Long-term stability of two-stage aerobic granular sludge system for combined organic matter and nitrogen removal from aerobically digested centrate wastewater

by

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Abstract

Nitrogen is an essential element for all living organisms and plays a key role in various biological processes. However, excessive nitrogen levels in the environment can lead to significant ecological issues, including water pollution and eutrophication, resulting in harmful algal blooms, oxygen depletion, and loss of aquatic life. Ammonia is the primary form of nitrogen compound in sewage and requires effective treatment in wastewater treatment plants. High ammonia and high chemical oxygen demand (COD) wastewater pose severe environmental threats, further exacerbating these issues. Effective and energy-efficient nitrogen removal technologies are essential to meet stringent discharge standards.

The overall objective of this thesis is to evaluate a two-stage moving bed biofilm reactor (MBBR) followed by an aerobic granular sludge (AGS) system developed to treat high COD and high ammonia biosolid autothermal thermophilic aerobic digestion (ATAD) centrate. The MBBR efficiently handled high organic loading rates (OLRs) exceeding 20 kg COD/m^3/d and reduced the carbon to nitrogen (C/N) ratios from 6 to 3. Treated effluent was then processed in the AGS system, where nitrogen removal was primarily accomplished via the nitritation/denitritation pathway, with removal efficiencies reaching 98.9% for NH_4^+-N and 91.7% for total inorganic nitrogen (TIN). After 200 days of operation, the AGS system had an optimized hydraulic retention time (HRT) of 10 hours and maintained a sludge volume index (SVI) between 40 to 80 mL/g, achieving a nitrogen treatment capacity of 1.77 kg N/m^3/d. Ammonia oxidizing bacteria (AOB) demonstrated substantial nitrogen conversion efficiency, evidenced by nitrogen conversion rates of 0.57 ± 0.02 g N/g VSS/day and denitritation rates of 1.27 ± 0.01 g N/g VSS/day.

Preface

This thesis is an original work by Lu Kong. All of the studies conducted in this thesis was designed and planned by me and supervised by Professor Yang Liu at the University of Alberta.

Chapter 4: Professor Yang Liu, Xin Zou contributed to the research planning; and Professor Yang Liu contributed to the manuscript edits.

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

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List of Abbreviations

NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
AGS	Aerobic granular sludge
AMO	Ammonia Monooxygenase
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
ATAD	Autothermal thermophilic aerobic digestion
CAS	Conventional activated sludge
COD	Chemical oxygen demand
Comammox	Complete ammonia oxidation
DO	Dissolved oxygen
FA	Free ammonia
FNA	Free nitrous acid
HAO	Hydroxylamine Oxidoreductase
HNO3	Nitric acid
HRT	Hydraulic retention time
MBR	Membrane bioreactor
MBBR	Membrane biofilm bioreactor
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
Nar	Nitrate reductase

NH_3	Ammonia
Nir	Nitrite reductase
NOB	Nitrite Oxidizing Bacteria
Nor	Nitrite Oxidoreductase
Nos	Nitrous oxide reductase
SBR	Sequencing batch reactor
SRT	Solid retention time
SVI	Sludge volume index
TIN	Total inorganic nitrogen
WWTPs	Wastewater treatment plant

Chapter 1 – Introduction

1.1 Background

Ammonia, commonly found in diverse aquatic environments, is integral to the synthesis of nitrogen-based products including fertilizers, cleaning agents, refrigerants, and polymers(Ishaq & Crawford, 2024). While it serves critical industrial and agricultural purposes, ammonia is also associated with significant ecological risks. It is toxic to aquatic organisms and contributes to eutrophication, which can degrade water quality and disrupt aquatic ecosystems. Furthermore, ammonia volatilization into the atmosphere plays a role in the formation of greenhouse gases, adding to its environmental footprint. Regulatory frameworks are essential for minimizing the impact of ammonia emissions. Canadian guidelines for the release of ammonia in wastewater effluents mandate that wastewater facilities maintain ammonia concentrations in water at or below 0.019 mg/L to mitigate its ecological impact (Canada, 1999). In Alberta, the Approval to Operate (#361975-00-00) sets specific seasonal effluent thresholds, limiting ammonium-nitrogen to 5 mg/L during warmer months and 10 mg/L in colder months. Ensuring ammonia is effectively treated and reduced in municipal wastewater before it enters natural waterways is a critical environmental safeguard.

Aerobic granular sludge (AGS) technology is highly regarded for its superior biological nutrient removal (BNR) capabilities and resilience against operational fluctuations. Featuring a compact, granular structure, AGS enhances biomass retention and fosters a diverse microbial environment (Abdullah et al., 2013; X. Liu et al., 2015; Miyake et al., 2022; Sarma & Tay, 2018). These features lead to significant reductions in energy consumption and operational costs, offering advantages over conventional activated sludge (CAS) systems. The granular form also ensures

excellent sedimentation and increased biomass concentration, enabling effective management of sudden high-load impacts (Chen et al., 2024). Moreover, AGS supports the simultaneous removal of organic pollutants, nutrients, and toxic contaminants from wastewater, addressing multiple environmental concerns efficiently. This capability makes AGS an essential tool in modern wastewater management strategies, effectively tackling a wide range of treatment challenges.

