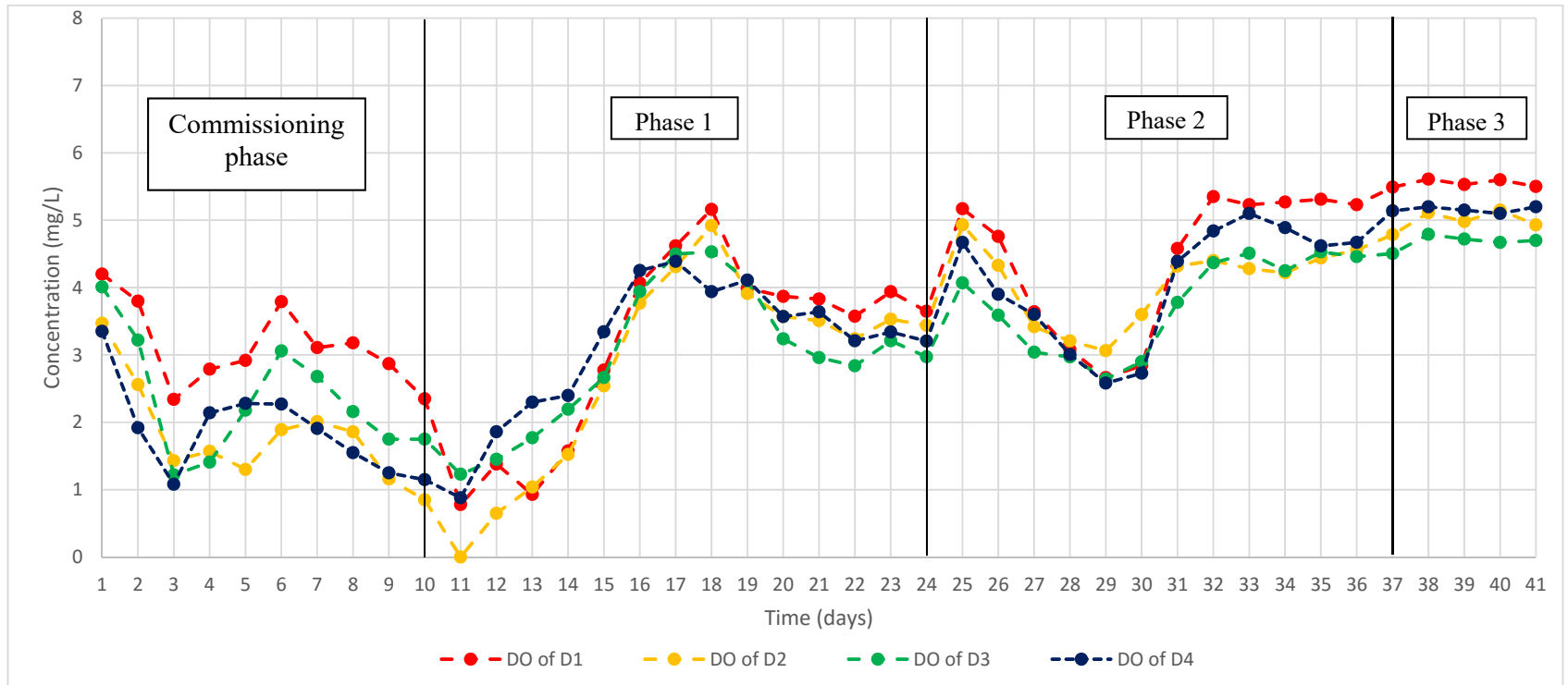
Based on the results indicating the benefits of utilizing spongy iron, the Experiment applied spongy iron into the denitrification system to remove dissolved oxygen. As mentioned above, 20 g of spongy iron was added to each of Column 2, 3 and 4. However, to re-evaluate and compare its effectiveness in the system, Column 1 was free of spongy iron. The changes in dissolved oxygen during the whole experimental time is shown in Figure 4.7. It can be seen from the graph that the performance of spongy iron was quite good in the Commissioning Phase. Dissolved oxygen concentrations in denitrifying columns reduced from average 3.6 mg L<sup>-1</sup> to over 1.2 mg L<sup>-1</sup>. The Figure 4.7 also indicates that dissolved oxygen concentration in columns

