ADVANCEMENT OF TOTAL AMMONIA NITROGEN REMOVAL TECHNOLOGIES FOR URBAN/PERI-URBAN AND RURAL WASTEWATER TREATMENT

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Thesis submitted to the University of Ottawa
in partial Fulfillment of the requirements for the
degree of Master of Applied Science in Environmental Engineering.

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Abstract

Due to the adverse effects of ammonia on the environment, many governments, including Canada, have imposed new regulations to reduce the discharge of ammonia wastewater effluent into natural receiving waters, which has resulted in the upgrade of ammonia removal at water resource recovery facilities (WRRFs) across the world. There is therefore a need to investigate present urban/peri-urban and rural challenges associated with municipal total ammonia (TAN) removal. In particular, there is a need to further advance and optimize technologies such as the moving bed biofilm reactor (MBBR) to meet these critical challenges. The first objective of this thesis is to validate an elevated loaded strategy for partial nitritation (PN) MBBR as an application for mainstream urban and peri-urban municipal wastewater treatment and to elucidate the mechanism of nitrite-oxidation suppression of this system. The second objective is to identify practical storage strategies for nitrifying MBBR units as rural municipal wastewater upgrade systems (lagoon systems), optimizing the TAN removal performance during seasonal discharge periods.

In the context of the present climate change crisis and sustainable development requirements, there is an increased need for efficient TAN removal from urban and peri-urban municipal wastewaters. The application of the energy and cost-efficient partial nitritation/anammox (PN/A) technology to mainstream urban and peri-urban municipal wastewater can prove challenging because of limited ability to achieve the stable PN. Hence, there is a need for the validation of the present strategies for achieving effective and stable PN in the mainstream portion of conventional urban and peri urban WRRFs. The 45 days operation of a laboratory-scale, elevated loaded PN MBBR with average surface area loading rate (SALR) of 5.2 ± 0.1 g TAN/m^2·d and a hydraulic retention time of 2h showed a successful and stable nitrite accumulation. The average surface area removal rate (SARR) of 2.3 ± 0.2 g TAN/m^2·d (theoretical performance objective of 2.7 g TAN/m^2·d), TAN removal efficiency of 43.1 ± 3.4% (theoretical performance objective of 53%) and NO_2^- / (NO_2^- + NO_3^-) ratio of 82.4 ± 4.8% (theoretical performance objective of 100%) meets the necessary requirement to support subsequent cost-efficient anammox process. Biofilm analyses of the laboratory-scale, elevated loaded PN MBBR indicated that the attached biofilm was thick and dense, stable biofilm that did not show and biofilm loss or washout. Biofilm cell viability analyses was indicative of an active biofilm. The ratio of AmoA gene targets of the ammonia oxidizing bacteria (AOB) in the MBBR biofilm to the targeted gene region of the Nitrospira nitrite oxidizing

bacteria (NOB) population demonstrates that NOB activity suppression of this technology was the dominant mechanism of nitrite-oxidation in the elevated loaded PN MBBR system.

In north America, the TAN removal performance of waste stabilization ponds (also termed wastewater treatment lagoon systems), which are widely applied as rural WRRFs, is often not stable due to seasonal temperature variations. Nitrifying MBBR as an upgrade TAN removal unit has been successfully applied to improve TAN removal during winter. However, re-seeding the nitrifying MBBR biofilm during each seasonal operation period is not sustainable. There is therefore an urgent need for optimizing storage strategies of nitrifying MBBR carriers when used as TAN removal upgrade systems of rural WRRFs. The study of storage strategies for nitrifying MBBR as lagoon upgrading systems indicated the batch storage of the nitrifying MBBR biofilms with intermittent aeration could be an effective storage strategy for short-term (12 weeks) storage. Carriers stored in continuous flow aerated condition was shown to be the second most suitable storage method for nitrifying MBBR carriers for systems exposed to less than 12 weeks of storage. Carriers stored in dry condition, batch aerated conditions without flow, and continuous flow aerated condition for long-term (over 18 weeks) failed to achieve full nitrification following 18 days of operation conditions. Carriers stored in dry condition did not successfully achieve full nitrification for short-term and long-term storage and may not be applied to store full nitrification MBBR carriers. The study suggested that, compared to re-seeding start up strategy of the lagoon upgrading nitrifying MBBR biofilm, the use of the appropriate storage strategies, such as batch aerated conditions without flow, has the potential to shorten the start-up time and save energy during the non-discharge periods.

Acknowledgements

First, I would like to express my sincere and heartfelt thanks to my supervisor Dr. Robert Delatolla for his full support, patient supervision, constant encouragement, and invaluable inspiration. I am also extremely grateful for Dr. Robert Delatolla giving me all the opportunities. I am very lucky and proud to be his student. What I learned from him was not only professional knowledge in the field, but his diligence, passion and rigorous attitude towards work, research and science deeply influenced me.

I also would like to thank Juliet Ikem for the lab skill training, her effort on the reactor and her patient assistance through the whole process of this thesis. We are in the same research group, and we overcame many difficulties together during the Covid-19 pandemic periods. I could not have completed this thesis without her participation and cooperation.

I am also grateful for Xin Tian, Sofia Perin and Gary Hatch. I would like to thank Xin Tian for her assistance in the ddpcr work and her advice on my study. I would like to thank Sofia Perin for teaching me how to use ddpcr. I would like to thank Gary Hatch for proving me the reagent for ddpcr.

I am very thankful to Patrick D'Aoust, environmental engineering Technician for all his assistance in the lab training and lab work. I would also like to thank Aaron Cowan for his advice on my writing.

Additionally, I would also like to thank all my friends for their encouragement and support. This thesis was finished during the Covid-19 pandemic. The pandemic changed everything. I would like to thank all the people who helped me during this hard time. Finally, I would like to express my heartfelt thanks to my family for their support, care and love.

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List of Acronyms

ANAMMOX	Anaerobic Ammonia Oxidation
AMO	Ammonia Monooxygenase
AOB	Ammonia-Oxidizing Bacteria
AS	Activated Sludge
BA	Biochemical Oxygen Demand
BAF	Batch & Aerated storage
BOD	Biological Aerated Filter
CA	Continuous &Aerated storage
C/N	Carbon to Nitrogen
CLS	Casselman Lagoon Systems
CLSM	Confocal laser scanning microscopy
COD	Chemical Oxygen Demand
D	Dry Storage
DN	Denitrification
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EPS	Extracellular Polymeric Substance
FA	Free Ammonia
FISH	Fluorescence In Situ Hybridization
FN	Full Nitrification
FNA	Free Nitrous Acid
HAO	Hydroxylamine Oxidoreductase

HRT	Hydraulic Retention Time
IFAS	Integrated Film-Activated Sludge
MBBR	Moving Bed Biofilm Reactor
MBR	Membrane Biofilm Reactor
MPN	Most Probable Number
NOB	Nitrite-Oxidizing Bacteria
NXR	Nitrite oxidoreductase
PCV	Polyvinyl chloride
PN	Partial Nitritation
PN/A	Partial Nitritation and Anammox
RBCs	Rotating Biological Contactors
S.D.	Standard Deviation
SAGR	Submerged Attached Growth Reactor
SALR	Surface Area Loading Rata
SARR	Surface Area Removal Rate
SEPS	soluble EPS
SRT	Sludge Retention Time
SS	suspended solids
T	Temperature
TAN	Total Ammonia Nitrogen
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids
WRRFs	Wastewater Resource Recovery Facilities

Chapter 1-Introduction

1.1 Background

Ammonia nitrogen in the form of both non-ionised (NH_3) and ionised (NH_4^+) ammonia is a common contaminant existing in the municipal wastewater (Karri et al., 2018; Ada et al., 2019). The ammonia concentrations in municipal wastewater usually range from 20 to 100 mg/L (Ashrafizadeh & Khorasani 2010; Zangeneh et al., 2021). The discharge of the municipal wastewater may cause the excessive presence of ammonia in the natural receiving water, which can accelerate the process of eutrophication in marine waters and be toxic to some aquatic life, resulting in gill damage, tremor, coma and even death (Wiesmann, 1994; Randall et al., 2002; Adam et al., 2020). Since 1999, ammonia has been identified as a toxic substance by the Canadian Environmental Protection Agency (CEPA). To limit ammonia discharged into the receiving water, many governments, including Canada, have imposed wastewater discharge regulations (Canadian Fisheries Act, 2012; US Clean Water Act, 2018). Thus, ammonia removal from wastewaters has also become a significant objective of many countries.

Total ammonia nitrogen (TAN) removal methods include air stripping (Zhu et al., 2017), breakpoint-chlorination (Aghdam et al., 2021), ion exchange (Al-Sheikh et al., 2021) and membrane processes (Krakat et al., 2017). However, these methods have limited applications in urban municipal wastewater TAN removal due to the large quantity of daily wastewater produced and the energy and chemical cost intensiveness of these methods. Traditional TAN removal is hence based on more cost and energy efficient biological treatment methods (Jorgensen & Weatherley, 2003; Liu et al., 2019). Nitrification (ammonia oxidation via nitrite to nitrate) is a key microbial process in the environmental nitrogen cycle (Alexander & Clark, 1965; Van et al., 2015) that has been widely applied in wastewater resource recovery facilities (WRRF) (Sharma & Ahlert, 1997). The combination of nitrification and denitrification (N/DN) is a mature technology that is the most frequently used technology for TAN removal from municipal wastewater.

More recently, anaerobic ammonium oxidation (anammox) as a promising alternative to the traditional TAN removal processes has attracted extensive attention since the process was first discovered in 1995 (Mulder et al., 1995). TAN is converted to nitrogen gas by autotrophic anammox bacteria during the anammox process with nitrite acting as an electron acceptor (Jin et al., 2012). The cost-efficient TAN removal anammox technology is usually required to be combined with an upstream partial nitritation process to convert a portion (i.e., the use of the term 'partial') of the TAN to nitrite (Van et al., 2010; Ali & Okabe, 2015). Compared with the traditional N/DN process, the application of partial nitritation/anammox (PN/A) leads to a significant reduction of aeration costs, added-carbon costs, added-alkalinity costs and added-sludge disposal costs.

Efficient wastewater treatment technologies are increasingly important with rapid global increase of urban and peri-urban populations. Further, within the context of the present climate change crisis, low energy and low carbon production wastewater solutions are urgently required. One of the critical challenges in wastewater treatment is hence to upgrade mechanical wastewater resource recovery facilities (WRRFs) in urban and peri-urban communities for efficient TAN removal. In this regard, the moving bed biofilm reactor (MBBR) technology is a compact, small footprint, low operational intensity mature technology that has been successfully applied as upgrade systems for nitrogen removal around the world (Ødegaard et al., 1994; Bassin et al., 2011; Bassin et al., 2012; Gong et al., 2012; Zhang et al., 2013; Bian et al., 2017; Liu et al., 2022). To date, the MBBR technology has recently been adopted for PN/A treatment with the PN/A MBBR technology having been successfully applied to treat sidestream wastewaters such as landfill leachate (Shalini, S. S., & Joseph et al.,2012; Podder & Goel et al., 2020) and sludge-digestion liquid (Joss et al., 2009; Wang et al., 2017). However, the application has been limited to industrial and sidestream wastewater and the application of the PN/A MBBR technology to the low-strength, urban and peri-urban mainstream municipal wastewaters have been restricted by the ability to produce stable nitrite accumulation during the PN process (Li et al., 2018). PN of mainstream municipal wastewaters, where these wastewaters are characterised by lower temperatures and higher carbon to nitrogen ratio (C/N ratio) compared to industrial and sidestream wastewater hence are difficult to prevent nitrite oxidation to nitrate, is challenging to maintain. To achieve effective PN in mainstream wastewater, some operational control strategies such as pH control, temperature control and metabolic kinetics control have been investigated in previous studies, but most of them failed to achieve the stable nitrite accumulation in the long-term. Recently, a stable nitrite accumulation has been successfully achieved in an elevated loaded PN MBBR with a TAN concentration of 125 mg TAN/L at a temperature of 19-21℃ without restricting DO for a period of 300 days, (Schopf et al., 2019). This approach has provided a potential strategy for achieving effective PN in the mainstream portion of conventional urban and peri-urban WRRFs. However, this finding has not been confirmed at conventional influent TAN concentrations of between 15 to 30 mg TAN/L and the mechanism of PN in these systems have yet to be identified.

In addition to the challenges facing urban and peri-urban wastewater treatment, there remains key challenges for upgrading current conventional rural WRRFs for removing TAN. In north America, waste stabilization ponds (also termed wastewater treatment lagoon systems) are usually applied as rural WRRFs. However, the TAN removal performance of lagoons is often not stable due to significant seasonal temperature variations that are observed in lagoon systems as opposed to urban and peri-urban WRRFs. The low temperatures of the wastewaters during winter operation usually have an adverse effect on the performance of the TAN removal of the lagoon systems. Some upgrade ammonia removal systems, such as the nitrification MBBR unit, have very recently been applied as an upgrading unit to improve TAN removal during winter operation, with the first installation in the world occurring in Casselman, Ontario Canada in 2020 (Wessman & Johnson, 2006; Almomani et al., 2014). These nitrification MBBR upgrade passive treatment systems are seasonally operated, meaning they operate intermittently during season discharge periods and subsequently designed to remain idle during non-discharge periods. Re-seeding the nitrifying MBBR biofilm in the upgrade units during each discharge period is not sustainable (Xu et al., 2018; Kowalski et al., 2019; Zhang et al., 2020) as re-seeding requires approximately 200 days to seed nitrifying MBBR biofilm (Young et al., 2017). Therefore, the upgrading nitrification MBBR systems require new knowledge to identify optimal storage strategies during non-operation periods to optimize their ability to re-start and perform nitrification during the required discharge periods of operation.

1.2 Research objective

The overall objective of this research is hence to address two important gaps of knowledge in the removal of TAN from (i) urban and peri-urban municipal wastewaters and (ii) rural municipal wastewaters. The first gap of knowledge, pertaining to urban and peri-urban municipal wastewater treatment, is the validation of the performance of an elevated loaded PN MBBR system under mainstream wastewater treatment conditions with conventional influent TAN concentrations and to identify the mechanism of nitrite-oxidation suppression of this system. The second gap of knowledge addressed in this research, pertaining to rural municipal wastewater treatment, is to identify practical storage strategies for nitrifying MBBR lagoon upgrade systems such that TAN removal performance is optimal during seasonal discharge periods.

1.3 Author contribution

1.3.1 Article 1

1. Huiyu Chen: Maintained the operation of the laboratory MBBR reactors, performed the chemical and microbial analyses, analyzed the data, and wrote the paper.

2. Robert Delatolla (supervisor): Developed the research question and the experimental design; directed the research; analyzed the results and reviewed manuscript.

3. Juliet Ikem: Maintained the operation of the laboratory MBBR reactors, performed the chemical and analyses, involved in writing and data analysis.

4. Xin Tian: Involved in writing and data acquisition.

5. Arron Cowan: Reviewed manuscript.

1.3.2 Article 2

1. Huiyu Chen: Analyzed the data and wrote the paper.

2. Robert Delatolla (supervisor): Developed the research question and the experimental design; directed the research; analyzed of results and reviewed manuscript.

3. Patrick Marcel D'Aoust: Performed the laboratory testing and data acquisition.

4. Elisabeth Mercier: Performed the laboratory testing and data acquisition.

5. Juliet Ikem: Reviewed manuscript.

6. Arron Cowan: Reviewed manuscript.

1.4 Thesis organization

This thesis consists of five chapters. Chapter 1 introduces the context of the research and presents the objectives, author contribution and organization of the thesis. Chapter 2 presents a literature review of biological wastewater treatment, biofilm technology, attached growth biological treatment systems, nitrogen cycling in wastewater treatment systems, and microorganisms involved in partial nitritation. Chapter 3 presents the validation study of an elevated loaded PN MBBR system under mainstream wastewater treatment conditions and describes the NOB inhibition mechanism. In Chapter 4, three storage strategies for nitrifying MBBR lagoon upgrade systems are compared with a practical, optimal storage strategy identified. Finally, in Chapter 5, the conclusions of this study are listed.

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Chapter 2-Literature Review

2.1 Biological wastewater treatment

The demands on effluent quality of wastewater are increasing (Wen et al.2015). The purpose of wastewater treatment is to reduce the contaminants in the water (Grady et al., 2011). Many technologies have been applied to the wastewater treatment field, including physical methods, chemical methods, and biological methods. The biological wastewater treatment is a kind of technology that degrades the harmful substances in wastewater through the metabolism of microorganisms. It can reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and effectively remove total nitrogen, total phosphorus, and other nutrients. Activated sludge process and biofilm process are common effective and widely used biological wastewater treatment technologies (Lazarova & Manem, 1995; Christensen et al., 2015). Biological wastewater treatment has more advantages in terms of capital investment, operating costs, and treatment efficiency than other treatment processes (Mittal, 2011).

2.1.1 Bacterial metabolism

The concept of metabolism refers to the biochemical reactions that occur in bacterial cells, including the ability of bacteria to live, function, and replicate in an appropriate chemical milieu (Jurtshuk et al., 1996). Metabolism is in forms of catabolism and anabolism. The schematic of bacterial metabolism is shown in figure 2.1. Nutrients, energy sources, terminal electron acceptors, and carbon sources are fundamental components for the bacterial metabolism process (Hoang, 2013). In catabolism process, bacteria can get energy through photosynthesis and oxidation of chemicals (Clayton, 1973). During in anabolism process, energy generated from the catabolism anabolism process will be consumed by bacteria for cell synthesis. Carbon substrate can provide the carbon atoms required in the formation of macromolecules such as proteins, lipids, carbohydrates, and nucleic acids.

Enzymes also play an essential role in bacterial metabolism. Environments have an influence on the bacterial metabolism and meanwhile on the types of enzymes. Microorganisms do not synthesize all the time all the enzymes but only those that are necessary for their metabolism under current physiological conditions and environment conditions (Gottschalk, 1986). According to the electron acceptor, organisms can be classified into five categories: Aerobic bacteria, Obligate bacteria, Aerobic bacteria, Anoxic Facultative bacteria, and Aerobic Anaerobic. Based on the energy source, and carbon source, bacteria can be classified into four categories: Organotrophic bacteria, Lithotrophic bacteria, Heterotrophic bacteria, and Autotrophic bacteria.

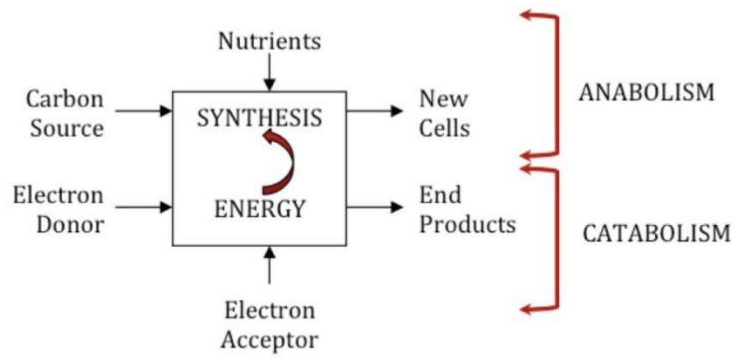


Figure 2.1 The schematic of bacterial metabolism (Ren, 2015).

2.1.2 Bacterial growth

Bacteria often grow at a specific phase in which the growth rate starts at a value of zero and then accelerates to a maximum value (Zwietering et al., 1990). The population of bacteria always exhibits growth dynamics. The whole growth process will go through the lag phase, log growth phase, stationary phase, and death phase (Figure 2.2). During the lag phase, the bacteria need to adjust to the new environment, and the growth rate is almost zero. The growth rate is the fastest during the log phase. The number of new cells equal to dead death cells is the characteristic of the stationary phase. The bacteria will lose the ability to divide at the dead phase.

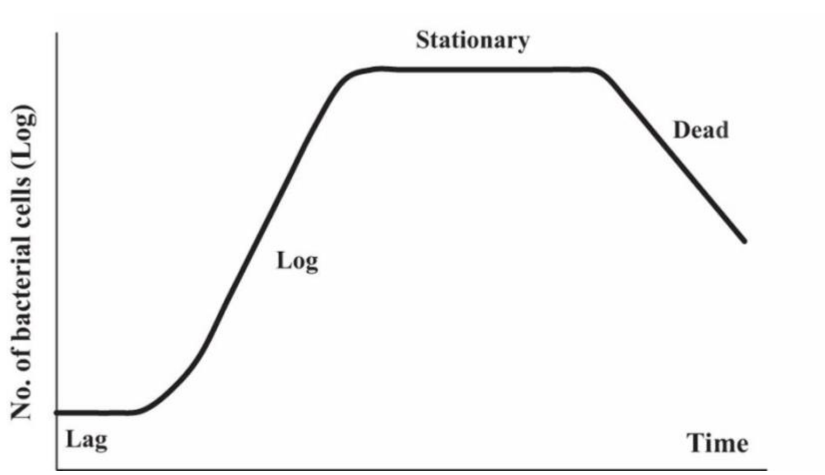


Figure 2.2 Graphic representation of typical bacterial growth curve in culture medium (Wang et al., 2015).

