Single-Reactor Nitritation-Denitritation for High Strength Digested Biosolid Thickening Lagoon Supernatant Treatment

By

XIN ZOU

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Department of Civil and Environmental Engineering
University of Alberta

Abstract

Nitrogen is an essential element for living organisms, accounting for 80% of chemical elements in the atmosphere. Nitrogen is also one of the most concerned elements in the environment. The significant sources of nitrogen that released into the Canadian environment is municipal wastewater, as well as the non-point sources. The supernatant of biosolid digestate thickening lagoon contains 25-30% of the total amount of nitrogen but only 1% of the flow in the influent in WWTPs. To meet the stringent discharge standards for nitrogen in water, highly effective and energy saving nitrogen removal technologies should be investigated. The conventional nitrogen removal method, nitrification-denitrification, has been widely used in mainstream treatment. Compared to that, nitritation-denitritation can save 25% aeration cost, and 40% external carbon demand.

The ammonia-rich lagoon supernatant with limited alkalinity is ideal for the single reactor nitritation-denitritation operation. In this work, an integrated fixed film activated sludge (IFAS) system was operated in sequencing batch mode at 21 ^{o}C. This thesis evaluates the feasibility and stability of single reactor nitritation-denitritation for treating ammonia rich lagoon supernatant, investigates the distribution of microbial community and predominant microbes that contribute to nitritation and denitritation. With a hydraulic retention time (HRT) of 2 days, the nitritation-denitritation reactor achieved a stable inorganic nitrogen removal rate at 98%. The dominant nitrifying and primary denitrifying genera were *Nitrosomonas* and *Thauera*, respectively. The relative abundance of both genera increased in suspension and biofilm after long-term operation. The suspended biomass exhibited higher activity than biofilm and suspended biomass were also proved to contribute more on both nitritation and denitritation process than biofilm.

Preface

The work presented in this thesis are my original work and supervised by Professor Yang Liu in the University of Alberta and Mr. Abdul Mohammed from EPCOR Water Services Inc and both of them have provided great contributions to all parts of this project.

Chapter 4:

Professor Yang Liu, Dr. Yun Zhou, Mr. Abdul Mohammed, and Dr. Yanxi Shao contributed to the researching planning and Professor Yang Liu and Dr. Yun Zhou contributed to the manuscript edits.

Lastly, a version of Chapter 4 has been prepared for journal publication.

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List of Abbreviations

NO_3^-	Nitrate
NO_2^-	Nitrite
NH_4^+	Ammonium
AMO	Ammonia Monooxygenase
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
CANDO	Coupled aerobic-anoxic nitrous decomposition operation
COD	Chemical oxygen demand
Comammox	Complete ammonia oxidation
CSTR	Completely stirred tank reactor
DNRA	Dissimilatory nitrate reduction to ammonia
DO	Dissolved oxygen
FA	Free ammonia
FH	Free hydroxylamine
FNA	Free nitrous acid
HAO	Hydroxylamine Oxidoreductase
HNO_3	Nitric acid
HRT	Hydraulic retention time
HZO	Hydrazine oxidase
IFAS	Integrated fixed film and activated sludge
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids

MLVSS	Mixed liquor volatile suspended solids
N_2O	Nitrous oxide
NA	Nitrite accumulation
Nar	Nitrate reductase
NH_3	Ammonia
NH_x	Reduced nitrogen
Nir	Nitrite reductase
NO	Nitric oxide
NOB	Nitrite Oxidizing Bacteria
Nor	Nitrite Oxidoreductase
Nos	Nitrous oxide reductase
NO_x	Nitrogen oxides
PBS	Phosphate buffered saline
SBR	Sequencing Batch Reactor
SCONA	Simultaneous COD oxidation, partial nitritation-denitritation and anammox
SRT	Solid retention time
SVI	Sludge volume index
TIN	Total inorganic nitrogen
TKN	Total Kjeldahl nitrogen
WWTPs	Wastewater treatment plant

Chapter 1 - Introduction

1.1 Nitrogen impacts and water discharge regulations

Nitrogen (N) accounts for ~ 80% by volume of dry air in the atmosphere (Camargo & Alonso, 2006). Nitrogen is an essential element for the synthesis of proteins, nucleic acids, and other cell components, and is therefore of high environmental concern (Madigan & Martinko, 2012). Anthropogenic activities that contribute to nitrogen pollution in the environment have altered the original nitrogen cycle. For example, the utilization of fertilizer has introduced a ten-fold higher nitrogen content in wastewater over the last 40 years (Van Hulle et al., 2010). Nature has the capacity to deal with a limited amount of nitrogen, but the discharge of untreated wastewater has exceeded the natural recovery capacity, increasing eutrophication, algae blooms, groundwater contamination, and other negative impacts on aquatic species and human health (Options, 2011). In Canada, municipal wastewater is the largest nitrogen source. However, non-point sources (e.g., atmospheric deposition, commercial fertilizer and animal manure) are also crucial nitrogen sources and are more difficult to control (Canada, 2004).

According to the Approval to Operate 361975-00-00 limits for the Gold Bar Wastewater Treatment Plant (WWTP) final effluent (EPCOR Water Service Inc., 2018), the discharge limits for ammonia-nitrogen were 5.0 mg/L in summer and 10 mg/L in winter. Due to the high NH_3-N (10-50 mg/L) and total Kjeldahl nitrogen (TKN) (30-70 mg/L) content in wastewater that enters the wastewater treatment plant (EPCOR Water Service Inc., 2018), nitrogen reduction is necessary before the wastewater is discharged into natural water bodies.

1.2 Gold Bar Wastewater Treatment Plant (WWTP)

The Gold Bar WWTP is located close to the North Saskatchewan River in Edmonton, Alberta, Canada. The plant received a total of 98,884 million litres (ML) of wastewater in 2018 (EPCOR Water Service Inc., 2019). Gold Bar treated wastewater, sewer overflows, and storm water outfalls are the three major pollutants in the North Saskatchewan River. Pre-treatments of wastewater at the Gold Bar WWTP focus on the removal of large particles, then biosolids are generated from solids where possible. Secondary treatments include the removal of nitrogen, phosphorus, and organics, and tertiary treatments consist of polishing and disinfection. Effluent from an anaerobic digester is settled in a lagoon to separate biosolids from the liquid. If a liquid fraction containing 25-30% of total ammonia (A. Mohammed, 2019) is sent back to the headworks, a significant increase in nitrogen load in the mainstream can be observed. To ensure nitrogen is removed in the mainstream, the ammonia rich liquid fraction is subjected to sidestream treatment with a focus on nitrogen removal.

1.3 Nitrogen removal processes

The conventional method for nitrogen removal is nitrification and denitrification. Nitrification is accomplished in two steps: (i) partial nitrification (nitritation), in which the oxidation of NH_4^+-N to NO_2^--N is performed by ammonia oxidizing bacteria (AOB), and (ii) nitratation, the conversion of NO_2^--N to NO_3^--N by nitrite oxidizing bacteria (NOB). Both AOB and NOB are chemoautotrophs that utilize inorganic carbon (CO_2 and bicarbonate) as a carbon source and utilize O_2 to oxidize inorganic compounds for cell growth. Denitrification is the reduction of NO_3^--N to NO_2^--N, which is an intermediate product, then further reduced to N_2 as the end product. Denitrification can be accomplished by many heterotrophic organisms, most of which are facultative anaerobic

organisms that utilize NO_3^--N, NO_2^--N, as well as organic carbon for cell growth. However, complete nitrification-denitrification requires an intensive energy supply and has a large carbon demand.

In comparison, nitritation-denitritation removes ammonia via nitrite instead of nitrate, which can save 25% of the aeration cost needed for the conversion of nitrite to nitrate, as well as 40% of the external carbon demand for nitrate reduction to nitrite, since nitrate is not produced. To date, most published studies have focused on a two-reactor nitritation-denitritation, which removes nitrogen through nitritation in a first reactor followed by denitritation in a second reactor (Sun et al., 2015). This process provides stability in nitritation control and the elimination of NOB, which competes with denitrifiers. However, lagoon supernatant has low alkalinity, and the addition of an inorganic carbon source is necessary to complete the nitritation if a two-reactor system is utilized. In comparison, only limited studies have reported the operation and optimization of single-reactor nitritation-denitritation (Erdirencelebi & Koyuncu, 2018; Gustavsson, Nyberg, & La Cour Jansen, 2011; Lemaire, Marcelino, & Yuan, 2008). To achieve a self-sufficient process, the single-reactor configuration should be explored, as it reduces the reactor footprint and eliminates the need to add alkalinity in the nitritation stage, as denitritation provides alkalinity and can be achieved through alternating aerobic/anoxic phases.

1.4 Overview of reactor types and operation strategies

Previous studies revealed that stable nitritation-denitritation can be accomplished with various reactor types (*i.e.*, fluidized-bed biofilm reactors, plug-flow reactors, sequencing batch reactors) (Aslan & Dahab, 2008; Ge, Peng, Qiu, Zhu, & Ren, 2014; L. Peng et al., 2017). However,

sequencing batch reactor (SBR) systems were utilized for most anaerobic supernatant treatments due to their high flexibility in various operational conditions; the duration of phases in time-based processes can be adjusted within SBR systems, and the online analyzers can be easily installed and monitored (Frison, Lampis, Bolzonella, Pavan, & Fatone, 2012; Katsou, Malamis, Frison, & Fatone, 2015; Mace & Mata-Alvarez, 2002; Malamis, Katsou, Di Fabio, Bolzonella, & Fatone, 2014). Integrated fixed-film activated sludge (IFAS) technology was developed in 1994 to enhance the efficiency of biological nutrient removal (Odegaard, Rusten, & Westrum, 1994). IFAS systems have been successfully demonstrated in mainstream treatments, utilizing bio-carriers to provide an environment for the coexistence of attached and suspended biomass in a single system. Bio-carriers enhance the reactor's capabilities to retain biomass, cope with shock loads, and remove contaminants (i.e., nitrogen) (Chan, Johansson, & Christensson, 2014; Singh & Kazmi, 2016). However, the stability and feasibility of nitritation-denitritation for lagoon supernatant treatment in an IFAS reactor was not tested. Therefore, this study investigates the performance of an IFAS reactor that operates under sequencing batch mode in the treatment of ammonia rich wastewater.

1.5 Research objectives and thesis structure

In this study, an integrated fixed-film activated sludge (IFAS) reactor was operated in sequencing batch mode to perform single reactor nitritation-denitritation for ammonia rich lagoon supernatant treatment. The objectives of this study are to:

1. Evaluate the stability and long-term feasibility of single reactor nitritation-denitritation treating ammonia rich lagoon supernatant;
2. Test the nitrogen removal kinetics in suspension and biofilm;

3. Elucidate the distribution of microbial communities in suspended and attached biomass in the IFAS system after long term operation.

Thesis structure

Chapter 1 introduces the relevant background information to the project and presents the scope, the research gap, the objectives, and the structure of the thesis. Chapter 2, a literature review, explains conventional and innovative nitrogen removal methods, and describes the microorganisms related to nitrogen removal. In chapter 3, methods of examining the water chemistry and the dynamics of the microbial community in the reactor are detailed, and the reactor operation period is described. Chapter 4 presents the nitritation-denitritation results after a long-term operation of the IFAS-SBR. Reactor performance, kinetic studies, and microbial dynamics regarding the IFAS-SBR operation are described. Chapter 5 summarizes the study and suggests methods of improving reactor performance. The results presented in this thesis will be published.

Chapter 2 – Literature Review

2.1 Overview of nitrogen in the environment

Over the past century, many reactive forms of nitrogen, such as nitrogen oxides (NO_x), reduced nitrogen (NH_x), nitrous oxide (N_2O), nitric acid (HNO_3), have been introduced into the natural environment via human activities (Stevens, 2019). Human activities and nature account for 210 Tg N/year and 203 Tg N/year, respectively, resulting in a doubling of nitrogen in the natural nitrogen cycle (Fowler et al., 2013). Major nitrogen circulation banks in the biosphere include the atmosphere, the hydrosphere, the earth's crust, and the tissue in live or dead organisms (EPA, 1993). Nitrogen exists in wastewater as ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and organic nitrogen (EPA, 1993). The ammonia is an emerging concern worldwide; without proper treatment, water containing ammonia can cause adverse effects on aquatic species, as well as eutrophication of natural water systems (Tchobanoglous, Burton, & Stensel, 2003). To minimize the impact on the environment of excess nitrogen, it is critical to reduce the nitrogen in wastewater. Biological processes are considered to be more effective than chemical and physical methods to remove nitrogen compounds (Zhu, Peng, Wang, Wu, & Ma, 2007; ZHU et al., 2007). Biological nitrogen removal processes rely on diverse microorganisms that use various pathways to convert aqueous inorganic nitrogen to a dinitrogen gas.

2.2 Biological nitrogen removal

2.2.1 Complete nitrification and nitritation

Complete nitrification can be accomplished in a two-step biological process conducted by two types of autotrophic bacteria: ammonia oxidizing bacteria (AOB) oxidize ammonia to nitrite (nitritation), then nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate (nitratation) (Metcalf &

Eddy, 2013). Recently found that complete nitrification can also achieved by one organism called Comammox bacteria (Metcalf & Eddy, 2013).