with spongy iron was lower than that in Column 1 without spongy iron. However, the dissolved oxygen concentration significantly increased in the first 8 days of Phase 1 and reached about 4.5 mg L<sup>-1</sup> on average before slightly declining until the end of this phase. The trend of changes in dissolved oxygen in Phase 1, 2 and 3 was not as clearly shown. Although dissolved oxygen concentrations in columns with spongy iron were still lower than that in Column 1 without spongy iron, the differences were not great. In short, the effectiveness of spongy iron in this study was limited. This could have been caused by the limited quantity of spongy iron employed. Leading to the suggestion that it might require more spongy iron to reduce and maintain low dissolved oxygen concentration in the denitrification system.





**Figure 4.6.** Change in total organic carbon in the denitrification system during whole experiment under following conditions: recirculation flow; hydraulic rate of 1.5, 1.2 and 1.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively in the first three experimental phases, hydraulic rate of 4.5, 3.6 and 4.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively in the last Phase; temperature of 20 – 21°C; and pH of 4.4 – 6.6

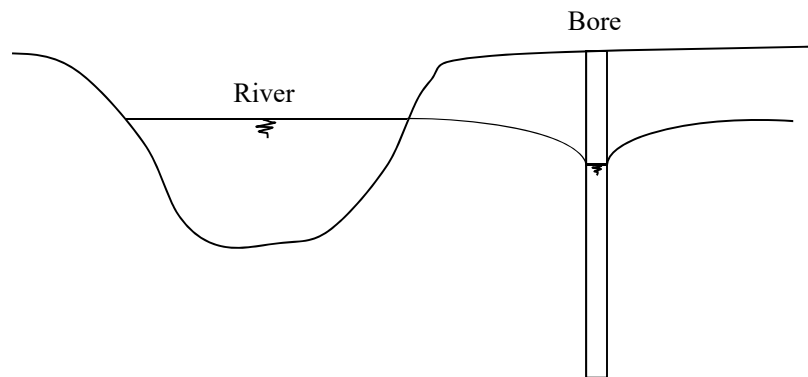


**Figure 4.7.** Change in dissolved oxygen in the denitrification system during whole experiment under following conditions: recirculation flow; hydraulic rate of 1.5, 1.2 and 1.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively in the first three phases, hydraulic rate of 4.5, 3.6 and 4.5 ml min<sup>-1</sup> for Group 1, 2 and 3 respectively in the last Phase; temperature of 20 – 21°C; pH of 4.4 – 6.6; barley straw and spongy iron in Column 2, 3 and 4; and only barley straw in Column 1

## 5. Integration of nitrification and denitrification model

### 5.1. Scenario

The public water utility supplying drinking water is required to build a water treatment plant for a small rural town with a population of 1,000 people. The standard water usage is 150 L day<sup>-1</sup> per person and the total water supply demand of the town is 150 m<sup>3</sup> d<sup>-1</sup>. Treated water must always meet the requirements of national drinking water standards and guidelines. The water source is pumped from a bore located near the river (Figure 5.1) which flows through a cultivated field.