2.1.3 Bioreactor configuration

According to different microorganism growth methods, the bioreactor can be divided into suspended growth bioreactor and attached growth bioreactor (Grady et al., 2011). Suspended growth treatment bioreactor usually grow as flocs in intimate contact with the wastewater they are treating (Horan, 2003). Activated sludge treatment, waste stabilization ponds and aerated lagoons are typically suspended growth bioreactor models (Gloyna & World Health Organization,1971; Nameche & Vasel, 1998; Henze et al., 2000). In the attached growth systems, microorganisms were retained on the surfaces of specialized carriers for wastewater treatment. As water flows through the surfaces of the carriers, organic materials can be consumed or degraded by the microorganisms. The attached growth bioreactor can maintain a high concentration of microorganisms and achieve high contaminants removal rates at relatively small hydraulic retention times (Loupasaki, E., & Diamadopoulos, 2012). The emergence of some new attached bioreactor technology like trickling filter, RBCs, BAF, and MBBR provides many new ideas for modern wastewater treatment.

2.2 Biofilm

Biofilms are widespread in nature environments (Azeredo et al., 2017). They may exist in water distribution pipe, copper water pipe, dental water lines, plastic, glass, human body, and some engineered media, which can be detrimental and beneficial depending on the situation. The active layer of a biofilm is thin, so it is not easy to study them. In the past years, many modern technologies like electron microscope and investigation of genes have helped people better understand the biofilm (Donlan, 2002). There are many definitions of biofilm. Until 1978, Costerton et al., first proposed the concept of biofilms. Bacteria in natural aquatic systems tend to predominately attach to surfaces (Geesey *et al.*, 1977). O'Toole et al. (2000) gave a broad and straightforward definition that biofilms are communities of microorganisms that are attached to a surface. Bacteria can adhere to carrier surfaces and form a slimy, slippery coat, called biofilm (Costerton et al., 1999).

Biofilm is composed of two parts, microbial cells and extracellular polymeric substance (EPS) (Kokare et al., 2009). Biofilm formation occurs step by step, which will go through bacterial attachment, growth, maturation, and detachment processes. Palmer & White (1997) reported that before forming a mature biofilm, cell-surface and cell-cell interactions could be observed at the early stages of biofilm formation. To achieve successful bacterial attachment, fimbriae, pilli, flagella, and EPS are necessary, which can be a bridge between bacteria and the conditioning film (Donlan, 2002). The performance of the biofilm is complex. Microbial conversion of substrates, volume expansion of biomass, and transport of substrates by molecular diffusion are three essential processes that can determine the microbial composition in biofilm (Wanner & Gujer, 1986). The spatial distribution of microorganisms and the microbial composition are crucial for the performance of the biofilm.

Biofilm technology has been widely used in wastewater treatment systems. The operational and maintenance cost of biofilm systems is low. Meanwhile, it performs well in nitrogen removal. Many studies have focused on the biofilm technology.

2.2.1 Bacteria cell attachment

Bacteria cell attachment is the beginning of the biofilm. The factors affecting the attachment of bacteria are elusive. Several parameters (such as surface conditioning, surface charge, hydrophobicity, and surface microtopography) are thought to have an influence on the attachment (Palmer et al., 2007). Bacteria need to be attached in two steps. A series of physical, biologic, and chemical processes were involved in biofilm attachment. At first step, the bacteria need to get close to the carrier to ensure the attachment occurs, during which time the bacteria remain in Brownian motion and are easily swept away by the current because they are not firmly attached (Marshall et al., 1971). Van der Waals forces, electrostatic forces, and hydrophobic interactions are main forces involved in bacteria attachment (Van et al., 1987; Carpentier & Cerf., 1993; Giovannacci et al., 2000). The stability of bacterial adhesion cannot be achieved without exo-polysaccharides and or specific ligands (Dunne, 2002). During the second step, some short-range forces like covalent and hydrogen bonding and hydrophobic interactions make attachment stronger (Kim & Frank, 1995). Poortinga & Busscher (2001) pointed out that bacteria either donated electrons to, or accepted electrons from the substratum during the attachment process. Bacteria attachment is a crucial and fundamental step in biofilm formation, because successful attachment can ensure different stable structure. Lappin-Scott & Bass (2001) found that slow-growing, anaerobic bacteria attach much more slowly than fast-growing, aerobic, pure cultures. In the early phase, bacteria attachment occurs randomly, and microbes are usually observed moving independently of fluid flow (frequently in the opposite direction to flow).

2.2.2 Bacteria growth and mature

When the bacteria attachment process is made, extracellular polymeric substance (EPS) will be produced (Gu & Ren, 2014). Mature bacteria attachment is stable, and they achieve this by using extracellular adhesive organelles, such as curli and fimbriae (Karatan & Watnick, 2009). As bacteria grow, these microbial communities can form different three-dimensional (3D) structure. EPS has stable viscosity, sufficient to support the spatial structure of biofilm, and realize the transfer of nutrients and excreta. Usually, the three-dimensional (3D) structure is not a mere homogeneous monolayer of slime but is heterogeneous, both in space and over time. The growth of bacteria is affected by many factors, such as oxygen, pH, temperature, nutrition. Donlan (2000) found that biofilm-associated microorganisms grow more slowly than planktonic organisms. Different biofilm bacteria behave differently in a specific environmental condition with different growth patterns (Hamilton, 1987). Mature biofilm owns a more complex structure and better physiological cooperativity (Costerton et al., 1995).

2.2.3 Detachment

Biofilm detachment when some particulate mass, such as bacteria cells and EPS, transfer from the biofilm to the bulk liquid phase (Bakke, 1986). Microbes growth on the surface and some detachment from the surface happen all the time (Lappin-Scott & Bass, 2001). The detachment process plays an important role in controlling the cell metabolic state (e.g., specific cellular growth rate). In wastewater treatment systems, shear force created as the fluid flows over a surface is usually considered as the principal physical force acting on the biofilm (Stoodley et al., 2002). Erosion and sloughing are two different processes involved in detachment. Erosion is caused by shear forces, which can lead to continuous removal of individual cells and small particles at the biofilm liquid interphase. Physiochemical condition change within the biofilm is also a primary factor that causes biofilm detachment. It can cause more significant portions of biofilm detachment. Erosion and sloughing may happen in biofilms at the same time. Besides, abrasion has an influence on biofilm detachment (Rochex et al., 2009). Abrasion is similar to erosion that both cause continual detachment of small particles from the biofilm, but abrasion is mainly caused by collisions of particles on the biofilm surface (Morgenroth & Wilderer, 2000). The phenomenon is a normal growth metabolism process.

2.2.4 Mass transfer in biofilm

Mass transfer influences the biofilm substrate uptake rate (Siegrist & Gujer, 1985). In a steady-state biofilm model, mass transfer inside the biofilm is dominated by molecular diffusion, while convective flow mainly controls the outside mass transfer (Yang & Lewandowski, 1995). Therefore, the structure of biofilm determined the mechanism of local mass transfer. The mass boundary layer above the biofilm surface is usually not uniform. When calculating the mass transfer rate from the bulk liquid to the biofilm surface needs to assume that it is uniform and stagnant. In fact, mass transfer rates are not consistent at different locations of biofilm. Complex interactions between local biofilm activity, structure, and hydrodynamics contribute to different mass transfer rates. Mass transfer in biofilm systems can be described by mass transfer coefficients (Brito & Melo, 1999). The higher the mass transfer coefficient is, the higher the nutrient transport rate is (Beyenal & Lewandowski, 2002). Yang & Lewandowski (1995) pointed out that the mass transfer coefficient in a biofilm varies horizontally and vertically. Mass transfer is a characteristic of biofilm.

2.2.5 Extracellular polymeric substance (EPS)

Bacterial EPS is a part of dissolved organic matter (Lignell, 1990). EPS consists of high molecular weight compounds with charged functional groups (Bhaskar & Bhosle, 2006). It is a kind of biomolecular hydrogel composed of excreted polymers, which has a significant influence on the biofilm structure and function. It contains polysaccharides, and in some strains, DNA, nucleic acids, lipids, and proteins but is thought to be predominantly polysaccharides (Lewandowski & Evans, 2000; Hornemann et al., 2008; Sheng et al., 2010). Usually, it is not easy for bacteria to produce EPS because the bacterial strains that produce EPS need to compete for nutrients and growth space with non-producers (Jayathilake et al., 2017). EPS production is also influenced by initial attachment, EPS production rate, ambient nutrient levels, and quorum sensing. EPS mediated adhesion is crucial for bacterial biofilm because it has a significant influence on the initial attachment to solid surfaces and the subsequent resistance to shear flows (Jayathilake et al., 2017). EPS can be classified into two types: soluble EPS (SEPS) and bound EPS. SEPS includes soluble colloids, slimes, and macromolecules. Bound EPS mainly consists of attached organic materials, loosely bound polymers, sheaths, condensed gels, and capsular polymers (Shi et al., 2017). One schematic of extracellular polymeric substances (EPS) structure is shown in figure 2.3. Bound EPS has a dynamic double-layer-like structure (Yuan et al., 2017). It also contains two types: as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). The former exists in the outer layer while the latter constitutes the inner layer. EPS has many functions. Generally, the EPS of biofilms enables the biofilm to operate normally, have a proper structure, good nutrient availability, and provide protection and communication pathways (Karatan and Watnik, 2009). Besides, it can act as an Electron donor or acceptor, permitting redox activity in the biofilm matrix, and result in the accumulation, retention, and stabilization of enzymes through their interaction with polysaccharides (Flemming & Wingender, 2010). EPS matrix makes biofilms one of the most successful forms of life on earth.

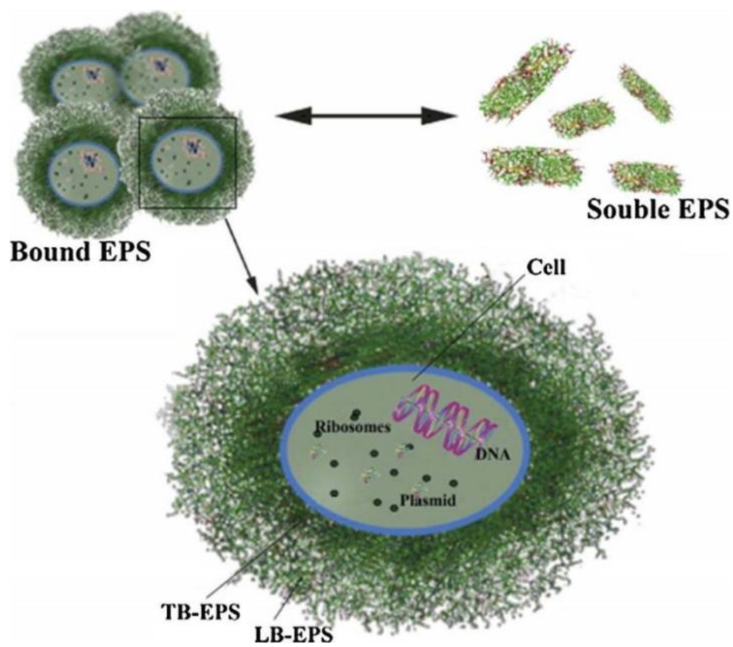


Figure 2.3 Schematic of EPS structure (Alaba et al., 2018).

2.3 Attached growth biological treatment systems

In the attached growth biological treatment systems, the hydraulic residence time and biological residence time can be effectively controlled, the loss of microorganisms on the biofilm caused by water flow will be significantly reduced, and the sludge residence time can be effectively extended. Besides, in the attached growth system, there are many kinds of microorganisms with complex structures. Meanwhile, biofilms play a protective role in microorganisms, which can effectively reduce the shear force of water flow and the influence of toxic and harmful substances on microbial activities. In this section, several attached growth biological technologies will be introduced.

2.3.1 Trickling filter

Trickling filter consists mainly of some adsorbents like rocks, coke, and gravel. It has been in use for about five decades. The filling material is laid on a fixed bed where the voids and porosity are so high that the wastewater flows from top to bottom. Biofilms can attach to the surfaces of these materials (Ahammad & Sreekrishnan, 2016). One schematic of the trickling filter is shown in figure 2.4. Trickling filter is suitable in areas where space is limited for a wastewater treatment system. Organic and inorganic matter, pathogens in the wastewater can be successfully removed by trickling filters (Dhokpande et al., 2014). In the trickling filter system, diffusion to the biofilm is the main parameter that controls the contaminants removal process. One advantage of trickling filter is that the maintenance requirements are low. Besides, it has a high resistance to upset from variations in wastewater volume and strength. However, the removal rate of pollutants is not high (Ahuja et al., 2014). It also has a high requirement for oxygen supply; otherwise, an odor problem may happen (Bennett, 2007). According to the loading, the trickling filter can be divided into low loading, medium loading, medium to heavy loading, and extremely high loading. According to the fill type, it can be divided into traditional packings and plastic packings. The former filler is mainly pozzolana, metallurgical coke, or crushed silica pebbles, and the latter is mainly plastic with Honeycomb Structure such as Polyvinyl chloride (PCV) (Lopez et al., 2015).

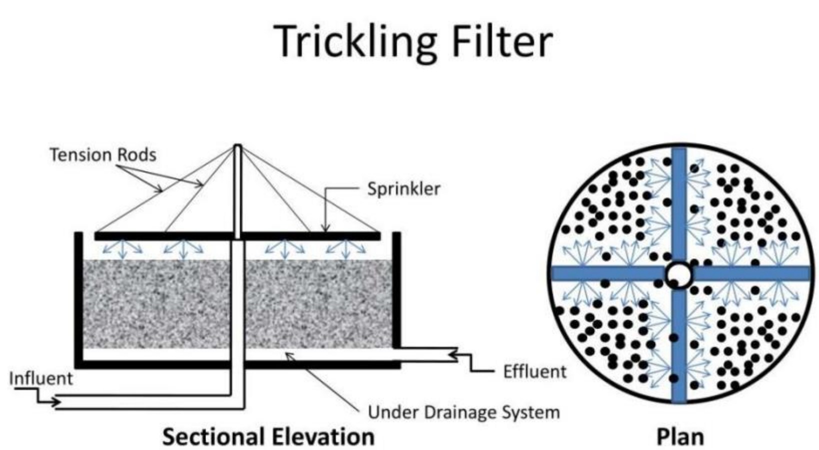


Figure 2.4 Trickling filter (Bressani-Ribeiro et al., 2018).

2.3.2 Rotating biological contactors (RBCs)

As a secondary (Biological) treatment process, RBCs improved the problem of insufficient contact area in the traditional active sludge process. It was firstly applied in the wastewater treatment systems in the 1970s. In RBCs systems, bacteria grow on a rotating disk. The Schematic view of an RBC is shown in figure 2.5. The disk is not completely submerged, but partly exposed to air. By rotating, the wastewater can be contacted with air. There are many kinds of disk media. In the beginning, the disk media consisted of a cylinder with wooden slats, and then they were replaced by metal disks. Now, polyethylene disks have been widely used (Patwardhan, 2003). Hassard et al. (2015) reported that the oxidation rates of RBCs could be 6 g m^{-2} d^{-1} for in municipal wastewater process. The energy costs are lower than the trickling filters. RBCs also perform well on nitrogen removal. However, the pathogen and TSS removal efficiency is low. RBCs have been applied in many areas like bioremediation of landfill leachate, leather tanneries effluents, nuclear and electronics industries effluents and decolorization of wastewaters, etc. (Cortez et al., 2009).

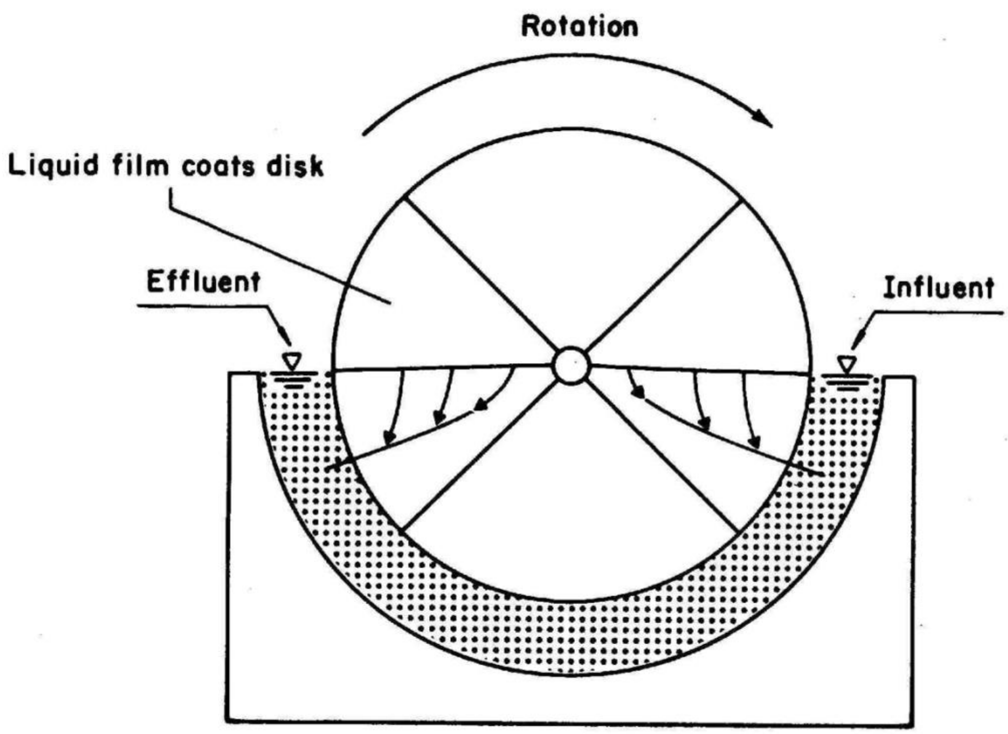


Figure 2.5 Schematic view of an RBC reactor (Hatzikioseyian & Remoundaki, 2012).

2.3.3 Biological aerated filter (BAF)

The first BAF appeared in the 1980s (Pujol et al., 1994). BAF combines traditional oxic biological treatment and biomass separation by depth filtration (Mendoza-Espinosa & Stephenson, 1999). It is submerged, different from RBCs. One schematic diagram of the Biological Aerated Filter (BAF) reactor is shown in figure 2.6. The media of BAF is granular or structured to act as a filter (Ryu et al., 2008). Organic matter, together with suspended solids, can be removed at the same time. Additional sedimentation is not required in the BAF process. However, the problem of clogging always happened in BAF. Wastewater containing a high concentration of suspended solids (SS) is not suitable to be treated by BAF. Trapped solids and biomass always block the filter pathways, so it is necessary to clear it by air-scouring or back-washing with treated effluent. It performs well in treating wastewater containing petroleum products (hydrocarbons), wastewater from the paper mill industry, the distillery industry wastewater, and industrial hazardous waste landfill leachate (Mendoza-Espinosa & Stephenson, 1999). The BAF materials are different. He et al., 2007 reported that BAF containing mineral media, such as expanded clay, is better for substrate removal.

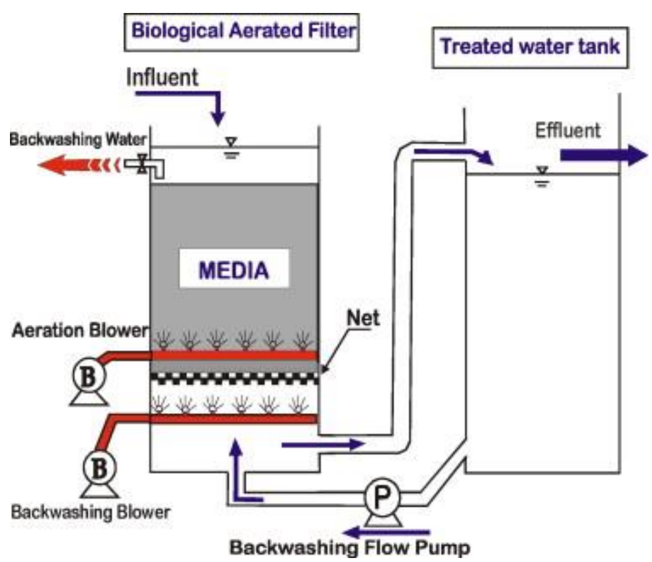


Figure 2.6 Schematic diagram of BAF reactor (Chang et al., 2009).

2.3.4 Moving bed biofilm reactor (MBBR)

MBBR firstly appeared in the late 1980s and developed and applied in the mid-1990s (Ødegaard et al., 1994). MBBR has the advantages of both traditional fluidized bed and activated sludge process and is an innovative and efficient wastewater treatment method.

