ABSTRACT

Title of Thesis: EVALUATING THE IMPACT OF DIFFERENT CARBON SOURCE AND COD/NO3-N RATIO ON COUPLING PARTIAL DENITRIFICATION AND ANAMMOX

Bo Peng, Master of Science, 2017

Thesis Directed By: Dr. Alba Torrents
Department of Civil and Environmental Engineering, University of Maryland

As the nitrogen discharge limit gets more stringent, ammonium and nitrate residual in the effluent of mainstream deammonification process are becoming an issue that need to be addressed. Thus, developing a post-deammonification polishing process is necessary to promote the implementation of mainstream deammonification. In this study, a final polishing process coupling anammox with partial denitrification is proposed to simultaneously remove ammonium and nitrate, and the long-term (120 days) impact of carbon type and COD/N ratio on promoting partial denitrification was evaluated. Results suggested that acetate and glycerol displayed a good potential for promoting partial denitrification. However, methanol showed challenges of establishing efficient partial denitrification in long-term operation. The key factor for a successful concurrent operation of anammox and partial denitrification lies on controlling the balance between denitrifier rate and AnAOB rate to simultaneously remove residual ammonium and nitrate.

Key words: partial denitrification; alternative external carbon sources, COD/N ratio; anammox; mainstream deammonification; final polishing;

EVALUATING THE IMPACT OF DIFFERENT CARBON SOURCE AND COD/NO3-N RATIO ON COUPLING PARTIAL DENITRIFICATION AND ANAMMOX

by

Bo Peng

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
[Master of Science]
[2017]

Advisory Committee:
Professor Alba Torrents, Chair
Dr. Birthe Kjellerup
Dr. Haydée De Clippeleir

Acknowledgements

I would first like to express my gratitude to my advisor Professor Alba Torrents of the Department of Civil and Environmental Engineering at University of Maryland and my supervisor Dr. Haydée De Clippeleir at DC Water. They have shown great support whenever I ran into a trouble or had a question about my research or writing.

I would also like to thank my working partners Tri Le who was involved in the project and providing solid support. Without his passionate participation and input, this study could not have been successfully conducted.

Finally, I must express my very profound gratitude to my parents, my partner Fan Yang, and my friends Chunyang Su and Qi Zhang for constantly showing their support and encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AOB – Ammonia oxidizing bacteria
AWTP – Advanced wastewater treatment plant
AnAOB – Anaerobic ammonia oxidizing bacteria
BNR – Biological nutrient removal
CAC – Citric acid cycle
COD – Chemical oxygen demand
DEAMOX – Denitrifying ammonium oxidation
DNRA – Dissimilatory nitrate reduction to ammonium
DO – Dissolved oxygen
FDN – Full denitrification
HRT – Hydraulic retention time
MLSS – Mixed liquor suspended solids
NOB – Nitrite oxidizing bacteria
PAO – Polyphosphate-accumulating organisms
PDN – Partial denitrification
PHA – Poly-b-hydroxyalkanoates
PHB – Polyhydroxybutyrate
SRT – Sludge retention time
TIN – Total inorganic nitrogen
TSS – Total suspended solid
VSS – Volatile suspended solid
WWTP – Wastewater treatment plant

Chapter 1 Introduction

1.1 Problem Statement

In wastewater treatment processes, the possibility of replacing conventional nitrogen removal processes with mainstream deammonification is being investigated, and it showed a promising potential for its energy effectiveness and nutrient removal efficiency (WERF, 2015). However, most of the proposed technologies and operational strategies had an effluent issue containing a total nitrogen residual higher than 5 mg N/L, mainly in the form of NO_3^- and NH_4^+ (Han et al. 2015; Regmi et al. 2015). As the nitrogen discharge limit is getting more stringent, a nitrogen polishing step for the effluent of mainstream deammonification process is essential. Conventionally, nitrogen polishing can be done through nitrification and denitrification. However, both of these processes are costly operations as aeration for nitrification requires a lot of energy, and denitrification needs the addition of an external carbon as energy source. Therefore, a more cost-effective final polishing step, which is driven by the concurrent operation of partial denitrification and anammox process, is proposed. Theoretically, 100% cost of aeration and up to 60% cost of external carbon can be saved by using this polishing step.

1.2 Objectives

The overall goal of this study is to investigate the feasibility of developing an anoxic nitrogen polishing step for the effluent of mainstream deammonification systems. Effluent from these systems usually contain ammonium and nitrate (Han et al. 2015; Regmi et al. 2015), which could be simultaneously removed by the proposed anoxic nitrogen polishing step performing partial denitrification and anammox process. In this polishing step, nitrate is expected to be only reduced to nitrite through denitrification and then the ammonium and nitrite are removed through anammox. Therefore, promoting partial denitrification over full denitrification is crucial to maintain the efficiency of the nitrogen polishing step. The objective of this study are:

1) Investigate the impact of type of external carbon (acetate, methanol, and glycerol) on selecting partial denitrification.
2) Estimate the impact of low COD/N ratio on selecting partial denitrification.
3) Evaluate the AnAOB activity on the concurrent operation of partial denitrification and anammox.

Chapter 2 Literature Review

2.1 Biological Nutrient Removal Processes

Nitrogen and phosphorus are the main reasons to cause eutrophication, which induces overgrowth of plants and algae and may reduce the dissolved oxygen content, in water bodies (U.S. EPA, 2007). It is important to reduce the content of these nutrients in treated wastewater before it is discharged. To alleviate the situation of nutrient excess in water bodies, many point source dischargers (e.g., wastewater treatment plant) have received stringent limits for nitrogen and phosphorus level in their effluent (U.S. EPA, 2007). Biological nutrient removal (BNR) processes remove total nitrogen and total phosphorus from wastewater with the use of microorganisms under different operation environment in the treatment processes (Metcalf and Eddy, 2003). In most facilities using BNR processes, both nitrogen and phosphorus are removed simultaneously (Williams & Wilson, 1994). In raw wastewater, the majority of nitrogen exists in the form of organic nitrogen, which may be bound in complex organic compounds, and ammonia. The mostly used biological treatment processes for removal of nitrogen is through nitrification and denitrification, which convert ammonia to nitrate and nitrogen gas consequently. Phosphorus, generally, in the influent wastewater is removed through two steps. Firstly, polyphosphate-accumulating organisms (PAOs) assimilate carbon sources and store them as poly-b-hydroxyalkanoates (PHAs) under anaerobic condition. Then PAOs use these PHAs for growth and at the meantime can store phosphate as intracellular polyphosphate, leading to the phosphorus removal in the wastewater (Metcalf and Eddy, 2003).

2.1.1 Nitrification

Nitrification is a two-step aerobic process to convert ammonia (NH_4^--N) to nitrate (NO_3^+-N) with nitrite (NO_2^--N) as an intermediate product. The conversion is mainly driven by two groups of autotrophic bacteria as follows: ammonia oxidizing bacteria (AOB) which oxidize ammonia to nitrite and nitrite oxidizing bacteria (NOB) which further oxidize nitrite to nitrate (Metcalf and Eddy, 2003). Most of the studied AOB belongs to the genera *Nitrosomonas* and NOB is mainly from the genus *Nitrobacter* and *Nitrospira* (Davis, 2011). The two-step oxidation processes are as described below:

First step by AOB:

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad (1)$$

Second step by NOB:

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (2)$$

The total reaction:

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad (3)$$

Based on equation (3), the theoretical oxygen demand for the total nitrification process is calculated as 4.57 g O_2/g N, where 3.43 g O_2 for ammonia conversion and 1.14 g O_2 for nitrite oxidation. Besides dissolved oxygen (DO), nitrification also requires sufficient alkalinity. Davis (2011) specified that 7.14 g of alkalinity as $CaCO_3$ is needed for oxidizing 1 g of ammonia.

Several environmental factors influence the nitrification process, such as pH, DO concentration, and temperature. Generally, nitrification process in wastewater treatment plant is controlled between pH of 7.0-7.2, while it can occur in the pH range of 6.8 to 8 (Davis, 2011; Metcalf and Eddy, 2003). DO level above 2.0 mg/L is recommended for nitrification process, and negative effect to the degree of nitrification is induced when DO level is below 0.5 mg/L (Grady et al., 1999). Temperature has an impact on the nitrification processes by affecting growth rate of nitrifiers. An exponential increase of specific growth rate can be observed between 5 and 30°C and the specific growth starts to decrease to zero when the temperature is higher than 35°C (Henze et al., 2002).

2.1.2 Denitrification

Denitrification is an anoxic respiration process in which nitrate is eventually reduced to nitrogen gas. It occurs whenever nitrate is present and oxygen is depleted. In wastewater treatment processes, nitrate can be reduced to nitrogen gas (N_2) with nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) as intermediate products after ammonia is converted to nitrate through nitrification process. The metabolic pathway is displayed as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (4)$$

A broad range of facultative anaerobic bacteria can perform denitrification, but the denitrifying ability of them varies (Alexander, 1961). Some of them are only capable of reducing nitrate to nitrite, some of them can only reduce nitrite to nitrogen gas, and some of them are able to reduce both nitrate to nitrite to nitrogen gas. Denitrifiers are presented in all main phylogenetic groups, most of them are heterotrophic bacteria, although autotrophic denitrifiers have also been discovered (Carlson and Ingraham, 1983; Zumft, 1997).

In each step of the denitrification process, oxidation of electron donors, organic matter in most cases, would happen corresponding to the reduction of oxidized form of nitrogen. In wastewater treatment plants, an external carbon source is added into denitrification process to provide sufficient chemical oxygen demand (COD). The total reaction can be written as nitrate-based half reaction, where e^- represents the donated electrons from organic matter, and complete reaction stoichiometry for the biodegradable organic matter below (U.S.EPA:

$$10e^- + 2NO_3^- + 12H^+ \rightarrow N_2 + 6H_2O \qquad (5)$$

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \qquad (6)$$

Based on equation (5), 5 mole of electrons, which is equivalent to the electrons provided by 1.25 mole of oxygen, are needed for reducing 1 mole of nitrate, thus 2.86 gram of oxygen is needed to reduce 1 g of NO3-N to nitrogen gas.

The main environmental factors influencing the denitrification process are pH, DO concentration, and temperature. Similar to nitrification process, pH also affects the growth rate of denitrifiers. The optimum pH for denitrification has been reported between 7.0 and 9.0 (Christensen and Harremoes, 1977; Dangcong et al., 2004). As shown in equation (6), pH increases in denitrification process as OH^- is produced. It is reported that 3.57 g of alkalinity (as $CaCO_3$) is produced for 1 gram of nitrate nitrogen being reduced (Metcalf and Eddy, 2003). Oxygen can affect the denitrification process by inhibiting the synthesis and activity of enzyme (Korner and Zumft, 1989). Additionally, Betlach and Tiedje (1981) demonstrated that the presence of DO might induce the accumulation of intermediate products. As denitrification is an anoxic process, the presence of oxygen could stop nitrate reduction as oxygen is a more active electron acceptor than nitrate. A DO level of lower than 0.2 mg O_2/L is recommended for optimizing denitrification process (Knowels, 1982). Temperature is an important factor affecting the growth rate and activity of microorganisms. The relationship between reaction rate and temperature for denitrification process has been reported as linear correlated in the range of 5 to 35°C, with an optimum of 40°C (Lewandowski, 1982).