**Figure 5.1.** Location of water source supplying the water treatment plant

Initial water analysis results from the bore reveals that, in general, the water quality satisfies requirements of raw water for a water treatment plant. However, ammonium and nitrate were detected in water samples with the ammonium concentration being around 3 mg NH<sub>4</sub> L<sup>-1</sup> (2.5 mg NH<sub>4</sub>-N L<sup>-1</sup>) and the value of nitrate being approximately 200 mg NO<sub>3</sub> L<sup>-1</sup> (45.2 mg NO<sub>3</sub>-N L<sup>-1</sup>). Both ammonium and nitrate concentration values exceed the standards in the national guidelines. In detail, the average value of ammonium is over 6 times higher than the guideline value of 0.5 mg NH<sub>4</sub> L<sup>-1</sup> (0.41 mg NH<sub>4</sub>-N L<sup>-1</sup>), while nitrate concentration is nearly 4 times above

with the national standard safety value of  $< 50 \text{ mg NO}_3 \text{ L}^{-1}$  ( $11.3 \text{ mg NO}_3\text{-N L}^{-1}$ ). The initial investigations indicate that poor agricultural activities are a main reason leading to ammonium and nitrate contamination. Although there are positive changes in agricultural activities in the field, there is a likelihood of ammonium and nitrate contamination still occurring in the near future. Therefore, units for removing ammonium and nitrate is required in the water treatment plant.

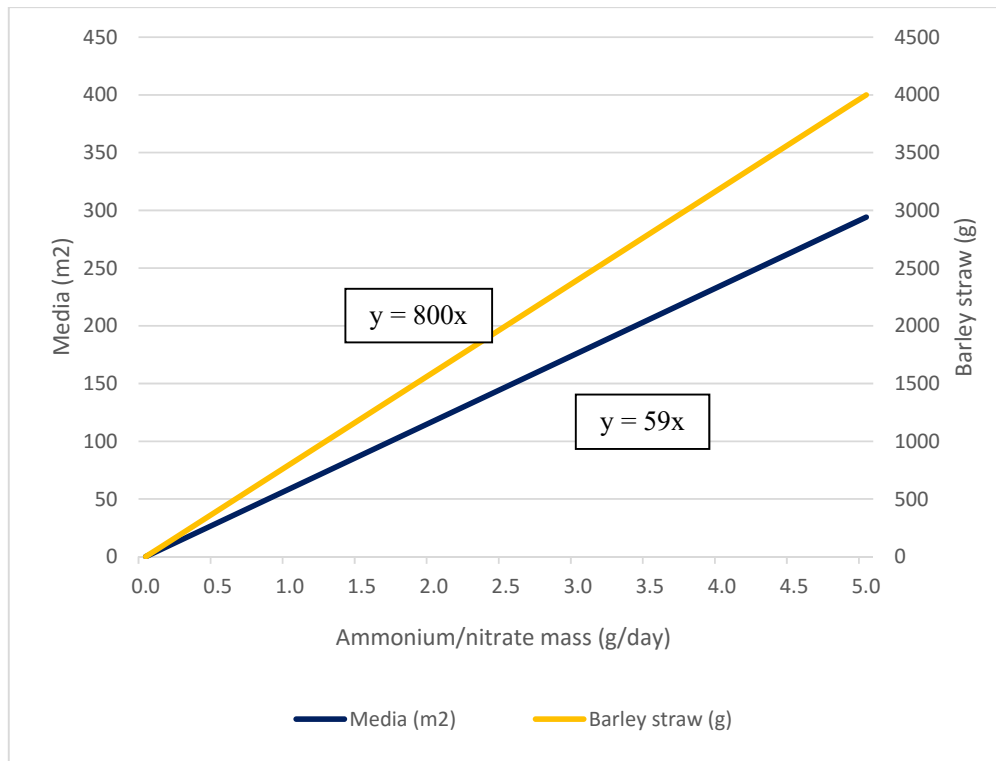
## 5.2. Hypotheses

In order to calculate the dimensions of decontamination units removing ammonium and nitrate in the water source, several hypotheses are proposed based on results of the laboratory study reported here including:

- (1) Relationship between ammonium concentration and nitrification is linear, in which nitrification is a mathematical function of ammonium concentration. Nitrification rate is proportional to polypropylene media surface area.
- (2) Relationship between nitrate concentration and denitrification is linear, in which denitrification is a mathematical function of nitrate concentration. Denitrification rate is proportional to weight of barley straw.
- (3) A nitrogen mass balance is maintained during nitrification and denitrification, subsequent differences in the total mass of soluble N is assumed to be due to loss of  $\text{N}_2$  via denitrification.