2.3.4.1 Characteristics of MBBR

By using the suspension carrier, the MBBR process can improve the reactor's biomass and biological species, thus improving the wastewater treatment efficiency of the reactor. The density of the carrier is close to that of water, so when aerated, it is thoroughly mixed with water, and the environment for microorganism growth is gas, liquid, and solid. When the air meets the carrier in the suspended water, the air bubbles will become smaller, increasing the utilization rate of oxygen. Comparing with other attached growth biological treatment systems, MBBR takes full advantage of the reactor space to provide microbial growth (Bassin & Dezotti, 2018). The head loss of MBBR is low, and block or clog seldom happen in MBBR filter medium. MBBR is suitable for both aerobic and anaerobic/anoxic systems. The possible configurations are shown in figure 2.7. Barwal & Chaudhary (2014) reported that the removal rate of COD and BOD could reach 90% and 95%, respectively, in MBBR systems. MBBR technology has been widely used in municipal and industrial wastewater treatment, aquaculture, potable water denitrification, and roughing, secondary, tertiary, and sidestream applications (McQuarrie & Boltz, 2011). It also has been used to treat medical wastewater containing coronavirus (Zhang et al., 2020). Besides, the application of MBBR for Nitrogen removal has a great prospect.

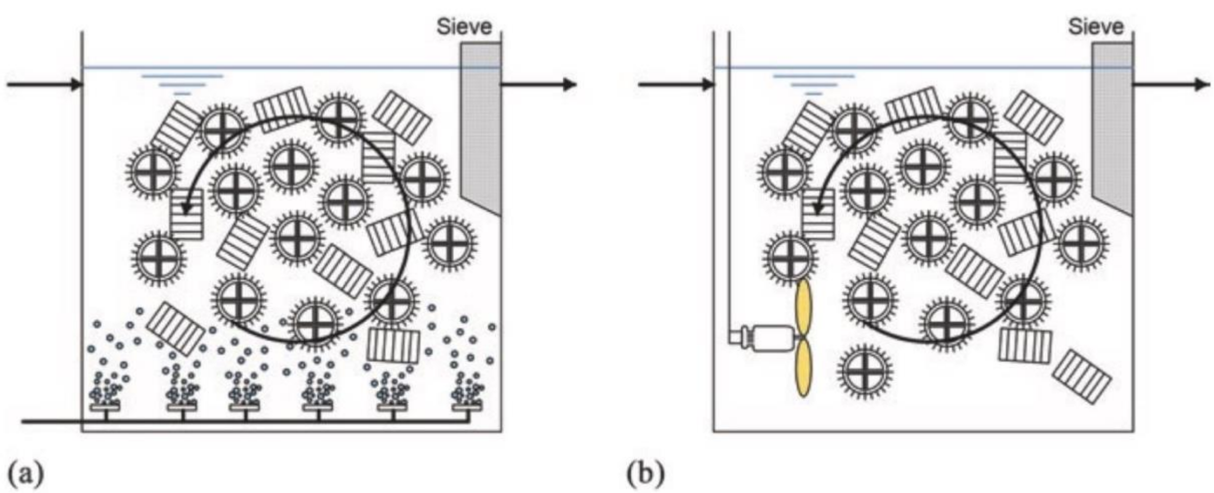
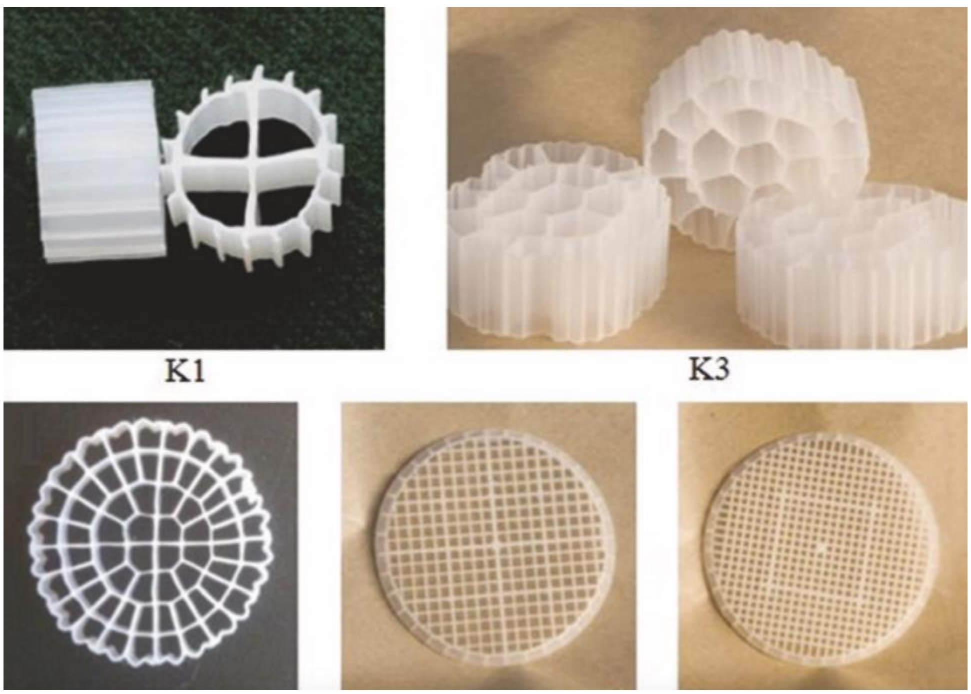


Figure 2.7 Functioning of the variants of the MBBR process. (a) Aerobic (aerated) reactor. (b) Anaerobic-anoxic reactor (Bassin & Dezotti, 2018).

2.3.4.2 Biofilm carriers used in MBBR systems

The MBBR carrier usually needs to be less dense than or close to water density to ensure that the carrier can be suspended in water. The carrier material and shapes are different. The thickness of the MBBR carrier disc is essential for active biofilm growth (Geiger & Rauch, 2017). The typical carriers on the market are different (such as hollow body carriers, tube-shaped, helical-shaped, and sponge carrier configuration). They have different wastewater treatment effects and can meet the requirements of each specific case (Levstek & Plazl, 2009). The kinetic energy can cause wear and abrasion of carriers. Plastic carriers are light and flexible, so they can effectively reduce the wear and abrasion. Figure 2.8 shows some commercial MBBR carriers. An optimal carrier geometry depends on the biological and physical requirements of biofilm.



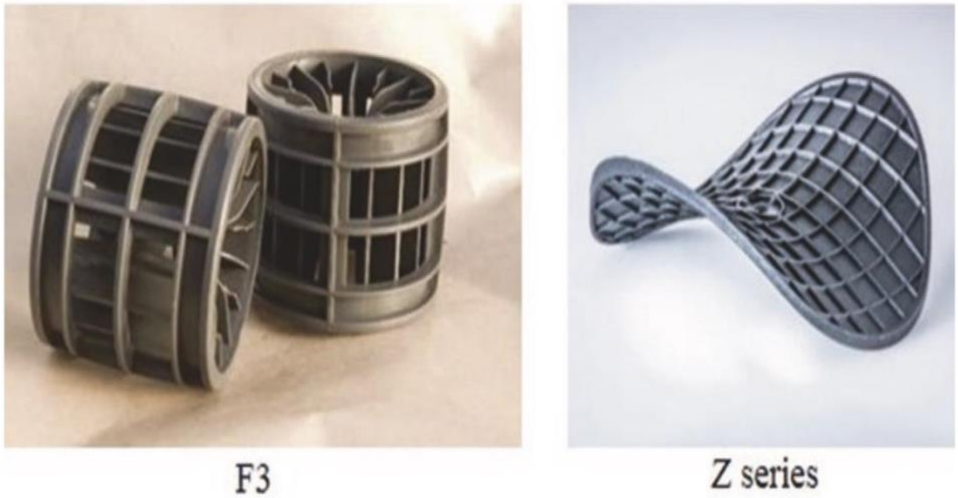


Figure 2.8 Some commercial MBBR carriers (Levstek & Plazl, 2009).

2.3.4.3 MBBR in nitrogen removal

There are many methods of nitrogen removal, which are described in detail in the following sections. Chu & Wang (2011) used biodegradable polymer as both carbon source and biofilm carriers to remove nitrogen from wastewater with a low C/N ratio, and they reported that the TAN removal rate could reach 74.6%. MBBR is also an ideal and efficient methods for nitrogen removal from municipal wastewater. Kermani et al. (2008) conducted an experiment with MBBR applied in series with anaerobic, anoxic, and aerobic units in four separate reactors for nitrogen removal from municipal wastewater and the average ammonium removal efficiency was up to 99.72% at optimum conditions. The ammonia removal rate from both the synthetic and industrial wastewaters is also high (>90%) (Zinatizadeh & Ghaytooli, 2015). When the temperature was 35-40 ℃, ammonia was effectively removed (Shore et al., 2012). Besides, Delatolla et al. (2010) reported that the ammonia removal rates of MBBR were shown to be significant at a low temperature (4℃) and low influent carbon. Hoang et al. (2014) found that during long term exposure to 1 °C, the nitrification rate of MBBR decreased significantly, but still had the effect of nitrogen removal (Rusten et al., 2006).

2.4 Nitrogen cycle in wastewater treatment systems

The conversion between nitrogen elements and nitrogen compounds in nature is called the nitrogen cycle (Canfield et al., 2010). Fixation, ammonification, anammox, nitrification, and denitrification are major processes of the nitrogen cycle (Stein& Klotz, 2016). As the fourth most abundant element in cellular biomass, nitrogen is critical for many organisms. (Bernhard, 2010; Stein& Klotz, 2016). Nitrogen compounds are also widely distributed in natural water (Feth, 1996). Nitrogen compounds in natural water are important to many areas. However, excessive nitrogen in water can be harmful to human health, aquatic animals, and aquatic plants (Blaas & Kroeze, 2016).

2.4.1 Nitrogen transformation

The transformation mechanisms between different nitrogen species are very complex (Paredes et al., 2007). Organic nitrogen compounds, ammonium (NH_4^+), trace amounts of nitrite (NO_2^-) and nitrate (NO_3^-) are the primary forms of nitrogen in the natural water (Soliman & Eldyasti, 2018). These forms of nitrogen can convert to each other. Among them, ammonium is very common and very important in natural water, while NO_2^- rarely builds-up in the ecosystem (Sinha & Annachhatre, 2007). Meanwhile, organic nitrogen can be converted to ammonium nitrogen by hydrolysis and mineralization (Soliman & Eldyasti, 2018). Nitrogen removal from water has been focused on by people recently. Figure 2.9 shows some significant pathways for nitrogen transformation (Paredes et al., 2007).

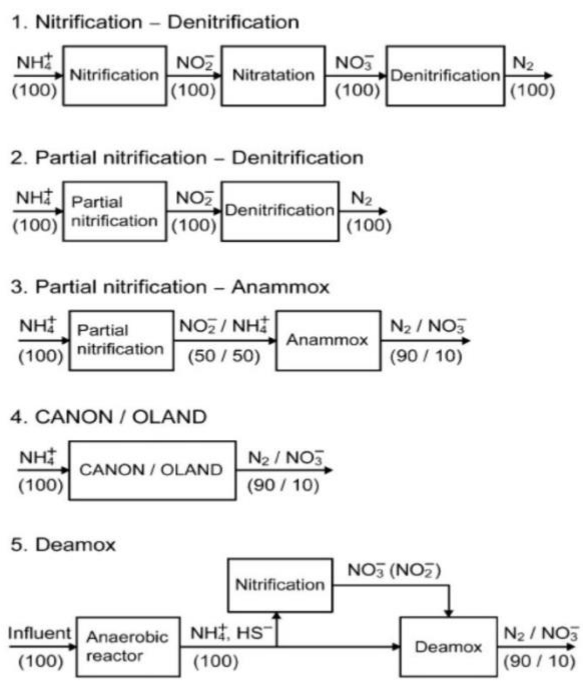


Figure 2.9 Some significant pathways for nitrogen transformation (Paredes et al., 2007).

2.4.2 Nitrification

Nitrification plays an important role in the global nitrogen cycle (Khin & Annachhatre, 2004). The two-step sequencing biological oxidation process that oxidation of ammonia via nitrite to nitrate is called nitrification (Eq. (2-1)) (Daims et al., 2015; Ge et al., 2015).

$$NH_4^+ \xrightarrow{AOB} NO_2^- \xrightarrow{NOB} NO_3^- \quad (2\text{-}1)$$

Most nitrification occurs in an aerobic environment and is carried out by prokaryotes (Bernhard, 2010). Ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are two necessary microorganisms for nitrification. They have been widely studied in many natural environments (Bernhard, 2010). The first step of nitrification needs the ammonia-oxidizing AOB) to oxidize the ammonium (NH_4^+) to nitrite (NO_2^-). In contrast, the second step needs the NOB to oxidize the nitrite (NO_2^-) to nitrate (NO_3^-) (Soliman & Eldyasti 2018).

In natural water, nitrification rates are affected by many factors like light, salinity, temperature, oxygen, pH, ammonium availability, organic carbon availability, and carbon to nitrogen ration (C/N ratio) (Wild et al., 1971; Dodds et al., 2017). Usually, nitrification process performs better in warmer seasons or climates (Sinha & Annachhatre, 2007). Nitrification rates are generally lower than other nitrogen cycle processes because energy gain from the aerobic chemoautotrophic process is generally low, while nitrification can almost operate with any ammonia concentrations, even deficient ammonium concentrations (Dodds et al., 2017).

Nitrification has a positive influence on the treatment of wastewater that requires ammonia removal (Kuenen & Robertson, 1994). Although ammonia is a nutrient form of nitrogen for many aquatic lives, it is toxic to many fish (Paredes et al., 2007; Ahuja et al., 2014). Nitrification can help to remove ammonia.

2.4.3 Partial nitritation

Partial nitritation (PN) is the first step of nitrification that converts NH_4^+ to NO_2^-. It is also called shortcut nitrification. This process also consists of two steps with hydroxylamine (NH_2OH) as the intermediate product (Ge et al. 2015). The conversion of NH_4^+ to NH_2OH via catalysis by ammonia monooxygenase (AMO) is the first step (Eq. (2-2)) (Soliman & Eldyasti 2018). The actual substrate is NH_3 rather than NH_4^+ (Paredes et al. 2007). The second step of PN is the oxidation of

NH_2OH to nitrite (NO_2^-) by the AOB with the hydroxylamine oxidoreductase (HAO) (Eq. (2-3)). Water provides one of the oxygen atoms, and oxygen molecule provides the other oxygen atoms for NO_2^- in this step (Eq. (2-4)) (Ge et al. 2015).

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{\text{AOB}} NH_2OH + H_2O \quad -120\ kJ\ mol^{-1} \quad (2\text{-}2)$$

$$NH_2OH + 0.5O_2 \xrightarrow{HAO} HNO_2 + 2H^+ + 2e^- \quad -114\ kJ\ mol^{-1} \quad (2\text{-}3)$$

$$NH_2OH + H_2O \rightarrow NHO_2 + 4H^+ + 4e^- \quad +23\ kJ\ mol^{-1} \quad (2\text{-}4)$$

PN can be well combined with many processes, such as anammox and denitrification to achieve the purpose of nitrogen removal (Ciudad et al., 2005; Paredes et al. 2007; Okabe et al., 2011; Ge et al., 2015; She et al., 2016). Compared to traditional biological nitrogen removal, PN can reduce nearly 25% oxygen requirement, 30% biomass production, and the carbon consumption can also be saved (Ruiz et al., 2003; Paredes et al., 2007). AOB and NOB are important in this process because PN needs to accumulate AOB and meanwhile reduce the activity of NOB (Ruiz et al., 2003; Soliman & Eldyasti 2018). Temperature is a significant factor that affects PN (Paredes et al., 2007). Some parameters like pH, DO, sludge retention time (SRT), inhibitor, free ammonia (FA), and nitrous acid concentrations can also be applied to control nitrification (Sinha et al., 2007; Ge et al., 2015).

2.4.4 Anammox

Mulder et al. (1995) firstly found anaerobic ammonium oxidation (Anammox) in a denitrifying fluidized bed reactor. The autotrophic anammox bacteria can oxidize ammonium to dinitrogen gas in anoxic environments (Jin et al., 2012). The electron acceptor in the anammox process is nitrite. The nitrogen cycle, including anammox, is shown in figure 2.10. As a promising, innovative, and sustainable technology, anammox has several advantages (Van et al., 2010). One advantage of the anammox process is that anammox bacteria do not need an additional carbon source. In conventional wastewater treatment systems, the carbon source is usually needed to remove nitrogen; meanwhile, aerobic conditions need to consume much energy (Kartal et al., 2010). Besides, the nitrogen removal rate of anammox is high and operational costs can be reduced by 90% (Jetten et al., 2005; Joss et al., 2009). Finally, the whole process will not produce nitrous oxide, so it can reduce greenhouse gas emissions (Ma et al., 2016).

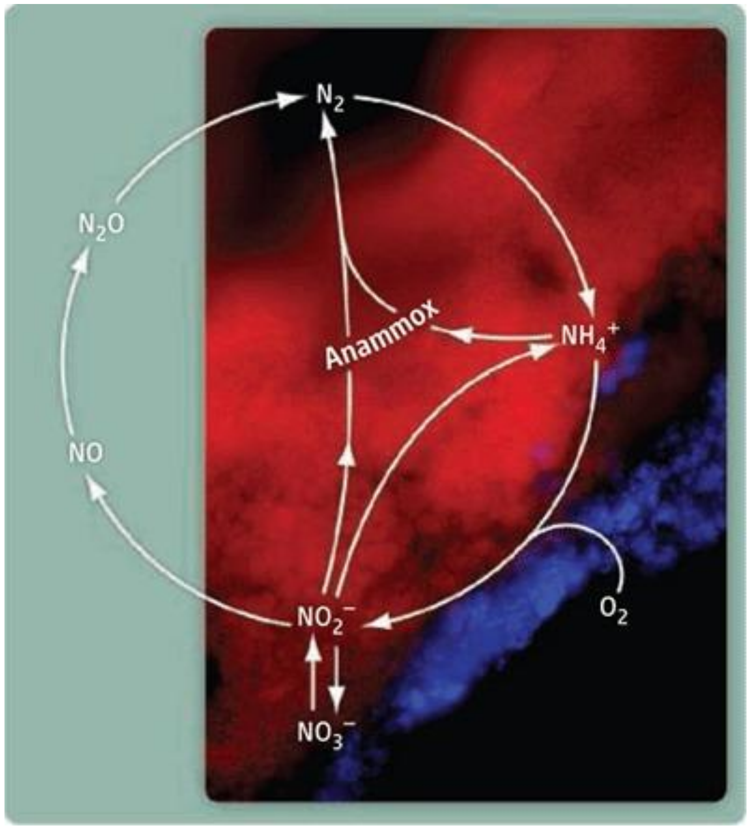


Figure 2.10 The nitrogen cycle including anammox. The anammox bacteria (red) are on the inside of the granule; AOB (blue) reside in a 40-µm-thick layer on the outside. (Kartal et al., 2010).

Anammox can well be combined with many other processes like PN, which has a high potential for more sustainable ammonium removal technology (Hao et al., 2002). Chemolithoautotrophic bacteria belonging to the order Planctomycetales are the main microorganism involved in the anammox process (Tsushima et al., 2007). Different temperatures, pH, dissolved oxygen (DO), substrate concentration, nitrite concentration and load, sludge acclimatization etc. also have an influence on anammox. Puyol et al. (2014) reported that in common pH conditions, nitrite concentration is a crucial parameter for anammox. Keeping the balance between the different microbial groups involved in the anammox process is critical to achieving a successful application (Lackner et al., 2014).

2.5 Microorganism involved in partial nitritation

AOB and NOB are the main bacteria involved in PN. AOB and NOB are common in nature and can be found almost everywhere (Sinha et al., 2007). Therefore, to achieve successful PN, many studies focus on these two bacteria.

2.5.1 Ammonia oxidizing bacteria

AOB was first isolated at the end of the 19th century (Sinha et al., 2007). AOB is responsible for the first step of nitrification in various environments, making them indispensable in the global nitrogen recycle process (Kowalchuk & Stephen, 2011).