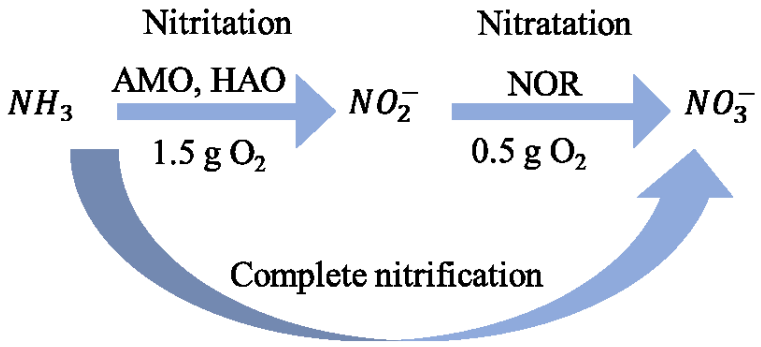


Fig. 1 Schematic diagram of ammonia oxidation. AMO is the ammonia monooxygenase; HAO is the hydroxylamine oxidoreductase; and NOR is the nitrite oxidoreductase.

2.2.1.1 Mechanism and principle of nitrification and nitritation

Complete nitrification (i.e., conventional nitrification) is carried out by two chemolithoautotrophic bacteria that grow on substrates with an inorganic carbon source such as CO_2, carbonate, or bicarbonate. The two steps of complete nitrification are shown in Fig. 1, they include the oxidation of nitrogen from (i) NH_3 to NH_2OH, catalyzed by the enzyme ammonia monooxygenase (AMO) (Eq. 1); (ii) NH_2OH to NO_2^-, catalyzed by the enzyme hydroxylamine oxidoreductase (HAO) (Eq. 2); and (iii) NO_2^- to NO_3^-, catalyzed by the enzyme nitrite oxidoreductase (NOR) (Eq.5) (Heil, Vereecken, & Brüggemann, 2016). The enzymes that catalyze the first two steps exist in AOB, such as *Nitrosomonas, Nitrosomonas europea*, and *Nitrosospira* (S.D., S.R., & D.F., 2007), which carry out the nitritation process. The following equations describe the nitritation reaction:

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O \qquad \text{Eq. 1}$$

$$NH_2OH + H_2O \xrightarrow{HAO} NO_2^- + 5H^+ + 4e^- \quad \text{Eq. 2}$$

$$0.5O_2 + 2H^+ + 2e^- \rightarrow H_2O \quad \text{Eq. 3}$$

$$\Sigma: NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O \quad \text{Eq. 4}$$

The enzyme that catalyzes the reaction in Eq. 5 resides in nitrite oxidizing bacteria (NOB) such as *Nitrobacter* and *Nitrospira* (Metcalf & Eddy, 2013), which conduct nitratation. Combining Eq. 3 and Eq. 5 give Eq. 6, and the sum of Eq. 4 and Eq. 6 give Eq. 7. The nitrification is the combination of nitritation and nitratation, the overall oxidation of nitrogen involved in nitrification is shown in Eq. 7.

$$NO_2^- + H_2O \xrightarrow{NOR} NO_3^- + 2H^+ + 2e^- \quad \text{Eq. 5}$$

$$\Sigma: NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad \text{Eq. 6}$$

$$\Sigma: NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O \quad \text{Eq. 7}$$

Based on the Eq. 4, Eq. 6, and Eq. 7, 4.57 g O_2/g NH_3-N is required for the complete oxidation of NH_3 to NO_3^- (Metcalf & Eddy, 2013). The partial oxidation of NH_3 to NO_2^- required 3.43 g O_2/g NH_3-N, and the oxidation of NO_2^- to NO_3^- required 1.14 g O_2/g NO_2-N (Metcalf & Eddy, 2013). Therefore, nitritation saves 25% oxygen compared to nitrification. The Eq. 8 and Eq. 9 are the stoichiometric equations that considered cell synthesis and buffer in nitrification and nitritation. It is known that alkalinity served as buffer in nitrogen oxidation, as well as inorganic carbon source for cell synthesis. The overall alkalinity requirement for nitrification (Eq. 8) and nitritation (Eq. 9) is 7.14 g alkalinity as $CaCO_3$/g NH_4^+-N oxidized (Metcalf & Eddy, 2013), and the cell production in nitrification is higher than that in nitritation.

$$NH_4(HCO_3) + 0.9852Na(HCO_3) + 0.0991CO_2 + 1.8675O_2 \rightarrow 0.01982C_5H_7NO_2 + 0.9852NaNO_3 + 2.9232H_2O + 1.9852CO_2 \quad \text{Eq. 8}$$

$$NH_4(HCO_3) + 0.9852Na(HCO_3) + 0.07425CO_2 + 1.4035O_2 \rightarrow 0.01485C_5H_7NO_2 + 0.9852NaNO_3 + 2.9406H_2O + 1.9852CO_2 \quad \text{Eq. 9}$$

2.2.1.2 Nitrifying organisms

Four nitrifying organisms, including ammonia oxidizing bacteria, nitrite oxidizing bacteria, ammonia oxidizing archaea, and complete ammonia oxidation bacteria that are responsible for the conversion from ammonia to nitrite or nitrate are described below (Fig. 2).

(i) Ammonia oxidizing bacteria (AOB)

Since 1890, numerous studies have been conducted to understand AOB. Five genera of AOB have been identified and classified. All five genera belong to the Proteobacteria class, four of them belongs the β-Proteobacteria subclass, which includes Nitrosomonas, Nitrosospira, Nitrosovibrio, and Nitrosolobus, and the remaining genus, Nitrosococcus, belongs to the γ-Proteobacteria subclass (Soliman & Eldyasti, 2018). Among the above five genera, Nitrosomonas is the predominant genus. A higher diversity of AOB was shown in municipal wastewater treatment plants (WWTPs) than in industrial or mixed WWTPs, which means the microbial community was influenced by different sources of influent (Chen et al., 2017). It was found that the *Nitrosomonas eutropha* is the most abundant species in the SHARON (single reactor system for high-rate ammonia removal over nitrite) process (Logemann et al., 1998), and the dominant species in salty industrial wastewaters was *Nitrosomonas mobilis* (Prosser, Head, & Stein, 2014). Previous studies have reported that *Nitrosomonas* is the dominant genus under high NH_3-N conditions, (Gonzalez-Martinez et al., 2016) and the *Nitrosomonas* lineage was considered to be fast-growing AOB, which showed higher abundance than other genus in a sequencing batch reactor (SBR). The

population of slow-growing AOB, *Nitrosospira sp.*, was enriched in a completely stirred tank reactor (CSTR) (Terada et al., 2013).

(ii) Nitrite oxidizing bacteria

Nitrite oxidizing bacteria (NOB) exist mainly in the genera *Nitrobacter*, *Nitrospira*, *Nitrotoga*, *Nitrococcus*, *Nitrospina*, and *Nitrolancetus* (Han et al., 2018). Among the six genera, only *Nitrobacter* and *Nitrospira* were found to play an important role in the ecosystem (Bartosch, Hartwig, Spieck, & Bock, 2002; Ke, Angel, Lu, & Conrad, 2013). NOB in the genus *Nitrobacter* have a low substrate affinity, preferring a high substrate concentration, whereas NOB in the genus *Nitrospira* showed an affinity to lower nitrite and oxygen concentrations (Blackburne, Vadivelu, Yuan, & Keller, 2007; Nowka, Daims, & Spieck, 2015). It was found that NOB in the genus *Nitrospira* are more dominant than NOB in the genus *Nitrobacter* in WWTPs that have low nitrite and oxygen concentrations (Nogueira & Melo, 2006).

(iii) Ammonia oxidizing archaea

Ammonia oxidizing archaea (AOA) belong to the phylum Thaumarchaeota, which was first isolated from a marine aquarium tank (Könneke et al., 2005). The discovery of AOA indicated that the oxidation of ammonia to nitrite is not only catalyzed by AOB, it is also performed by AOA (Limpiyakorn et al., 2013). The main difference between AOA and AOB is that hydroxylamine oxidoreductase (HAO) enzymes have not been found in AOA genomes (Simon & Klotz, 2013; Walker et al., 2010). Studies have reported that during the oxidation of hydroxylamine to nitrite, catalyzed by AOA, nitric oxide (NO) is a necessary intermediate. NO together with hydroxylamine served as co-substrates for a copper containing enzyme to produce nitrite (Hatzenpichler, 2012;

Kozlowski, Stieglmeier, Schleper, Klotz, & Stein, 2016). Accordingly, AOA could be enriched in water with a low concentration of dissolved oxygen (DO) (< 0.2 mg/L) and a long solids retention time (SRT), in wastewater with low concentrations of toxic compounds (Bai, Sun, Wen, & Tang, 2012; Giraldo, Jjemba, Liu, & Muthukrishnan, 2012; R. N. Mohammed, Abu-Alhail, & Xi-Wu, 2014; Park, Wells, Bae, Griddle, & Francis, 2006).

(iv) Complete ammonia oxidation

Comammox is a process that conducted by an organism, which can perform both steps in the oxidation of ammonia to nitrate. Normally two organisms are needed to accomplish this reaction. To date, all the identified complete nitrifiers, including *Candidatus Nitrospira inopinata*, *Candidatus Nitrospira nitrosa*, *Candidatus Nitrospira nitrificans*, and *Nitrospirae sp. genome_bin_8*, are in sub-lineage II of the *Nitrospira* genus (Daims et al., 2015; Palomo et al., 2016; Pinto et al., 2016; Van Kessel et al., 2015; Wang et al., 2017; Bartelme, McLellan, & Newton, 2017). The Comammox genome contains genes that enable it to oxidize ammonia and nitrite (Daims et al., 2015). Comammox *Nitrospira* are not only widespread in nature (Pjevac et al., 2017), they have also been detected in a number of engineering systems, for instance, aquaculture biofiltration units (Bartelme et al., 2017; Palomo et al., 2016; Van Kessel et al., 2015); drinking water treatment plants with low temperature and high DO (Daims et al., 2015; Pinto et al., 2016; Wang et al., 2017), water distribution system (Berry, Xi, & Raskin, 2006), and wastewater treatment plants (Chao, Mao, Yu, & Zhang, 2016; Pjevac et al., 2017).

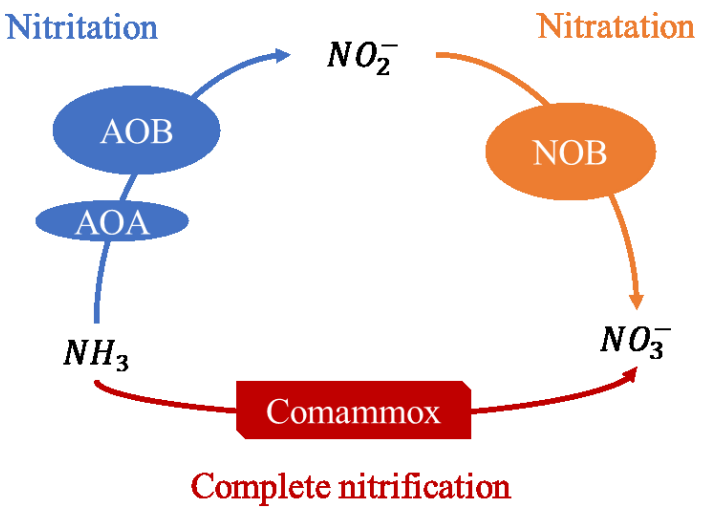


Fig. 2 Overview of different microorganisms involved in ammonia oxidation. AOB is ammonia oxidizing bacteria, AOA is ammonia oxidizing archaea, NOB is nitrite oxidizing bacteria., and Comammox stands for COMplete AMMonia OXidiser.

2.2.2 Complete denitrification and denitritation

Denitrification is a biological process that reduces nitrate to nitrogen gas via heterotrophic bacteria (Metcalf & Eddy, 2013). The biological reduction of nitrite to nitrogen gas is termed denitritation.

2.2.2.1 Mechanism and principle of denitrification and denitritation

Complete denitrification is carried out by heterotrophic bacteria, which convert nitrate to nitrogen gas using organic carbon as a carbon source. Another pathway for nitrate respiration is termed ammonification, which reduces nitrate to nitrite then to ammonia (Shapleigh, 2013). A series of intermediates are generated during the denitrification process, including nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) (Fig. 3).

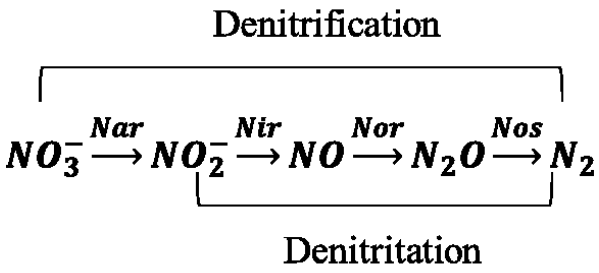


Fig. 3 Intermediates in complete denitrification and nitritation pathways. Nar is nitrate reductase, Nir is nitrite reductase, Nor is nitric oxide reductase, and Nos is nitrous oxide reductase.

In denitrification, methanol, acetate, glucose, and ethanol are commonly used as external carbon sources (Ahn, 2006). Piggery manure (J. Yang, Wang, & Luo, 2019) and raw sewage (Shen, Yang, Wu, Zhang, & Zhang, 2019) are utilized as carbon sources in denitrification or denitritation as well. Methanol (CH_3OH) has been widely used in denitrification because it is relatively inexpensive (Rittmann & McCarty, 2001), but it is flammable, which introduces safety concerns. In comparison, acetate is safer to use, and at temperatures lower than 20 ^oC, the denitrification rate with acetate was nearly double the rates with other carbon sources (Cherchi et al., 2009). It was also found that sludge can be denitrified only by acetate and not by other carbon sources, if they have been fed with acetate (Cherchi, Onnis-Hayden, El-Shawabkeh, & Gu, 2009).