2.1.3 Anammox

Anaerobic ammonium oxidation (anammox) is an important process that exists in many natural ecosystems and has a tremendous contribution in global nitrogen cycle (Kartal et al., 2007). In this biological process, anammox bacteria are attracted to presence of ammonium and nitrite. Ammonium acts as an electron donor to reduce nitrite to nitrogen gas with hydrazine as an intermediate under anoxic condition. The total reaction is simplified as follows:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (7)$$

Anammox was always occurring in wastewater treatment plants. However, people were not always aware that they were there and did not know what was responsible for the process (Boumann et al. 2009; Kuenen, 2008). In 1995, anammox was discovered in a denitrifying fluidized bed reactor for the first time (Mulder et al., 1995). Since then, anammox process had caught researchers’ attention for applying it into industry. Later in the 2000s, anammox became the trademarked name for the anammox-based ammonium removal technology (van der Star et al. 2007). To date, anammox process has proven with a higher nitrogen removal rate, lower operating costs, and less space demands (Jetten et al., 2005; Joss et al., 2009; van der Star et al., 2007).

There are five anammox genera have been identified: *Brocadia, Kuenenia, Scalindua, Anammoxoglobus*, and *Jettenia*. Each genus of anammox dominate different ecosystems, suggesting that anammox is highly adaptable to form ecological niches (Staunton, 2014). Anammox bacteria, or anaerobic AOB (AnAOB), has an extremely slow overall growth rate with a doubling time of approximately 2 weeks (Kuenen, 2008; Staunton, 2014) and they are sensitive to the changes of ambient environment (Strous, Kuenen, & Jetten, 1999), thus it is not easy to cultivate and enrich these organisms. Similar to nitrification and denitrification processes, several factors are also affecting anammox process. High free ammonia (NH_3) concentration has been found to be an inhibitor to AnAOB activity, and the optimal free ammonia concentration was reported to be less than 20 -25 mg/L (Fernández et al., 2012). pH can decrease the free ammonia concentration, thus a neutral pH is recommended. Strous et al. (1999) reported a pH range of 6.7-8.3 is preferable, while Egli et al. (2001) stated that AnAOB remained well activity within a pH range of 6.5 and 9, with the optimum pH of 8. Nitrite (NO_2^-) concentration has also been widely studied on its inhibition to AnAOB activity. An influent nitrite concentration of 280 mg N/L and an effluent nitrite concentration of 100 mg N/L is generally considered as an accepted warning threshold (Jin, Yang, Yu, & Zheng, 2012). As anammox process is anoxic, a lower DO level is critical to guarantee the AnAOB activity. High concentration of DO (>18% air saturation) has been reported to have irreversible inhibition on AnAOB activity (Egli et al., 2001). Additionally, temperature also impact the AnAOB activity, the optimum range is between 30 and 40 °C (Jin et al., 2012).

2.1.4 Deammonification

Deammonification is a short-cut nitrogen removal pathway in the nitrogen-cycle and a two-step biological process where AOB aerobically convert about half the amount of ammonia to nitrite, performing partial nitrification, and the residual ammonia and nitrite are removed by AnAOB through anammox process under anoxic condition (Wett, 2007). Theoretically, 62.5% less of oxygen is required for deammonification compared to complete nitrification based on equation (1) and (3). Additionally, this process do not need external carbon source to perform heterotrophic denitrification. Comparing to conventional nitrification and denitrification in wastewater treatment processes, deammonification is more energy efficient in treating ammonia-rich wastewater. It is promising for wastewater treatment plant to become more sustainable, cost effective, and energy positive by deploying deammonification.

Table 1. Effluent quality of several mainstream deammonification systems

References	NH4-N (mg N/L)	NO2-N (mg N/L)	NO3-N (mg N/L)	Total Nitrogen (mg N/L)
Laureni et al., 2016	1.8 ± 0.4	<0.2	3.6 ± 1.4	5.7 ± 1.3
Lotti et al., 2014	6.8 ± 5.4	2.3 ± 0.4	9.1 ± 3.1	18.2 ± 8.9
Regmi et al., 2015	4.4 ± 2.9	0.14 ± 0.2	3.6 ± 2.2	8.14 ± 5.3
Trojannowicz et al., 2016	6.5	0.3	19.1	25.9
Han et al., 2015	2.5	0.2	3.9	6.6

Many strategies and systems have been proposed and studied regarding mainstream deammonification, and the effluent from these systems would have at least 1-2 mg/L of ammonium residual and the nitrate residual depends on the efficiency of NOB out-selection. Table 1 lists several researches studying mainstream deammonification, and the effluent from these systems contained a total nitrogen higher than 5 mg N/L, mainly in the form of ammonium and nitrate. To meet a stringent discharge limit for nitrogen removal (3 – 5 mg N/L), a nitrogen polishing process is needed for a broader application of mainstream deammonification.

Chapter 3 Manuscript: Polishing Effluent Mainstream Deammonification in Concurrent Operation of Anammox and Denitrifier

This paper is in preparation and planned to submit to *Applied Microbiology and Biotechnology*.

Abstract

This study addresses the need to develop a nitrogen polishing process capable of removing both ammonium and nitrate. The impact of carbon sources (acetate, methanol, and glycerol) and COD/NO3-N ratio in concurrent operation of anammox and partial denitrification was assesses under long-term (120 days) operation. Results suggested that both acetate and glycerol displayed a good potential for partial denitrification selection. The contribution of partial denitrification to nitrate removal in both reactors is higher than 90% after acclimation. However, methanol reactor showed challenges of establishing efficient partial denitrification. The key factor for a successful concurrent operation of anammox and denitrification lies on controlling the balance between denitrifier rate and AnAOB rate to simultaneously remove residual ammonium and nitrate.

Key words: partial denitrification; alternative external carbon sources; COD/N ratio; anammox; mainstream deammonification; final polishing

3.1 Introduction

Meeting a stringent discharge limit for nitrogen removal (3 – 5 mg-N/L Total Inorganic Nitrogen - TIN) requires wastewater treatment plants (WWTPs) in U.S. to design post denitrification polishing process in mainstream treatment. Conventional post denitrification polishing process is operated under anoxic condition with external carbon addition as electron donor for denitrifier to reduce nitrate to nitrogen gas. However, with a rapid implementation of deammonification in mainstream in many plants across U.S., residual ammonium ends up in effluent along with nitrate. Laureni et al. (2016) have shown 1.8 ± 0.4 mg NH4-N/L and 3.6 ± 1.4 mg NO3-N/L in the effluent; while the effluent quality from Lotti et al. (2015) was 6.8 ± 5.4 mg NH4-N/L and 9.1 ± 3.1 NO3-N/L; Regmi et al. (2015) reported effluent of 4.4 ± 2.9 mg NH4-N/L and 3.6 ± 2.2 NO3-N/L; and pilot in Han et al. (2015) was operated with deammonification with effluent quality of 2.5 mg NH4-N/L and 3.9 NO3-N/L. Therefore, development of novel post denitrification polishing process capable of removing both ammonium and nitrate is necessary. Under anoxic condition, anoxic ammonium oxidizing bacteria (anammox - AnAOB) is the only bacteria capable of removing ammonium at the expense of nitrite (Strous et al., 1997), and denitrifier is able to reduce nitrate to nitrite; thus combining these two sludge system can simultaneously remove ammonium and nitrate under anoxic condition. Two sludge system using moving bed biofilm reactor (MBBR) (Regmi et al. 2015; Gilbert, Agrawal, Schwartz, Horn, & Lackner, 2015; Pérez, Lotti, Kleerebezem, Picioreanu, & van Loosdrecht, 2014; Vlaeminck, De Clippeleir, & Verstraete, 2012) can be used not only to tackle energy problem but also enhance the activity of AnAOB. However, these designs require constructions of additional infrastructure to accommodate two separated sludge system. A concurrent operation of partial denitrification and anammox can be feasible and economical to achieve the same goal. In this approach, nitrate is expected to only be reduced to nitrite (partial denitrification) by denitrifiers, then AnAOB can remove residual ammonium and nitrite under anoxic condition. Therefore, the key factor of successful post denitrification polishing process relies on controlling the balance between denitrifier rate and anammox rate to remove both ammonium and nitrate completely.

Denitrification is usually considered as a two-step process, in which nitrate is first reduced to nitrite ($NO_3^- \rightarrow NO_2^-$) and then comes to the reduction of nitrite to molecular nitrogen ($NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (McCarty et al., 1969). Alexander et al. (1961) stated that the extent of denitrification may vary as a function of the microorganism community, while some groups of bacteria only capable of reducing nitrate to nitrite, others are only able to nitrite to nitrogen gas, and some are able to convert both nitrate and nitrite to nitrogen gas. Nitrate and nitrite reductases in denitrification accept electrons from several carbon sources. The accumulation of nitrite in denitrification process can be explained by two mechanisms: (1) repression of nitrite reductase but not nitrate reductase and/or (2) selection and enrichment of microorganism only capable of reducing nitrate to nitrite. Wilderer et al. (1987) stated that nitrite accumulates as a result of enrichment of nitrate reducing bacteria, not as an intracellular competition between denitrification enzymes. Both nitrate and nitrite reductases can be inhibited by oxygen, but research has shown that nitrite reductase is more severely affected, resulting in nitrite accumulation (Tiedje, 1988). Limited amount of carbon might also can cause partial denitrification by limiting substrate electron flow to nitrite reductase (Almeida et al., 1995). In this case, carbon will be consumed quickly by denitrifying bacteria to reduce nitrate to nitrite and there is not sufficient carbon left to convert nitrite to nitrogen gas, resulting in the nitrite accumulation. Type of carbon source and COD/N ratio also affect the nitrite accumulation. The nitrite accumulation of 23%, 21% and 17% for glucose, acetic acid and methanol, respectively, was reported when limited concentrations of the carbon sources were used (Her and Huang, 1995). Van Rijn et al. (1996) observed high nitrite accumulation when acetate was used in denitrifying cultures of Pseudomonas stutzeri. Oh et al. (1999) observed complete denitrification at a value of 3.4 mg N/g VSS/hr with negligible nitrite accumulation at a COD/N ratio of 2.0. When the COD/N ratio decreased to 1.0, significant nitrite accumulation was observed and slowed down the denitrification rate to 2.3 mg N/g VSS/hr.

Different type of external carbon source has been discovered to have significant influences on the denitrification process, such as bacteria development, nitrate reduction rate, and nitrite accumulation (Obaja, Macé, & Mata-Alvarez, 2005). Generally, methanol is selected as the carbon sources for denitrification due to its low cost (Bill, Bott, & Murthy, 2009), but it has several backwards connected to transportation, handling, and storage due to its toxicity and reactivity (Cherchi, Onnis-Hayden, El-Shawabkeh, & Gu, 2009), which encourages a consideration of using alternative carbon sources. Ethanol, acetate, and glycerol have been studied as alternative external carbon sources for denitrification. Acetate and ethanol has shown a higher denitrification rate and are more readily available for denitrifying bacteria compared to methanol (Christensson, Lie, & Welander, 1994; Gavazza dos Santos, Amâncio Varesche, Zaiat, & Foresti, 2004; McCarty, 1969). Regmi et al. (2015) demonstrated that nitrite produced within a nitritation-denitritation process using acetate can supply anammox bacteria in a separate post-polishing step. Systems using glycerol for denitrification have also observed nitrite accumulation (Bill et al., 2009; Ledwell, Fabiyi, & Farmer, 2011). These alternative carbon sources have shown potential for denitrification, however, most of the studies were perform in simple sludge system (without anammox bacteria) and short-term batch tests. Additionally, information impact of carbon type on performing partial denitrification under long-term operation is limited.