As mentioned, results of Experimental Phase 2 revealed that the average ammonium removal rate of the nitrification system was  $83 \text{ mg NH}_4\text{-N d}^{-1}$ , while  $4.88 \text{ m}^2$  of polypropylene media was utilized for the removal of this ammonium amount. It corresponds with an ammonium unit surface removal of  $17 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$ . Based on this unit surface removal of ammonium

a mathematical relationship between ammonium removal mass per day and media surface area was developed and is shown in Figure 5.2. Similarly, the Experimental Phase 3 indicated that 716 mg NO<sub>3</sub>-N day<sup>-1</sup> was removed by three groups of the denitrification system, in which, 43, 22 and 35% of total nitrate removal mass were contributed by Group 1, 2 and 3 respectively. Although Group 1 accounts for 43% removal, the highest efficiency belonged to Group 3 with 35% because Group 1 is comprised of two columns. The relationship between nitrate removal mass and barley straw mass was developed based on the highest efficiency of Group 3 (Figure 5.2). From the figure, it can be seen that in order to remove 1 mg NO<sub>3</sub>-N day<sup>-1</sup>, it requires 0.8 g of barley straw. Accordingly, Figure 5.2 can be used to define necessary media polypropylene volume and barley straw mass once the ammonium/nitrate concentration is known.



**Figure 5.2.** Relationship among loading Ammonium/Nitrate mass, media and barley straw

### 5.3. Ammonium and nitrate removal units

#### 5.3.1. Nitrification unit

Nitrification rate and percentage nitrification closely depend on ammonium surface load and hydraulic surface load. If these factors change, the efficiency of the nitrification system could significantly increase or decline (van den Akker 2008). Hence the necessary polypropylene media volume for the nitrification system was calculated by two different methods including ammonium surface load and hydraulic surface load methods.

##### *a. Ammonium surface load method*

Total ammonium nitrogen mass loaded to the nitrification system is defined as Equation 5.1 below.

$$C = Q \times C_0 = 375 \text{ (g NH}_4\text{-N day}^{-1}\text{)} \quad \text{(Eq. 5.1)}$$

Where  $C$  is total ammonium nitrogen mass per day ( $\text{g NH}_4\text{-N day}^{-1}$ );  $Q$  is total water supply demand of the town ( $Q = 150 \text{ m}^3$ );  $C_0$  is initial ammonium nitrogen concentration ( $C_0 = 2.5 \text{ mg NH}_4\text{-N L}^{-1}$ ).

The plastic media area required to remove ammonium from the water source is  $20,059 \text{ m}^2$  (Equation 5.2) below. It corresponds to approximately  $92 \text{ m}^3$  of plastic media which has a surface area of  $240 \text{ m}^2 \text{ m}^{-3}$ . This result is similar to the result referred from Figure 5.2.

$$A_M = \frac{C}{M_{\text{asl}}} = 20,509 \text{ (m}^2\text{)} \quad \text{(Eq. 5.2)}$$

Where  $A_M$  is the necessary area of plastic media for ammonium removal ( $\text{m}^2$ );  $M_{\text{asl}}$  is mass of ammonium nitrogen surface load achieved in the Experiment ( $M_{\text{asl}} = 17 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$ ).

Based on the required plastic media volume, the primary inside dimensions of the nitrification unit are 2.9 m in height and 7.0 m in diameter. The height of plastic media is 2.4 m which equals the total height of media in experimental nitrifying columns. The overall height of the nitrification tank would include an additional 0.3 m at the top of the column to reduce overspray and 0.2 m at the bottom of the nitrification tank used to collect water before pumping to denitrification system; overall height would therefore be about 2.9 m. The irrigation rate of the nitrification tank is  $3.9 \text{ m}^3 \text{ m}^{-2} \text{ day}^{-1}$  (Equation 3.2).