Equation 10 shows denitrification with acetate:

$$NO_3^- + H^+ + 0.33NH_4^+ + 1.45CH_3COO^- \rightarrow 0.5N_2 + 0.33C_5H_7O_2N + 1.6H_2O + 1.12HCO_3^- + 0.12CO_2 \quad \text{Eq. 10}$$

Equation 11 shows denitritation with acetate:

$$NO_2^- + H^+ + 0.24NH_4^+ + 0.98CH_3COO^- \rightarrow 0.5N_2 + 0.24C_5H_7O_2N + 1.24H_2O + 0.74HCO_3^- + 0.008CO_2 \quad \text{Eq. 11}$$

The COD of acetate can be determined via calculating the amount of oxygen that is required to oxidize acetate, and the result indicated that 1 g acetate equals to 0.78 g COD. According to Eq. 10 and Eq. 11, complete denitrification requires 3.7 g COD/g N and complete denitritation requires 2.3 g COD/g N. Therefore the amount of COD required for denitritation is only 62% of the amount of COD required for complete denitrification (Metcalf & Eddy, 2013; Rittmann & McCarty, 2001).

2.2.2.2 Denitrifying organisms

(i) Heterotrophic nitrate/nitrite removal

Denitrification capacity has been widely observed in both bacteria and archaea. A higher diversity of bacteria and archaea was observed in the environment than in wastewater treatment plants (WWTPs) (Noredal Throbäck, 2006). The genera of denitrifiers such as *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Thiobacillus* are within the α, β, or γ class of the Proteobacteria. Some Gram-positive bacteria like *Bacillus*, as well as some halophilic archaea like *Halobacterium* are also capable of performing denitrification (Shapleigh, 2013). Denitrification bacteria such as *Hyphomicrobium* (Martineau, Villeneuve, Mauffrey, & Villemur, 2013), *Paracoccus* (Chakravarthy, Pande, Kapoor, & Nerurkar, 2011), *Pseudomonas* (J. J. Su, Liu, & Liu, 2001), and *Comamonas* spp. (Gumaelius, Magnusson, Pettersson, & Dalhammar, 2001) in Proteobacteria are found in bioreactors. In terms of denitrification activity, *Hyphomicrobium* spp. are complete denitrifiers, and can convert nitrate and nitrite to N_2 (Sperl & Hoare, 1971); Methyloversatilis spp. are incomplete denitrifiers, reducing nitrate or nitrite to NO (H., M., & K., 2012); and some strains of *Pseudomonas* spp. are incomplete nitrite reducers, which convert only nitrite to NO (Vangnai & Klein, 1974).

(ii) Autotrophic nitrate/nitrite removal

Autotrophic denitrifiers derived energy from the oxidation of inorganic compounds, such as inorganic sulfur compounds, hydrogen, and Fe (II), facilitated with the reduction of nitrate and nitrite (Chung et al., 2014; Mora, Guisasola, Gamisans, & Gabriel, 2014; Sahinkaya & Dursun, 2012; J. F. Su et al., 2015; W. Zhou et al., 2011). *Pseudomonas sp.* SZF15 has the capacity to oxidize ferrous ion while reducing nitrate (J. F. Su et al., 2015). Additionally, studies reported that both nitrite and nitrate can be removed by autotrophic denitrifiers (such as *Thiobacillus denitrificans*) under anaerobic conditions, utilizing thiosulfate as an electron donor (Chung et al., 2014; Mora et al., 2014).

Aerobic denitrification, or autotrophic nitrate/nitrite removal, was first found in *Paracoccus* (Shapleigh, 2013) and *Thiosphaera pantotropha*, which were detected in well mixed aerobic cultures (Robertson & Kuenen, 1984). *Pseudomonas carboxydohydrogena* was detected when H_2 was present in the water sample (Shapleigh, 2013). *Pseudomonas stutzeri* SU2 was isolated from activated sludge (J. J. Su et al., 2001), and *Magnetospirillum magnetotacticum* was found to consume O_2 while performing denitrification (BAZYLINSKI & BLAKEMORE, 1983). However, the DO concentration should be well controlled for aerobic denitrifiers, otherwise, Nir or Nor gene expression might be inhibited in wastewater with a high concentration of dissolved oxygen (DO) (Bergaust, Shapleigh, Frostegård, & Bakken, 2008), and NO was generated when insufficient DO was provided (Shapleigh, 2013). If an air supply is needed for nitrate or nitrite removal, more energy would be consumed.

2.2.3 Anaerobic ammonia oxidation (Anammox)

Another promising nitrogen removal process is anaerobic ammonia oxidation (anammox), which was defined as a lithoautotrophic biological process carried out by *Planctomycete* bacteria (Strous et al., 1999). This organism was first discovered in a denitrifying fluidized bed reactor (Mulder, van de Graaf, Robertson, & Kuenen, 1995). *Planctomycete* consume ammonia and nitrite (electron donor) in the absence of O_2 and generate N_2 and nitrate as end products, as shown in Eq. 12.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad \text{Eq. 12}$$

Anammox organisms grow with inorganic carbon such as CO_2. To date, there are six known anammox genera and over 20 known species. *Candidatus Brocadia* (*C. Brocadia*), *C. Kuenenia*, *C. Jettenia*, *C. Anammoxoglobus*, *C. Scalindua*, and *C. Anammoximicrobium* were found in WWTPs or laboratory scale reactors (Jetten et al., 2001; Kartal et al., 2007; Schmid et al., 2005). However, *C. Scalindua* is normally found in natural ecosystems (Jetten et al., 2009). Compared to conventional nitrification-denitrification, the merits of anammox are lower operational costs, no need for carbon addition (Veuillet et al., 2014), and 90% less sludge production (Van Dongen, Jetten, & Van Loosdrecht, 2001). However, the long start up time (10 months) is needed to achieve stable anammox performance, except when the reactor is seeded with enriched anammox sludge (Ni et al., 2010; Rikmann, Zekker, Tenno, Saluste, & Tenno, 2018; van der Star et al., 2007), and widespread inhibitors of anammox are commonly found in wastewaters (Jin, Yang, Yu, & Zheng, 2012). Therefore, in terms of the process stability in larger scale application, denitrifiers are much more stable than anammox bacteria.

2.2.4 Dissimilatory nitrate reduction to ammonium (DNRA)

In nitrogen cycle, dissimilatory nitrate reduction to ammonia (DNRA) was considered as one of the least investigated process (Kuypers, Marchant, & Kartal, 2018). DNRA reduces nitrate to nitrite then further to ammonia. The conversion from nitrite to ammonia was catalyzed by the cytochrome c nitrite reductase (Friedl et al., 2018). Compared to denitrification, DNRA does not remove nitrogen as N_2, but retained the nitrogen in the form of ammonia (Friedl et al., 2018; Rahman, Roberts, Grace, Kessler, & Cook, 2019). Recently, DNRA has been detected in the eco-systems, where denitrification was also occurred (Friedl et al., 2018) and under high C/N condition, DNRA rates was higher than denitrification (Jahangir et al., 2017).

2.3 Co-existence of nitritation-denitritation organisms in wastewater treatment

In WWTPs, the most widely applied technology is complete nitrification-denitrification. However, complete nitrification-denitrification requires high energy for aeration during nitrification, and external carbon addition for denitrification is costly (H. Wang, Xu, Qiu, Zhou, & Liu, 2019). A less expensive procedure is nitritation-denitritation. Compared to nitrification-denitrification, nitritation-denitritation used 25% less oxygen, saved 60% of the energy cost associated with aeration and 40% of the COD associated chemical cost, and emitted 20% less CO_2 (Yong Zhen Peng & Zhu, 2006; Regmi et al., 2014; Van Kempen, Mulder, Uijterlinde, & Loosdrecht, 2001). Additionally, the reaction rate of denitritation via nitrite is 1.5 to 2 times higher the reaction rate of denitrification via nitrate, and 33-35% and 55% lower sludge generation could be achieved via nitritation and denitritation, respectively (Yong Zhen Peng & Zhu, 2006; Picioreanu, Van Loosdrecht, & Heijnen, 1997).

2.3.1 Factors that influence nitritation-denitritation

The main challenge to achieve nitrite accumulation is the inhibition or washout of nitrite oxidizing bacteria (NOB). Strategies implemented to tackle the challenge include control of: dissolved oxygen (DO), temperature, pH, free ammonia (FA), free nitrous acid (FNA), free hydroxylamine (FH), and the solid retention time (SRT); different operation strategies can also be used to find the optimal conditions (Ge et al., 2015; Yong Zhen Peng & Zhu, 2006). Additionally, the microbial community in a reactor might shift with the addition of different carbon sources (Cherchi et al., 2009).

2.3.1.1 DO concentration

As the microbial community can change under different dissolve oxygen (DO) concentrations, DO is an important parameter in nitritation-denitritation. The oxygen half saturation coefficients for AOB and NOB are 0.2-0.4 mg/L and 1.2-1.5 mg/L, respectively (Picioreanu et al., 1997). Therefore, AOB was dominant under low DO conditions, while NOB was restricted due to its the lower affinity for oxygen (Y. Z. Peng et al., 2004). A literature search revealed that nitrite accumulation has been observed in laboratory reactors with DO levels at 1.5 mg/L, 0.4-0.7 mg/L, and 0.3-0.5 mg/L. Some studies indicated that high DO (1-1.5 mg/L) was critical for the oxidation of ammonia and accumulation of nitrite (Yong Zhen Peng & Zhu, 2006; Ruiz, Jeison, Rubilar, Ciudad, & Chamy, 2006). However, successful nitritation at 98% nitrite accumulation has been achieved with 0.7 mg/L DO (Katsou et al., 2015; Kulikowska & Bernat, 2013). In a laboratory-scale system, over 85% nitrite accumulation was achieved with DO concentrations of 0.5-1.0 mg/L(Guo, Peng, Yang, Gao, & Wang, 2013). High DO concentrations can also restrict denitrification activity, because nitrite reductases (Nir) and nitrous oxide reductases (Nos) are

sensitive to DO, thus toxic NO and N_2O can accumulate in the system. (Lu & Chandran, 2010; Otte, Grobben, Robertson, Jetten, & Kuenen, 1996). Therefore, performing nitritation at a low DO concentration (0.5-1 mg/L) could be an energy effective method for wastewater treatment plants (WWTPs) (Ge et al., 2015).

2.3.1.2 Temperature

The nitritation and nitratation organisms, AOB and NOB are temperature sensitive (Hellinga et al., 1998). The growth rate of AOB was much higher than the growth rate of NOB when the operating temperature was higher than 15 ºC (Ge et al., 2015). Accordingly, 25 ºC is the suggested temperature to eliminate NOB and enrich AOB (Paredes et al., 2007). However, if nitritation is performed at lower temperature, NOB elimination can be achieved by controlling other parameters, such as DO, FA and the solid retention time (SRT). Additionally, the difference in growth rates of NOB and AOB become more significant with temperature increase (Bougard, Bernet, Chèneby, & Delgenès, 2006). The optimum temperature for denitritation is 20-30 ºC, and if the temperature lies outside that range, the denitritation process decreases significantly (Y. Ma, Peng, Wang, Yuan, & Wang, 2009).

2.3.1.3 pH

The pH value in a reactor can influence the activity of microorganisms and the concentrations of free ammonia (FA) and free nitrous acid (FNA) (Anthonisen, Loehr, Prakasam, & Srinath, 1976). The optimum range of pH for nitritation is 7.5-8.0 (Metcalf & Eddy, 2013), and the optimum range of pH for denitritation is 7-9 (Grady, Daigger, Love, & Filipe, 2011). At pH values lower than 7, nitritation activity is 80-90% lower than nitritation activity at pH 7.5-8.0 (Metcalf & Eddy, 2013).

Sodium bicarbonate or lime can be added to the reactor to keep the pH in a suitable range (Metcalf & Eddy, 2013; Shao, Shi, Mohammed, & Liu, 2017). The impacts of FA and FNA on AOB and NOB are discussed in the following section.

2.3.1.4 Free ammonia (FA), free nitrous acid (FNA), and free hydroxylamine (FH)

Eq. 13 and Eq. 14 show the relationships of FA and FNA, respectively, to the pH and temperature in a reactor (Anthonisen et al., 1976).

$$FA = \frac{17}{14} \times \frac{TAN \times 10^{pH}}{e^{\left(\frac{6344}{273+T}\right)} + 10^{pH}}, \quad \text{Eq. 13}$$

$$FNA = \frac{46}{14} \times \frac{TNO_2}{e^{\left(\frac{-2300}{273+T}\right)} + 10^{pH}}, \quad \text{Eq. 14}$$

where TAN is the total NH_4^+-N (mg/L), TNO_2 is the total NO_2^--N (mg/L), T is the temperature in ^oC, and the pH is equal to the value measured in the reactor.