The denitrification potential is mainly determined by the stoichiometric ratio between the organic compound used and nitrate, which is usually expressed as the COD/N ratio. In co-existing system, with high COD/N ratio, denitrifier can out-compete AnAOB for nitrite, resulting a decrease in ammonium removal. Therefore, COD/N ratio can also be used as a control parameter to balance denitrification rate with AnAOB rate. Thus, a low COD/N ratio lower than 2 is evaluated in the suspended sludge system; not only to minimize the suppression of COD on AnAOB but also favor the partial denitrification.

Although many studies covered partial denitrification under different operational conditions, yet not many have investigated the concurrent operation of partial denitrification and anammox. In this study, the impact of three carbon sources (acetate, methanol, and glycerol) and COD/N ratio on selecting partial denitrification was investigated; and the efficiency concurrent operation of partial denitrification and anammox was evaluated.

3.2 Material and Methods

3.2.1 Reactor set-up

20 L reactors (Figure 1 and Figure 2) and with internal clarifier were operated under anoxic conditions in an incubator where temperature was maintained constant at 20 ^{o}C. Magnetic mixer was used to assure the well-mixed condition in each reactor. pH was controlled between 7-7.5 by dosing sulfuric acid intermittently. Synthetic wastewater and COD stock solutions stored in separate containers were fed continuously at the top of the reactors with a hydraulic retention time (HRT) of 15 hours. Reactors were inoculated with AnAOB granules from a sidestream DEMON system (Strass, Austria) collected from the cyclone underflow. This fraction accounted for 70% of the mixed liquor concentration and to other 30% was provided by nitrification/denitrification sludge from Blue Plains AWTP operating at a total SRT of 25 days and acclimated to methanol dosing for denitrification. Due to reactor design deficiency and floating granules in the clarifier, all three reactors had effluent challenge in maintaining good effluent quality and thus retain enough granules (Table 2, 3, and 4). To alleviate the situation of loss of biomass, effluents from three reactors were collected separately then pour through a 53 µm sieve every day and brought back to the reactors to maintain the MLSS stability.

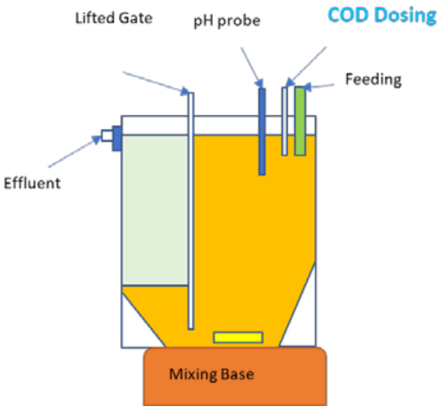

Figure 1. Schematic diagram of reactor

Figure 2. Photograph of reactor

3.2.2 Synthetic Wastewater Preparation

Synthetic wastewater feed was prepared in separate feeding tanks (70L) for each reactor, and the composition contained 22 mg NH_4^+-N/L (NH_4HCO_3), 44 mg NO_3^--N/L (KNO_3), 200 mg CO_3^{2-}/L ($NaHCO_3$), 0.5 mg PO_4^{3-}-P/L (K_2HPO4). Ammonium and nitrate loading rates of 36 and 72 mg N/L/d were maintained throughout the experiment, which are the same as secondary effluent loading of Blue Plains Advanced Wastewater Treatment Plant (AWTP). Thus in these bench-scale systems, ammonium and nitrate concentrations were non-limited to evaluate only the impact of COD. The synthetic wastewater was prepared with plant effluent from Blue Plains AWTP and pretreated with nitrogen gas to lower the dissolved oxygen before fed into the anoxic reactors. After the initial nitrogen gas flushing, the dissolved oxygen concentration the values fluctuated between 3.5 and 7.0 mg O_2/L. COD stock solutions were concocted with pure methanol, pure glycerol, and sodium acetate, respectively, in separate containers (2L) with COD concentration of 1400 mg COD/L.

3.2.3 Nutrient Concentration Analysis

System performance was evaluated on a daily basis (after 1 HRT) and concentration of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^-), soluble COD and TSS/VSS were analyzed.

Samples for nutrient (nitrogen and phosphorus) and COD analysis were taken and immediately filtered through 0.45 μm membrane filters. Then they were analyzed by HACH vials (Loveland, CO). Procedures strictly followed the HACH method (Appendix A.1). TSS/VSS were measured according to Standard methods (ALPHA, 1998).

3.2.4 Determination of Contribution to Nitrate Removal

Contribution of AnAOB, nitrite accumulation, and full denitrification are calculated based on the nitrate specific removal rate. AnAOB contribution to nitrate removal is determined as follows:

$$AnAOB\% = \frac{(1.32-0.26) * NH_4{}^{+}\ removal\ rate}{NO_3{}^{-}\ removal\ rate} * 100\% \qquad (8)$$

Where (1.32-0.26) is the conversion factor of ammonium removal rate to nitrate removal rate based on the stoichiometry of anammox reaction.

Nitrite accumulation contribution is determined as:

$$\text{Nitrite\%} = \frac{NO_2^- \ accumulation\, rate}{NO_3^- \ removal\, rate} * 100\% \qquad (9)$$

Partial denitrification (PDN) contribution is the sum of AnAOB and nitrite accumulation contribution.

Full denitrification (FDN) contribution to nitrate removal is determined as:

$$\text{FDN\%} = 100\% - \text{PDN\%} = 100\% - (\text{AnAOB\%} + \text{Nitrite\%}) \qquad (10)$$

3.3 Results

3.3.1 Methanol Reactor

Throughout the period, COD/NO3-N addition was maintained around a ratio of 1.0 (Table 2). Initially, 49 ± 16% of the reduced NO_3^--N was available for AnAOB achieving a specific removal rate of ammonium of 5.3 ± 1.4 mg N/gVSS/d (Table 2). The observed yield of the system was 4.6 g COD added per TIN (total inorganic nitrogen) removed during the first two weeks of operation. Due to inefficient retention of the AnAOB granules, a continuing decrease of AnAOB contribution to nitrate removal was observed. Unlike other reactors where a loss of AnAOB biomass resulted in nitrite accumulation (Figure 4 and 5), in the methanol reactor nitrite accumulation was never observed and decreasing AnAOB contribution lead to increased full denitrification contribution (Figure 3B). At the end of the experimental period, only 32 ± 12% of the reduced nitrate nitrogen was used by AnAOB. Ammonium specific removal rates thus decreased from 5.3 ± 1.1 mg N/gVSS/day (day 1 to 30) to 4.5 ± 1.1 mg N/gVSS/day (day 31 to 40). Due to the continuous loss of AnAOB contribution without observation of any nitrite, the reactor was dismantled after day 40.

Table 2. Summary results of methanol reactor

Parameter	Period 1 (day 16-40)
COD/NO3-N dosing	1 ± 0.1
MLSS (mg TSS/L)	1096 ± 233
Effluent TSS (mg TSS/L)	6 ± 3
Effluent nitrite (mg N/L)	0.15 ± 0.19
Effluent COD (mg sCOD/L)	N/A
NO3 removal (mg N/gVSS/d)	11.8 ± 4
Partial denitrification (mg N/gVSS/d)	5.3 ± 1.5
NH4 removal (mg N/gVSS/d)	5.3 ± 1.4
N balance	
Full DN (%)	51 ± 16
Partial DN (%)	49 ± 16
NO2 accumulation (%)	0 ± 0
AnAOB contribution (%)	49 ± 16

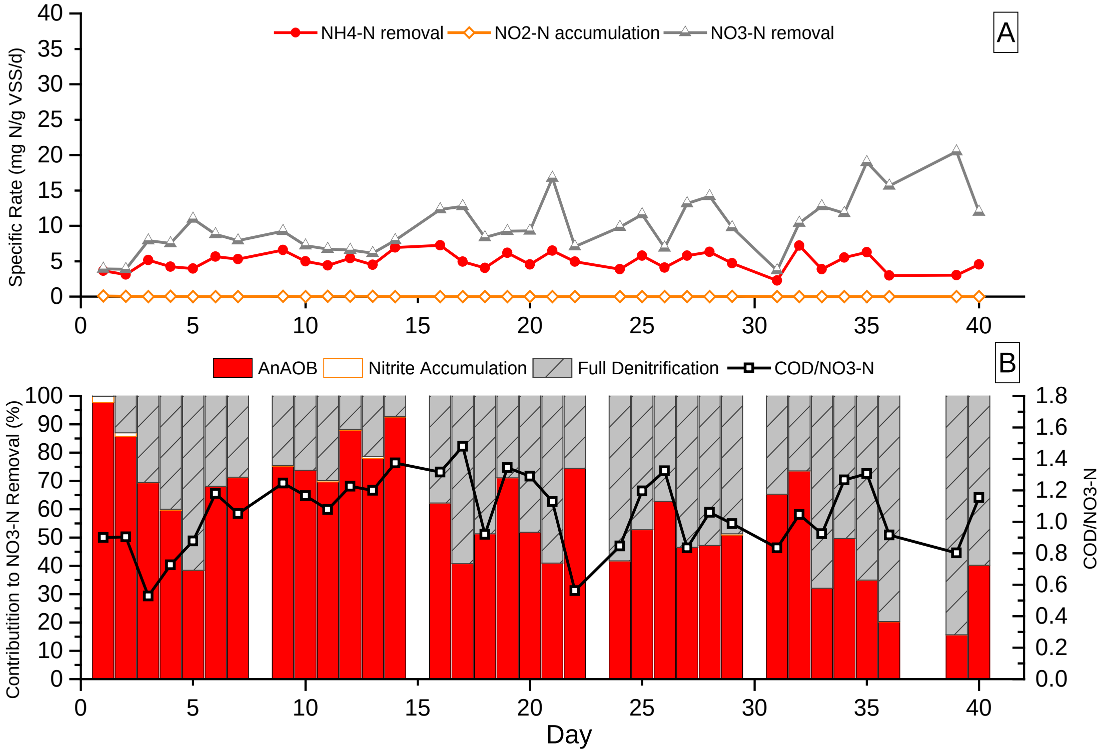

Figure 3. Trends of parameters assessing methanol reactor performance: A) NH4+-N and NO3--N specific removal rate and NO2--N specific accumulation rate; B) Relative contribution of AnAOB, Nitrite accumulation, and Full Denitrification to NO3--N removal.

3.3.2 Glycerol Reactor

During the first 27 days of operation, glycerol reactor was running at similar COD/NO3-N addition of 1.0 compared to the methanol reactor. The initial observed AnAOB contribution to nitrate removal was 77 ± 13%. The observed yield of the system was 3.1 g COD added per TIN removed and overall nitrate specific removal rate was 9.5 mg N/gVSS/day (Table 3). During this period, no nitrite accumulation was observed (Figure 4B), indicating a good balance between AnAOB rates and partial denitrification and resulting in partial denitrification rates reached of 7.1 ± 1.6 mg N/gVSS/day. Due to a sudden loss of AnAOB activity, probably caused by increased chlorine levels in the Blue Plains effluent, nitrite started to accumulate from day 28 onwards. AnAOB specific removal rates decreased from 7.2 mg NH4-N/gVSS/day at day 28 to 2.4 mg NH4-N/gVSS/day at day 34. Despite the decreased AnAOB rates, partial denitrification increased to approximately 100% (period 2-II, Figure 4B).