2.5.1.1 Morphologic and phylogenetic diversity of AOB

Now, five genera with several species in each are recognized and classified in the Proteobacteria class. It can be divided into two subclasses: the β-Proteobacteria subclass and the γ-Proteobacteria subclass. Nitrosomonas (including Nitrosococcusmobilis), Nitrosospira, Nitrosovibrio, and Nitrosolobus four of them lie in the β-Proteobacteria subclass, while one cluster of Nitrosococcus elongs within the γ-Proteobacteria subclass (Soliman & Eldyasti 2018). There are also many types of AOB classifications. As shown in Figure 2.11, according to the morphological, AOB can also be classified by cell shape, cell size, flagellation of motile cells, and arrangement of intracytoplasmic membranes (Soliman & Eldyasti 2018). Till now, about 25 kinds of AOB species were cultured from the environment, but recent studies mainly focus on the Nitrosomonas and the Nitrosospira (Ge et al. 2015; Soliman & Eldyasti 2018). Most AOB grow in aerobic conditions, while some of them can also survive in both aerobic and anaerobic conditions such as brackish water (Schmidt et al., 2003; Kowalchuk & Stephen, 2011).

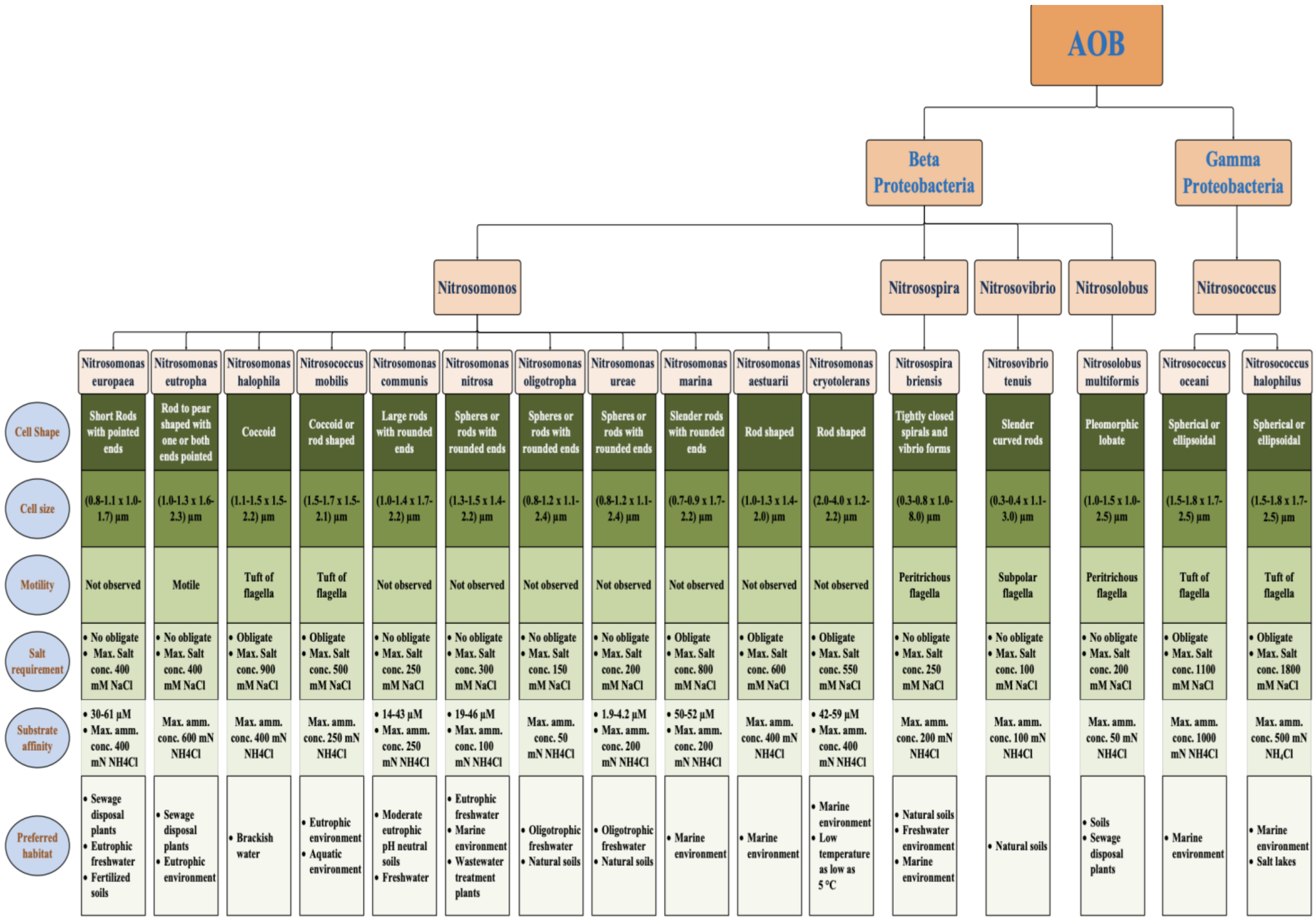


Figure 2.11 Morphological and eco-physiological characteristics of reported AOB's species (Soliman & Eldyasti 2018).

2.5.1.2 Key enzymes of AOB

The hydroxylamine oxidoreductase (HAO) and the ammonia monooxygenase (AMO) are two key enzymes of AOB (Monteiro et al., 2014). As introduced in the previous part, the ammonia oxidation to nitrite should go through two steps: ammonia should be oxidized to hydroxylamine first and then further oxidized to nitrite (Soliman & Eldyasti 2018). In the first step, AOB need to use the ammonia monooxygenase (AMO) to oxidize ammonia to hydroxylamine (Norton et al., 2002). amoC (31.4 kDa), amoA (31.4 kDa), and amoB (38 kDa) are the operon of AMO (Junier et al., 2010), but usually only a portion of the gene amoA is used to study the AOB (Rotthauwe et al. 1997).

During the second step, hydroxylamine oxidoreductase (HAO) is involved in the oxidation of NH_2OH to nitrite (Soliman & Eldyasti 2018). HAO can be found in the periplasm and has multi-c-heme and homotrimer (64 kDa) subunits (Arp et al., 2002). HAO is soluble and is considered the best-studied meaningful part of AOB (Igarashi et al. 1997). HAO transport the electrons to the ubiquinone pool by using two cytochromes, c554 and cM552 (Hooper et al. 2005).

2.5.1.3 Parameters affecting AOB

To achieve PN successfully, AOB must be grown in a suitable environment. AOB accumulation depends upon many factors. It may include temperature, dissolved oxygen, FA, free nitrous acid (FNA), pH, and inhibitors.

2.5.1.3.1 Temperature

Temperature is a critical parameter for AOB. Usually, AOB is active at higher temperatures. Nitrite can accumulate when AOB grows faster than NOB. High temperatures would be more beneficial for AOB to outcompete NOB and achieve nitrite accumulation (Ge et al. 2015). It has been observed in an activated sludge plant that nitrite tends to accumulate, especially during the summer (Tonkovic 1998). The optimum temperature conditions for nitrite accumulation vary from reactor to reactor. The ammonium oxidizers can effectively outcompete the nitrite oxidizers at temperatures above 25 °C (Paredes et al., 2007).

Temperature can promote FA and FNA's chemical equilibriums, especially for the high-strength wastewater (Ge et al. 2015). The influence of FA and FNA concentrations on AOB activity will be discussed in the following section.

2.5.1.3.2 Dissolved oxygen

The oxygen saturation coefficients of Monod kinetics for nitritation and nitratation are 0.3 and 1.1 mg/L, respectively (Wiesmann, 1994). Therefore, NO_2^- can be accumulated at low DO concentration. Hanaki et al. 1990 found that low ammonia oxidation can occur at a low DO (< 0.5 mg/L) environment as a whole in the pure nitrification system in a suspended growth reactor at 25°C. DO concentration below 1.0 mg/L is sufficient to ensure the dominance of the ammonia oxidizer (Sinha & Annachhatre, 2007). Nitrite accumulation rate can get to 96% with AOB population 5.33 x 10^8 cell/mL in a suspended growth system with a DO concentration of 0.4–0.5 mg/L in an activated sludge reactor (Mohammed et al., 2014). Besides, AOB recovered faster from aerobic conditions; thus, alternating anoxic and aerobic operation can be applied to accumulate nitrite (Ge et al. 2015).

2.5.1.3.3 Free ammonia and nitrous acid

FA (at high concentrations) can inhibit the activity of AOB considerably (Vadivelu et al., 2006). Abeling and Seyfried (1992) found that at pH = 8.5 and Temperature = 20℃, when then FA is about 5 mg l^{-1}, the AOB is active. The influence of FA on AOB is specific and depends on the concentration of bacteria as upon the threshold concentration of FA causing inhibition (Rols et al., 1994; Villaverde et al., 2000). When the concentration of FA gets to a certain value, the effect FA will dominate (Fdz-Polanco et al., 1994)

FNA can also inhibit the AOB metabolisms. Claros et al., 2013 found that as nitrite gradually accumulated, the AOB activity gradually decreased. Their results neutralized the effect of pH. Different AOB species also have different tolerance levels to FNA. N. europaea was found to have a tolerance to high nitrite concentrations because it possesses a gene that encodes a functional copper-type NirK (Beaumont et al., 2002).

2.5.1.3.4 pH

The optimal pH condition for PN is on the alkaline side. Many researchers found that nitrite accumulation happened at high pH. Suthersan and Ganc- zarcczyk (1986) found that it is possible to achieve nitrite accumulation at pH 8.0. According to the acid-base reaction theory, pH will affect the substrate concentration. The distribution of NH_4^+/NH_3 and NO_2/HNO_2 will change at different pH values (Eq. (2-5) (2-6)). AOB is also adaptive to different pH values: the optimal pH for AOB usually depends on the prevailing environment conditions in the reactor (Claros et al., 2013).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \quad \text{(2-5)}$$

$$H^+ + NO_2^- \leftrightarrow HNO_2 \quad \text{(2-6)}$$

2.5.1.3.5 Inhibitors

The presence of some compounds can inhibit AOB. $NaClO_2$ (sodium chlorite) is demonstrated to have a strong inhibition on AOB (Hooper & Terry, 1973). In gold-mine service industry, it was shown that chlorine (3–13 mg/L), chlorine dioxide (2–8 mg/L), bromine (>8 mg/L) and cyanide

(> 2 mg/L) all inhibit AOB to varying degrees (Jooste, 1993). Some metal-binding agents such as allylthiourea and potassium, carbon monoxide, catalase, peroxidase, some amine oxidases such as thiosemicarbazide, ethylxanthate, and iproniazid, uncouplers of oxidative phosphorylation and some electron acceptors such as phenazine methosulfate are also confirmed to influence AOB to some extent (Hooper & Terry, 1973). Also, light can act as inhibitors. The kind of inhibitors is diverse. It is necessary to control their concentration to achieve successful nitrification.

2.5.2 Nitrite oxidizing bacteria

NOB are essential in the biogeochemical nitrogen cycle. They can oxidize nitrite to nitrate, so NOB must be inhibited in the PN process. NOB is as diverse as AOB, different from each other in fundamental physiological and molecular traits (Daims et al., 2016). However, comparing with other microbes related to the nitrogen cycle, research about NOB is limited.

2.5.2.1 Morphologic and phylogenetic diversity of NOB

The process of culturing NOB is complicated and time-consuming (Lebedeva et al., 2008). NOB has eight pure cultures with different ecophysiological requirements (Koops & Pommerening-Röser, 2011). Now, the NOB found by people can be divided into seven different genera in four bacterial phyla, shown as following Figure 2.12. Among them, Candidatus Nitromaritima is a new candidate genus of uncultured marine NOB (Daims et al., 2016; Kitzinger et al., 2018). All NOB are Gram-negative except Nitrolancea hollandica which stains Gram-positive (Sorokin et al., 2012).

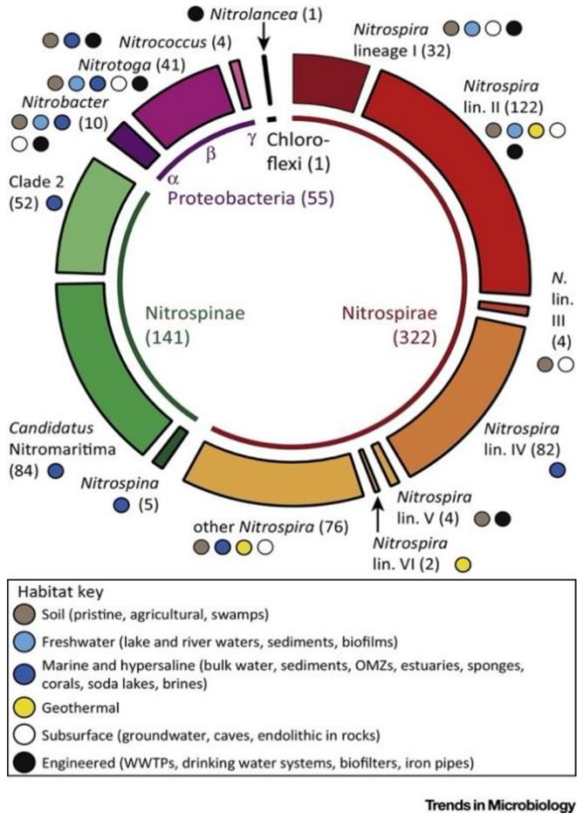


Figure 2.12 Phylogenetic affiliation, species-level diversity, and habitats of NOB (Daims et al., 2016).

Nitrospira and Nitrobacter are the most common NOB in the natural environment (Sinha & Annachhatre 2007). Nitrospira was considered more valuable and specialize in many water systems. The kinetics and biochemistry of nitrite oxidation can explain the ecophysiological differences between major NOB groups (Abeliovich, 2006; Daims et al., 2016). Most Nitrospira are obligately chemolithotrophic, while Nitrobacter can also grow with organic compounds for energy (Bock 1976). Usually, Nitrospira prefers growing in low nitrite concentrations environments; in other words, they have a low substrate consumption (Ehrich et al. 1995). Both Nitrospira and Nitrobacter can tolerate temporarily higher nitrite concentrations (Wagner and Loy 2002). NOB can generate energy by oxidizing nitrite (Sinha & Annachhatre, 2007). NOB are sensitive to inorganic nutrients, so it is necessary to control all nutrients to culture NOB. Figure 2.13 shows some NOB derived from activated sludge.

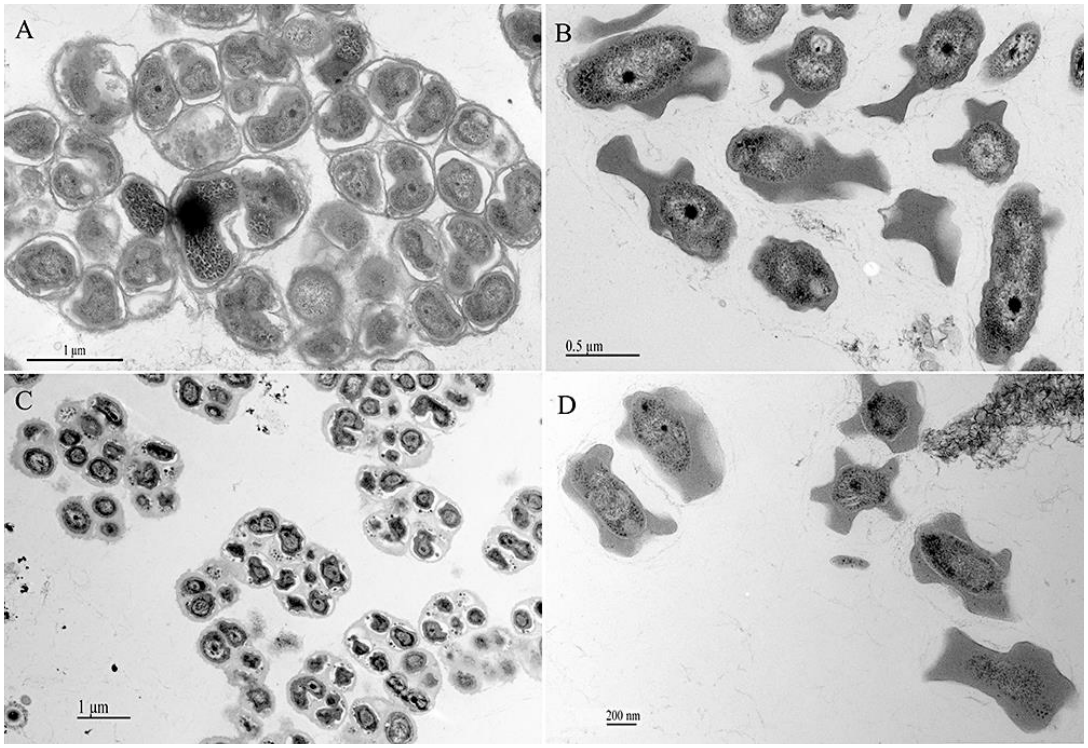


Figure 2.13 Electron micrographs of NOB derived from activated sludge without further cultivation (A and B) and after selective enrichment in mineral nitrite medium at 10°C (C and D). Microcolonies of Nitrospira (A and C) and cells of Nitrotoga (B and D). Bars: A=1.

2.5.2.2 Key enzymes of NOB

Nitrite oxidoreductase (NXR) is the key enzyme involved in the oxidization of the nitrite. As is shown in figure 2.14, it oxidizes nitrate, meanwhile shuttles two electrons into every respiratory chain reaction. They are the type II DMSO reductase-like family of molybdopterin-binding enzymes (Daims et al., 2016). NXR usually occurs in two phylogenetically distinct forms, which contains three subunits NxrA (α), NxrB (β), and NxrC (γ) (Sundermeyer-Klinger et al., 1984; Lücker et al., 2010). The substrate-binding subunit NxrA exists in the periplasmic space in Nitrospira, Nitrospina (Lücker et al., 2013), and Candidatus Nitromaritima (Ngugi et al., 2016), but in the cytoplasm in Nitrobacter, Nitrococcus, and Nitrolancea (Figure 2.14). Besides, the nxrA and especially the nxrB gene can act as markers to identify NOB. Cytoplasmic membrane is important for NXR and the transportation of nitrite and nitrate is achieved by it (Daims et al., 2016).

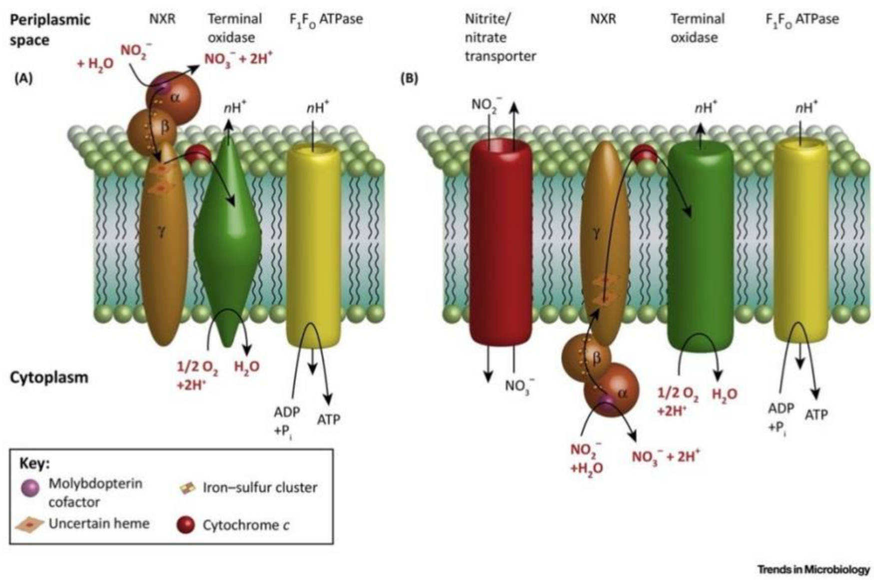


Figure 2.14 Schematic illustration of the NXR types and the assumed electron flow during nitrite oxidation (Daims et al., 2016).

2.5.2.3 Parameters affecting NOB

As mentioned before, like AOB, several parameters influence NOB. Controlling these parameters in a PN system to inhibit NOB growth is critical. When selecting conditions, these parameters should have as little impact on AOB as possible.

2.5.2.3.1 Temperature

Temperature is an important parameter for AOB, but also critical for NOB. Many types of research results have revealed that the population structure of nitrite-oxidizing bacteria responds strongly to temperature changes. Nitrobacter is more active than Nitrosomonas when temperature is between 10 and 20 °C (Knowles et al., 1965). Grunditz & Dalhammar (2001) found that the temperature for the highest activities was 38 °C for NOB-Nitrobacter. AOB is more like a high temperature than NOB. In other words, the optimum temperature for oxidizing ammonia is higher than that for oxidizing nitrite (Wortman & Wheaton, 1991). Mulder & Kempen (1997) found that at higher temperatures, the growth rate of the AOB is higher than that of the NOB (Figure 2.15). NOB can be washed out by controlling temperature. Jetten et al. (1997) enriched some novel nitrite oxidizers at temperatures of 10°C and 17°C, among which Nitrospira and were able to grow in a broad temperature range. As introduced before, temperature can affect the FA and FNA's chemical equilibriums and further affect the activity of NOB.