It was suggested that FA influences the activities of AOB and NOB by different extents: 10-150 mg N/L and 0.1-1.0 mg N/L were reported to inhibit AOB and NOB activities, respectively, and AOB and NOB are inhibited at an FNA concentration of 0.22-2.8 mg/L (Anthonisen et al., 1976). A high FA concentration can inhibit the activity of nitrite oxidoreductase (NOR) (L. Yang & Alleman, 1992). FNA can affect the pH gradient in a cell and further influencing the synthesize of adenosine triphosphate (ATP) that required for all microbial activities (Glass, Silverstein, & Oh, 1997). Another potential inhibitor is free hydroxylamine (FH), which was characterized as a toxic intermediate generated from AOB activity. FH showed acute toxicity to NOB when 0.42 mg NH_2OH N/L was present (Hu, 2002).

2.3.1.5 SRT

At temperatures lower than 15 ºC, longer solids retention time (SRT) was needed for AOB growth than for NOB growth, however, this trend was reversed at temperatures > 25 ºC and the recommended temperature for nitritation was 30 ºC (Hellinga et al., 1998). The reported minimum doubling times for AOB and NOB are 7-8 h and 10-13 h, respectively, thus based on the doubling time, AOB and NOB can be selectively enriched by adjusting the SRT (Hellinga et al., 1998). To date, successful nitritation has been achieved under various SRTs, for instance, 6 days at 25 ºC (Regmi et al., 2014) and 30 days at temperatures lower than 13 ºC (Peng & Zhu, 2006). However, to achieve stable nitritation-denitritation, the control of SRT should cooperate with other control parameters.

2.3.1.6 External carbon sources

The carbon source has a strong impact on the denitrification microbial community, for instance, *Methylophilus*, *Paracoccus*, *Methyloversatilis*, and *Hyphomicrobium* spp. are detected in methanol fed systems (Baytshtok et al., 2009; Hallin, Throbäck, Dicksved, & Pell, 2006); ethanol enriched populations included *Paracoccus*, *Thauera*, and *Azoarcus* spp. (Baytshtok et al., 2009); acetate utilizers were closely related to *Comamonas*, *Acidovorax*, and *Thauera* spp., which belong to *Comamonadaceae* and *Rhodocyclaceae* families (Ginige, Keller, & Blackall, 2005; Osaka et al., 2006); *Comamonas* sp. dominated in glycerol fed systems (Lu, Chandran, & Stensel, 2014), and the abundance of *Aquaspirillum*-related bacteria was highest (20%) in complex carbon mixtures (Lu et al., 2014).

2.3.1.7 Operational strategies for nitritation: Aeration control

Aeration control was suggested as an alternative to achieve nitritation (Hidaka, Yamada, Kawamura, & Tsuno, 2002). The time length of aeration is closely related to the extent of nitritation, because nitrite is converted to nitrate if a long aeration period is provided (Turk & Mavinic, 1989). Nitritation has been successfully achieved via a strategy of intermittent aeration in mainstream wastewater (Regmi et al., 2014), raw landfill leachate (Li, Zhou, Huang, & Xu, 2013), domestic wastewater with a high concentration of DO (Bao, Wang, Ma, Zhang, & Peng, 2017), and ammonia rich wastewater (Y., C., B.G., & B.F., 2017). Thus, aeration control via intermittent aeration was shown to be an effective strategy to achieve the nitrite accumulation.

2.3.2 Nitrous oxide emission

Nitrous oxide (N_2O) is a greenhouse gas with 265 times the impact of CO_2 (IPCC, 2014). N_2O not only contributes to the greenhouse effect, it also depletes the ozone layer (Q. Yang et al., 2009). It has been reported that the nitritation-denitritation process could be a potential source of N_2O emission. Three pathways that could lead to N_2O emission include hydroxylamine oxidation, nitrifier denitrification, and heterotrophic denitrification (Fig. 4) (Ni & Yuan, 2015; Wunderlin et al., 2013; Wunderlin, Mohn, Joss, Emmenegger, & Siegrist, 2012). Hydroxylamine oxidation might be related to an imbalance in AOB metabolic activity (Yu, Kampschreur, Van Loosdrecht, & Chandran, 2010), or to chemical decomposition or chemical oxidation (Stüven, Vollmer, & Bock, 1992). Nitrifier denitrification occurred when limited oxygen was available and a high nitrite concentration was present (Colliver & Stephenson, 2000; Wrage, Velthof, Beusichem, & Oenema, 2001). The production of N_2O from heterotrophic denitrification is induced by insufficient

biodegradable organics (Itokawa, Hanaki, & Matsuo, 2001), oxygen inhibition (H. & K., 2010), or nitrite accumulation (Von Schulthess, Wild, & Gujer, 1994).

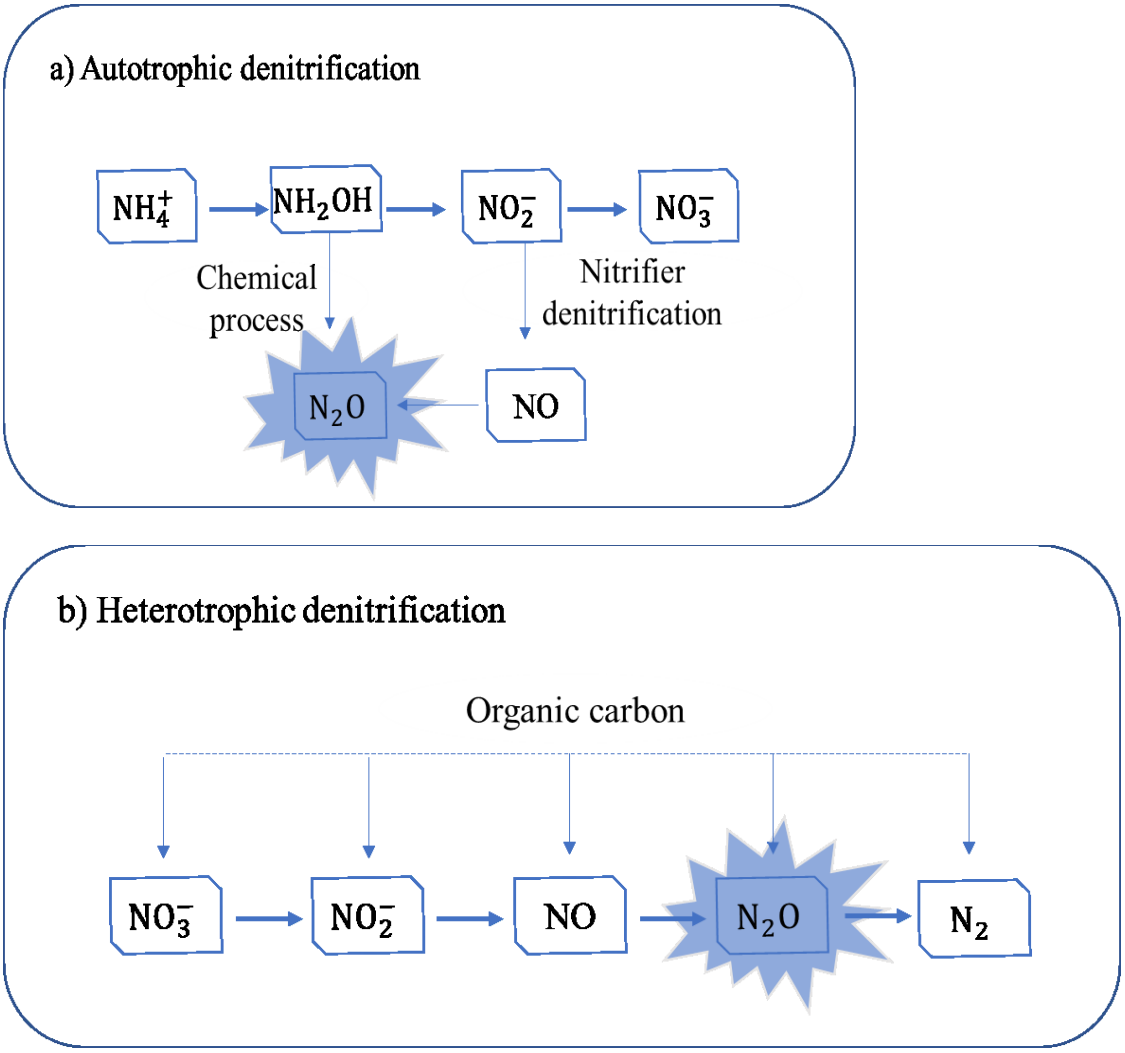


Fig. 4 Schematic diagram of N_2O production from (a) autotrophic denitrification and (b) heterotrophic denitrification.

The production of N_2O from nitritation-denitritation was 1.5 times higher than the production of N_2O from nitrification-denitrification, and the main contributor to the production of N_2O is the conversion of ammonia to nitrite. N_2O production was favored when high ammonia and low nitrite concentrations were present; therefore, it was suggested that a step-feed strategy of influent could

reduce 50% of the N_2O emission during the nitritation-denitritation process (Q. Yang et al., 2009), and that N_2O emission could be reduced when sufficient biodegradable carbon and DO were provided while less nitrite was present (Wunderlin et al., 2012). An innovative application of N_2O has been termed "coupled aerobic-anoxic nitrous decomposition operation" (CANDO). CANDO converts ammonia to nitrite which is then reduced to nitrous oxide, which can be harvested and co-combusted with biogas to recover energy (Scherson et al., 2013; Scherson, Woo, & Criddle, 2014).

This measurement was not conducted in this study, but for future nitrogen removal operations, optimal operational strategies and a validated model with various N_2O generation pathways are critical for mitigating the emission of N_2O (Massara et al., 2017).

2.3.3 Integrated fixed film activated sludge (IFAS) - sequencing batch mode

Integrated fixed film activated sludge (IFAS) is a modified activated sludge system, which contains both microbial flocs and biofilms. This hybrid system can provide a long SRT for biological wastewater treatment. A long SRT would allow relatively slower growing bacteria (AOB) to be enriched in an IFAS system (Mahendran, Lishman, & Liss, 2012). There are two types of media in IFAS, fixed media and mobile media. The drawback of fixed media is the unexpected occurrence of worms during nitrogen treatment (Copithorn, Sturdevant, Farren, & Sen, 2014). To eliminate worm growth, mobile medias were investigated. To date, the most popular mobile media, marketed by AnoxKaldns, was produced by polyethylene and a 10 years life span was suggested. The formation of biofilm is mostly developed on the interior surface of media (Mahendran et al., 2012). The merits of IFAS technology compared to activated sludge treatment

of wastewater include a higher retention of biomass in the system due to the increase in surface area, and better settleability, which enhances the reactor capacity without increasing the volume. The biomass attached to the media can be used as seed for future work, and a longer SRT can be achieved with an IFAS system compared to activated sludge treatment of wastewater (Kim et al., 2010; Saknenko, Nazareth, Gibb, Devlin, & Thomas, 2015).

To date, the sequencing batch reactor is known for its high flexibility, which meets the needs of most operational conditions. For instance, the operational mode of the sequencing batch reactor can easily be adjusted over the duration of time-based processes (Frison et al., 2012; Katsou et al., 2015; Mace & Mata-Alvarez, 2002; Malamis et al., 2014). Therefore, in this nitritation-denitritation study, mobile medias made by polyethylene were utilized in an IFAS reactor and operated under sequencing batch mode.

2.3.4 Other processes related to nitritation-denitritation

Nitrogen removal through nitritation, denitritation, and anammox, and phosphorus removal were accomplished in a single system in the treatment of carbon limited wastewater (Zeng, Li, Wang, Bai, & Peng, 2014). In that study, nitrite was used as an electron accepter instead of oxygen to perform phosphorus uptake. The results indicated a 90% of nutrient removal, including 60% nitrogen and 88% phosphorus removal. Another study integrated simultaneous COD oxidation, partial nitritation-denitritation and anammox (SCONA) in a single system, and the result showed 94.3%, 92.6% and 88% of COD, ammonia nitrogen, and total nitrogen removal, respectively (X. Zhou, Zhang, Zhang, & Liu, 2018). Additionally, anammox was often used as a polishing step after nitritation-denitritation (Gu, Yang, & Liu, 2018; Regmi et al., 2015, 2016).

Overall, the combination of nitritation, denitritation and anammox in a single reactor is a promising technology for reducing the addition of external carbon source, and thus chemical costs related to external carbon source can be further reduced.

Chapter 3 – Reactor Performance analysis and Microbial Related Measurement Method Development

3.1 Introduction

To understand the reactions happened in the reactor, and microbial community in both activated and attached sludge, methods for all the parameters related to performance and DNA extraction from attached and suspended biomass should be investigated. This chapter elucidates the wastewater characteristics, reactor operation strategy, and materials and methods utilized to analyze the reactor performance, microbial activity, biofilm thickness as well as DNA extraction process.

3.2 Materials and methods

3.2.1 Lagoon supernatant collection and characteristics

Raw wastewater from an anaerobically digested sludge lagoon in Edmonton, Alberta, was collected monthly and stored in a cold room at 4 ^{o}C to reduce the microbial activity before bioreactor treatment. The basic characteristics of the lagoon supernatant are shown in Table 1.

Table 1. The basis composition of lagoon supernatant

Parameter	Range
NH_4^+-N (mg/L)	800-1000
NO_2^--N (mg/L)	5-10
NO_3^--N (mg/L)	1-5
COD (mg/L)	400-600
Alkalinity (mg $CaCO_3$/L)	2500-3000
pH	7.5-8

3.2.2 Reactor configuration and operation

An IFAS reactor (working volume 5.5 L) (Fig. 5) was equipped with a spiral-shaped impeller, a fine bubble air diffuser and 40% of apparent volume was filled with polyethylene carriers. The polyethylene biocarriers are a cylindrical shape, and their effective surface area is approximately 463 m^2/m^3. An air flowmeter was equipped to adjust the air flow rate and the whole process was controlled by time-based.