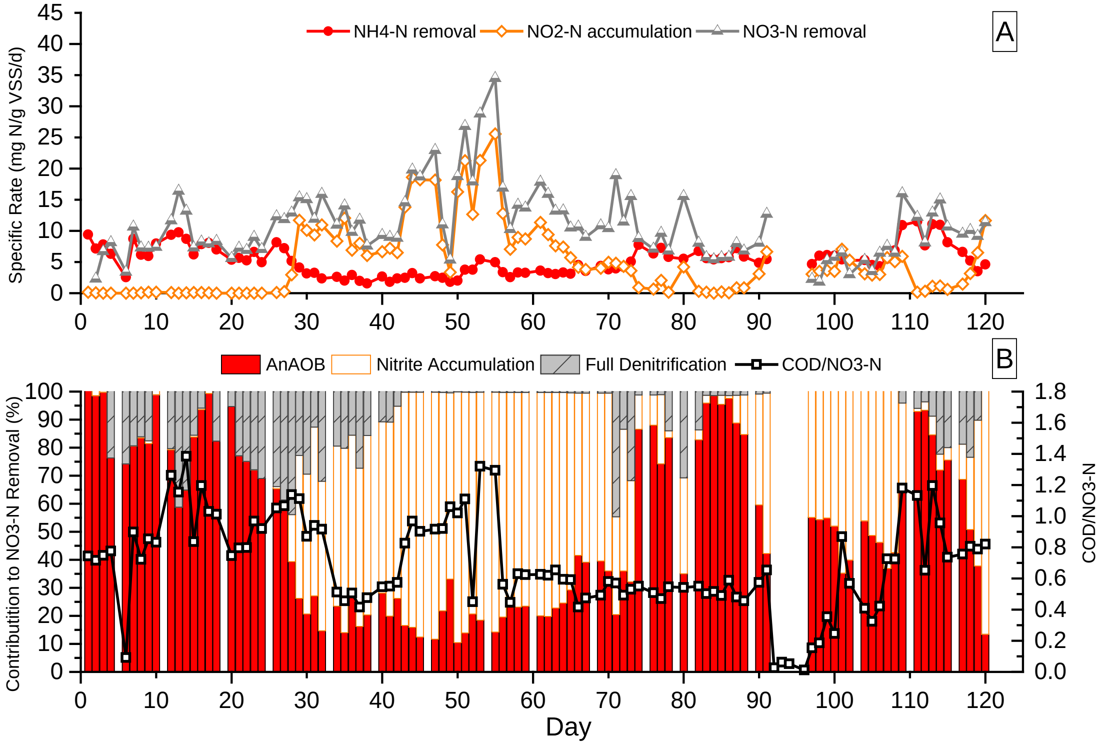

Figure 4. Trends of parameters assessing glycerol reactor performance: A) NH4+-N and NO3--N specific removal rate and NO2--N specific accumulation rate; B) Relative contribution of AnAOB, Nitrite accumulation, and Full Denitrification to NO3--N removal.

To investigate the impact of nitrate removal rate on the partial denitrification selection and its balance with AnAOB rates, COD/NO3-N ratios were modified between day 28 and 57 by increasing or decreasing the carbon dosing rate. In period 2-I, nitrate specific removal rate was higher than in period 1 even though lower COD/NO3-N ratios (0.5 instead of 1) were dosed (Table 3). An increased residual soluble COD was observed in the effluent accounting for 6.1 ± 1.4 mg COD/L, when subtracting the non-biodegradable COD in the feeding influent from the total soluble COD fraction in the effluent (period 2-I, Table 3). Later in period 2-II, COD/NO3-N ratio was raised back to 1.0 and nitrate removal rates doubled as a result of this. Nitrite accumulation kept increasing and the partial denitrification finally reached almost 100% with an average partial denitrification rate of 20.7 ± 9.5 mg

N/gVSS/day during this period. COD residual in the effluent also increased to 12.4 ± 4.9 mg COD/L as more carbon was dosed. Within these two periods, similar ammonium removal rates and AnAOB contribution was observed (Figure 4. A, B).

As the nitrite residual in the effluent reached a peak of 15 mg NO2-N/L in the effluent on day 55, COD/NO3-N ratio was decreased to 0.5 in an attempt to better balance the partial denitrification rates with AnAOB rates. However, AnAOB rate remained limited (Table 3) and thus additional AnAOB biomass (5g of AnAOB-TSS) was added to restore the balance and investigate if nitrite accumulation was needed to maintain proper partial denitrification selection (day 73, Figure 4 A, B). Nitrite accumulation decreased for the first 4 days, but started to increase quite rapidly on day 80 and thus an extra addition of AnAOB biomass (10 g AnAOB-TSS) was added to the system. Consequently, nitrite residual in the effluent was eliminated by the additional AnAOB biomass in the first 5 days, then it started to increase rapidly, which was similar as previous observation. By comparing period 3 and 2-I (Table 3), a lower nitrate removal rate in period 3 was observed, which can be explained by the loss of denitrifiers biomass as confirmed by mixed liquor concentration drop from 1304 ± 92 to 1097 ± 106 mg TSS/L. Additionally, lower nitrite accumulation and partial denitrification rate were also observed, coupling with less soluble COD in the effluent. At the meantime, ammonium specific removal rate increased to 5.8 ± 0.7 mg NH4-N/gVSS/day, leading a rise of AnAOB contribution to 78 ± 24%. However, the partial denitrification percentage remained relatively stable and a huge contribution, with an average of 94 ±11% (Table 3-Period 3). On day 108, another 10 g AnAOB-TSS biomass was added to confirm the observation of previous two time AnAOB biomass addition. As expected, a trend of nitrite re-accumulation was clearly observed after five days of operation and the partial denitrification remained as the major removal pathway of nitrate, with an average of 88 ± 9% (Table 3-Period 4).

Table 3. Summary results of glycerol reactor

Parameter	**Period 1 (day 12-27)**	**Period 2-I (day 34-42)**	**Period 2-II (day 47-55)**	**Period 3 (day 80-91)**	**Period 4 (day 109-120)**
COD/NO3-N dosing	0.9 ± 0.2	0.5 ± 0.1	1.0 ± 0.3	0.5 ± 0.1	0.9 ± 0.2
MLSS (mg TSS/L)	1689 ± 189	1304 ± 92	995 ± 52	1097 ± 106	1402 ± 60
Effluent TSS (mg TSS/L)	37 ± 19	32 ± 15	38 ± 19	26 ± 5	19 ± 6
Effluent NO_2^- (mg N/L)	0.09 ± 0.05	4.6 ± 1.02	9.6 ± 4.5	1.3 ± 1.4	2.2 ± 2.3
Effluent COD (mg sCOD/L)	1.18 ± 1.49	6.1 ± 1.4	12.4 ± 4.9	3.5 ± 3.6	4.2 ± 6.4
NO_3^- removal (mg N/gVSS/d)	10 ± 3	10.2 ± 2	20.8 ± 9.6	8.2 ± 3.4	11.5 ± 2.6
Partial denitrification (mg N/gVSS/d)	7.1 ± 1.6	8.5 ± 1.3	20.7 ± 9.5	7.5 ± 2.4	10.2 ± 2.6
NH_4^+ removal (mg N/gVSS/d)	7.1 ± 1.6	2.2 ± 0.5	3.3 ± 1.3	5.8 ± 0.7	8.0 ± 3.0
N balance					
Full DN (%)	23 ± 13	16 ± 7	0 ± 0	6 ± 11	12 ± 9
Partial DN (%)	77 ± 13	84 ± 7	100 ± 0	94 ± 11	88 ± 9
NO2 accumulation (%)	0.4 ± 0.5	62 ± 6	82 ± 7	17 ± 20	23 ± 28
AnAOB contribution (%)	77 ± 13	22 ± 6	18 ± 7	78 ± 24	66 ± 26

3.3.3 Acetate Reactor

During period 1 (Table 4), nitrate specific removal rate of 14.6 mg NO3-N/gVSS/day, fastest among three carbon sources, and extremely small low nitrite accumulation (0.08 ± 0.02 mg NO2-N/L) was achieved under a similar COD/NO3-N ratio with other two reactors. Although acetate reactor displayed a lower partial denitrification percentage compared to glycerol reactor, a lower yield of 2.5 g COD added per TIN removed was observed. An ammonium removal rate of 7.8 ± 1.8 mg N/gVSS/day (Table 4) was similar to the observed rate in the glycerol reactor (Table 3) but was faster than the methanol reactor (Table 2). As low nitrite accumulation was observed (< 0.2 mg NO2-N/L), AnAOB contribution to the nitrate removal equaled the partial denitrification contribution and was 54 ± 9% during the first 27 days (Table 4-Period 1).

Table 4. Summary results of acetate reactor

Parameter	**Period 1 (day 12-27)**	**Period 2 (day 47-55)**	**Period 3 (day 73-120)**
COD/NO3-N dosing	0.9 ± 0.2	1.3 ± 0.2	0.5 ± 0.1
MLSS (mg TSS/L)	1421 ± 249	565 ± 96	470 ± 89
Effluent TSS (mg TSS/L)	41 ± 39	25 ± 7	27 ± 12
Effluent nitrite (mg N/L)	0.08 ± 0.02	6.9 ± 2.0	0.4 ± 0.3
Effluent COD (mg sCOD/L)	0.7 ± 1.0	9.1 ± 2.8	1.9 ± 4.6
NO3 removal (mg N/gVSS/d)	14.6 ± 3.2	26.9 ± 7.2	10 ± 3.4
Partial denitrification (mg N/gVSS/d)	7.7 ± 1.8	22.4 ± 6.2	8.9 ± 2.9
NH4 removal (mg N/gVSS/d)	7.8 ± 1.8	6.9 ± 1.5	8.8 ± 2.3
N balance			
Full DN (%)	46 ± 9	15 ± 15	8 ± 13
Partial DN (%)	54 ± 9	85 ± 15	92 ± 13
NO2 accumulation (%)	0.4 ± 0.3	58 ± 12	12 ± 8
AnAOB contribution (%)	53 ± 9	26 ± 5	79 ± 18

Similar to the glycerol reactor, a drop of AnAOB activity was also observed on day 28 due to increased chlorine concentrations in the feed. However, in this system the loss of AnAOB activity and the resulting nitrite accumulation showed some delay with only a small change during day 28 and 34, but increased nitrite accumulation up to 9.3 mg NO2-N/L from day 34 onwards (Figure 5, Table 4). The latter was also induced by operation at increased COD/NO3-N at day 40 (Figure 5B). In period 2, partial denitrification contribution increased to 85 ± 15% on average and up to 100% (Figure 5B, Table 4). During this period, high nitrite residual in the effluent (6.9 ± 2.0 mg NO2-N/gVSS/day) under increased partial denitrification rates (26.9 ± 7.2 mg NO3-N/gVSS/day) were observed. Moreover, similar to the glycerol reactor, increased soluble COD concentration in the effluent (9.1 ± 2.8 mg COD/L) was observed. Due to the sudden modification of COD/NO3-N ratio from 1.5 to 0.5 at day 56, a raise of AnAOB contribution and reappearance of full denitrification was observed (Figure 5B). System performance returned to similar results as previous days after a few days of acclimation (day 66), with nitrite re-accumulation and almost 100% partial denitrification contribution (Figure 5B).