*b. Hydraulic surface load method*

The plastic media area required to remove ammonium in water source is  $1667 \text{ m}^2$  and calculated as Equation 5.3 below. It corresponds to approximately  $7 \text{ m}^3$  of plastic media which has surface area is  $240 \text{ m}^2 \text{ m}^{-3}$ .

$$A_{M1} = \frac{Q}{Q_s} = 1,667 \text{ (m}^2\text{)} \quad (\text{Eq. 5.3})$$

Where  $A_{M1}$  is the necessary area of plastic media for ammonium nitrogen removal ( $\text{m}^2$ );  $Q$  is total water supply demand of the town ( $Q = 150 \text{ m}^3$ );  $Q_s$  is hydraulic surface load of the Experiment ( $Q_s = 0.09 \text{ L m}^{-2} \text{ day}^{-1}$ ).

The primary inside dimensions of the nitrification unit are calculated based on required polypropylene media volume of  $7 \text{ m}^3$ . Its dimensions are 2.9 m in height and 2 m in diameter. Height of plastic media is 2.4 m which equals to total height of media in experimental nitrifying columns. An additional 0.3 m collar to prevent overspray from the top of the filter and 0.2 m at the bottom of the nitrification tank was used to collect water before pumping to denitrification system. The irrigation rate of the nitrification tank is nearly  $48 \text{ m}^3 \text{ m}^{-2} \text{ day}^{-1}$  and it is calculated as Equation 3.2. Meanwhile, ammonium surface load is approximately  $225 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$  as Equation 5.4 below.

$$M_{\text{asll}} = \frac{C}{A_{\text{M1}}} = 225 \text{ (mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}) \quad (\text{Eq. 5.4})$$

Where  $M_{\text{asll}}$  is mass of ammonium nitrogen surface load per day (mg NH<sub>4</sub>-N m<sup>-2</sup> day<sup>-1</sup>);  $C$  is total ammonium nitrogen mass per day ( $C = 375 \text{ g day}^{-1}$ );  $A_{\text{M1}}$  is required plastic media area for ammonium nitrogen removal ( $A_{\text{M1}} = 1,667 \text{ m}^2$ ).

It can be seen from this data that the required polypropylene media volume is very different between the two methods described. While the ammonium nitrogen surface load method required up to 92 m<sup>3</sup> of media, this number in the hydraulic surface load method is only 7 m<sup>3</sup>. Furthermore, results in each method has limitations. In the former method, although removal rate of ammonium nitrogen 17 mg NH<sub>4</sub>-N m<sup>-2</sup> day<sup>-1</sup> is consistent with the Experimental phase results, its irrigation rate is around 13 times lower than that in the experiment. Meanwhile, in the latter method, the irrigation rate is quite similar to the experimental value, however, the ammonium nitrogen surface load is over 13 times higher than that in the experimental result. In order to obtain good results in the nitrification process, a larger nitrification unit which has dimensions with 2.9 m in height and 7 m in diameter is selected for the water treatment plant.

### 5.3.2. Denitrification unit

Total nitrate nitrogen including nitrate in water source 45.2 mg NO<sub>3</sub>-N L<sup>-1</sup> and nitrate converted from ammonium nitrogen 2.5 mg NO<sub>3</sub>-N L<sup>-1</sup> was 47.7 mg NO<sub>3</sub>-N L<sup>-1</sup>. Total nitrate nitrogen needed to be removed is 7,155 g NO<sub>3</sub>-N day<sup>-1</sup> and it is defined as Equation 5.5 below.

$$C_{\text{N}} = Q \times C_{\text{N0}} = 7,155 \text{ (g NO}_3\text{-N day}^{-1}) \quad (\text{Eq. 5.5})$$

Where  $C_{\text{N}}$  is total nitrate nitrogen mass per day (g NO<sub>3</sub>-N L<sup>-1</sup>);  $Q$  is total water supply demand of the town ( $Q = 150 \text{ m}^3$ );  $C_0$  is the initial nitrate nitrogen concentration in water source ( $C_0 = 47.7 \text{ mg NO}_3\text{-N L}^{-1}$ ).