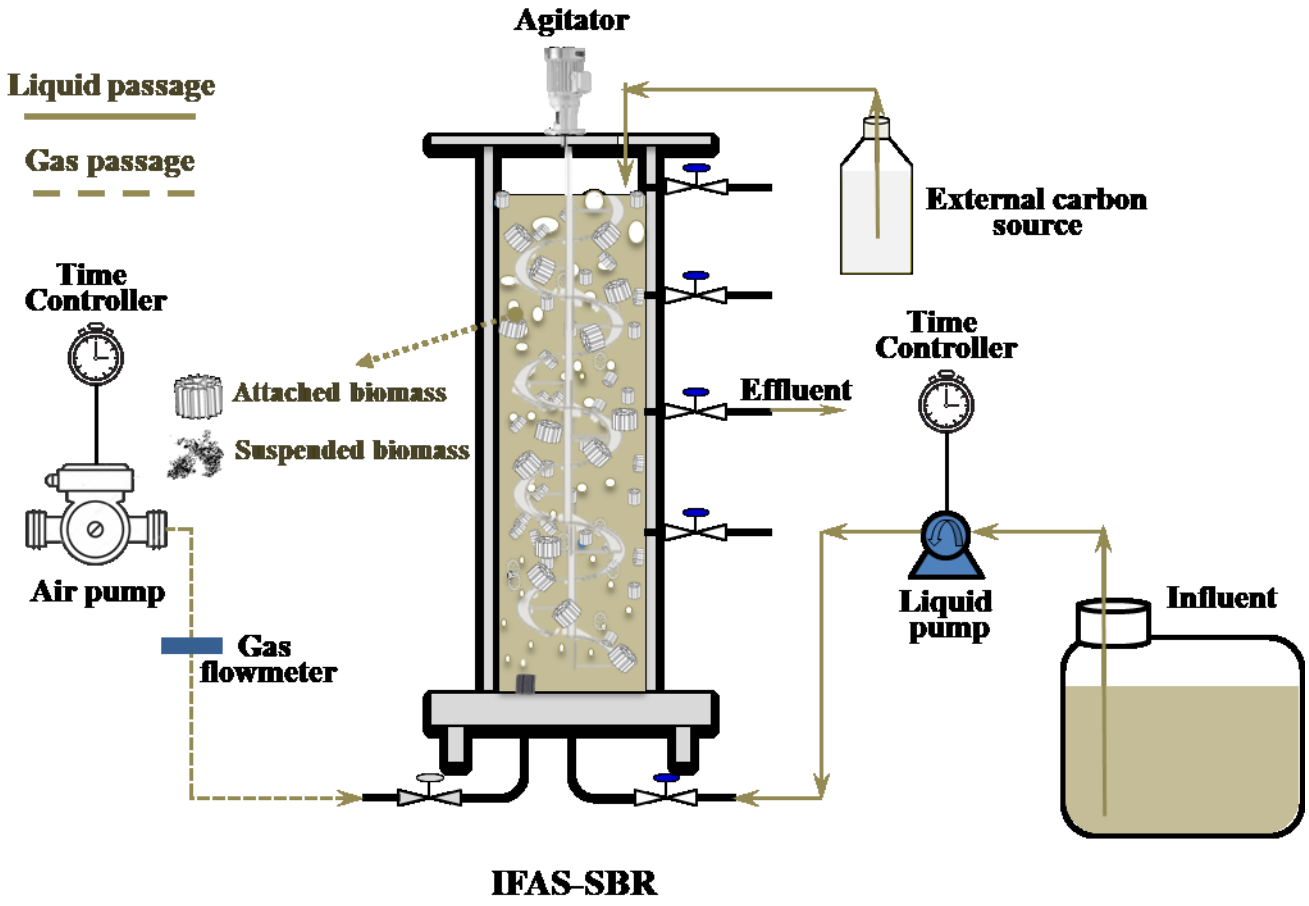


Fig. 5 Schematic overview of IFAS-SBR nitritation-denitritation reactor

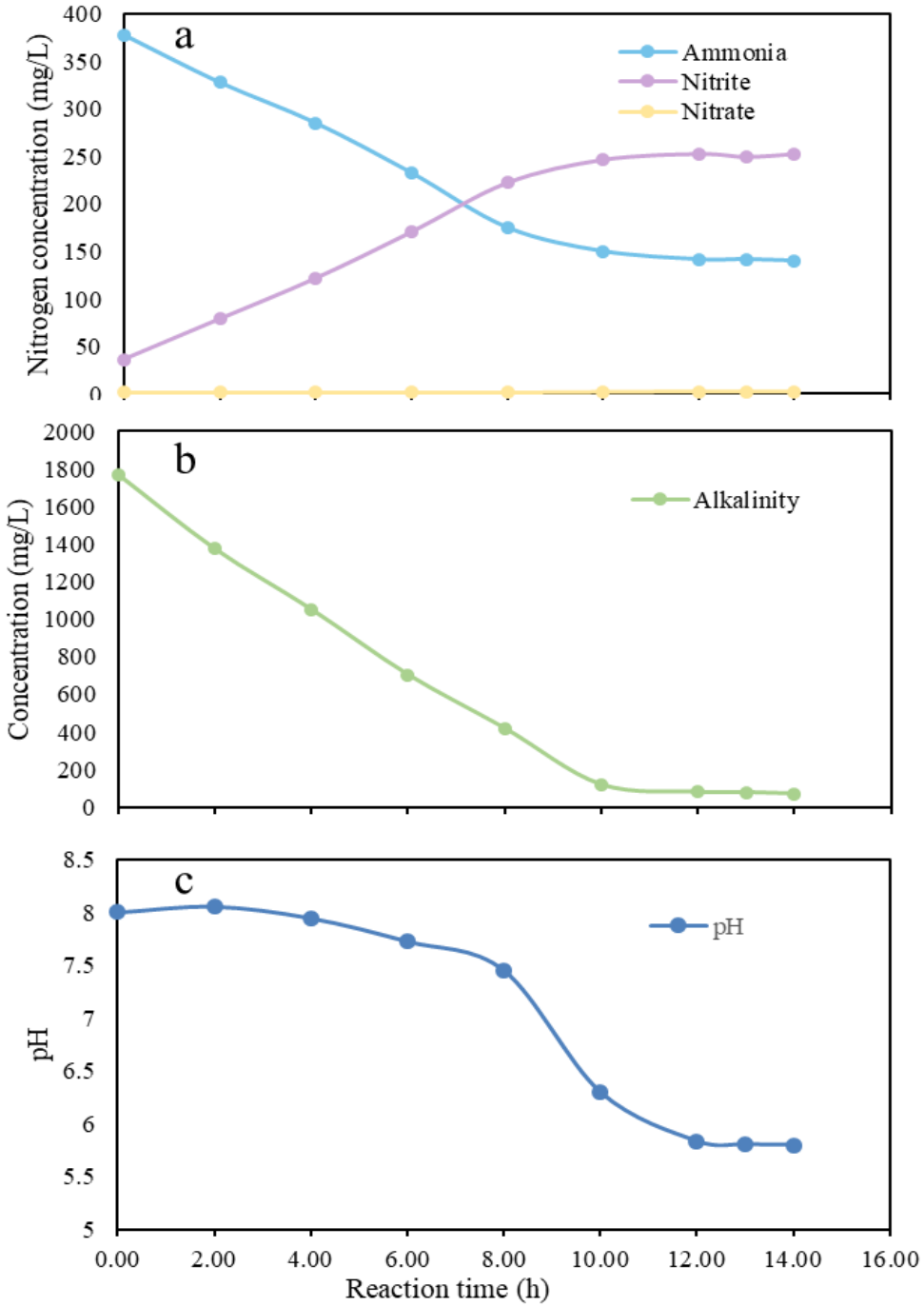


Fig. 6 Results of a) dynamics of nitrogen species, b) alkalinity concentration, and c) pH values along the preliminary test

The IFAS reactor was seeded with sludge that was taken from a nitritation and denitritation dual reactor in the lab. The IFAS reactor was operated in sequencing batch mode with a 50% exchange

ratio of influent at 21 ºC. The hydraulic retention time (HRT) was 2 days and the solid retention time (SRT) was 17 days. Prior to the startup, a preliminary test was conducted to determine the length of aeration required for each cycle, based on ammonia conversion rate and alkalinity availability. The preliminary test results are shown in Fig. 6, which indicated that the ammonia conversion rate decreased after 8 hours aeration, thus the duration of aerobic period should not exceed 8 hours. It also can be noticed that after 7 hours aeration, about 50% of ammonia was oxidized and the corresponding pH was 7.5 and alkalinity was 600 mg/L. To ensure a high nitritation reaction rate throughout the aeration period, 7 hours aeration was determined as the alkalinity and pH are within the reasonable range, and half of initial ammonia concentration was oxidized. Therefore, the IFAS-SBR was operated in 2 alternating aerobic (7 h) and anoxic cycles (3 h 50 min), followed by settling and decanting in sequence (shown in Fig. 7). During the nitritation process, the airflow meter control the amount of air that was diffused into the reactor, so that the DO can be maintained at 0.5 – 0.7 mg/L. Sodium acetate, at an average concentration of 4 g/L, was continuously introduced (0.3 mL/min) into the system during the denitritation process as an external carbon source; no air was pumped into the reactor during this stage. The reactor was operated for over 100 days to test the process stability and microbial community dynamics in the single-reactor system.

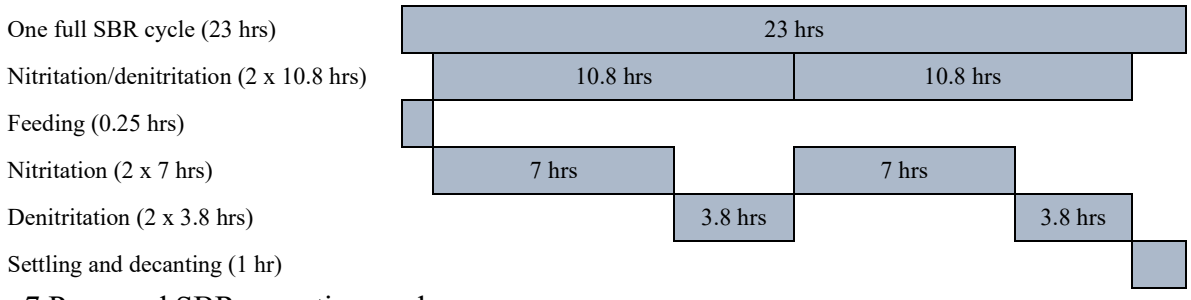


Fig. 7 Proposed SBR operation cycle

3.2.3 Chemistry measurement

Raw effluent was collected to measure the effluent pH, using a benchtop pH meter. Prior to the measurement, the influent and effluent water samples were collected through the effluent port, were filtered through 0.45 μm filters. The chemical parameters, for instance, NH_4^+-N, NO_2^--N, NO_3^--N, and alkalinity were analyzed by Hach kits (HACH company) and read via a DR3900 benchtop Spectrophotometer (DR3900, HACH, Germany). The method for NH_4^+-N is 8038 and termed Nessler, while method 8153 (Ferrous sulfate), TNT 835, and TNT 870 were used for NO_2^--N, NO_3^--N, and alkalinity measurement, respectively. The COD measurement was based on the *Standard method* 5220 D (APHA, 2000), which is a closed reflux, colorimetric method. This method uses the digestion solution (a mixture of $K_2Cr_2O_7$, H_2SO_4, $HgSO_4$), sulfuric acid and samples, heating under 150 ^oC for 2 h, the samples were read at 600 nm by the DR3900 benchtop Spectrophotometer once the sample cooled down.

3.2.4 Solids measurement

In terms of solid concentrations, the mixed liquor suspended solids (MLSS) was measured based on *Standard method* 2540 D (APHA/WEF/AWWA, 2018). The samples were filtered through a glass fiber and dried at 105 ^oC overnight. For the mixed liquor volatile suspended solids (MLVSS) measurement, *Standard method* 2540 E (APHA/WEF/AWWA, 2018) was used, which ignites the 105 ^oC dried sample at 550 ^oC for 30 min (based on the amount of residue) to determine the MLVSS concentration. For the measurement of the attached biomass, firstly biomass should be sonicated down from the media, and then follow the MLSS and MLVSS procedures to get the results. Another parameter, sludge volume index (SVI) was tested according to the *Standard*

method 2710 D (APHA, AWWA, 2004), depending on the settled sludge volume after 30 min settling of 100 mL mixed liquor.