On day 73, additional AnAOB biomass (5 g AnAOB-TSS) was added in an attempt to better balance the partial denitrification rates with AnAOB rates and thus avoiding nitrite accumulation, leading to an increase of ammonium specific removal rate up to 8.8 ± 2.3 mg N/gVSS/day (Table 4-Period 3) and AnAOB contribution from 26 ± 5% (Period 2) to 79 ± 18% in period 3, while efficient partial denitrification (92 ± 13%) has been maintained since period 2 (Table 4). While nitrite levels were maintained low (0.4 ± 0.3 mg NO2-N/L) and partial denitrification was maintained efficiently, decreased effluent soluble COD levels were achieved (1.9 ± 4.6 mg COD/L) under COD/NO3-N ratio dosing of 0.5 ± 0.1.

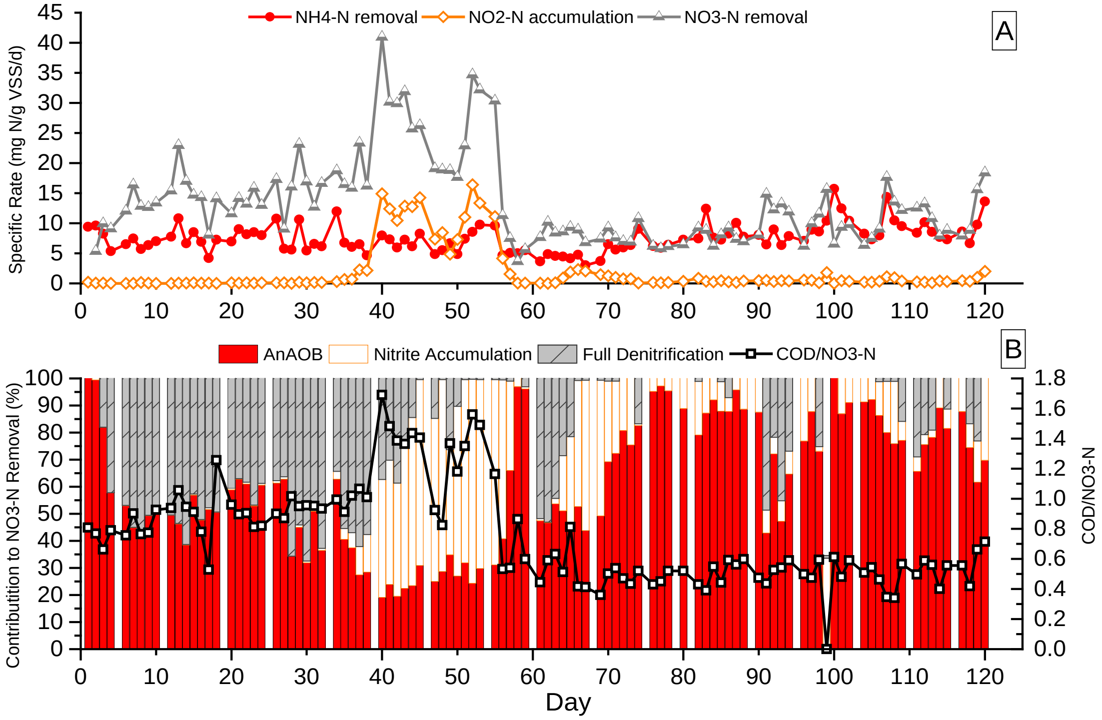

Figure 5. Trends of parameters assessing acetate reactor performance: A) NH4+-N and NO3--N specific removal rate and NO2--N specific accumulation rate; B) Relative contribution of AnAOB, Nitrite accumulation, and Full Denitrification to NO3--N removal.

On day 108, due to an additional washout of mixed liquor concentration caused by technical failure, leaving only 373 mg TSS/L in the system, additional AnAOB biomass (5 g AnAOB-TSS) was added. This allowed for restoring reactor performance including low nitrite accumulation (<0.4 mg NO2-N/L), stable ammonium specific removal rates (9.15 ± 1.9 mg NH4-N/L), and stable nitrate specific removal rates (11.8 ± 3.4 mg NO3-N/L). Both AnAOB biomass (day 73 and day 108) addition have helped to maintain an efficient partial denitrification during the last period (48 days) (Figure 5, Table 4).

3.4 Discussion

3.4.1 Impact of COD source

All carbon sources tested were able to achieve a combination of partial denitrification and AnAOB activity, especially when AnAOB rates were sufficient. Methanol showed the least potential for partial denitrification followed by acetate and glycerol. Within period 1 of the long-term experiments, glycerol showed higher partial denitrification selection (77 ± 13%) compared to acetate (54 ± 9%), which indicates that reactors using glycerol or acetate as carbon source are tended to perform partial denitrification instead of full denitrification, thus creating considerable nitrite availability and an efficient nitrite assimilation through AnAOB. Intracellular carbon storage may be a reason allowing for a better partial denitrification selection. The storage of polyhydroxyalkanoates (PHA) can be commonly observed in heterotrophic bacteria and it happens as the electron donor and electron acceptor may not be steadily provided in the ambient environment (Carucci, Dionisi, Majone, Rolle, & Smurra, 2001; Krasnits, Beliavsky, Tarre, & Green, 2013). In such cases, bacteria with the ability to store carbon could outcompete the bacteria without such ability (van Loosdrecht, Pot, & Heijnen, 1997). Acetate and glycerol have been verified that they can be converted to poly-3-hydroxybutyrate (PHB) and stored intracellularly for future growth and serving as effective electron donors for denitrification (Carucci et al., 2001; Moralejo-Gárate, Mar'atusalihat, Kleerebezem, & van Loosdrecht, 2011). Turning to carbon cycle of acetate and glycerol, the carbon storage can potentially lead to a faster electron pool for nitrate reduction. Additionally, reduction of nitrate to nitrite and nitrite to nitric oxide in denitrification process are catalyzed by nitrate- and nitrite-reductase, respectively (Bedmar, Robles, & Delgado, 2005). Nitrate reductase has a higher electron capacity than nitrite reductase as two electrons are transferred per mole nitrate reduced while one electron is transferred per mole nitrite reduced. With the carbon storage, or electron storage, nitrate reductase could take up more electrons, thus creating sufficient nitrite availability for anammox. In this result, different nitrate removal rate among three carbon sources was observed (period 1 in Table 2, 3, and 4), and acetate reactor displayed the fastest nitrate removal rate and partial denitrification rate, even though glycerol reactor showed a higher partial denitrification selection. Both acetate and glycerol demonstrated a better partial denitrification potential than methanol.

Although there was a difference in the partial denitrification selection in period 1, partial denitrification specific rates were similar between glycerol and acetate reactors (7.1 ± 1.6 vs 7.7 ± 1.8 mg N/gVSS/d) as acetate reactor performed a faster nitrate specific removal rate. Acetate is an effective substrate for energy production because it can be directly used to produce acetyl-CoA before starting the CAC (citric acid cycle) to produce energy (Ge et al., 2012). Comparing to acetate, glycerol is converted to pyruvate before going through pyruvate oxidation to produce acetyl-CoA for the citric acid cycle. The difference in metabolism pathway explains why acetate performed the fastest nitrate specific removal rate and lowest COD addition per TIN removal (2.5 g COD added per TIN removed).

Despite of same operational conditions, acclimation of the mixture of BNR and AnAOB sludge to different carbon sources behaved differently in period 1 (Figure 3, 4 and 5), implying that carbon source involved in selecting for partial denitrification. The partial denitrification selection difference between the carbon sources tested might be related to how and where electrons are donated. Competition for electron donor between nitrate and nitrite reductases has also been reported (Almeida, Reis, & Carrondo, 1995; Thomsen, Geest, & Cox, 1994; van Rijn, Tal, & Barak, 1996), and as advocated by van Rijn et al. (1996), electrons are transferred from either ubiquinone or cytochrome b in the upstream region of electron transfer chain to nitrate reductase whereas nitrite reductase accepts electrons from cytochrome c in a more downstream region. If electrons are donated in the upstream region, an increase of nitrite availability is expected. In this study, glycerol and acetate have displayed a better potential for partial denitrification and AnAOB contribution compared to methanol in period 1 (Table 2, 3, and 4). Acetate donates electrons closer to nitrate reductase in the upstream region instead of nitrite reductase in the downstream region, leading to a faster nitrate reduction rate and thus more nitrite availability (van Rijn et al., 1996). Similarly, glycerol has also been found as a readily electron donor to cytochrome b in the upstream region (Stewart, 1988). Methanol, however, has been studied that cytochrome c plays an important role in oxidizing methanol and the electron-donating location of methanol is majorly close to downstream region (Van Verseveld & Stouthamer, 1978). Accordingly, the partial denitrification percentage of methanol reactor in the last week of operation was only 32 ± 12%.

In later periods, high partial denitrification selection was achieved in both the acetate as well as the glycerol reactor. It is possible that this might be linked to the effect of types of carbon source on the community of denitrifying bacteria. Glass and

Silverstein (1998) identified certain strains of bacteria that can only reduce nitrate to nitrite, known as nitrate respiring bacteria, while true denitrifying bacteria can reduce both nitrate and nitrite. In general, these two bacteria can coexist, but the population of each community would change along with the alteration of ambient conditions. Under certain condition, nitrate respiring bacteria has been found to enrich at the expense of true denitrifying bacteria (Wilderer, Jones, & Dau, 1987). Nitrate respiring bacteria could possibly dominate the denitrifying bacteria population in both glycerol and acetate reactor, leading to an exceptional partial denitrification selection. However, based on the performance after additional AnAOB was added, glycerol reactor always displayed a considerable nitrite re-accumulation within a week whereas acetate displayed a relatively low nitrite accumulation, indicating glycerol may not be a better choice for coupling anammox with partial denitrification.

Similar ammonium removal rates have been observed in glycerol and acetate reactors, while methanol reactor displayed a lower ammonium removal rate (Table 2, 3, and 4). The possible reasons could be the methanol inhibition on AnAOB activity (Güven et al., 2005) , or the competition between AnAOB and denitrifiers as nitrite accumulation was not observed throughout the experiment. Studies have found out that AnAOB are capable of dissimilatory nitrate reduction to ammonium (DNRA) with nitrite as intermediate product at the expense of organic compounds (Kartal et al., 2007; Strous et al., 2006). In this study, it is possible that AnAOB and denitrifying bacteria competed for the carbon, intriguing an unclear impact on partial denitrification selection. Organic compounds could be a key factor on successful DNRA of AnAOB (Kartal et al., 2007), but the effect of carbon type on this process is still unclear. Since period 2, glycerol reactor has displayed a slower ammonium specific removal rate than acetate reactor, which allowed for a higher nitrite residual in the effluent. Regarding AnAOB activity, acetate seems to be a better choice for coupling anammox with partial denitrification. Additionally, the ability Possible reasons for selecting a better partial denitrification are provided above, more researches need to be implemented regarding to carbon metabolism.