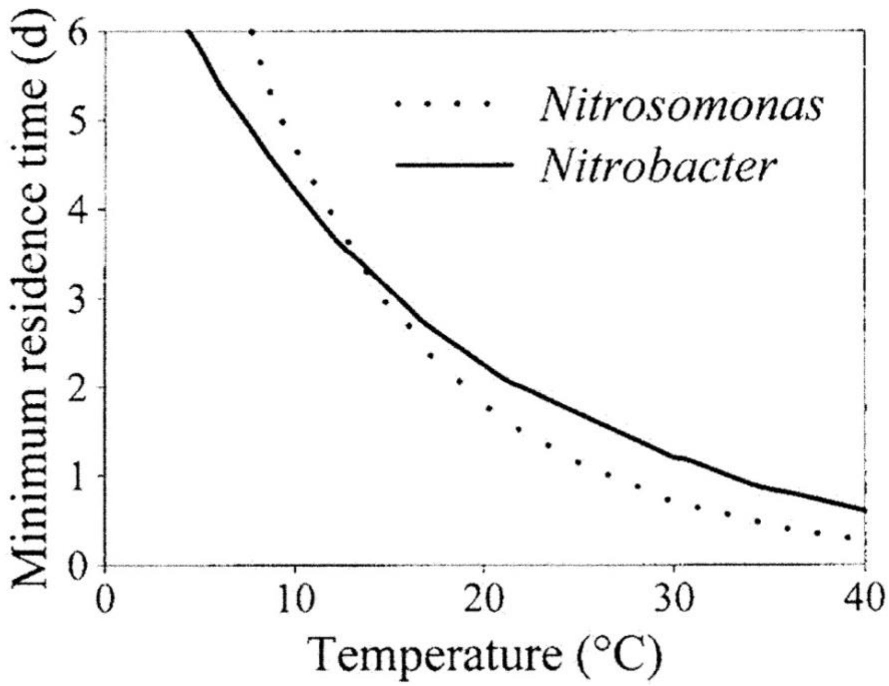


Figure 2.15 Effect of temperature on the minimal required cell residence time for ammonia and nitrite oxidation (Sinha & Annachhatre, 2007). (NOB can be washed out while maintaining the AOB when temperature is over ℃ (Jetten et al. 1997).

2.5.2.3.2 Dissolved oxygen

NOB need to use oxygen to generate energy for metabolism. However, low dissolved oxygen concentration does not always inhibit the NOB (Park et al., 2008). Leu et al. (1998) found that in deep mixed biofilms, NOB is more sensitive to low DO under low organic matter environment. Compared with AOB, low DO has a more significant influence on the activity of NOB (Leu et al. 1998). Because of the lower affinity of the NOB for oxygen, it is possible to wash out NOB by control the low DO condition (Garrido et al. 1997). Sinha & Annachhatre (2007) found that In SHARON process, NOB can be washed out selectively at a temperature between 30 ℃ and 40 ℃. Goreau et al. (1980) also found that pure cultures of Nitrosomonas sp. will produce less NO_2^- at low oxygen concentrations.

2.5.2.3.3 Free ammonia and nitrous acid

FA and FNA are the main parameters that affect the activity of NOB. FA is considered as a bacterial inhibitor (Jiménez et al., 2011). Anthonisen et al., (1976) firstly found that only 0.1-1.0 mg FA/L can inhibit NOB. Bae et al., 2001 also found the influent FA concentrations at 0.1–4.0

mg/L can lead inhibitory to NOB. The threshold FA inhibition concentrations of NOB is lower than AOB. FNA is a key parameter of inhibition for NOB when pH is lower than 7.5, while FA is the main inhibitor when pH is higher than 8 (Sinha & Annachhatre, 2007). Glass et al. (1997) reported that FNA acted as uncouplers and donated a proton inside the cell, and meanwhile it interfered with the transmembrane pH gradient required for ATP synthesis. FA and FNA influence on NOB were reversible, and FA or FNA cannot be used as a single parameter for nitrite oxidizing control (Han et al., 2003). The inhibiting effect of FA is the result of a combination of several parameters like the pH, SRT, substrate concentrations, and the temperature.

2.5.2.3.4 pH

As mentioned in the last section, pH influences FA, FNA, and AOB. pH is also an important factor for NOB. Jiménez et al. (2011) found that NOB were strongly inhibited by low pH values (no activity was detected at pH 6.5). However, pH values have little influence on them (activity was nearly the same for the pH range 7.5–9.9) in an activated sludge reactor. Experiment results indicate that the wastewater's pH is the decisive parameter in NOB activity inhibition (Surmacz-Gorska et al., 1997). Although pH at 7.5 to 8.5 did not significantly inhibit NOB, it was beneficial for nitrite accumulation (Balmelle et al., 1992; Villaverde et al., 1997). The effect of pH on FA and FNA also affects the activity of NOB.

2.5.2.3.5 Inhibitors

Several chemicals can cause inhibition to NOB. The efficiency of PN can be increased by selecting inhibitors with an inhibitory effect on NOB but not AOB. Tomlinson et al. (1966) found that chlorate, cyanate, azide and hydrazine were useful inhibitors for NOB, but cause less inhibition to AOB. Only 50% at 0.3 μM in vivo azide were demonstrated to have potent inhibition on NOB (Ginestet et al. 1998). Lees & Simpson (1957) reported that chlorate is a specific inhibitor of NOB. Besides, the salt content (NaCl) also has a more adverse influence on NOB than AOB (Dincer & Kargi 1999). Some metals, like nickel, also strongly inhibit the activity of NOB (Randall, C. W., & Buth). However, some metals like Cd, Cr, Pb, Cu, and Fe were seen not to inhibit the AOB (Kamath et al. 1991). As mentioned before, sunlight is an inhibitor for AOB, but NOB is more sensitive to it (Olson & RJ, 1981; Vanzella et al., 1989). Future research could focus more on selective inhibitors.

2.5.3 Partial nitritation microbiome

The AOB species present in the partial-nitrification bioreactors are related to the environments. Gonzalez-Martinez et al. (2014) found that HRT has a significant influence on the nitrifying microbial community. When HRT is changed, there will be two different clusters of AOB, most of which were grouped in the Nitrosomonaseuropaea/eutropha or in the Nitrosomonas marina/oligotropha groups. Besides, sequences of Nitrosospira and Nitrosovibrio were also detected. PN is dominated by bacteria of the genera Nitrosomonas and Nitrosospira (Utåker et al., 1995). Chen et al. (2017) used qPCR analyses for AOB and NOB, founding that in inoculums, the copy numbers were 4.87×10^5, and 9.14×10^2/ng DNA, respectively in packed bag reactor. With different HRT, the numbers of AOB and NOB are also different. Pal et al. (2012) reported that in the municipal wastewater treatment process, community structure did not change much and Nitrosomonas Europaea lineage dominated WW. At the same time, the influence of temperature on the PN microbiome is not great. Young et al. (2017) found about 2000 species of bacteria in nitrifying biofilm at 1℃. Nitrosomonads were proved to be the dominant AOB. The primary population of AOB did not change at 20 ℃ and 1℃. Cell viability and biofilm thickness were observed to increase at low temperatures. According to FISH analysis, AOB were the dominant bacteria group among the microbial compositions in PN systems (et al., 2009). Compared to an activated sludge system, the proportion of AOB in the biofilm system is lower (Zhang et al., 2016).

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Chapter 3-Elevated Loaded MBBR System Achieving Stable Mainstream Partial Nitritation via Nitrite Oxidizing Bacteria Activity Suppression

3.1 Abstract

This study investigates the performance of an elevated loaded, mainstream partial nitritation moving bed biofilm reactor (MBBR) to elucidate the nitrite-oxidation suppression mechanism resulting in stable partial nitritation performance. A lab-scale MBBR system was operated for 45 d at an elevated surface area loading rate (SALR) of 5.2 ± 0.1 g TAN/m^2·d and a hydraulic retention time of 2h. The average surface area removal rate (SARR) is 2. 3 ± 0.2 g TAN/m^2·d (theoretical performance objective of 2.7 g TAN/m^2·d). TAN removal efficiency was 43.1 ± 3.4 % (theoretical performance objective of 53%) and the NO_2^- / (NO_2^- + NO_3^-) ratio was 82.4 ± 4.8% (theoretical performance objective of 100%). Biofilm thickness, biofilm density, and biomass viability analyses indicated a stable, robust, thick, and dense biofilm attached to the MBBR bio-carriers with elevated cell viability during long-term operation of the system. The copies of ammonia-oxidizing bacteria (AOB) population to copies of nitrite-oxidizing bacteria (NOB) population of the bio-carrier biofilm is shown to be 3.4 for the elevated loaded partial nitritation MBBR system. As the attached growth mass of the system is negligible in comparison to the biofilm growth, the observed biofilm AOB/NOB ratio is indicative of the NOB activity being suppressed, as opposed to the quantity of the NOB population itself being low or suppressed in the embedded biofilm. Thick biofilms induced by elevated TAN loading likely limits the mass transfer of oxygen to the NOB populations and thus led to the NOB activity suppression responsible for the effective partial nitritation of the system.

3.2 Introduction

The discharge of nitrogen promotes eutrophication in marine environments and subsequently may cause water toxicity in receiving natural waters (Ryther & Dunstan, 1971; Driscoll et al., 2003). The effect of ammonia discharge may lead to ammonia toxicity on fish, which can be chronic or acute that results in gill damage, reduced reproductive capacity, or death (Randall & Tsui, 2002). As a result of these concerns numerous countries have imposed regulations to limit the discharge of ammonia in wastewater effluent to receiving water bodies (US EPA, 2013). Thus, the removal of ammonia from wastewater has become essential for modern wastewater resource recovery facilities (WRRFs).

Total ammonia nitrogen (TAN) of wastewaters is conventionally removed through combined nitrification and denitrification processes in WRRFs. Nitrification is the aerobic oxidation of TAN to nitrite by ammonia oxidizing bacteria (AOB) followed by the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB). Denitrification is performed by heterotrophic bacteria (denitrifiers) under anoxic conditions and involves the reduction of nitrate to nitrogen gas where denitrifiers using carbon as an electron donor (Farazaki & Gikas, 2019). Although conventional nitrification and denitrification can effectively removal TAN, it has specific drawbacks, including, intensive energy requirements, organic carbon demand and significant sludge production (Bueno et al., 1999; Jianlong & Ning, 2004; Ruiz et al., 2006; Xu et al., 2019). Considering these limitations, other alternative cost-effective TAN removal pathways such as partial nitritation and anammox (PN/A) have become attractive solutions for WRRFs.

In the PN/A process, TAN is partially converted to nitrite via AOB and subsequently TAN and nitrite are converted to nitrogen via anammox bacteria (Strous et al., 1998; Cao et al., 2017). Compared to conventional nitrification and denitrification, PN/A in theory saves approximately 60% of aeration costs, almost 100% of added carbon costs and 50% of added alkalinity costs (Van et al., 1995; Kuenen, 2008; Ali & Okabe, 2015; Ge et al., 2015; Agrawal et al., 2018). Hence PN/A as a low-cost biological TAN removal technology has gained popularity (Kartal et al., 2010; Yang et al., 2013; Liu & Ni, 2015; Miao et al.,2016; Xu et al., 2019; Tian et al., 2020). To date, PN/A has been successfully applied at over 100 WRRFs worldwide for the treatment of sludge centrate produced from the dewatering of anaerobic digester sludge, also referred to as sidestream municipal wastewater (Lackner et al., 2014; Cui et al., 2009). The application of PN/A to mainstream municipal wastewater has been less successful to date, where the lower TAN concentrations, lower temperatures, higher carbon to nitrogen ratios (C/N ratios) of mainstream wastewaters compared to sidestream wastewater limit AOB and anammox bacteria (AnAOB) growth rate and do not necessarily promote effective suppression of the NOB populations or activity (Anthonisen et al., 1976; Hill, 2003; Vlaeminck et al., 2012; Cao et al., 2013; Lackner et al., 2014; Sánchez et al., 2014; Xu et al., 2015; Cao et al., 2017; Li et al., 2018; Trinh et al., 2021).

Several studies have shown the possibility of employing various operational control strategies to achieve effective NOB populations or activity suppression. Many PN studies notably focus on the influence of the temperature, dissolved oxygen (DO), pH, hydraulic retention time (HRT) and inhibitory compounds on the growth and activity of the AOB and NOB (Hanaki et al.,1990; Abeling & Seyfried, 1992; Kuai et al., 1998; Zekker et al., 2011; Gu et al., 2012; He et al.,2012; Wang et al., 2014; Li et al., 2019; Cui et al., 2020;). Based on the discrepancy in metabolic kinetics, recent studies have investigated the strategies of reducing DO (Cui et al., 2020) and applying a short hydraulic retention time (HRT) (Zekker et al., 2011). These studies have provided significant strategies for decreasing nitratation in short term by reducing the NOB population but failed to inhibit NOB in the long-term. Recently, an elevated TAN loaded PN moving bed biofilm reactor (MBBR) strategy has been studied by Schopf et al., (2019) to investigate the potential of achieving stable PN. A stable PN MBBR was successfully run for a period of 300 days and fed with a TAN concentration of 125 mg TAN/L at a temperature of 19-21℃ without restricting DO. The study by Schopf et al., (2019) may provide a potential knowledge base for further investigation of an effective mainstream PN MBBR system. However, there are no studies that have employed the elevated TAN loading rate PN MBBR system to mainstream treatment conditions.

The aim of this study is to validate the performance of an elevated loaded PN MBBR system under mainstream wastewater treatment conditions and to identify the mechanism of nitrite-oxidation suppression of this system. In particular the study will quantify the microbial community of the nitrifying bacteria and will characterize the biofilm and bacterial viability in the PN MBBR system to further understand the relation between the design of the system, operation of the system, characteristics of the biofilm and the mechanism of nitrite-oxidation suppression.

3.3 Materials and methods

3.3.1 Reactor operation

A 2 L-volume, lab-scale, elevated loaded MBBR reactor, cylindrical in shape, was operated as a continuous flow system at room temperature (20.2℃) (Figure 3.1). The reactor was operated at a fill fraction of 9.5% of cylindrical AnoxK™5 (K5) carriers (AnoxKaldnes, Lund, Sweden), with the carriers having a diameter of 2.5 cm, depth of 0.4 cm, and a specific biofilm surface area of 800 m^2/m^3. The design strategy of the system, and in particular the use of a lower than conventional fill fraction to achieve elevated loading within the reactor, was adopted from Schopf et al. (2019).

Aeration was supplied using an aeration tube installed at the bottom of the reactor, with aeration providing DO for the aerobic microbial activity of the system and also providing the dynamic flow rate in the reactor to maintain the carriers in motion. The HRT was 2 hours, with the flow rate controlled by a peristaltic pump. The airflow applied to the reactor was 1.5L/min, with the rate of aeration controlled by a rotameter. The seeded K5 carriers were harvested from a full-scale, secondary, municipal integrated film-activated sludge (IFAS) wastewater treatment system located in Hawkesbury, Ontario, Canada. The lab reactor was initially filled with seeded and clean K5 carriers at a ratio of 1:25 to start-up the reactor and inoculate the clean carriers. The PN MBBR reactor was operated for over two years at elevated loaded PN MBBR before being used in this study.

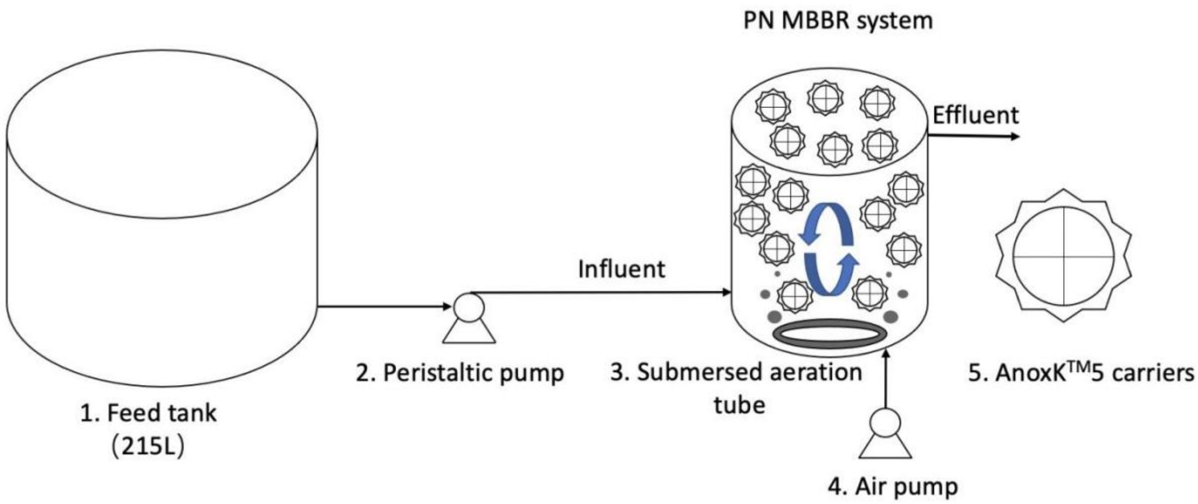

Figure 3.1 Schematic of the elevated loaded partial nitritation MBBR: 1. Feed tank; 2. Peristaltic pump; 3. Submersed aeration tube; 4. Air Pump; 5. AnoxK^{TM}5 carriers.

3.3.2 Synthetic wastewater

Synthetic wastewater was used to feed the reactor and provide stable loading rates. The feed recipe (per 100L of synthetic wastewater) was based on the research of Hoang et al. (2014b), Young et al. (2017), Tian et al. (2019) and Schopf et al. (2019): 14.1 g $(NH_4)_2SO_4$, 39.0 g $NaHCO_3$, 5.5 g $MgSO_4 \cdot 7H_2O$, 2.2 g $CaCl_2 \cdot 2H_2O$, 5.8 g K_3PO_4 and 0.4 g $FeSO_4 \cdot 7\ H_2O$. The carbon stock solution consisted of glucose (D-glucose/Dextrose) at 45.0 g/L, sodium acetate at 24.0 g/L, and peptone at 45.0 g/L. The trace solution consisted of $MnCl_2.4H_2O$ at 200.0 μg/L, $NaMoO_4 \cdot 2H_2O$ at 49.6 μg/L, $CuSO_4.5H_2O$ at 205.1 μg/L, $CoCl_2.6H_2O$ at 2.0 μg/L, and $ZnSO_4.7H_2O$ at 59.8 μg/L. Feed was produced twice a week, and the feed basin was cleaned between all batches. The characteristics of the synthetic wastewater are shown in Table 3.1. Each time the effluent sample was measured, the ammonia, nitrite, nitrate, temperature, pH, DO, alkalinity, COD, TSS and VSS of the feed solution were measured. An alkalinity of 331 ± 3.6 mg/L was supplied in the feed to supply alkalinity in excess of the stoichiometric ratio of 1 mg of oxidized TAN requiring approximately 7.3 mg alkalinity (Li & Irvin, 2007).

Table 3.1 Characteristics of the synthetic, feed wastewater

Type	Average (average ± stdev)
Ammonia (NH_4^+/NH_3—N)	32.8 ± 2.8 mg/L
Nitrite (NO_2^-—N)	0.7 ± 0.1 mg/L
Nitrate (NO_3^-—N)	0.02 ± 0. 2 mg/L
Temperature	20.2 ± 2.4 °C
pH	8.0 ± 0.2
DO	5.7 ± 1.9 mg/L
Alkalinity	331 ± 3.6 mg/L
COD	20.3 ± 1.2 mg/L
TSS	7.6 ± 2.1 mg/L
VSS	2.3 ± 2.3 mg/L

TSS: Total Suspended Solids

VSS: Volatile Suspended Solids

3.3.3 Constituent analysis

Standard methods (APHA, 1998) were used to measure the feed and effluent concentrations of nitrogen (TAN, NO_2^--N, and NO_3^--N), alkalinity, chemical oxygen demand (COD), total suspended solids (TSS), and volatile suspended solids (VSS). Before analyzing, all wastewater samples were filtered through a 0.45 μm filter (Fisher, Ontario, Canada) using a vacuum filter (Marathon Electric, WI, US). All samples were analyzed immediately upon collection, and all samples were tested in triplicates. The DO and temperature were measured using a symphony

Multi-Parameter Meter with relative probes (VWR, Ontario, Canada). pH was measured with a pH meter (HACH, CO, US).

3.3.4 Microbial analysis

3.3.4.1 Biofilm thickness

Biofilm thickness was measured using an Axiocam 105 colour stereoscope (ZEISS, VS, USA). The samples were not pretreated prior to analysis to preserve the integrity of the biofilm (Delatolla et al., 2009). Meanwhile, two samples were selected at random, and five images of each carrier were acquired for biofilm analysis at magnifications of approximately ×5. MedCalc Software Digimizer Image Analysis Software (v4.6.1 Ostend, Belgium) was used to measure the thickness. As the biofilm thickness was not uniform, 1000 measurements of thickness were acquired at different random locations along the surface of the biofilm attached to the MBBR carriers. The biofilm thicknesses of clogged pores, empty pores or corners of the carriers were not measured.