3.2.5 Cycle test and activity test

To gain a better understanding of the reactions that might happen together with nitritation and denitritation during aerobic and anoxic phases, cycle tests were performed. The cycle tests were conducted when the stable condition was reached. The stable condition means inorganic nitrogen removal rate remained unchanged for more than a week. Mixed liquor samples were collected at 2-hr intervals during aeration phase and 1-hr intervals during anoxic phase. The harvested samples were filtered through 0.45 µm filters immediately and stored at 4 °C for NH_4^+-N, NO_2^--N, NO_3^--N, and alkalinity analysis. The value of FA and FNA were determined using the collected data (*i.e.*, pH, temperature) and the Eq. 13 and Eq. 14 presented by Anthonisen et al. (1976). To evaluate the nitrite accumulation (NA) and NOB suppression in a cycle, the degree of nitrite accumulation was calculated based on Eq. 15 (Guo et al., 2009). The equations are shown as follows:

$$FA = \frac{17}{14} \times \frac{TAN \times 10^{pH}}{e^{\left(\frac{6344}{273+T}\right)} + 10^{pH}} \quad \text{Eq. 13}$$

$$FNA = \frac{46}{14} \times \frac{TNO_2}{e^{\left(\frac{-2300}{273+T}\right)} + 10^{pH}} \quad \text{Eq. 14}$$

$$NA = \frac{[NO_2\text{-}N]}{[NO_2\text{-}N] + [NO_3\text{-}N]} \times 100\% \quad \text{Eq. 15}$$

Where TAN is total NH_4^+-N (mg/L), TNO_2 is total NO_2^--N (mg/L), T is temperature in °C, pH is measured value in reactor, and $[NO_2^-\text{-}N]$ and $[NO_3^-\text{-}N]$ are the concentrations at the end of nitritation phase.

The activity tests were performed to assess the nitrogen conversion contribution from suspended and attached biomass during nitritation and denitritation, and to determine the specific microbial activity. Based on the reactor biomass concentration and the amount of media/L reactor volume, a certain volume of synthetic water coupled with an exact number of carriers or amounts of suspended sludge were utilized for batch tests. The batch tests consisted of four parallel conditions: control (no attached biofilm or suspended sludge); suspended sludge only (sludge in 200 mL mixed liquor from IFAS reactor); attached biofilm only (36 carriers from IFAS reactor); and both attached biofilm and suspended sludge (sludge in 200 mL mixed liquor and 36 carriers). Suspended and biofilm sludge were collected from the IFAS reactor on day 92. The conditions in the batch experiments were set to mimic the environment within the reactor. Prior to the experiment, sludge samples should be aerated for at least 1 hr and washed three times by 1× phosphate-buffered saline (PBS) solution to remove remaining COD in sludge. All assays were performed in triplicate in 500 mL bottles in a shaker (150 rpm) at room temperature (21 ºC).

In terms of the AOB activity test, the synthetic substrate contained 400 mg/L NH_4^+-N (as ammonium chloride) and 1250 mg/L alkalinity as $CaCO_3$ (as sodium bicarbonate), and the DO value along the test was maintained at the same level as in reactor (0.6 mg/L). Collected samples were filtered through 0.45 μm filters and measured for NH_4^+-N, NO_2^--N, and alkalinity concentrations. The ammonia oxidizing bacteria (AOB) activity was determined as the NH_4^+-N conversion rate divided the biomass concentration in biofilm or flocs.

For denitrifiers' activity test, the synthesized medium contained 160 mg/L NO_2^--N (as sodium nitrite) and 350 mg/L COD (as sodium acetate). Before adding the sludge, the medium was purged

with N_2 to create an anoxic environment for the denitritation to occur. The samples were collected every 30 min in the first 1 hour and then collected every 1 hr. All samples were filtered and analyzed for NO_2^--N and COD concentration. The denitrifiers' activity was determined by dividing the reduction rate of NO_2^--N to the biomass concentrations in biofilm or suspension.

3.2.6 DNA extraction and microbial community measurement

To understand the microbial community after long term operation, DNA extraction and Illumine Miseq sequencing were performed. The genomic DNA was extracted from fresh suspended sludge (1 mL) and attached biomass in duplicates via DNeasy PowerSoil® DNA Isolation Kits (QIAGEN, Hilden, Germany). For sample preparation, suspended sludge was centrifuged to separate solid from liquid, while because it is hard to sonicate all the biomass down from media, therefore the media with attached biomass was cut into small pieces to fit in the PowerSoil kit. Afterwards, the DNA samples were quantified by NanoDrop One (ThermoFisher, Waltham, MA) and then stored at -20^{o}C for future analysis on the Illumine Miseq sequencing platform.

3.2.7 Biofilm thickness

To evaluate the biofilm formation on the media, the biofilm thickness measurement was conducted. Biofilm samples were obtained by cutting the interior part of media with a sterile scalpel, washing by PBS to remove unattached biomass, then stained by LIVE/DEAD ® BacLightTM Bacterial Viability Kits immediately and incubated at a dark condition for 1h. The stain was made of 3 μL dye mixture (SYTO9 and propidium iodide) for each mL. The distribution of live and dead cells could be observed via fluorescent, because the live cells stain fluorescent green by dye mixture and stain fluorescent red of dead cell. Before putting biofilm samples under

the microscope, they should be washed by 0.85% NaCl to remove dye residues, then Confocal Laser Scanning Microscope (CLSM, Zeiss LSM 710, Carl Zeiss Micro Imaging GmbH, Germany) with tracks Cy3 (red) and FITC (green) were used to analyze the stained biofilm samples. The biofilm thickness measurements were conducted with 20× objective, and three randomly chosen spots were evaluated for each sample. The biofilm thickness can be obtained by setting the original position and the top surface position, then the difference between these two positions is the measured biofilm thickness.

Chapter 4 – Results and Discussion[1]

4.1 Overall nitrogen removal performance

Fig. 8 (a) displays the chemical characteristics of influent and effluent and removal efficiencies along the reactor's operation period. The average concentration of TIN in the feeding was 800 mg/L, and that remained in effluent was below 30 mg/L, and the corresponding TIN removal efficiency was consistently over 90 %. With COD addition during denitritation, the COD removal efficiency (Fig. 8 (b)) ranged from 75 – 85% and the average COD to denitrified N ratio (COD:N) was 2 g COD/g $N_{denitrified}$ (Fig. 8 (c)), which falls into the previously reported COD:N range for complete denitritation (1.56 – 2.2) (Abeling & Seyfried, 1992; Akunna, Bizeau, & Moletta, 1993; Fux, Velten, Carozzi, Solley, & Keller, 2006). The MLSS was maintained at 3.5 – 4 g/L in the IFAS reactor. The biofilm thickness was stable at 56 μm after one-month operation in the single reactor. The average SVI increased gradually from the beginning of operation (120 mL/g) before stabilizing between 260 – 270 mL/g. Compared to the SVI in the previously operated nitritation reactor (without denitritation process), which was around 240 mL/g (Shao, Yang, Mohammed, & Liu, 2018), no significant increase of SVI was found in nitritation-denitritation reactor, therefore, it is likely that combined nitritation-denitritation would not negatively affect the sludge settleability.

[1]This chapter was included in this paper: Zou, X., Zhou, Y., Guo, B., Shao, Y., Yang, S., Mohammed, A., Liu, Y., Single reactor nitritation-denitritation for high strength digested biosolids thickening lagoon supernatant treatment. This paper will be submitted to biochemical engineering journal.

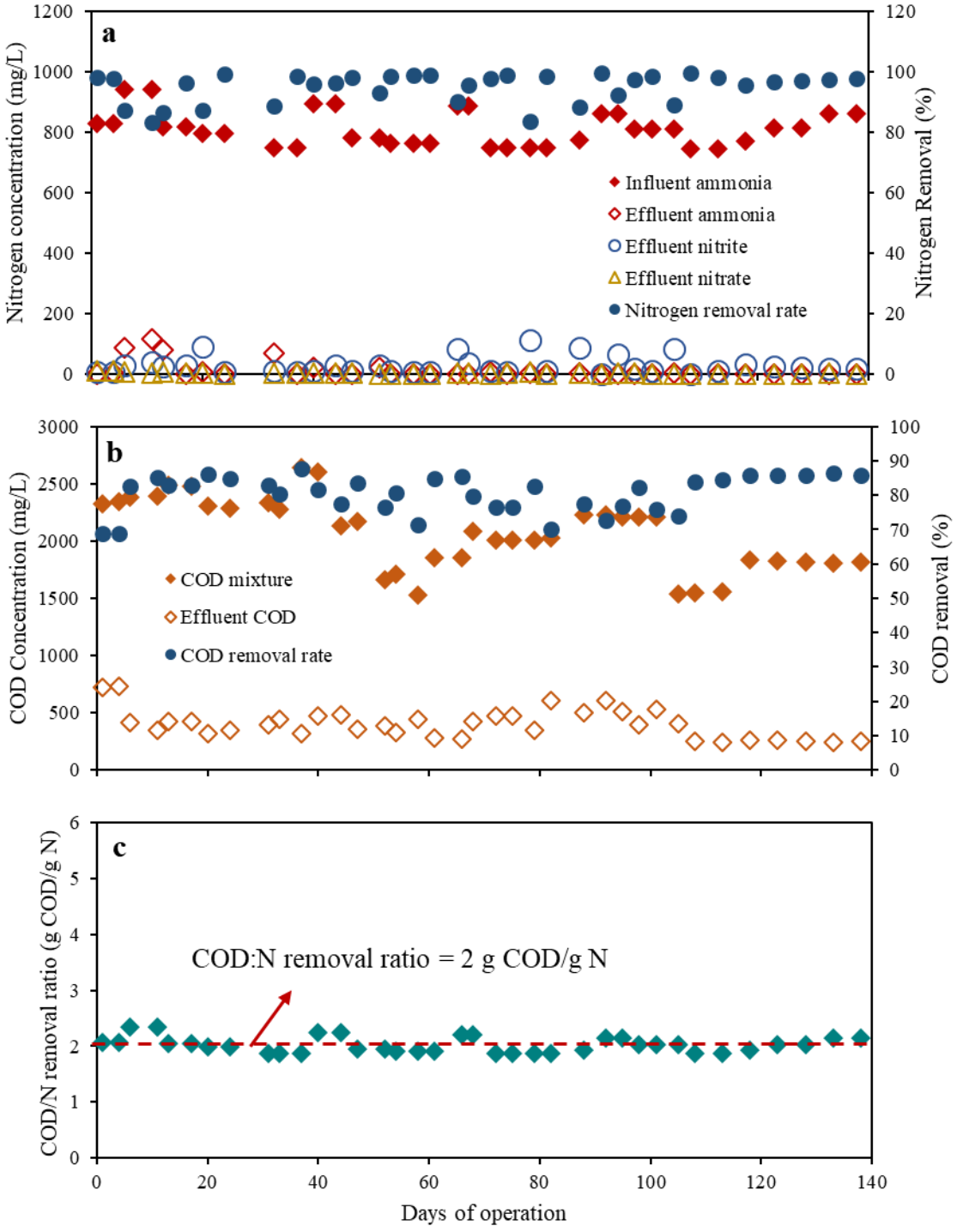


Fig. 8 (a) Dynamics of nitrogen, (b) COD removal efficiencies, and (c) COD:N removal ratio in the single-stage nitritation-denitritation process along the entire operational period. COD mixture was characterized as the total concentration of COD (including influent and external carbon source) introduced into the system.

4.2 Cycle test study

Fig. 9 shows the variation of NH_4^+-N, NO_2^--N, NO_3^--N, alkalinity, and pH in one typical cycle in the single reactor (day 74). Half reduction of NH_4^+-N was achieved within 7 hours of aeration, meanwhile, the NO_2^--N concentration (228 mg NO_2^--N/L) was significantly higher than NO_3^--N concentration (1.68 mg NO_3^--N/L), and the remaining alkalinity was only 500 mg $CaCO_3$/L. According to Metcalf and Eddy (2003), when the alkalinity lower than 400 mg/L, the corresponding pH is 6.8, and the nitritation rate would significantly reduce and even cease the nitritation process; further this would result in a slight decrease in the denitritation reaction rate. Thus 7 hours aeration was confirmed to be a suitable length for aeration phase. The highest nitritation rate observed in this study was 0.74 kg NH_4^+-N/m^3/d. According to Fig. 9 (a), some ammonia was consumed during the anoxic phase, one possible reason of that is biomass growth utilize ammonia, another might be the AOB consumed the remaining oxygen is the bulk liquid. The end of aeration phase indicates the occurrence of denitritation process. As shown in Fig. 9 (a), the denitritation rate was relatively stable in both sub-cycles and the highest denitritation rate observed in this study was 1.2 kg NO_2^--N/m^3/d.

4.2.1 Stable nitritation performance

During nitritation process, conversion efficiencies from NH_4^+-N to NO_2^--N or NO_3^--N were > 99 %. The calculated degree of nitritation accumulation (NA) in this study was over 98%, which represents the success of nitrite accumulation and NOB suppression through the operation. Previous studies have demonstrated that *Nitrosomonas* can be inhibited once the FA concentration reaches 10 – 150 mg/L, while the threshold to *Nitrobacters* is 0.1-1 mg/L (Anthonisen et al., 1976; Aslan & Dahab, 2008; Regmi et al., 2014). According to the calculated average FA concentration

(21 mg/L at the beginning of each cycle), the *Nitrobacter* was significantly inhibited under this operating condition. The highest FNA value was 6.57 x 10^{-5} mg/L in this system, which was lower than the reported inhibition level for both *Nitrobacter* and *Nitrosomonas*. The short SRT might be another reason for NOB suppression, because under ammonia rich, low DO, and low temperature conditions, the growth of NOB was slower than AOB, and thus short SRT could wash out the NOB in this system.