3.4.2 Impact of COD/NO3-N ratio

COD/NO3-N ratio has been reported to have an impact on the extent and rate of denitrification process (Ge et al., 2012; Her & Huang, 1995; Yang, Wang, & Zhou, 2012). By dosing a lower COD/NO3-N ratio, Tseng, Potter, and Koopman (1998) observed that partial denitrification occurred and Oh and Silverstein (1999) found out nitrite started to accumulate when the COD/N ratio was 1 whereas no nitrite accumulated with a COD/NO3-N ratio in the range of 2 to 3. In this study, reactors were conducted under COD/NO3-N ratio from 0.5 – 1.5, both glycerol and acetate demonstrated an exceptional performance of partial denitrification after day 40 (Figure 4B and 5B). The impact of carbon source might help to determine a very strict COD/NO3-N ratio, or at least a range, for coupling denitrification with AnAOB. Ever since day 40, changing COD/NO3-N ratio in glycerol reactor did not show an obvious impact on the partial denitrification selection, which may be related to the biocommunity selection in the initial period. Under low carbon dosing, true denitrifying bacteria might be outcompeted by the partial-denitrifying bacteria as they do not have enough electrons provided. Martienssen and Schöps (1999) reported that COD/NO3-N ratio and HRT were closely related to the denitrifying communities.

With both low HRT (lower than 1.5 days) and COD/NO3-N ratio (lower than 2.5), denitrifying community were enriched with nitrate respiring bacteria and bacteria which reduced nitrate with nitrite as an intermediate product. In this study, HRT was around 15 hours and COD/NO3-N ranged from 0.5 to 1.5, which could help to shape the denitrifying community.

Furthermore, denitrifying community selection could also be completed through Sludge Retention Time (SRT) control. Cao et al. (2013) proposed that the dominant factor for nitrite accumulation in denitrification process seems to be the species composition within the denitrifying community itself. Du et al. (2017) studied a DEAMOX (DEnitrifying AMmonium OXidation) process using seeding sludge from inoculated partial-denitrification sludge and partial denitrification of 96.7% using acetate and 89.3% using ethanol, respectively, were achieved with COD/N ratio of 3. Although SRT control was not performed purposely in this study due to reactor design deficiency, both acetate and glycerol performed a partial denitrification percentage of higher than 90% after 40 days of operation (Table 3 and 4). Full denitrifying bacteria may have been out-selected due to factors such as short HRT, low COD/NO3-N, etc. It is possible that once the partial denitrifying bacteria compose the majority of denitrifying community in the sludge system, COD/NO3-N ratio may not be a crucial factor to more nitrite availability for AnAOB. Nevertheless, COD/NO3-N ratio could be more important in a mixed denitrifying community as a method to control extent and rate of denitrification process, thus creating sufficient nitrite availability to AnAOB. It is essential to find the balance between these two bacteria community, depending on the operating condition.

A certain amount of soluble COD in the effluent has been noticed since nitrite started to accumulate from period 2 in both acetate and glycerol reactor (Table 3 and 4). Both reactors were performing efficient partial denitrification and seem to share a pattern that more soluble COD left in the effluent coupling with higher PDN selection and nitrite accumulation. A higher nitrite accumulation was caused by raising COD/N ratio from 0.5 to 1.0, thus a faster partial denitrification rate was obtained and more COD was leaving the reactor within the same hydraulic retention time (HRT). In this study, partial denitrifying bacteria were possibly selected and these organisms, however, were not efficient enough to consume all electron donors, which possibly could be due to low affinity to the substrate. It is presumed that if a good partial denitrification is performed; organisms with a high affinity to the substrate are out-selected, thus increasing the COD residual in the effluent. From a cost-efficiency perspective, another nutrient treating step focused on full denitrification after final polishing is recommended to eliminate the potential nitrite and COD residual.

Chapter 4 Conclusions

This study was designed to evaluate the impact of carbon type and COD/NO3-N ratio on selecting partial denitrification and the concurrent operation of partial denitrification and anammox. Results suggested that both glycerol and acetate showed a great potential in promoting partial denitrification, while methanol did not.

In glycerol reactor, partial denitrification percentage could remained higher than 90% after day 45, but most of it was contributed by nitrite accumulation. Each time after AnAOB biomass addition, nitrite started to re-accumulate in a short period. Furthermore, ammonium specific removal rate was always slower in glycerol reactor despite of available nitrite present, indicating that glycerol may not be a good choice for coupling partial denitrification with anammox process. However, glycerol reactor always had a higher percentage of partial denitrification, implying that it is the best carbon source among three for selecting partial denitrification.

Similar as glycerol, acetate also displayed a good potential for partial denitrification. Due to the chlorine incident in plant effluent, AnAOB activity was impaired, leading to nitrite accumulation. Unlike glycerol reactor, nitrite did not re-accumulate in acetate reactor after AnAOB biomass addition. Furthermore, ammonium removal rate in acetate was the fastest among three carbon sources. Overall, acetate could be a carbon source for selecting partial denitrification, not as efficient as glycerol though, and showed the greatest potential for the concurrent operation of partial denitrification and anammox.

As the most wildly used carbon source for denitrification in full-scale systems, methanol did not show a good potential in promoting partial denitrification, with decreasing AnAOB activity observed.

In this study, COD/NO3-N ratio did not show a clear impact on partial denitrification selection, especially after the partial denitrification contribution reached a high percentage in both glycerol and acetate. However, as a low carbon to nitrogen ratio was maintained from the beginning, which may contribute to the selection process. Furthermore, the property of the carbon source might help to determine a very strict COD/NO3-N ratio, or at least a range, for promoting partial denitrification, which is beneficial for this nitrogen polishing step.

As acetate has been identified as the most beneficial carbon source for coupling partial denitrification with anammox, it is used as external carbon in the final polishing step of an ongoing study of mainstream deammonification pilot in DC Water for further research.

Appendices

A.1 Detailed Test Procedures

In this study, concentration of ammonia, nitrite, nitrate, phosphate, and soluble COD were measured with HACH test vials. Detailed test procedures are as follows:

1. Ammonia, high range (2-47 mg/L NH_3-N)

After sample is collected and filtered into a clean culture tube, 0.2 mL of filtered sample is pipetted into the test vial. Shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Ammonia-Salicylate HR TNT Method 10205)

2. Nitrite, low range (0.015 to 0.600 mg/L NO2-N)

After sample is collected and filtered into a clean culture tube, 2 mL of filtered sample is pipetted into the test vial. Shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Nitrite Diazotization LR Method 10207)

3. Nitrite, high range (0.6 to 6.0 mg/L NO2-N)

After sample is collected and filtered into a clean culture tube, 0.2 mL of filtered sample is pipetted into the test vial. Shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Nitrite Diazotization HR Method 10237)

4. Nitrate, low range (0.23 to 13.50 mg/L NO3-N)

After sample is collected and filtered into a clean culture tube, 1 mL of filtered sample and 0.2 mL of solution A are pipetted into the test vial. Shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Nitrate Dimethylphenol LR Method 10206)

5. Nitrate, high range (5 to 35 mg/L NO3-N)

After sample is collected and filtered into a clean culture tube, 0.2 mL of filtered sample and 1 mL of solution A are pipetted into the test vial. Shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Nitrate Dimethylphenol HR Method 10206)

6. Phosphorus, Reactive (Orthophosphate) and Total (0.05 to 1.50 mg/L PO4-P)

After sample is collected and filtered into a clean culture tube, 2 mL of filtered sample and 0.2 mL of solution B are pipetted into the test vial. Put a grey DosiCap C on the vial and shake the test vial two to three times, make sure all the reagent in the cap has dissolved and wait for 15 mins. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Ascorbic Acid LR Method 10209 (Reactive) and Method 10210 (Total))

7. COD, low range (3 to 150 mg COD/L)

Preheat the DRB200 Reactor to 150°C. After sample is collected and filtered into a clean culture tube, 2 mL of filtered sample is pipetted into the test vial. Close the vials tightly and shake it several times. Put the vial into the preheated DRB200 Reactor for 2 hours and invert the vial every 30 mins. After 2 hours, place the vial in a clean tube rack for cooling to room temperature. Before inserting the test vial into the DR2800 photo analyzer for reading, clean the vial with vial-tissue and invert the test vial two to three times. (HACH, Chemical-Reactor Digestion Method 8000)

8. TSS/VSS Standard methods, (ALPHA, 1998)

Preparation: Place the metal containers with filters installed into a 550°C furnace for 20 minutes. Cool the crucibles down for 20 minutes, Store them in a rack in the oven at 104°C overnight. Remove the metal plates from the oven and let them cool in a desiccator for at least 20 minutes before use.

Procedure: Take an initial weight of each plate with its installed filter. Utilizing a vacuum flask with the filter on. Depending on MLSS concentration of samples, different volume of well mixed sample is poured through the filter until the entire sample has been filtered, rinse the cylinder two times with distilled water. After the filter is completely drained, move the filter back to the metal plates. Place the plates with filters and solids into the oven at 104°C for one-hour. Remove the plates from the oven and cool them in the desiccator for 30 mins and record the weight. The volume of sample used plus the dry weight of the sample are used to calculate the TSS, usually as mg/L. To obtain the VSS, place the plates with the filter and dried sample into the furnace for 20 mins at 550°C. Remove the plates from the furnace and allow them to cool in the air for 5 minutes. Once again weigh the plates; the VSS is then calculated, using the volume of the original sample and the furnace dried sample weight.

A.2 Inorganic Nitrogen Profile in Reactor

In this study, reactor was fed with synthetic wastewater, which was made with Blue Plains' plant effluent, with ammonium loading rate of 36 mg N/L/d and nitrate loading rate of 72 mg N/L/d, respectively. The loading rate was chosen based on the designed loading of denitrification reactors in Blue Plains. But the reactor had a HRT limitation of 15 hours, which was the minimum can be achieved, otherwise the flocs would be washed out. With this HRT limitation, high concentration of ammonium and nitrate was incurred. Concentration profile of ammonium, nitrite, and nitrate in the influent and effluent of each reactor is shown as follows.