3.3.4.2 Biofilm mass

The biofilm mass measurement method was adopted from a protocol reported by Forrest et al. (2016), Young et al. (2016) and Delatolla et al. (2008). Carriers were placed in a dry oven (VWR, IL, US) at 103-105 ℃ for 24 h after being harvested from the reactor. The carriers were kept dry and were cooled to room temperature in a desiccator (Fisher, Quebec, Canada) for a minimum of 1 h and subsequently weighed. A brush was used to remove the dried biofilm and clean the carrier. The clean carriers were dried in the oven again at the same temperature for 24 h. The carriers were then cooled in the desiccator for a minimum of 1 h and subsequently weighed a second time. The difference in the weights was used to quantitate the biofilm mass on the carriers.

3.3.4.3 Cell viability

Cell viability assays were used to determine the quantity of embedded bacteria in the biofilm attached to the MBBR carriers. Viable cells were quantified based on the procedures described in Ren et al. (2016) and Young et al. (2016). Carriers were harvested on day 45 of operation, a mid-point of operation with steady performance for analysis. The carriers were cut into five segments with a feather surgical blade (Fisher, Ontario, Canada) to expose the inner surfaces. SYTO9 and propidium iodide of the biofilm Tracer™ LIVE/DEAD® biofilm viability kit (Life Technologies, CA, US) were used for cell viability staining. Confocal laser scanning microscopy (CLSM) images were acquired using a Zeiss LSM 510/AxioImager M.1 confocal microscope (ZEISS, VA, USA) with an argon and helium-neon laser at 488, 514 and 543 nm wavelengths using a ×63 water immersion objective. Five stacks of 5 images with a depth interval of 5-6 μm were captured. The CLSM images were analyzed by NI Vision Assistant 7.1 software (LabView 8.0-National Instruments Canada). The percentage of viable embedded cells was calculated by dividing live cells by the (live cells + dead cells).

3.3.4.4 DNA extraction and droplet digital PCR assay

DNA of the nitrifying bacteria was extracted by using a FastDNA Spin Kit for soil (MP Biomedicals, CA, US). Prior to extraction, biofilm samples were stored at -20°C. Fifty to 150 mg of biofilm was collected into a sterilized eppendorf tube (1.5 mL). The extracted DNA concentration was measured using a Qubit 3 Fluorometer (Thermo Fisher Scientific, MA, US). The concentration of DNA samples was assured to be equal to or greater than 90 μg/μL. All DNA samples were stored at −80 °C until analyzed using ddPCR.

Three sets of primers were used to amplify the nitrifying bacteria population (AOB and NOB) DNA (Table 3.2). Each ddPCR reaction mixture consisted of 5 μL of DNA template (after suitable dilution) and 11.5 μL of QX200™ ddPCR™ EvaGreen Supermix (Bio-Rad, CA, US), including 0.23 μL of each primer set (10 μmol/L) and 6.04 μL of nuclease-free water. Each reaction consisted of a 20 μL PCR reaction mixture, and 65 μL droplet generator oil that was added to the droplet generator (Bio-Rad, CA, US). Droplet generation processes were completed in the Droplet Generator unit (Bio-Rad, CA, US), and approximately 40 μL of droplet emulsion was generated from each reaction. Using a pipette, the droplets were transferred to a 96-well PCR plate (Eppendorf, Hamburg, Germany). The 96-well PCR plate was sealed in a Plate Sealer unit (Bio-Rad, CA, US) and run using a T100™ Thermal Cycler (Bio-Rad, Hercules, CA). The ddPCR amplification program consisted of denaturation for 5 min at 95°C followed by 50 cycles of denaturing at 95°C for 30 s., annealing at 53℃ for 30 s (56℃ for *Nitrobactor*), and at 72°C for 30 s. Following this, the reactions were cooled down at 4℃ for 5 min, stabilized at 90°C for 5 min, and then held at 12°C. The amplificated droplets were read using a QX200 droplet reader (Bio-Rad, CA, US). The QuantaSoft analysis software (Bio-Rad, version 1.7.4, CA, US) was used to analyze the amplification curves. Every sample was run in triplicate.

Table 3.2 Primer sets targeting AOB and NOB populations

Target	Primer	Primer Sequence (5'-3')	T ℃	Reference
amoA (AOB)	amoA-1f	GGGGTTTCTACTGGTGGT	53	Rotthauwe et al. (1997)
	amoA-2r	CCCCTCKGSAAAGCCTTCTTC		
Nitrospira (NOB)	NSR1113f	CCTGCTTTCAGTTGCTACCG	53	Bao et al. (2017)
	NSR1264r	GTTTGCAGCGCTTTGTACCG		
Nitrobacter (NOB)	FGPS872f	CTAAAACTCAAAGGAATTGA	56	Bao et al. (2017)
	GPS1269r	TTTTTTGAGATTTGCTAG		

3.4 Statistical analysis

The student's t-test (p < 0.05) was applied to validate the statistical significance between concentrations, removal efficiencies, operation conditions, and dd-PCR results. Error bars in figures indicate 95% confidence intervals.

3.5 Results and discussion

3.5.1 Elevated loaded PN MBBR performance

The PN MBBR was operated with DO concentrations of 6.8 ± 0.3 mg O_2/L, pH of 7.8 ± 0.2 and an operating temperature of 20.2 ± 0.4 (Table 3.3) for 45 days. Stable pH and DO conditions are critical for nitrifying bacterial activity (Prinčič et al., 1998) and are common in many WWRFs. As such, the DO was controlled using set airflow rates. Due to the slight changes in the room temperature and the central air supply unit, the DO fluctuated slightly in the system between 6.4 and 7.2 mg/L, with a mean value of 6.8 ± 0.3 mg O_2/L. pH fluctuated slightly between 7.4 and 8.1 mg/L, with a mean value of 7.8 ± 0.2 mg O_2/L. The optimum pH condition for AOB is between 7.0 and 8.0 (Liu et al., 2020) and conventional pH values for mainstream wastewaters is 6.5-7.5 (Metcalf et al., 1991).

Table 3.3 Elevated loaded PN MBBR system operational parameters (average ± stdev)

Temperature (℃)	pH	DO (mg O_2/L)	Alkalinity (mg/L)
20.2 ± 0.4	7.8 ± 0.2	6.8 ± 0.3	225 ± 8.1

The surface area loading rate (SALR) and surface area removal rate (SARR) were monitored across the 45 d of operation (Figure 3.2). The target SARR is 2.7 g TAN/m^2·d to achieve the stoichiometrically precise 53% TAN oxidization to nitrite for subsequent anammox processing. The achieved average SARR was approximately 80% of the target SARR. The average SALR of the elevated loaded PN MBBR system was 5.2 ± 0.1 g TAN/m^2·d, with the SALR being considered stable throughout the study. The measured SARR fluctuated between 2.0 and 2.8 g TAN/m^2·d with a mean SARR of 2.3 ± 0.2 g TAN/m^2·d. The SARR of the mainstream operated elevated PN MBBR system was approximately 1.0 g TAN/m^2·d lower than the Schopf et al. (2019). Although the Schopf study was operated at a similar SALR, the system was not operated under mainstream conditions and hence was fed with a higher influent TAN concentration and hence higher fill fraction.

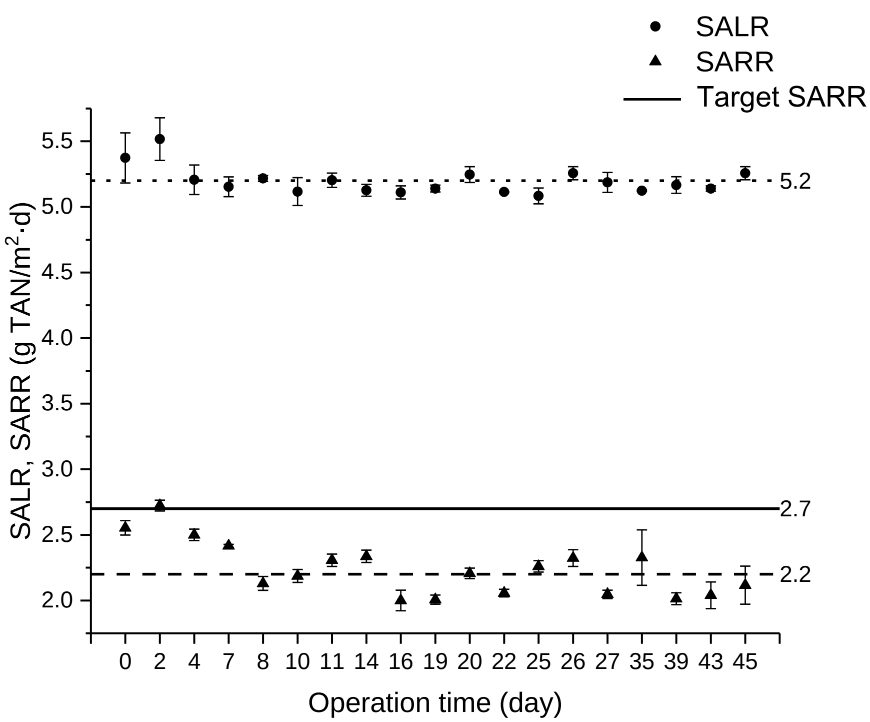

Figure 3.2 Elevated loaded PN MBBR system TAN, SALR and SARR values; solid horizontal line indicates target SARR; dashed horizontal lines indicate average SALR and SARR values.

The influent and effluent TAN, NO_2^- and NO_3^- concentrations and percentage of TAN removal along with NO_2^- as NO_x^- were quantified approximately every second or third day of the study (Figure 3.3). The average influent TAN concentration was 32.9 ± 0.7 g TAN/L (Figure 3.3a). Since NO_2^- and NO_3^- were not directly added to the synthetic feed wastewater, the concentrations of

NO_2^- and NO_3^- were negligible, with likely small quantities of TAN being oxidated to NO_2^- and NO_3^- within the feed tank (Figure 3.3a). The average TAN concentration of the effluent was 18.6 ± 1.4 mg TAN/L (Figure 3.3a) and the average percentage of TAN removal was 43.1 ± 3.4 % (Figure 3.3b). Significant nitrite accumulation was observed in the PN MBBR with an average effluent nitrite concentration of 10.5 ± 1.8 mg NO_2^-/L (Figure 3.3a), while the system showed limited nitrate accumulation with an effluent nitrate concentration of 2.3 ±0.7 mg NO_3^-/L (Figure 3.3a). The average NO_2^- / (NO_2^- + NO_3^-) ratio was 82.4 ± 4.8% and 70% of the measured NO_2^- / (NO_2^- + NO_3^-) ratio data was equal to or above 80% during the 45 d of operation (Figure 3.3b). Based on the stable TAN removal efficiency and the significant NO_2^- / (NO_2^- + NO_3^-) ratio, it can be concluded that PN was successfully achieved in the PN MBBR system.

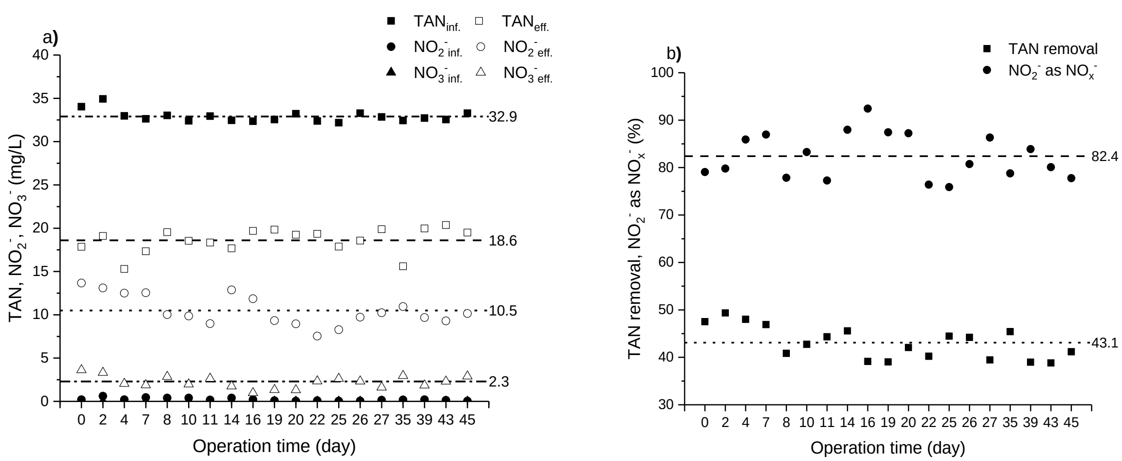

Figure 3.3 Elevated loaded PN MBBR system TAN, NO_2^- and NO_3^- values; (a) TAN, NO_2^- and NO_3^- concentrations; dashed horizontal lines indicate average values (b) Percentage of TAN removal and NO_2^- as NO_x^-; dashed horizontal lines indicate average values horizontal lines indicate average values.

3.5.2 Biofilm characteristics

Biofilm thickness, mass, density and biomass viability measurements of the PN MBBR carriers were quantified from samples collected on day 45 of operation (Table 3.4), after the system was operating under steady state conditions for an extended period and the microbial characteristics were allowed sufficient time to respond to the operational conditions. The collection and examination of the carriers to characterize the biofilm was limited to the one date of sampling to limit the effects of carrier removal from the performance of the system. The biofilm thickness of the PN MBBR was 434 ± 166.7 μm (Table 3.4). An example of profile measurements is shown in

Figure 3.3S1. The standard deviation of biofilm thickness also reflected the uneven, heterogeneous growth of the biofilm

Table 3.4 Biofilm characteristics of the PN MBBR system

Carrier Type	Partial Nitritation (PN) /Full Nitrification (FN)	Biofilm Thickness (µm)	Biofilm Mass (mg/carrier)	Biofilm Density (kg/m^3)	SALR (g TAN/m^2·d) Influent & Ammonia Concentration (mg TAN/L)	Temperature (°C)	Fill Fraction (%)	Citation
K5	PN	434 ± 166.7	49.8 ± 3.4	47.50 ± 3.3	SALR = 5.5 ± 0.2	20.2 ± 0.4	9.5	This study
K5	PN	338 ± 62	35.6 ± 2.2	43.3 ± 11	SALR = 5	20.5 ± 0.2	60	Schopf et al., 2019
K5	PN	572 ± 148	39.4 ± 3.0	28.3 ± 9.5	SALR = 6.5	20.9 ± 0.6	60	Schopf et al., 2019
K5	PN	367 ± 146	-	-	TAN = 65 ± 9	22 ± 1	15	Meng et al., 2021
K5	PN	406 ± 58	15.1	-	TAN = 63.2 ± 2.3	22 ± 1	30	Wang et al., 2020
K5	FN	108 ± 348	-	-	TAN = 60	30	30	Ashkanani et al., 2019
K5	FN	229 ± 6	10.8	58.6	TAN = 40	10	45	Ahmed & Delatolla, 2020
K5	FN	366 ± 16	6.8	23.2	TAN = 40	1	45	Ahmed & Delatolla, 2020

Note: Dashes in the table means the study does not report that specific information.

Biofilm thickness is related to the performance of the system and itself characterizes the PN MBBR performance (Table 3.4). The biofilm thickness of the system was statistically similar with that observed by Schopf et al., (2019) at SALR 5 g TAN/m^2·day with a biofilm thickness of 338 ± 62 µm (p>0.05). MBBR systems with low fill fractions may have fewer collisions between the carriers, and hence this may cause the different biofilm thicknesses. However, the biofilm thickness of 434 ± 166.7 µm observed in this study (Table 3.4) was still relatively thick compared with that in other studies. For example, the closest designed system to date (Schopf et al., 2019) demonstrated the biofilm thickness of 572 ± 148 µm with an SALR of 6.5 g TAN/m^2·day. Under these conditions, complete PN was achieved, which is similar to high NO_2^- / (NO_2^- + NO_3^-) ratio of 82.4 ± 4.8% observed in this study.

Although NOB growth has been shown to be suppressed in thinner MBBR biofilms (Piculell et al., 2016), a thicker biofilm, may suppress NOB growth or activity by creating DO concentration gradients in the inner portions of the thicker biofilm that can restrict the mass transfer of DO to the embedded NOB population. As such NOB populations or activity could be suppressed as well in thicker biofilms due to the limited diffusion of DO into depths of the biofilm where NOB populations, or a significant portion of them, may exist. As the thick biofilm of the elevated loaded PN MBBR system in this study did not significantly suppress TAN oxidation to NO_2^- in the system, the AOB population may be less embedded at depth in the biofilm and less susceptible to DO mass transfer limitations compared to the NOB populations.

In this study, the biofilm mass per carrier was 49.8 ± 3.4 mg/carrier with a biofilm density of 47.5 ± 3.3 kg/m^3. The biofilm mass per carrier was significantly higher compared with previous MBBR partial nitritation and full nitrification studies (Table 3.4), which is likely expected due to the elevated loaded design of the system (Gerardi, 2002; Wijeyekoon et al., 2004). Hence, the SALR of 5.2 ± 0.1 g TAN/m^2·d likely resulted in the dense, thick biofilm characteristic of the elevated loaded PN MBBR system in this study. However, it should be noted that although the system was highly loaded completely clogged carriers were not observed in this system, rather all carriers showed thick, dense biofilms attached to all carriers. Previous research by Young et al (2016) observed that clogged nitrifying carriers were characterized by a low biofilm density and a significant decrease in SARR. Thus, the biofilm characteristics induced by the design and operation of this study’s mainstream, elevated loaded PN MBBR designed system supports an appropriate SARR for subsequent anammox processing.

3.5.3 Viable cells

The presence of viable cells often has a direct association with the performance of nitrifying systems, with the percentage of viable cells embedded in the biofilm of the elevated loaded PN MBBR system of this study being 75.1 ± 15.5 % (Table 3.5). The standard deviation of 15% may reflect to some extent the restricted growth of NOB at certain locations in the biofilm, where increased variance was observed at depths within the biofilm. Figure 3.S2 shows a CLSM image with viability staining; cells illuminated in green are viable (or deemed live), whereas cells that are illuminated red are not viable (deemed dead). The live and dead cell coverages of the biomass were quantified at different depths within the PN biofilm. Some cells existed as clustered colonies in the biofilm attached to the carriers, while some existed as single distinct cells embedded in the biofilm. Table 3.5 displays viable cell measurements observed in previous partial nitritation or full nitrification attached growth research. It can be seen here that the percentage of viable cells ranged from 50-90% within partial nitritation and full nitrification systems.

Table 3.5 Viability measurements of bacterial cells embedded in biofilm attached to MBBR carriers

Carriers Type	Partial Nitritation (PN) /Full Nitrification (FN)	SALR (g TAN/m^2·d) Influent &TAN Concentration (mg TAN/L)	Viable Cells (%)	Temperature (℃)	Fill Fraction (%)	Citation
K5	PN	SALR = 5.2 ± 0.1	75.1 ± 15.5 %	20.2 ± 0.4	9.5	This study
K3	PN	TAN = 150	70-90	10	67	Wang et al., 2021
K5	FN	TAN = 60	79	30	30	Ashkanani et al., 2019
-	FN	-	50	1	-	Minh et al., 2020
K5	FN	SALR = 2.4	72	20	22	Young et al., 2017b
K5	FN	SALR = 1.4	83	1	22	Young et al., 2017b
K5	FN	TAN = 40	94	22 ± 1	30	Ahmed & Delatolla, 2021

Note: Dashes in the table means the study does not report that specific information.

3.5.4 Quantification of nitrifying bacteria

Suppression of NOB growth or activity while maintenance and performance of AOB is necessary for achieving successful PN (Turk & Mavinic, 1989; Jianlong & Ning, 2004; Ciudad et al., 2007; Sinha & Annachhatre 2007; Blackburne et al., 2008; Pérez et al., 2014; Ma et al., 2015; Wang et al., 2016; Cui et al., 2019). The AOB and NOB populations in the biofilms attached to the MBBR carriers were hence quantified under steady state operation of the elevated loaded PN MBBR system to elucidate the mechanism of NO_2^- suppression in the system. The AOB population is shown to be the dominant nitrifying bacteria in the PN MBBR compared to the NOB population (AOB - amoA: $8.3 \pm 0.2 \times 10^7$ cells/g biofilm; NOB - *Nitrospira*: $2.4 \pm 0.3 \times 10^7$ cells/g biofilm; NOB - *Nitrobacter*: non-detect). As shown in previous wastewater treatment systems, very few *Nitrobacter* cells were found in the PN MBBR system, so *Nitrobacter* cells were ignored in this research (Kindaichi & Okabe, 2004; Desloover et al., 2011; De et al., 2013; Gilbert et al., 2014; Gilbert et al., 2015; Cao et al., 2017; Gustavsson et al. 2020). The number of AmoA gene targets of the AOB population is statistically distinct to the targeted gene region of the *Nitrospira* NOB population (P=0.002).