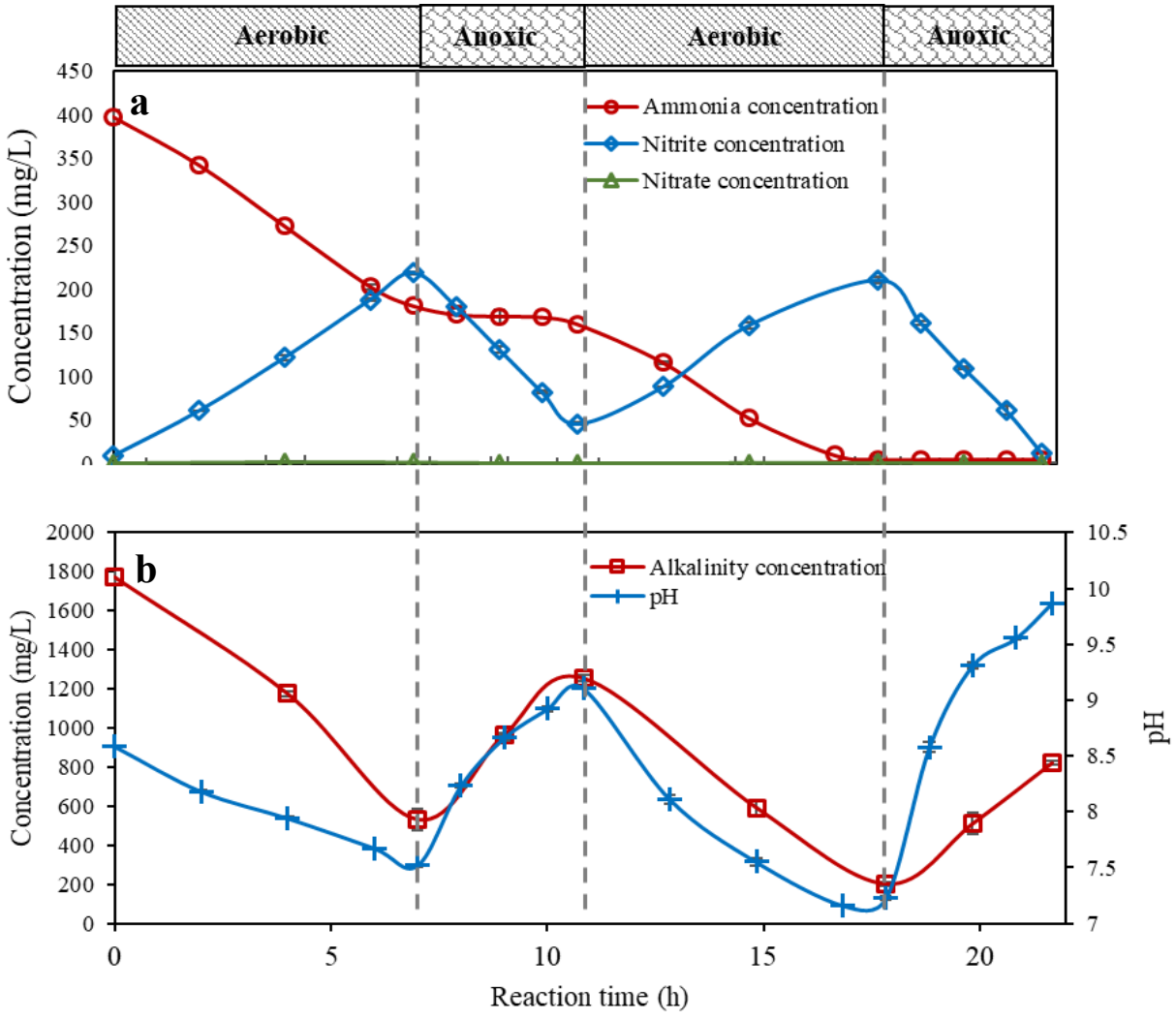


Fig. 9 (a) Variation of NH_4^+-N, NO_2^--N and NO_3^--N concentrations and (b) the change of pH and alkalinity concentration during the cycle test. Cycle test was carried out directly in the reactor after 74-day operation (error bars represents the standard deviation).

4.2.2 Stable denitritation performance

Due to the lack of COD in the raw wastewater, sodium acetate was continuously introduced as an external carbon source during denitritation. As Fux et al. (2006) mentioned, a more stable reactor performance could be achieved with continuous COD supply, rather than pulse input. In this study, the denitritation process achieved a consistent nitrite removal rate with continuous COD addition.

4.2.3 Nitrogen balance

According to the cycle test results, the amount of ammonia converted in the first two hours of aeration was 302 mg N, and the corresponding nitrite production was 274 mg N, from which we could hypothesis that denitritation or anammox might be happened during aerobic phase as well. The denitritation rate in the first hour of anoxic phase was 214.5 mg/h, which was lower than the average denitritation rate (269.5 mg/h), and a slight decrease of ammonia concentration was also observed. From the observation, we could hypothesis that the nitritation might be also happened in the anoxic period. Thus, nitritation and denitritation were not the only reaction that happened in aerobic and anoxic period, respectively.

During aerobic phase, there are many possible nitrogen pathways, including nitritation (Eq. 4), nitrification (Eq. 7), oxidation of influent COD (Eq. 16); EPS hydrolysis (Merkey, Rittmann, & Chopp, 2009) (Eq. 17); comammox (Eq. 7); denitritation (Eq. 18); denitrification (Eq. 19), denitratation (Eq. 20) and; anammox (Eq. 21).

Nitritation: $NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O$ Eq. 4

Nitrification/Comammox: $NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O$ Eq. 7

Influent COD oxidation: $COD + O_2 \rightarrow CO_2 + H_2O$ Eq. 16

EPS hydrolysis: $EPS + H_2O \rightarrow organic\ carbon$ Eq. 17

Denitritation: $2NO_2^- + 4e^- + 4H^+ \rightarrow N_2 + 2H_2O$ Eq. 18

Denitrification: $2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$ Eq. 19

Denitratation: $NO_3^- + 2H^+ \rightarrow NO_2^- + H_2O$ Eq. 20

Anammox: $NH_4^+ + 1.32NO_2^- \rightarrow 2.06N_2 + 0.26NO_3^- + 2H_2O$ Eq. 21

Additionally, the pathways that might be happened during anoxic period includes denitrification (Eq. 19), denitratation (Eq. 20), anammox (Eq. 21), COD reduction (Eq. 22), dissimilatory nitrate reduction to ammonia (DNRA) (Eq. 23), nitrification (Eq. 7), and nitritation (Eq. 4).

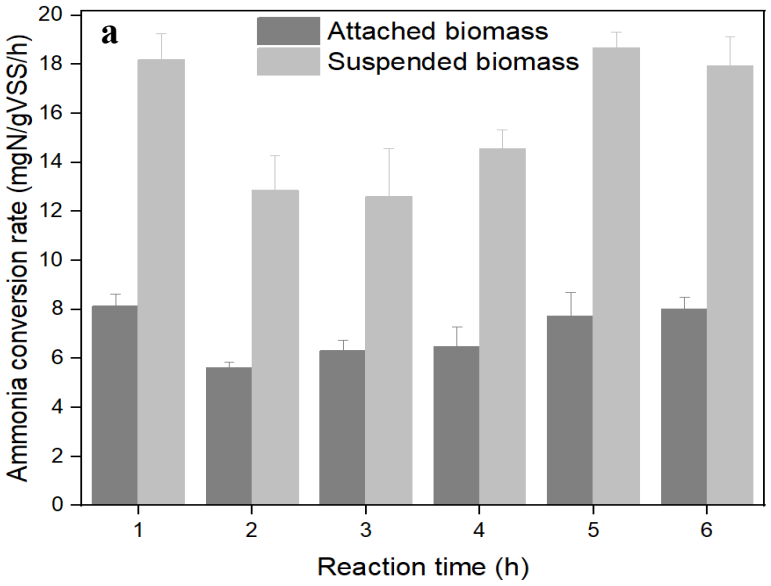
COD reduction: $COD \rightarrow CO_2 + H_2O$ Eq. 22

DNRA: $NO_3^- \rightarrow NO_2^- \rightarrow NH_3$ Eq. 23

Due to the low DO concentration on the inner side of biofilm, anammox that attached on the biofilm might contribute to the reduction of nitrite and ammonia in both aerobic and anoxic phases. However, anammox activity might be inhibited by the COD that added during anoxic phase. To figure out if anammox was performing N reduction, one option is to detect the hydrazine oxidase (HZO) and heme, which are the indicators for the anammox activity (Lin et al., 2019). Without the addition of sodium acetate during aerobic condition, the organic carbon might be introduced with feeding or from EPS hydrolysis, assisting the denitritation and denitrification. Based on the observations in the cycle test, many reactions other than nitritation and denitritation might be involved in aerobic and anoxic period, thus to determine the existence of certain reaction, certain enzymes or functional genes can be detected as the indication of the activity of corresponding bacteria. However, the detection of enzymes and functional genes, is not sufficient to understand the nitrogen dynamics in aerobic and anoxic condition. To determine that, ^{14}N or ^{15}N isotope can be used to trace the dynamics of nitrogen elements throughout the operation period.

4.3 Microbial activity in flocs and biofilm

Batch experiments were conducted to evaluate the contribution from suspension and biofilm on nitrogen conversion. Fig. 10 (a) shows the NH_4^+-N conversion rate in suspension and biofilm. The highest nitritation activity of 19 mg N/g VSS/h was achieved in suspension, while the highest activity achieved in biofilm was 8 mg N/g VSS/h. Fig. 10 (b) presents the NO_2^--N conversion rates in biofilm and suspension. The result showed the highest denitrifying capacity was 25 mg N/g VSS/h in suspension, which was about 12 folds higher than that in biofilm (2 mg N/g VSS/h). High NO_2^--N conversion rates at the beginning of batch test might be attributed to the high available bCOD concentration under such condition, and the lower NO_2^--N after 2 hrs. Thus, the biomass in suspension showed higher activity in both the nitritation and denitritation process. The variation of activities in biofilm and suspension were closely related to the microbial community composition in biofilm and flocs.



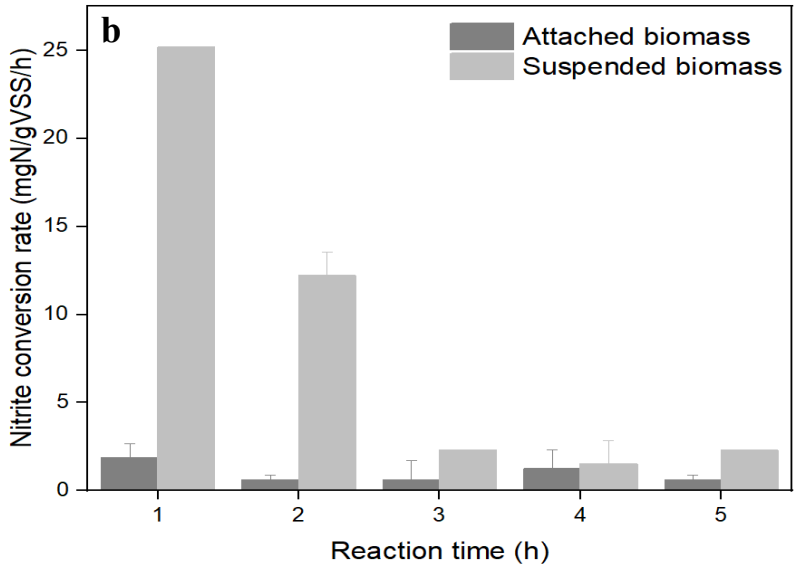


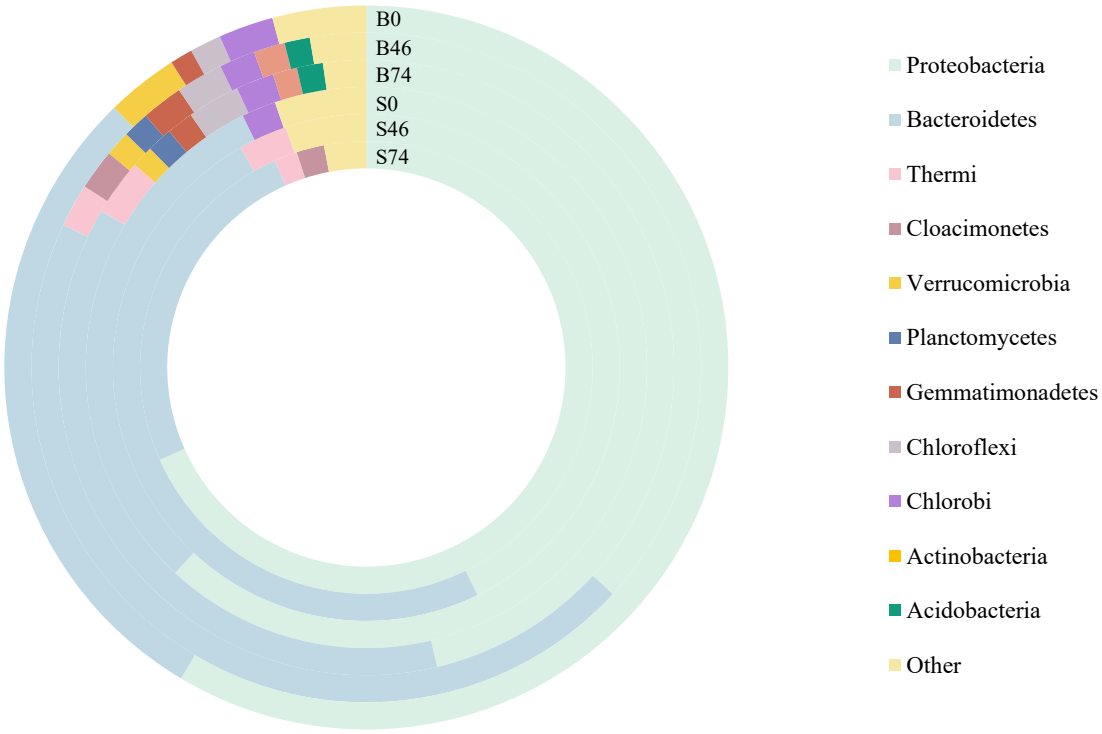
Fig. 10 (a) NH_4^+-N and (b) NO_2^--N conversion rate in the biofilm and suspended biomass in batch test (error bar represents the standard deviation).