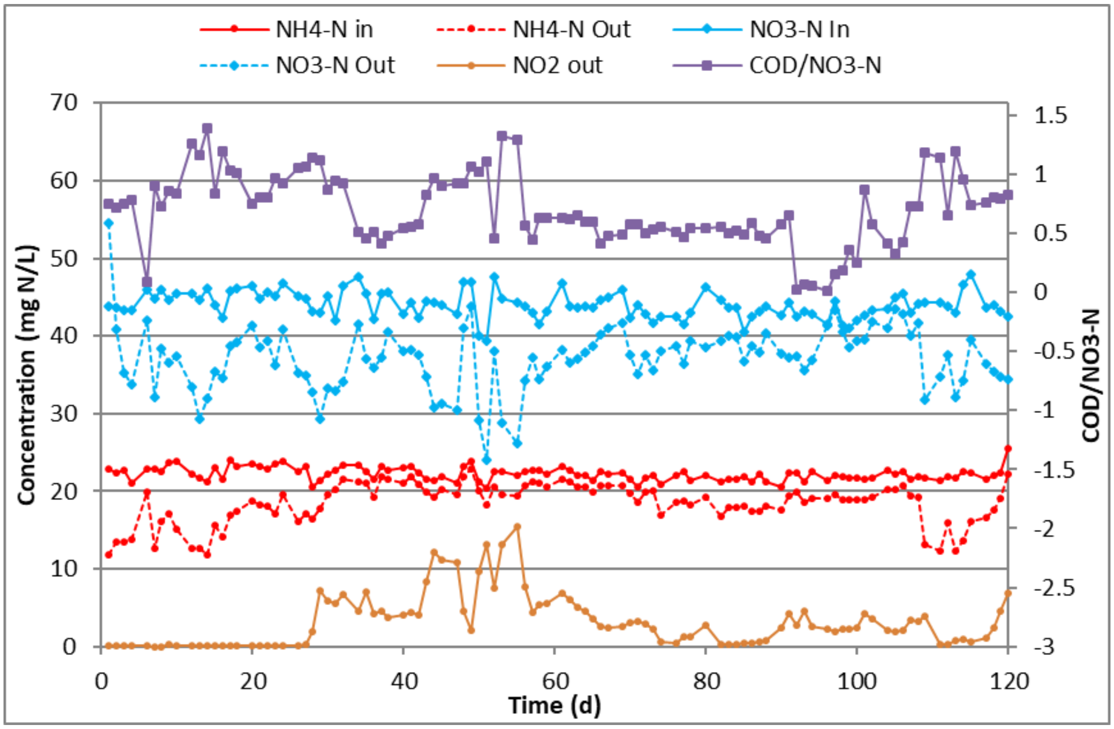

Figure A-1 Concentration profile of ammonium, nitrite, and nitrate in the influent and effluent of glycerol reactor

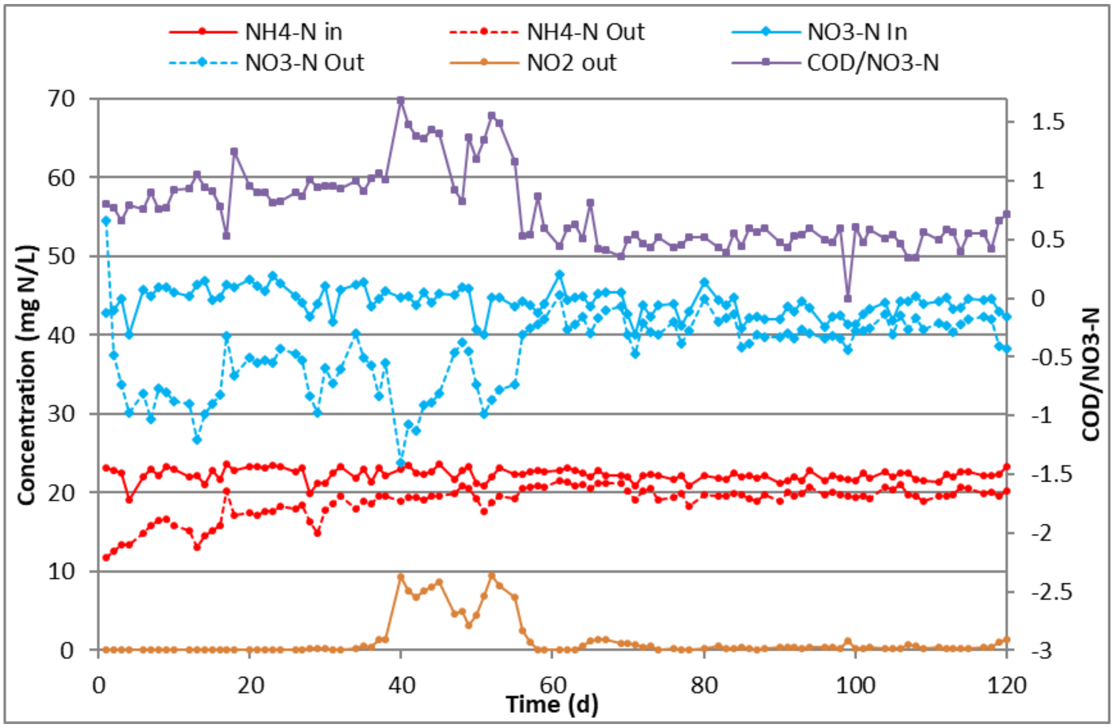

Figure A-2 Concentration profile of ammonium, nitrite, and nitrate in the influent and effluent of acetate reactor

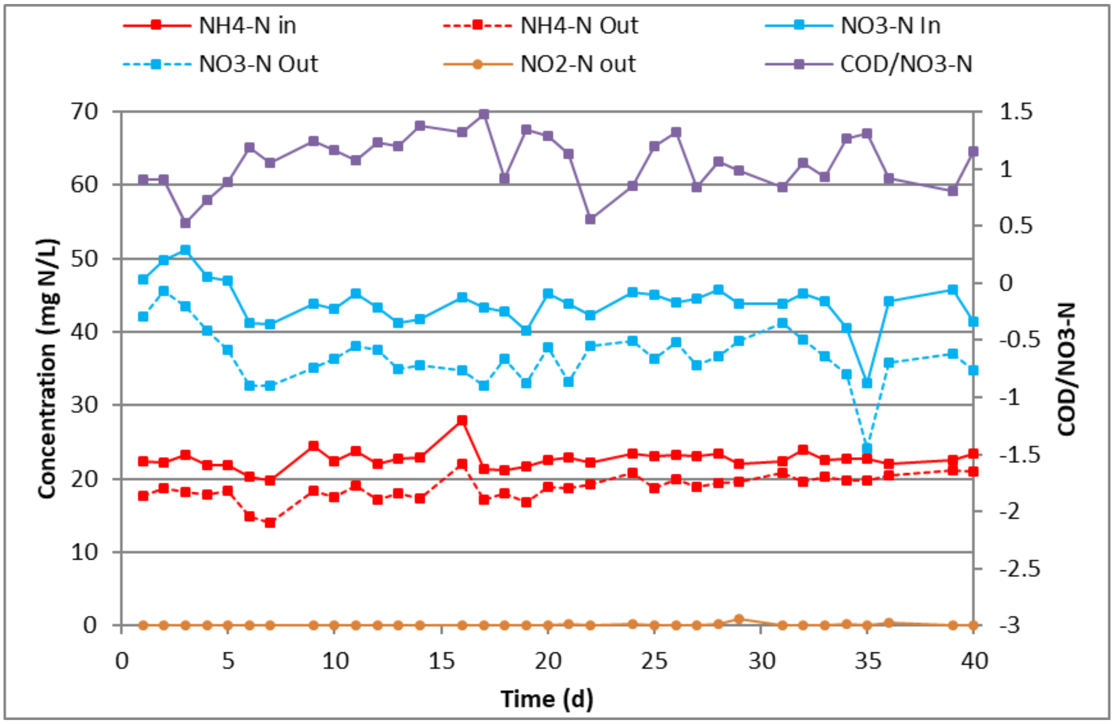

Figure A- 3 Concentration profile of ammonium, nitrite, and nitrate in the influent and effluent of methanol reactor

Bibliography

Alexander, M. (1961). "Soil Microbiology." JOHN WILEY, New York, 293-308.

Almeida, J. S., Reis, M. A., & Carrondo, M. J. (1995). Competition between nitrate and nitrite reduction in denitrification by Pseudomonas fluorescens. *Biotechnol Bioeng, 46*(5), 476-484. doi: 10.1002/bit.260460512

Bedmar, E. J., Robles, E. F., & Delgado, M. J. (2005). The complete denitrification pathway of the symbiotic, nitrogen-fixing bacterium Bradyrhizobium japonicum. *Biochemical Society transactions, 33*(Pt 1), 141-144.

Betlach, M., and Tiedje, J. (1981). "Kinetic Explanation for Accumulation of Nitrite, Nitric Oxide, and Nitrous Oxide during Bacterial Denitrification." *Applied and Environmental Microbiology*, 42(6), 1074-1084.

Boumann, H. A., Stroeve, P., Longo, M. L., Poolman, B., Kuiper, J. M., Hopmans, E. C., . . . Schouten, S. (2009). Biophysical properties of membrane lipids of anammox bacteria: II. Impact of temperature and bacteriohopanoids. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1788*(7), 1452-1457. doi:10.1016/j.bbamem.2009.04.005

Bill, K. A., Bott, C. B., & Murthy, S. N. (2009). Evaluation of alternative electron donors for denitrifying moving bed biofilm reactors (MBBRs). *Water Sci Technol*, *60*(10), 2647-2657. doi: 10.2166/wst.2009.622

Cao, S., Wang, S., Peng, Y., Wu, C., Du, R., Gong, L., & Ma, B. (2013). Achieving partial denitrification with sludge fermentation liquid as carbon source: The effect of seeding sludge. *Bioresour Technol*, *149*, 570-574. doi: http://doi.org/10.1016/j.biortech.2013.09.072

Carucci, A., Dionisi, D., Majone, M., Rolle, E., & Smurra, P. (2001). Aerobic storage by activated sludge on real wastewater. *Water Research, 35*(16), 3833-3844. doi: http://doi.org/10.1016/S0043-1354(01)00108-7

Carlson, C. A.; Ingraham, J. L. (1983). "Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *Paracoccus denitrificans*". *Appl. Environ. Microbiol*. **45**: 1247–1253.

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., & Gu, A. Z. (2009). Implication of Using Different Carbon Sources for Denitrification in Wastewater Treatments. *Water Environment Research, 81*(8), 788-799.

Christensen, M. H., and Harremoes, P. (1977). "Biological Denitrification of Sewage: A Literature Review." *Progress in Water Technology*, 8(4-5), 509-555.

Christensson, M., Lie, E., & Welander, T. (1994). A comparison between ethanol and methanol as carbon sources for denitrification. *Water Science and Technology, 30*(6), 83.

Davis, Mackenzie Leo. Water and Wastewater Engineering: Design Principles and Practice. New York: McGraw Hill, 2011. Print

Dangcong, P., Yi, W., Hao, W., and Xiaochang, W. (2004). "Biological Denitrification in a Sequencing Batch Reactor." *Water Science and Technology*, 50(10), 67-72.

Du, R., Cao, S., Li, B., Niu, M., Wang, S., & Peng, Y. (2017). Performance and microbial community analysis of a novel DEAMOX based on partial-denitrification and anammox treating ammonia and nitrate wastewaters. *Water Research, 108*, 46-56. doi: http://doi.org/10.1016/j.watres.2016.10.051

Egli, K., Fanger, U., Alvarez, P. J. J., Siegrist, H., van der Meer, J. R., & Zehnder, A. J. B. (2001). Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Archives of Microbiology, 175*(3), 198-207. doi: 10.1007/s002030100255

Fernández, I., Dosta, J., Fajardo, C., Campos, J. L., Mosquera-Corral, A., & Méndez, R. (2012). Short- and long-term effects of ammonium and nitrite on the Anammox process. *Journal of environmental management, 95*(Suppl), S170-174.

Gavazza dos Santos, S., Amâncio Varesche, M. B., Zaiat, M., & Foresti, E. (2004). Comparison of Methanol, Ethanol, and Methane as Electron Donors for Denitrification. *Environmental Engineering Science, 21*(3), 313-320.