Table 3.6 Summary of quantitative analysis of nitrifying bacteria

Technology	Analytical Methods	Partial Nitritation (PN) or Full Nitrification (FN)	AOB/NOB	Citation
MBBR	ddPCR	PN	3.4	This study
MBBR	FISH	PN	5.4	Abzazou et al., 2016
MBBR	FISH	PN	3.3	Gustavsson et al., 2020
MBBR	qPCR	PN	3.7	Miao et al., 2016
MBBR	qPCR	PN	2.9	Li et al., 2019
MBR	MPN	FN	0.2	Canziani et al., 2006
AS	qPCR	PN	1.6-7.7	Ge et al., 2014
AS	qPCR	PN	3.1	Zhang et al., 2019

AS	qPCR	PN	4.0	Li et al., 2021
AS	qPCR	PN	10.0	Ahn et al., 2011
AS	qPCR	FN	0.5	Ahn et al., 2011

MBR: Membrane biofilm reactor

AS: Activated Sludge

MPN: Most probable number

FISH: Fluorescence in situ hybridization

The ratio of AmoA gene target of the AOB to targeted gene region of the *Nitrospira* NOB population in this research is 3.4 (Table 3.6), which is indicative of the non-statistical difference between the AOB and NOB populations. Hence, it can be concluded that NOB activity suppression is the dominant mechanism for PN in this mainstream MBBR system.

The findings of this study are supported by previous research, where Abzazou et al. (2016) demonstrated that PN was successfully achieved in a partial nitritation MBBR pilot plant with a ratio of AOB to NOB of 5.4, with similar AOB/NOB ratios also being reported in recent wastewater treatment system studies (Table 3.6). Further, Miao et al (2016) studied a PN MBBR system and reported a low AOB/NOB ratio of 3.7 with stable AOB activity and significantly lower NOB activity; 10.4 mg-N/h·gVSS for AOB and 4.0 mg-N/h·gVSS for NOB. Similar results were also found by Li et al. (2021) for the PN of domestic wastewater, where the NOB activity was shown to be significantly inhibited within a system with an AOB/NOB ratio of 4.0.

3.6 Conclusion

Stable nitrite accumulation was successfully observed during the long-term operation of a lab-scale, mainstream, elevated loaded PN MBBR reactor with an average SARR of 2.3 ± 0.2 g TAN/m^2·d (theoretical performance objective of 2.7 g TAN/m^2·d), TAN removal efficiency of 43.1 ± 3.4% (theoretical performance objective of 53%) and NO_2^- / (NO_2^- + NO_3^-) ratio of 82.4 ± 4.8% (theoretical performance objective of 100%) at SALR of 5.2 ± 0.1 g TAN/m^2·d. Biofilm analysis of the laboratory PN MBBR in this study indicated the presence of a stable, robust, thick and dense biofilm with effective cell viability, and without biofilm loss or washout, during long-term operation. The measured AOB/NOB ratio of 3.4 is not indicative of statistical differences between the AOB and NOB populations in the attached growth system, demonstrating that NOB activity suppression as opposed to suppression of growth was the dominating mechanism for the elevated loaded PN MBBR system. The NOB activity is likely to be inhibited by the limited mass transfer in the thick biofilm. This study contributes to the current knowledge of on the development of elevated TAN loading as a design strategy for PN MBBR mainstream treatment.

3.7 Appendix: supporting material

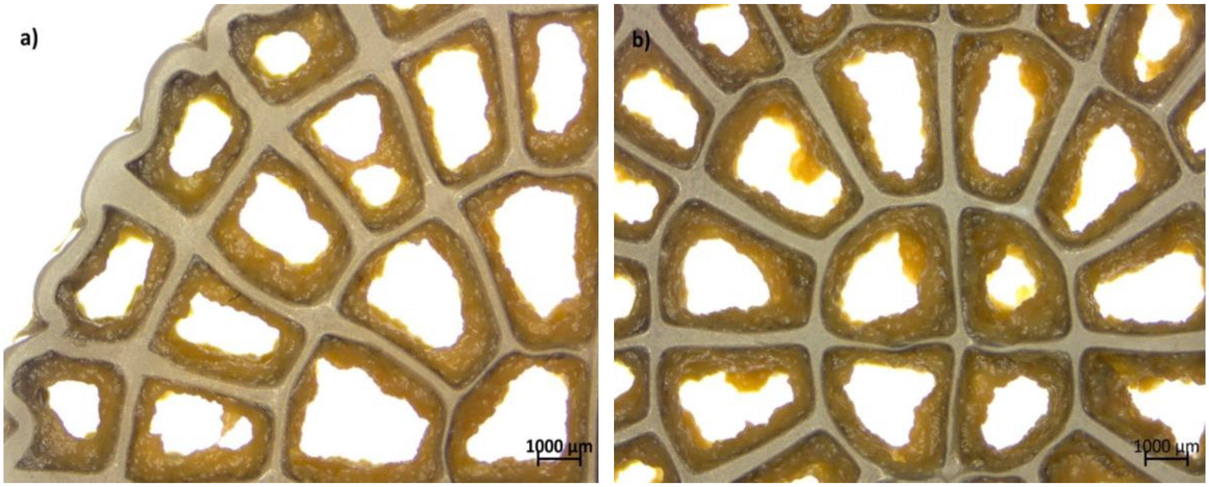

Figure 3.S1 Stereoscope images of partial nitritation biofilm attached to a single K5 carrier sampled on day 45. a) Edge area of the carrier and b) Center area of the carrier.

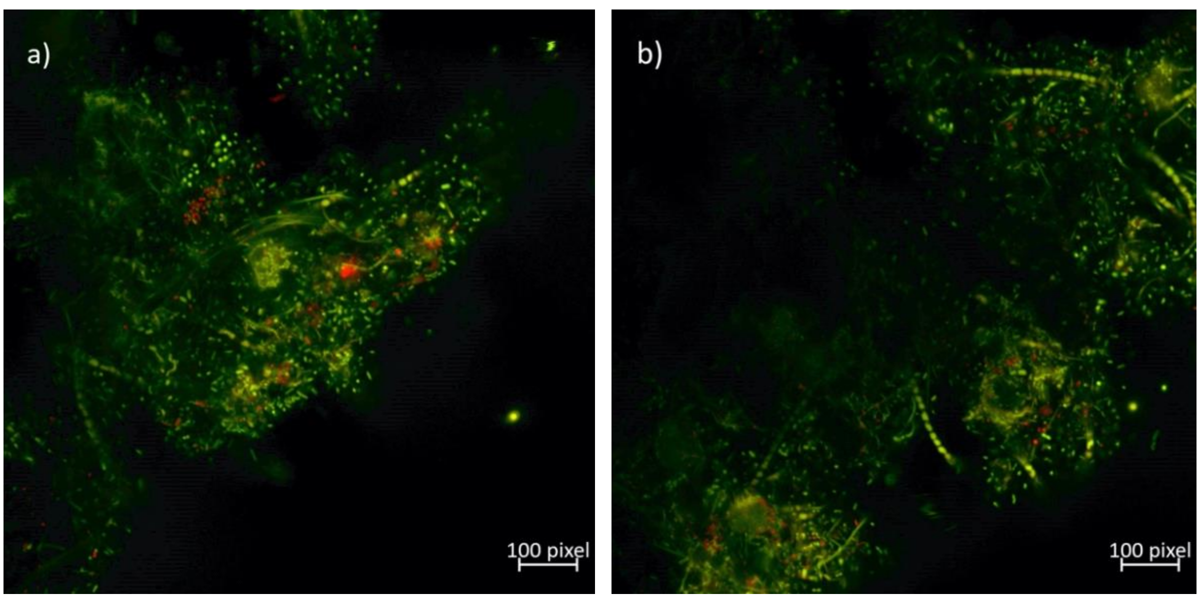

Figure 3.S2 CLSM images of PN biofilm sampled on day 45. Live and dead cells are illuminated green and red respectively.

3.8 Reference

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Chapter 4-Optimal Storage of Nitrifying Biofilm for Treatment of Seasonally Discharged Lagoon Effluent

4.1 Abstract

The recent development and installation of biofilm nitrification systems for ammonia removal from seasonally discharged lagoon wastewater treatment systems necessitates the storage of biofilm carriers for extended periods of time each year during non-discharge periods. This study investigates three nitrifying moving bed biofilm reactor (MBBR) carrier storage strategies to simulate full-installation storage of carriers during the seasonal operation of an add-on nitrifying MBBR unit to a seasonally discharged lagoon wastewater treatment facility. The storage strategies were evaluated to identify the best nitrification performance following storage. As such, carriers harvested from a nitrification/denitrification MBBR pilot system operating as an add-on lagoon treatment system were stored for 6, 12 and 18 weeks under (i) dry conditions, (ii) batch aerated conditions without flow, and (iii) continuous flow aerated conditions. System performance was analyzed following storage of the samples for a start-up period. The dry condition storage carriers did not successfully achieve full nitrification. Batch aerated storage without flow and continuous flow aerated conditions are effective strategies for short-term (6 to 12 weeks) storage of full nitrification MBBR carriers of seasonal operation systems. Batch aerated storage without flow demonstrated to be an effective and economical method to restore full nitrification MBBR carriers even up to 12 weeks of storage.

4.2 Introduction

Waste stabilization ponds (also termed lagoon systems) in North America account for about 50% of existing public municipal water resource recovery facilities (WRRFs) (Jeke *et. al.*, 2019; LeBlond *et. al.*, 2020; D'Aoust *et. al.*, 2021) due to their low cost, low sludge generation, and low energy consumption (Muga & Mihelcic, 2008; LeBlond, 2020). Many small communities apply lagoon systems to treat the municipal wastewater where land is not a limiting factor (Roya *et. al.*, 2020). Although lagoons have been shown to be able to significantly remove common wastewater deleterious substances such as total suspended solids (TSS) and biological oxygen demand (BOD), the inherent drawback of total ammonia-nitrogen (TAN) removal poses a significant problem in regard to modern wastewater treatment regulations in many countries (Van *et. al.*, 2003; Crites *et. al.*, 2006). This is especially true in some northern and cold climate countries, where low wastewater temperatures in lagoons during winter operation decreases the metabolic activity of nitrifying bacteria communities and severely restricts nitrification (Krkosek *et. al.*, 2012; D'Aoust *et. al.*, 2021). Nitrification kinetics are significantly reduced at low temperatures as the metabolism of ammonia-oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are temperature-sensitive (Hwang, & Oleszkiewicz, 2007; Ducey*et. al.*, 2010).

Several upgrade technologies have been applied to enhance nitrogen removal of lagoons to meet the requirements of rapidly increased populations while meeting strict wastewater effluent regulations. For instance, Wang *et. al.* (2012) used a combination of a surface area media made of polyethylene fibres and an upgraded fine-bubble aeration system to improve BOD and TAN removal performance. Biological aerated filtration (BAF) biological technology has been applied to upgrade the performance of the traditional lagoon systems in TAN removal (Spellman, 2014). Submerged attached growth reactor (SAGR) has shown remarkable performance in upgrading lagoon systems for nitrification at near freezing temperatures (Mattson *et. al.*, 2018; Anderson *et. al.*, 2020). Further, Delatolla *et. al.* (2010) used the moving-bed biofilm reactor (MBBR) technology as an upgrade unit to improve the TAN removal efficiency of the lagoon, with Houweling *et. al.* (2007), Hoang *et al.* (2014a; 2014b) and Young *et al.* (2017b) demonstrating the ability of the MBBR system to achieve efficient nitrification and hence TAN removal of lagoon effluent during low wastewater temperature operation.

Although the MBBR technology has been shown to be effective at low temperature nitrification of lagoon effluent, many lagoon systems around the world are designed for seasonal discharge. Hence, the systems intermittently discharge wastewaters during specific time period of the year, usually when the receiving natural waters will experience the least impact from the discharged effluent. Thus, nitrifying MBBR units installed to treat the effluent of seasonally discharged lagoon systems must be designed to operate solely during the discharge period of the lagoon system and remain idle (with the MBBR carriers in a storage condition) during all subsequent periods of operation. As such, it is necessary identify strategies to keep the MBBR system idle and to store the nitrifying MBBR carriers (with the attached nitrifying biofilm) during non-discharge periods of the lagoon system operation for the rapid subsequent start-up of the nitrifying MBBR biofilm during the discharge period. Based on the long re-seeding times of 180 to 240 days for nitrifying MBBR systems (Young *et. al.*, 2017a), re-seeding the nitrifying carriers each discharge period as a start-up strategy is not feasible. An appropriate, simple, cost-effective carrier storage strategy is required to maintain the nitrifying MBBR biofilm for rapid start-up. There are no studies to date that have reported on storage strategies of nitrifying MBBR biofilms.

The objective of the study is to identify storage strategies for nitrifying MBBR lagoon upgrade systems during seasonal periods of non-discharge such that the nitrifying biofilm attached to the MBBR carriers rapidly restarts TAN removal during periods of lagoon discharge. Nitrifying MBBR carriers in this study were stored under three distinct strategic storage conditions at a pilot scale operation of an MBBR system treating lagoon effluent. Subsequent to storage for periods of 6, 12 and 18 weeks, the carriers stored under various conditions were restarted in the laboratory units to simulate periods of lagoon discharge to identify optimal storage conditions for post-lagoon, nitrifying MBBR systems.

4.3 Materials and methods

4.3.1 Study site

The Casselman Lagoon system (CLS), located in Eastern Ontario, was constructed to treat the wastewater of the village of Casselman. The designed average rated flow capacity is 2,110 m^3/day with a peak rated flow capacity of 6,250 m^3/day. The CLS consist of two facultative treatment cells (Cells A and B), an aerated reaction cell (Cell C) and a nitrifying MBBR plant (Cell D) (Figure 4.1). Wastewater is transported to a large capacity pumping station by gravity sewer and then conveyed to the lagoon (Cell A) via an upstream pumping station. The wastewater is treated by passing through Cell A, Cell B and Cell C.

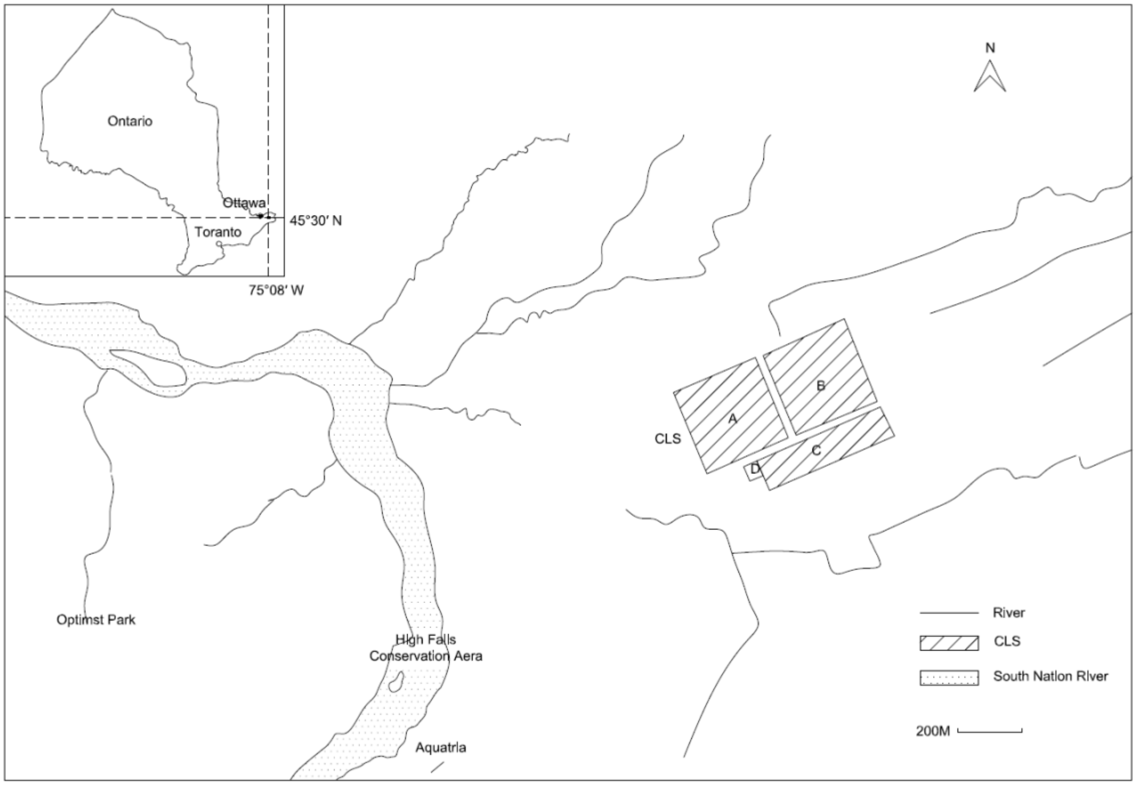

Figure 4.1 Location of the Casselman lagoon system (CLS).

To enhance the TAN removal efficiency and meet the Canadian wastewater systems effluent regulations (WSER) (Canada Gazette, 2012), a pilot nitrifying MBBR system was installed to treat the final lagoon effluent prior to discharge, and hence was fed from Cell C of the lagoon system during discharge. The nitrifying MBBR pilot system was installed and operated at CLS between November 2017 and June 2018. It was operated at an HRT of four hours with surface area loading rate (SALR) of 0.38 g TAN/m^2·d and surface area remove rate (SARR) of 0.21 g TAN/m^2·d.

4.3.2 Storage methods

MBBR carriers were harvested from the nitrifying MBBR and stored under three distinct storage conditions during non-discharging periods of operation of the CLS. As wet storage, dry storage along with batch and continuous flow storage conditions are common bacterial storage methods (Swift, 1921; Stamp, 1947; Lapage *et al.*, 1970; Gherna & Reddy, 2007), combinations of these strategies were tested in this study. In addition to considering conventional methods, practical implications and energy efficiency were considered as well. For example, if wet storage conditions were considered than aeration, intermittent or continual, would be necessary to prevent anaerobic and septic conditions from developing in during storage. The three storage strategies investigated in this study were as follows:

Dry storage (D): Carriers were collected from the pilot nitrifying MBBR system and stored in a container with black plastic share hexagons (Uline, Canada, ON) placed over the container and carriers to prevent ultraviolet degradation of the dry plastic carriers. The use of the plastic coverage simulates conditions that a full-scale system would be exposed to during dry storage. The carriers in the container were stored outdoors, open to seasonal conditions and precipitation to simulate full-scale storage conditions. This storage strategy is a zero energy and zero operating cost strategy.

Batch & aerated storage (BA): Carriers were collected from the pilot nitrifying MBBR system and placed in a second 200L nitrification reactor filled with wastewater from the CLS. This reactor was operated in batch operation with no flow through. Pulse aeration was supplied for five minutes every three hours. This storage strategy is a low energy and low operating cost strategy.

Continuous & aerated storage (CA): Carriers were collected from the pilot nitrifying MBBR system and placed in another 200L nitrification reactor basin. The storage reactor was operated on recirculation loop with cell C of the CLS. The HRT of the system was four hours, and continuous aeration was supplied to the basin. This storage strategy is a moderate energy and moderate operating cost strategy. The characteristics of the influent flow are shown in Table 4.1.