4.4 Microbial community analysis

In both biofilm and suspension, the primary dominant phylum in the six samples (biofilm and sludge samples, sampled on Day 0, 46 and 74; labeled as biofilm [B0, B46, B74] and sludge [S0, S46 and S74]) was *Proteobacteria*, followed by *Bacteroidetes*. This observation is consistent with the findings from Shao et al. (2019) and Zhang, Shao, and Ye (2012), both indicated that *Proteobacteria* and *Bacteroidetes* are commonly found primary phyla in activated sludge systems. The number of phyla that accounted for > 1 % abundance within biofilm samples (B0, B46, B74) increased from sample B0 to B74, indicating the microbial community became less homogeneous throughout the reactor operation (Fig. 11 (a)). Compared to sample B0, the prevalence of *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Planctomycetes* and *Thermi* increased in samples B46 and B74, while a reduced abundance of proteobacteria was detected. Compared to biofilm samples, the suspended biomass samples (S0, S46, S74) were less diverse. The higher diversity in

attached biomass may be attributed to the higher diffusion resistance, longer SRT and the retaining of slow growing microorganisms in biofilms. Among the phylum that was shown in Fig. 11 (a), *Proteobacteria* is the major phylum that responsible for nitrogen removal. It has to be noticed that *Planctomycetes*, which is the phylum of anammox bacteria, was detected in sample B46 and B74, which verified the existence of anammox in this system, however, the activity of anammox should be further investigated. Other than *Proteobacteria* and *Planctomycetes*, other phyla are not capable to remove nitrogen.

a



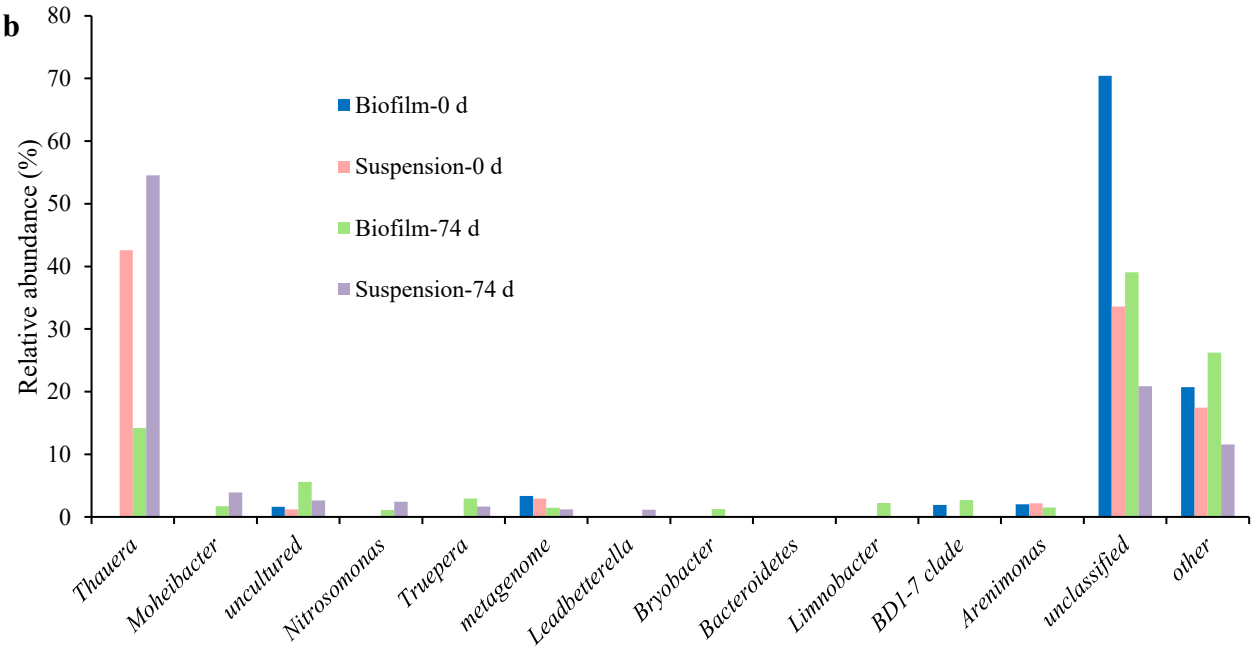


Fig. 11 The relative abundance of sequences (> 1%) at the (a) phylum level and (b) genus level during the long-term operation of the IFAS-SBR and (a) represented the samples of Suspension-74d (S74), Suspension-46d (S46),, Suspension-0d (S0), Biofilm-74d (B74), Biofilm-46d (B46) and Biofilm-0d (B0) from inside to outside circle, respectively.

Fig. 11 (b) shows the microbial composition in two biofilm (B0, B74) and two flocs samples (S0, S74) at the genus level. The unclassified genera in the families *Rhodocyclaceae* (36.8%) *Saprospiraceae* (18.6%) were the primary and secondary abundant genus in B0. In sample B74, the unclassified genera in the families *Saprospiraceae* (15.2%) and *Burkholderiaceae* (12.7%) and the genus *Thauera* (14.2%) were the three most dominant genera. The genus *Thauera* was dominant in samples S0 (42.6%) and S74 (54.6%), and the second-most abundant genera were unclassified genera in the families *Saprospiraceae* (12.8%) and *Chitinophagaceae* (8.5%) for the two samples, respectively. The genus *Thauera*, which is a primary heterotrophic bacterium, was dominant in this nitritation-denitritation system in the presence of an external carbon supply. This result is supported by Ma et al. (2017) who found that *Thauera* could survive well under relatively

low oxygen conditions. Miao, Zhao, and Wu (2017) examined the microbial communities in flocs under different carbon-source conditions in a nitritation-denitritation SBR system; they found that *Thauera*, an uncultured genus in the *Cyclobacteriaceae* family, and *paludibacter* were the dominant genera that facilitated the denitrifying process with sodium acetate as an external carbon source. Ma et al. (2017) analyzed the microbial community in biofilm in a sequencing batch biofilm reactor, and the results indicated that *Thauera* and *Pseudomonas* were the dominant genera in biofilm samples. Further, Ma et al. (2017) found the relative abundance of *Thauera* in suspension was greater than that in biofilm. Other than *Thauera*, the *Limnobacter* is a heterotrophic bacteria that was considered to have the capacity to protect anammox bacteria from extreme environments, such as high COD condition (C. Wang et al., 2018). Other genus, such as *Truepera* and *Metagenome*, were not responsible for the nitrogen reduction.

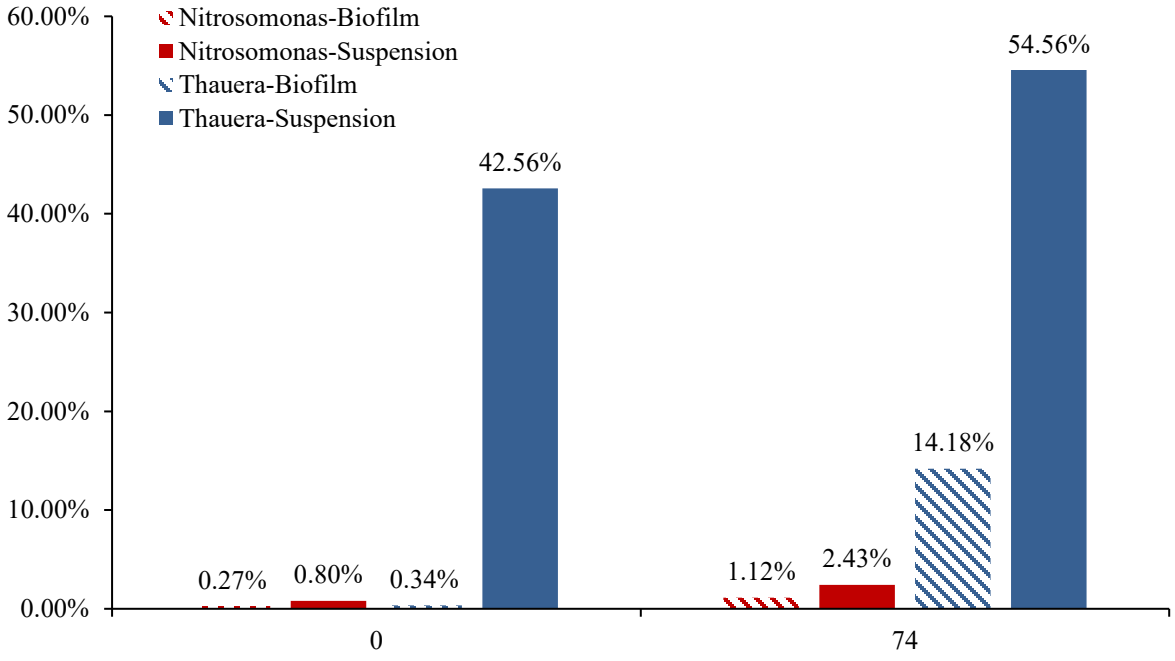


Fig. 12 The relative abundance of *Nitrosomonas* and *Thauera* in both biofilm and suspended flocs on day 0 and 74.

To achieve successful nitritation, high relative abundance of AOB and low relative abundance of NOB are crucial. *Nitrospira* has previously been reported as the predominant NOB in WWTPs (Harms et al., 2003). In this study, the activity of *Nitrospira* was very low. Combined with our observation in that no NO_3-N was detectable at the end of each aeration phase, we can conclude successful nitritation was maintained in the combined system; Laureni et al. (2019) and Wang et al. (2019) have reported similar results, in which the disappearance of NOB was observed in combined nitritation-denitritation systems. Among AOB species, *Nitrosomonas* was the only one that was detected in this study and it is the most commonly found AOB species in wastewater treatment processes (Harms et al., 2003; Mobarry, Wagner, Urbain, Rittmann, & Stahl, 1996). Throughout the reactor operation, the relative abundance of *Nitrosomonas* increased in both biofilm and suspension (Fig. 12). However, the increase of *Nitrosomonas* was more substantial in suspended flocs than in biofilm, similar to biomass distribution of *Nitrosomonas* in an IFAS system (nitritation only reactor) reported by Shao et al. (2019), indicating that higher relative abundance of *Nitrosomonas* was detected in suspension, rather than in biofilm.

Chapter 5 – Conclusions and Directions for Future Work

5.1 Conclusions

The major focus of this study was to evaluate the feasibility and stability of using single reactor nitritation-denitritation to treat ammonia rich lagoon supernatant. In this study the single reactor nitritation-denitritation has been successfully demonstrated in IFAS-SBR system, and stable TIN removal rate at 90% was achieved with external carbon (sodium acetate) addition. Additionally, there is no need to provide the chemical addition that related to alkalinity, since the denitritation recover the alkalinity for the following nitritation process. The keys to achieve stable nitritation-denitritation is the high initial FA (21 mg/L) concentration and the low DO (0.5-0.7 mg/L) concentration, which eliminates the growth of NOB. The activity test indicated that the suspended sludge contributes 2.4 and 13 folds more on both nitritation and denitritation process, respectively, compared to attached biomass. Additionally, the microbial community analysis indicated higher relative abundance of major AOB (*Nitrosomonas*) and denitrifier (*Thauera*) microbes in suspended biomass, suggesting the microbial distribution might be the core reason for the difference of contribution on nitritation denitritation from two biomass aggregates.

5.2 Future work

This work solely focused on the feasibility and stability of single-reactor nitritation-denitritation to treat ammonia rich lagoon supernatant. However, the detection of functional genes or ^{14}N, ^{15}N isotope experiment, as well as the nitrous oxide emission from nitritation-denitritation process are necessary to be determined. Additionally, there are some other technologies can be investigated for nitrogen removal, for instance, the combination of nitritation, denitritation and anammox in a single reactor, or the combination of nitrogen and phosphorus removal.

The nitrous oxide emission from wastewater treatment plant are often seen, and the nitritation-denitritation process has the potential to generate more nitrous oxide than conventional nitrification-denitrification. Therefore, for the future work, the monitoring of nitrous oxide production should be involved in this study. The detection of functional genes and ^{14}N, ^{15}N isotope experiment should be conducted to understand the nitrogen dynamics and pathways during reactor operation. Modelling could be investigated to model the process, then based on the modelling, modifications of reactor or process design could be conducted.

For pilot scale reactor, heating blanket will be needed to maintain the temperature at around 20 ºC, and DO adjustment, the degree of mixing should be investigated prior to the startup.

The combination of nitritation, denitritation and anammox in a single reactor could reduce the amount of external carbon that need to add in carbon limited wastewater, and biodegradable COD can be removed by this technology as well. Therefore, compared to nitritation-denitritation process, this technology can save more on chemical cost related to carbon addition.

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