Based on the relationship between nitrate concentration and barley straw mass (Figure 5.2), it could be inferred that in order to remove  $7,155 \text{ g NO}_3\text{-N day}^{-1}$ , it requires 5,724 kilogram of barley straw. Moreover, barley straw density is indicated to be  $112 \text{ kg m}^{-3}$  in the study of Lerner and Goode (2000). Therefore, the required volume of barley straw is about  $51 \text{ m}^3$ . The primary inside dimensions of the denitrification unit are 7 m in length, 3.7m in width and 1.8 m in height, within which the height of barley straw is 1.5 m. The freeboard of the denitrification tank is 0.3 m. The denitrification tank is covered to limit the dissolution of oxygen into water in this tank. The retention time in the denitrification tank is 8.2 hours, while this number is over 7 hours in denitrifying Column 3, which was selected to build a relationship between nitrate nitrogen concentration and barley straw mass.

Consequently, in order to remove  $3 \text{ mg NH}_4 \text{ L}^{-1}$  ( $2.5 \text{ mg NH}_4\text{-N L}^{-1}$ ) and  $200 \text{ mg NO}_3 \text{ L}^{-1}$  ( $45.2 \text{ mg NO}_3\text{-N L}^{-1}$ ) in  $150 \text{ m}^3$  water per day, nitrification and denitrification units need to be built in the water treatment plant. The dimensions of the nitrification tank are 2.9 m in height and 7.0 m in diameter, while the dimensions of the denitrification tank are 7 m in length, 3.7m in width and 1.8 m in height. Total polypropylene media volume is  $92 \text{ m}^3$  and barley straw mass is 5,724 kilograms. Nitrification and denitrification units are installed after the bore and before other units of the water treatment plant.

#### **5.4. Limitation**

Although a model which integrates nitrification and denitrification systems was described in item 5.3 above, it is only a concept or beginning of the design which needs to be developed further before application in the field. There are several limitations of both nitrification and denitrification systems which should be considered to improve the model quality, such as utilization of recirculation flow and experimental time.

Firstly, utilization of recirculation flow could be a main reason leading to low capacity of the nitrification system. Only 17 mg NH<sub>4</sub>-N m<sup>-2</sup> day<sup>-1</sup> were removed by nitrifying columns in the Experiment and this result might be quite low and under its real capacity. A number of previous studies showed that the capacity of nitrification is quite high from 300 to 1000 NH<sub>4</sub>-N m<sup>-2</sup> day<sup>-1</sup> in comparison with the results of the experiment (Timmons & Summerfelt 1998; Tucker & Hargreaves 2004). Similarly, in a study about removal of ammonia by biological nitrification in a fixed film reactor, van den Akker (2008) also indicated that a maximum nitrification rate can be achieved between 800 and 1000 mg NH<sub>4</sub>-N m<sup>-2</sup> day<sup>-1</sup>. Low nitrification capacity in the experiment led to significantly increasing the polypropylene media volume. As a result, dimensions of the nitrification tank are greater. The main reason for limited nitrification capacity could be total organic carbon in recirculation of the denitrification flow. The analysis of results revealed that total organic carbon in effluent flows of the denitrification system are quite high from around 50 mg L<sup>-1</sup> to nearly 900 mg L<sup>-1</sup>. Meanwhile, negative effects of organic carbon on the nitrification process have been reported in many researches (Fdz-Polanco et al. 2000; Gupta & Gupta 2001; Jie et al. 2009; Ling & Chen 2005; Okabe et al. 1996). Parker and Richards (1986) concluded that nitrification is prohibited if organic carbon concentration is greater than 20 mg sBOD<sub>5</sub> L<sup>-1</sup>. Similarly, van den Akker (2008) and van Den Akker et al. (2010) indicated that percentage nitrification is a function of organic carbon load. It can achieve 90 to 100 % of nitrification if the organic load is under 4 mg sBOD<sub>5</sub> L<sup>-1</sup>. And this number will decline if organic carbon concentration increases over 5.5 mg sBOD<sub>5</sub> L<sup>-1</sup>.