Ge, S., Peng, Y., Wang, S., Lu, C., Cao, X., & Zhu, Y. (2012). Nitrite accumulation under constant temperature in anoxic denitrification process: The effects of carbon sources and COD/NO3-N. *Bioresour Technol, 114*, 137-143. doi: http://doi.org/10.1016/j.biortech.2012.03.016

Gilbert, E. M., Agrawal, S., Schwartz, T., Horn, H., & Lackner, S. (2015). Comparing different reactor configurations for Partial Nitritation/Anammox at low temperatures. *Water Research, 81*, 92-100.

Glass, C., & Silverstein, J. (1998). Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Water Research, 32*(3), 831-839. doi: https://doi.org/10.1016/S0043-1354(97)00260-1

Güven, D., Dapena, A., Kartal, B., Schmid, M. C., Maas, B., van de Pas-Schoonen, K., . . . Schmidt, I. (2005). Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. *Applied and Environmental Microbiology, 71*(2), 1066-1071.

Grady, C. P. L., Barth, F. S., and Barbeau, D. S. (1996). "Variability in Kinetic Parameter Estimates: A Review of Possible Causes and a Proposed Terminology." *Water Research*, 30(3), 742-748.

Han M., Vlaeminck S.E., Al-Omari A., Wett B., Bott, C., Murthy, S., and De Clippeleir H. (2016). Uncoupling the solids retention times of flocs and granules in mainstream deammonification: A screen as effective out-selection tool for nitrite oxidizing bacteria. *Bioresource Technology*. 221 (2016), 195-204.

Henze, M., Harremoes, P., Arvin, E., and Jansen, J. L. C. (2002). *Wastewater Treatment: Biological and Chemical Processes*, Springer.

Han, M., De Clippeleir, H., Al-Omari, A., Stewart, H., Wett, B., Vlaeminck, S., . . . Murthy, S. (2015). NOB out-selection in mainstream deammonification–A resilience evaluation. *Proceedings of the Water Environment Federation, 2015*(13), 2237-2242.

Her, J.-J., & Huang, J.-S. (1995). Influences of carbon source and C/N ratio on nitrate/nitrite denitrification and carbon breakthrough. *Bioresour Technol, 54*(1), 45-51. doi: http://dx.doi.org/10.1016/0960-8524(95)00113-1

Jetten, M. S., Cirpus, I., Kartal, B., van Niftrik, L., van de Pas-Schoonen, K. T., Sliekers, O., . . . Strous, M. (2005). 1994-2004: 10 years of research on the anaerobic oxidation of ammonium. *Biochemical Society transactions, 33*(Pt 1), 119-123.

Jin, R.-C., Yang, G.-F., Yu, J.-J., & Zheng, P. (2012). The inhibition of the Anammox process: A review. *Chemical Engineering Journal, 197*, 67-79. doi: https://doi.org/10.1016/j.cej.2012.05.014

Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., . . . Siegrist, H. (2009). Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitritation and Anammox in One SBR. *Environmental science & technology, 43*(14), 5301-5306. doi: 10.1021/es900107w

Kartal, B., Kuypers, M. M., Lavik, G., Schalk, J., Op den Camp, H. J., Jetten, M. S., & Strous, M. (2007). Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. *Environmental microbiology, 9*(3), 635-642.

Krasnits, E., Beliavsky, M., Tarre, S., & Green, M. (2013). PHA based denitrification: Municipal wastewater vs. acetate. *Bioresour Technol, 132*, 28-37. doi: http://doi.org/10.1016/j.biortech.2012.11.074

Kartal B. et al. 2007. “Anammox Bacteria Disguised As Denitrifiers: Nitrate Reduction to Dinitrogen Gas Via Nitrite and Ammonium.” *Environmental Microbiology* 9(3):635–42.

Korner, H., and Zumft, W. G. (1989). "Expression of Denitrification Enzymes in Response to the Dissolved Oxygen Level and Respiratory Substrate in Continuous Culture of Pseudomonas Stutzery." *Applied and Environmental Microbiology*, 55, 1670-1676.

Knowles, R. (1982). "Denitrification." *Microbiological Reviews*, 46(1), 43-70.

Kuenen, J. G. (2008). Anammox bacteria: from discovery to application. *Nature Reviews Microbiology, 6*(4), 320-326. doi:10.1038/nrmicro1857

Laureni, M., Falås, P., Robin, O., Wick, A., Weissbrodt, D. G., Nielsen, J. L., . . . Joss, A. (2016). Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures. *Water Research, 101*, 628-639.

Ledwell, S. A., Fabiyi, M., & Farmer, G. (2011). Optimizing denitrification with non-methanol carbon sources in deep-bed denitrification filter technologies. *Proceedings of the Water Environment Federation, 2011*(1), 406-419.

Lewandowski, Z. (1982). "Temperature Dependency of Biological Denitrification with Organic Materials Addition." *Water Research*, 16, 19-22.

Lotti, T., Kleerebezem, R., Hu, Z., Kartal, B., De Kreuk, M., van Erp Taalman Kip, C., . . . Van Loosdrecht, M. (2015). Pilot-scale evaluation of anammox-based mainstream nitrogen removal from municipal wastewater. *Environmental technology, 36*(9), 1167-1177.

Martienssen, M., & Schöps, R. (1999). Population dynamics of denitrifying bacteria in a model biocommunity. *Water Research, 33*(3), 639-646. doi: https://doi.org/10.1016/S0043-1354(98)00222-X

Metcalf, and Eddy. (2003). Wastewater Engineering: Treatment and Reuse, McGraw Hill, New York.

Mulder, A., Vandegraaf, A.A., Robertson, L.A., and Kuenen, J.G. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *FEMS Microbiol Ecol* **16:** 177–183.

McCarty, P. L., Beck, L. and Amant, P. S. (1969). *Biological denitrification of wastewater by addition of organic materials*. Paper presented at the Proceedings of the 24th Industrial waste conference, Purdue University, West Lafayette, Indiana.

Moralejo-Gárate, H., Mar'atusalihat, E., Kleerebezem, R., & van Loosdrecht, M. C. M. (2011). Microbial community engineering for biopolymer production from glycerol. *Applied Microbiology and Biotechnology, 92*(3), 631-639.

Obaja, D., Macé, S., & Mata-Alvarez, J. (2005). Biological nutrient removal by a sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. *Bioresour Technol, 96*(1), 7-14.

Oh, J., & Silverstein, J. (1999). Acetate Limitation and Nitrite Accumulation during Denitrification. *Journal of Environmental Engineering, 125*(3), 234-242. doi: doi:10.1061/(ASCE)0733-9372(1999)125:3(234)

Pérez, J., Lotti, T., Kleerebezem, R., Picioreanu, C., & van Loosdrecht, M. C. (2014). Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: a model-based study. *Water Research, 66*, 208-218.

Regmi, P., Holgate, B., Fredericks, D., Miller, M. W., Wett, B., Murthy, S., & Bott, C. B. (2015). Optimization of a mainstream nitritation-denitritation process and anammox polishing. *Water Science and Technology, 72*(4), 632-642.

Regmi, P., Holgate, B., Miller, M. W., Park, H., Chandran, K., Wett, B., Murthy, S. and Bott, C. B. (2015), Nitrogen polishing in a fully anoxic anammox MBBR treating mainstream nitritation–denitritation effluent. *Biotechnology and Bioengingeering*.

Staunton, E. (2014). INTEGRATED BIOLOGICAL TREATMENT OF SWINE WASTE FOR NITROGEN REMOVAL AND ENERGY RECOVERY (M. Aitken, M. Deshusses, A. Matthysse, J. Stewart, G. Walters, & S. Whalen, Eds.). *Integrated Biological Treatment of Swine Waste for Nitrogen Removal and Energy.*

Stewart, V. (1988). Nitrate respiration in relation to facultative metabolism in enterobacteria. *Microbiological reviews, 52*(2), 190-232.

Strous, M., Kuenen, J. G., & Jetten, M. S. (1999). Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology, 65*(7), 3248-3250.

Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M. W., . . . Le Paslier, D. (2006). Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature, 440*(7085), 790-794.

Thomsen, J. K., Geest, T., & Cox, R. P. (1994). Mass Spectrometric Studies of the Effect of pH on the Accumulation of Intermediates in Denitrification by Paracoccus denitrificans. *Applied and Environmental Microbiology, 60*(2), 536-541.

Tseng, C.-C., Potter, T. G., & Koopman, B. E. N. (1998). Effect of influent chemical oxygen demand to nitrogen ratio on a partial nitrification/complete denitrification process. *Water Research, 32*(1), 165-173. doi: http://doi.org/10.1016/S0043-1354(97)00195-4

U. S. EPA. 1993. "Process Design Manual for Nitrogen Control." Washington, DC.

U.S. EPA. 2007. Biological Nutrient Removal Processes and Costs. Online at https://www.epa.gov/nutrient-policy-data/biological-nutrient-removal-processes-and-costs

van der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., . . . van Loosdrecht, M. C. M. (2007). Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research, 41*(18), 4149-4163. doi: http://dx.doi.org/10.1016/j.watres.2007.03.044

van Loosdrecht, M. C. M., Pot, M. A., & Heijnen, J. J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Science and Technology, 35*(1), 41-47. doi: http://dx.doi.org/10.1016/S0273-1223(96)00877-3

van Rijn, J., Tal, Y., & Barak, Y. (1996). Influence of Volatile Fatty Acids on Nitrite Accumulation by a Pseudomonas stutzeri Strain Isolated from a Denitrifying Fluidized Bed Reactor. *Applied and Environmental Microbiology, 62*(7), 2615-2620.

Van Verseveld, H. W., & Stouthamer, A. H. (1978). Electron-transport chain and coupled oxidative phosphorylation in methanol-grown <i>Paracoccus denitrificans</i>. *Archives of Microbiology, 118*(1), 13-20.

Vlaeminck, S., De Clippeleir, H., & Verstraete, W. (2012). Microbial resource management of one‐stage partial nitritation/anammox. *Microbial biotechnology, 5*(3), 433-448.

Wett, B. (2007). Development and implementation of a robust deammonification process. *Water Sci Technol, 56*(7), 81-88.

Wilderer, P. A., Jones, W. L., & Dau, U. (1987). Competition in denitrification systems affecting reduction rate and accumulation of nitrite. *Water Research, 21*(2), 239-245. doi: http://dx.doi.org/10.1016/0043-1354(87)90056-X

WEF and WERF. (2015) Shortcut nitrogen removal-nitrite shunt and deammonification. Water Environment Federation and Water Environment Research Foundation.

W. R. L. van der Star et al., Startup of reactors for anoxic ammonium oxidation: experiences from the first full-scale anammox reactor in Rotterdam, Water Res. 41, 4149–63 (2007).

Williams, S., & Wilson, A. W. (1994). Beckton Demonstration Biological Nutrient-Removal Plant. *Water and Environment Journal, 8*(6), 664-670.

Yang, X., Wang, S., & Zhou, L. (2012). Effect of carbon source, C/N ratio, nitrate and dissolved oxygen concentration on nitrite and ammonium production from denitrification process by Pseudomonas stutzeri D6. *Bioresour Technol, 104*, 65-72. doi: 10.1016/j.biortech.2011.10.02

Zumft, W (1997). "Cell biology and molecular basis of denitrification". *Microbiol. Mol. Biol. Rev*. **61**: 533–616.