Table 4.1 Characteristics of CLS wastewater during storage conditions, used to fill the BA storage reactor and continuous flow into the CA storage reactor

Component	Average Concentration (mg/L)
TAN	0.55 ± 0.46
Nitrite (NO_2^-—N)	0.24 ± 0.42
Nitrate (NO_3^-—N)	0.24 ± 0. 17
Alkalinity	143.18 ± 42.10
Dissolved oxygen (DO)	0.54 ± 1.14
Chemical oxygen demand (COD)	94.85 ± 56.18
Total suspended solids (TSS)	42.16 ± 62.87
Notes: Temperature:21.68 ± 2.64 °C; pH: 7.12 ± 0.70	

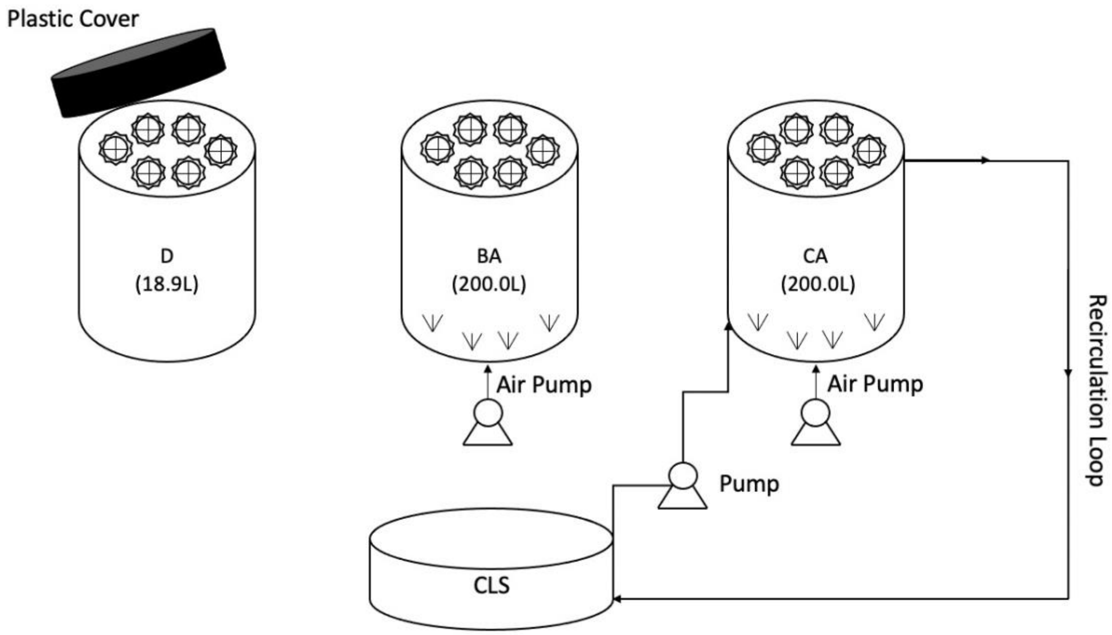

Figure 4.2 Schematic of the storage container and reactors.

4.3.3 Carrier performance post storage

Carriers were collected from the three storage conditions for performance testing post storage after 6, 12 and 18 weeks of storage and were placed in laboratory-scale performance testing nitrifying reactors (Figure 4.2). The post storage, performance testing reactors were 5.4 L continuous flow reactors that were operated at an HRT of four hours each and fed with effluent CLS discharging wastewater. Performance testing experiments were conducted at 22 ± 2°C with the discharged wastewater characteristics shown in Table 4.2.

Table 4.2 Characteristics of CLS discharge wastewater fed to performance testing reactors

Component	Average Concentration (mg/L)
TAN	18.39 ± 2.78
Nitrite (NO_2^-—N)	0.83 ± 1.25
Nitrate (NO_3^-—N)	1.68 ± 0. 79
Alkalinity	210.77 ± 15.43

Dissolved oxygen (DO)	6.92 ± 1.04
Chemical oxygen demand (COD)	36.85 ± 4.45
Total suspended solids (TSS)	112.49 ± 12.37

Notes: Temperature:22.13 ± 0.38 ℃; pH: 7.87 ± 0.20

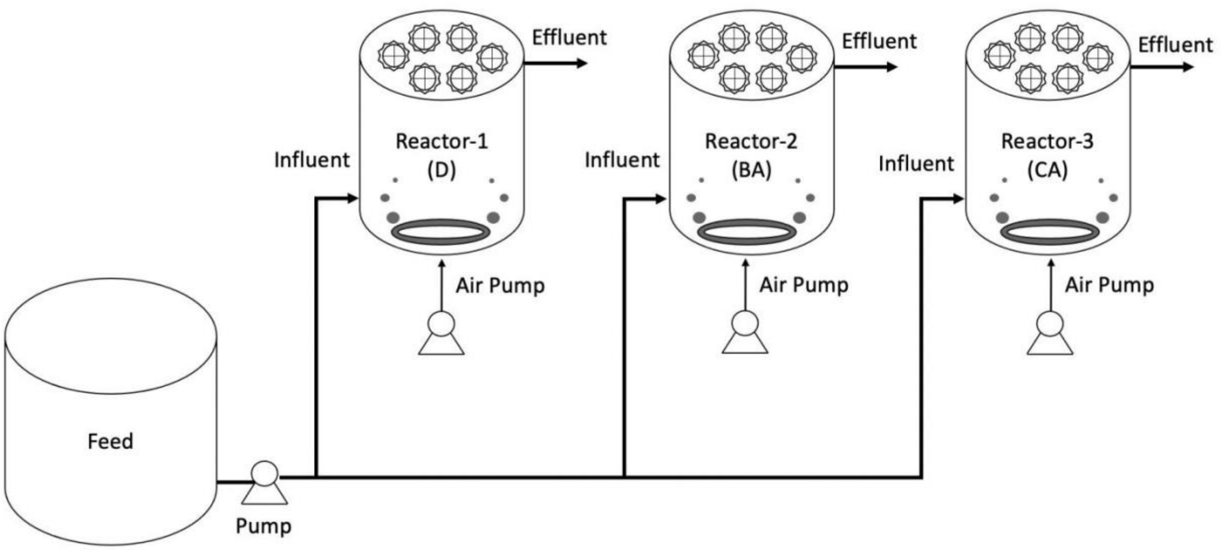

Figure 4.3 Schematic showing laboratory-scale, performance testing reactors of stored carriers harvested from the pilot plant storage reactors after 6, 12 and 18 weeks.

4.3.4 Constituent analysis

Standard methods (APHA, 1998) were used to measure the TAN, nitrite, nitrate, alkalinity, COD and TSS concentrations of the discharged CLS effluent, which also served as the feed to the performance testing reactors. The DO and temperature were measured using a symphony Multi-Parameter Meter with relative probes (VWR, Canada, ON). pH was measured with a pH meter (HACH, US, CO). Before performing the analyses, all samples were filtered through a 0.45 μm filter (Fisher, Canada, ON) using a vacuum filter (Marathon Electric, US, WI)). All samples were analyzed immediately upon being collected and were tested in triplicate.

4.4 Statistical analysis

The Student's *t*-test ($p < 0.05$) was applied to validate the statistical significance between the percentage TAN concentration of the post storage, performance testing reactors/total nitrogen concentration of the CLS discharge wastewater ($TAN/TN_{inf.}$), nitrite concentration of the post storage, performance testing / total nitrogen of the CLS discharge wastewater ($NO_2^-/TN_{inf.}$), and nitrate concentration of the post storage, performance testing reactor/total nitrogen of the CLS discharge wastewater ($NO_3^-/TN_{inf.}$).

4.5 Result and discussion

The $TAN/TN_{inf.}$, $NO_2^-/TN_{inf.}$ and $NO_3^-/TN_{inf.}$ were monitored after 6, 12 and 18 weeks of storage (Figure 4.4). Stable and complete TAN removal (90%) was observed in all three post storage reactors, associated with D, BA and CA storage conditions, after 6 weeks of operation (Figure 4.4a). In particular, stable and complete TAN removal was observed on day 11 after D storage, day 6 after BA storage, and day 10 after CA storage. All three storage conditions resulted in carriers that were successfully re-started for TAN removal, with no statistical difference between the re-start time of the DS and CA storage carriers ($p = 0.45$). As such, the BA practice of storing the carriers provided quickest TAN removal re-start time (six days) after six weeks of storage. Full nitrification (i.e., complete oxidation to nitrate) was successfully achieved in the BA and CA reactors after 6 weeks of storage (Figure 4.4b). Instead of full nitrification, partial nitritation (PN) was only observed after 6 weeks of storage and within 18 days of re-start time by the D stored carriers. Hence, after 6 weeks of storage under D conditions, a pronounced impact on the NOB activity was observed. This is of significance as the discharge of nitrite concentrations may be more strictly restricted due to its higher toxicity to aquatic life when compared to nitrate.

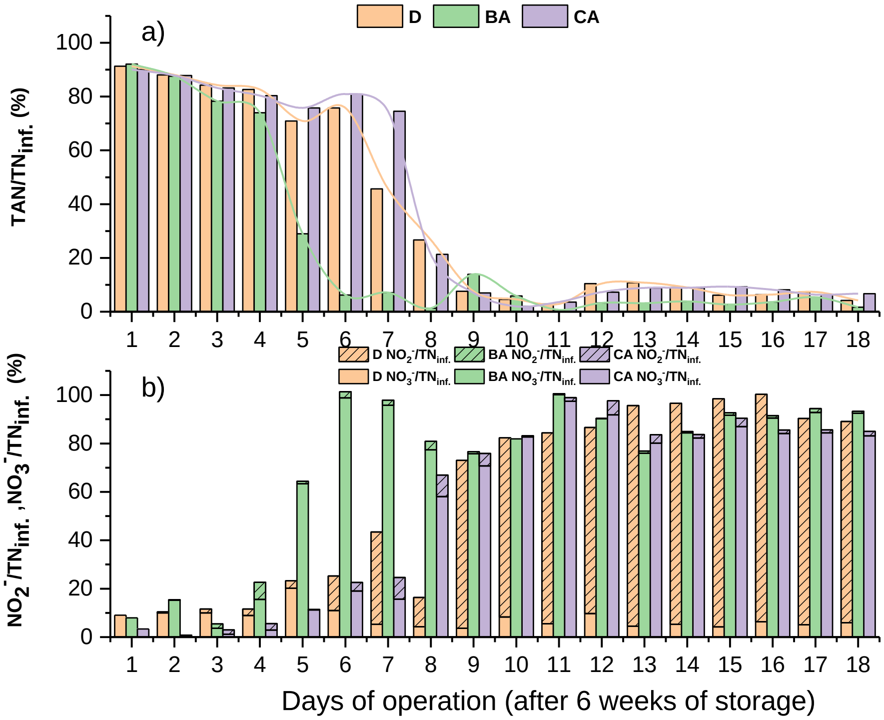

Figure 4.4 Performance testing of the stored carriers after 6 weeks of storage. Percentage of $TAN/TN_{inf.}$, $NO_2^-/TN_{inf.}$, and $NO_3^-/TN_{inf.}$ of the D, BA and CA stored carriers.

After 12 weeks of storage, stable and complete TAN removal (90%) was observed by the D, BA and CA stored carriers on day 12, day 7, and day 13 of performance testing, respectively (Figure 4.5a). As seen after 6 weeks of storage, after 12 weeks of storage the BA stored carriers provided the quickest TAN removal re-start time (seven days). Full nitrification was successfully achieved in the BA and CA reactors within the 18-day performance testing period after storage (Figure 4.5b). Although TAN removal was successfully decreased by the D stored carriers, full nitrification was not observed within the performance testing time period of 18 days for these stored carriers (Figure 4.4b). As such, after 12 weeks of storage, a pronounced impact on the NOB activity of the D stored carriers was again observed. The observed impact of D storage on NOB activity is observed starting after 6 weeks of storage and is again observed after 12 weeks of storage.

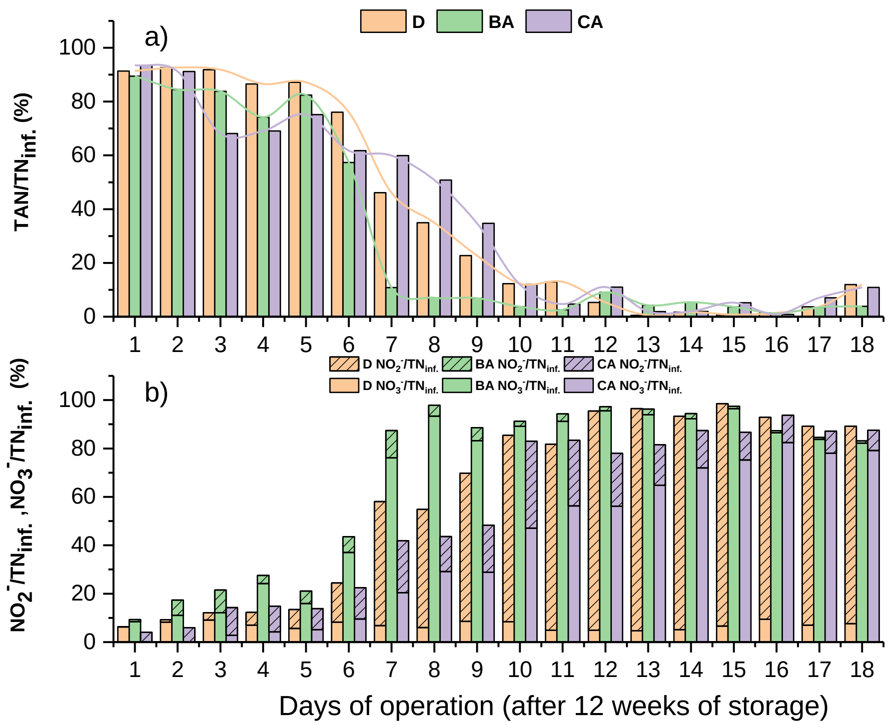

Figure 4.5 Performance testing of the stored carriers after 12 weeks of storage. Percentage of TAN/TN$_{inf.}$, NO_2^-/TNinf., and NO_3^-/TN$_{inf.}$ of the D, BA and CA stored carriers.

After 18 weeks of storage, stable and complete TAN removal (90%) was observed in the D and BA stored carriers (Figure 4.6a). The TAN removal efficiency of these reactors peaked on days 10 and day 11, respectively, and then stabilized on days 14 and 15, respectively. (Figure 4.6a). There was no statistical distinction between the TAN removal performance of the D and BA stored conditions (p= 0.40). During the 18 days of performance testing, stable and complete TAN removal was not observed by the CA stored carriers, which marks an effect of storage time on TAN removal by the CA stored carriers and an effect of 18 weeks of storage time on the activity of the AOB population of the CA stored biofilm. In addition, full nitrification was not observed in all three stored carrier conditions, indicating that NOB active was not activated after 18 weeks of storage at any of the storage conditions applied in this study. Thus, NOB populations present themselves as more susceptible to being impacted during non-discharge seasons.

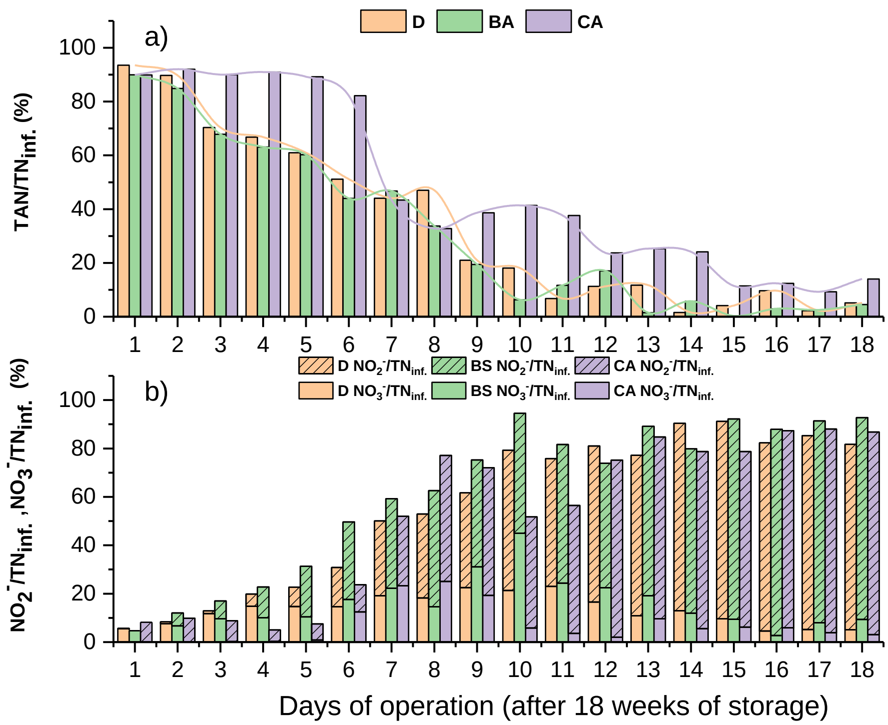

Figure 4.6 Performance testing of the stored carriers after 18 weeks of storage. Percentage of $TAN/TN_{inf.}$, $NO_2^-/TNinf.$, and $NO_3^-/TN_{inf.}$ of the D, BA and CA stored carriers.

The BA stored carriers showed no significant difference between TAN removal after 6 or 12 weeks of storage (*p = 0.33*). The D and CA stored carriers on the other hand showed similar TAN removal results after 6 and 12 weeks of storage ($p = 0.39$, $p = 0.18$, respectively). However, for all storage conditions investigated in this study, carriers stored for 18 weeks took longer to achieve complete TAN removal as compared to those same carriers stored for 6 or 12 weeks. As such, BA storage conditions demonstrated the best performance, however even with the use of this storage condition, the AOB population activity is affected by storage periods in excess of 18 weeks.

Complete nitrification was not observed in the D stored carriers even after 6 weeks of storage, indicating that NOB populations due not recover their activity quickly after storage under dry conditions. This finding is supported by previous studies that showed that PN biomass can be preserved for periods without nitrite oxidation to nitrate (Zhu *et. al.*, 2022), with the AOB population activity being more likely to be activated after storage than NOB population activity. The two wet storage conditions of BA and CA in this study also do not seem to be feasible for the long-term preservation of NOB population activity (longer than 18 weeks) and hence for complete nitrification. Due to the lack of stable preservation methods observed in this study and others

(Vekeman *et. al.*, 2013), a long-term and cost-effective strategy to storing full nitrifying MBBR carriers remains a challenge. This study however provides short-term (6-12 weeks) storage strategies for full nitrification MBBR.

4.6 Conclusion

BA and CA storage methods are effective strategies for the short-term storage of nitrifying MBBR carriers for less than 12 weeks. In particular, the quickest significant TAN removal activity was observed when stored carriers were stored in wastewater with aeration for five minutes every three hours (BA conditions). CA was shown to be the second most suitable storage method for nitrifying MBBR carriers. However, for long-term storage (greater than 18 weeks), D, BA and CA stored carriers were unable to achieve full nitrification following 18 days of re-starting the nitrifying MBBR system. The D storage method is not recommended to be applied to store full nitrification MBBR carriers for short-term and long-term storage.

4.7 Reference

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Chapter 5 – Conclusion

This thesis addresses two specific research goals that address important gaps of knowledge relating to total ammonia nitrogen (TAN) removal from urban, per-urban and rural municipal wastewaters. The first objective was to validate the performance of an elevated loaded partial nitritation (PN) moving bed biofilm reactor (MBBR) system for urban and peri-urban municipal wastewaters to achieve efficient TAN removal and to identify the mechanism of nitrite-oxidation suppression of this system. The second objective was to investigate practical storage strategies for nitrifying MBBR lagoon upgrade systems that are being applied as TAN removal upgrade systems to conventional, rural wastewater treatment lagoon systems. With these storage strategies being evaluated to identify storage conditions that result in the optimal TAN removal performance during seasonal discharge periods.

The elevated loaded PN MBBR technology was confirmed as an applicable system for mainstream TAN removal at urban and peri-urban wastewater resource recovery facilities (WRRFs). Additional conclusions from this work were as follows:

- Average surface area removal rate (SARR) of 2.3 ± 0.2 g TAN/m^2·d, TAN removal efficiency of 43.1 ± 3.4% and NO_2^- / (NO_2^- + NO_3^-) ratio of 82.4 ± 4.8% at surface area loading rate (SALR) of 5.2 ± 0.1 g TAN/m^2·d indicated a stable and successful partial nitritation.
- Biofilm analysis of the elevated loaded PN MBBR system showed that the biofilm was stable and robust, and the cell viability was effective and healthy, which could support an appropriate SARR for subsequent anammox processing.
- The ratio of 3.4 of AmoA gene target of the ammonia oxidizing bacteria (AOB) to the targeted gene region of the Nitrospira nitrite oxidizing bacteria (NOB) population demonstrated that NOB activity suppression as opposed to suppression of NOB growth was the dominant mechanism of nitrite oxidation in the elevated loaded PN MBBR system.

The nitrifying MBBR carrier storage strategy study demonstrated that batch storage of the nitrifying MBBR biofilms with intermittent aeration demonstrated successful short-term (12 weeks) storage. Additional conclusions from this work were as follows:

- For long-term storage (over 18 weeks), carriers stored in dry condition, batch aerated conditions without flow, and continuous flow aerated condition failed to achieve full nitrification following 18 days of operation conditions.
- Carriers stored in dry condition did not successfully achieve full nitrification and cannot be applied to store full nitrification MBBR carriers for short-term and long-term storage.
- Compared to re-seeding the nitrifying MBBR biofilm as a start-up strategy, the carriers stored in batch aerated conditions without flow, and continuous flow aerated condition for less than 12 weeks demonstrated a significantly shorted start-up time.

This research advances the application of the cost-effective PN/Anammox MBBR TAN removal technology for urban and peri-urban municipal WRRFs. The knowledge produced supports future investigation of this technology at the pilot scale. In addition, this research provided new information on how to improve the performance of nitrifying MBBR upgrade units to rural, seasonally discharged, wastewater treatment lagoon systems. It provides new knowledge on addressing the challenge of upgrading conventional rural treatment systems and provides information that can be used to investigate further storage strategies.