In addition, the experiment was conducted within a short period of time (25/05/2016 to 7/08/2016), therefore, its results might be limited. For example, nitrification bacteria did not reach maximum capacity because they

require more time for their development. Verstraete, Vanstaen and Voets (1977) and Vallés-Morales et al. (2004) reported that it requires up to 100 days for nitrification bacteria to develop and achieve stability. When the development of bacteria is stable, percentage nitrification and nitrification rate are notably increased. Furthermore, the nitrate and nitrite removal ability of the denitrification system was only evaluated in limited time conditions rather than over a long period. This evaluation may not offer definitive evaluation of performance. Furthermore, the study did not indicate how long before barley straw remained active and effective and how often barley straw replacement should occur. Several studies concluded that it requires at least 14 days to activate barley straw and 30 days to make it effective. According to research, the effective period of barley straw is from 4 to 6 months (Barrett, Littlejohn & Curnow 1999; Holmes, Plant & Water 2010). Therefore, increasing period of observation to further determine the capacity and longevity of barley straw should be considered for further study.

### **5.5. Future improvement**

In order to improve the reliability of the integration of nitrification with denitrification model, further research should be conducted with a larger scale pilot and should consider utilization of single pass loading and an expansion of the experimental time period. Indeed, results from a larger scale pilot systems are more precise than those from smaller scale pilot systems (Leon, Davis & Kraemer 2011). In reality, larger scale pilots more accurately reflect the characteristics of the full scale systems.

In terms of flow, a single pass loading could assist in eliminating the effects of total organic carbon from recirculation of denitrification flow which contains a huge amount of organic carbon from barley straw. When the effects of organic carbon are removed from the denitrification system, nitrification rate and percentage nitrification results will be determined more

accurately. Additionally, a variety of hydraulic surface loads should be deployed because this could enhance the accuracy of the relationships among ammonium/nitrate mass, polypropylene media volume and barley straw mass (Figure 5.2). This is a necessary precursor for integration of nitrification with denitrification systems.

Furthermore, the experimental time should be increased so that analysis results from nitrification and denitrification systems are more precise. With a suitable length of experimental time, the nitrifying bacteria could achieve more stable development, while necessary information about barley straw, such as active time and longevity, would also be more accurately determined.

## 6. Conclusion

In conclusion, this study indicated that the nitrification system can remove  $83 \text{ mg NH}_4\text{-N day}^{-1}$  with  $4.88 \text{ m}^2$  of polypropylene media. This corresponds to  $17 \text{ mg NH}_4\text{-N m}^{-2} \text{ day}^{-1}$  of ammonium nitrogen removal per unit surface area of the filter. While,  $716 \text{ mg NO}_3\text{-N}$  was eliminated by the denitrification system, Column 1 and 2 accounted for over 43% of the total nitrate nitrogen removal mass. These figures were nearly 22% and 35% contributed to by Column 3 and 4 respectively. Based on the experimental results and several hypotheses, the relationship among ammonium/nitrate mass, polypropylene media volume and barley straw mass was established (Figure 5.2). This graph is a useful tool to measure the required polypropylene media surface area in the nitrification system and barley straw mass in the denitrification system when the initial ammonium/nitrate mass removal is pre-defined. Furthermore, the application of this graph can enable the development of suitable water treatment model for integrating a nitrification unit and a denitrification unit that can efficiently remove ammonium and nitrate from surface water and groundwater.

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