Given that chemical oxygen demand (COD) is a crucial component of wastewater, effectively removing both nitrogen and COD simultaneously can greatly enhance the cost-effectiveness and energy efficiency of the biological nutrient removal process. AGS technology is well-suited for treating biosolids aerobically digested centrate wastewater that exhibits high levels of ammonia and COD. In optimizing AGS technology for such applications, it is important to consider the C/N ratio, which significantly impacts the efficacy of nitrogen removal processes. For the treatment of biosolids autothermal thermophilic aerobic digestion (ATAD) centrate with C/N ratios greater than 6, a two-stage treatment approach is recommended. By initially using a moving bed biofilm reactor (MBBR) to reduce biodegradable COD, this configuration stabilizes the C/N ratio, enhancing the subsequent performance of the AGS in nitritation/denitritation processes and optimizing the treatment of high COD and high ammonia wastewater.

1.2 Research objectives

This study evaluated the performance of a lab-scale, two-stage MBBR-AGS system for the simultaneous removal of COD and nitrogen from ATAD centrate wastewater. The present study delineates three principal objectives:

(1) to develop and optimize a two-stage treatment system that integrates an MBBR and an AGS to process high COD and high ammonia wastewater efficiently;

(2) to investigate the nitrogen removal pathways within this system, assessing both the COD and nitrogen removal efficiencies and the long-term operational stability;

(3) to conduct a detailed analysis of the microbial community present within the biofilms, flocs, and granular sludge to understand the microbial dynamics and their contributions to the treatment process.

1.3 Thesis structure

Chapter 1 delineates the background and objectives of this study. Chapter 2 is a literature review of previous studies concerning nitrogen and COD removal. Chapter 3 detailed the experimental methods for analyzing reactor performance and microbial community dynamics, accompanied by an overview of the reactor's operational parameters and the wastewater's characteristics. Chapter 4 details the findings from long-term operations of MBBR and AGS, discussing the reactors' performance and microbial community analysis during the two-stage MBBR-AGS process. Chapter 5 encapsulates the research outcomes and outlines recommendations for future implementation. The results presented in this thesis will be published.

Chapter 2 – Literature Review

2.1 Overview of nitrogen and COD in the environment

Nitrogen is an essential element naturally occurring in the environment and is critical for the growth and reproduction of living organisms. It is found in various forms in wastewater, including ammonia (NH_3), ammonium (NH_4^+), nitrogen gas (N_2), nitrite (NO_2^-), nitrate (NO_3^-), and organic nitrogen (EPA, 1993). The removal of nitrogen from wastewater is typically managed through physical, chemical, and biological methods. Physical treatment techniques such as ammonia stripping, ion exchange, and adsorption are implicated in the retention of organic pollutants, which leads to substantial operational costs and the production of secondary pollutants requiring further treatment (Gupta et al., 2012; Jiang et al., 2022; Priya et al., 2022; Scandelai et al., 2020). Chemical processes like breakpoint chlorination and magnesium ammonium phosphate hexahydrate precipitation, though quick and simple, also entail significant expenditures and result in by-products that necessitate further processing and considerable labour (Aghdam et al., 2021; Huang et al., 2017; Yi et al., 2020). In contrast, biological methods are recognized for their high efficiency in degrading pollutants without generating secondary pollution, establishing them as a mature and preferable approach in nitrogen management.

The most widespread biological method for nitrogen removal from wastewater is the activated sludge process, which combines nitrification and denitrification. The COD content critically affects the performance of this nitrogen removal process. It has been observed that high COD levels detrimentally impact autotrophic nitrification due to the competitive consumption of oxygen by both autotrophic and heterotrophic microorganisms (Zhao et al., 2013). Additionally,

the efficiency of the denitrification process is closely dependent on the carbon to nitrogen (C/N) ratio, which provides essential organic carbon for the denitrifying bacteria to function effectively.

2.2 Complete nitrification and denitrification

Nitrification is a two-stage aerobic biological process that converts ammonia first to nitrite and then to nitrate. This conversion is facilitated by two types of aerobic chemoautotrophs: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). These bacteria are distinct from heterotrophic bacteria that consume organic materials, as chemoautotrophs use carbon dioxide as their carbon source and inorganic compounds as their energy source for the growth (Tchobanoglous et al., 2014). Complete nitrification consists of five sub-steps:

Step 1: $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$ (Equation 1)

Step 2: $NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$ (Equation 2)

Step 3: $0.5O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (Equation 3)

Σ: $NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O$ (ΔG=-274.91KJ/mol N)

Step 4: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ (Equation 4)

Step 5: $0.5O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (Equation 5)

Σ: $NO_2^- + 0.5O_2 \rightarrow NO_3^-$ (ΔG=-74.16KJ/mol N)

Fig. 1 illustrates the two stages of the nitrification process, highlighting the specific enzymes and oxygen needs associated with each step. In AOB, the enzymes ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO) catalyze the conversion of NH_3 to NO_2^-. Meanwhile, NOB utilizes nitrite oxidoreductase (NOR) to convert NO_2^- to NO_3^-.

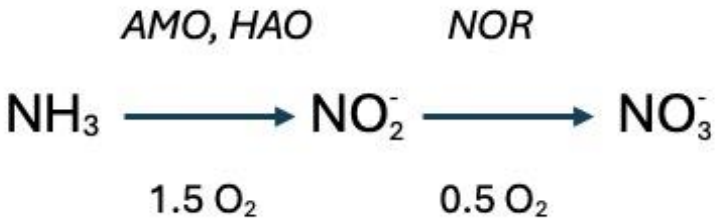

Fig. 1. Complete nitrification diagram. Key enzymes include AMO for ammonia monooxygenase, HAO for hydroxylamine oxidoreductase, and NOR for nitrite oxidoreductase.

Denitrification is an anoxic biological process that sequentially reduces nitrate and/or nitrite to nitrogen gas. This process is initiated by converting nitrate to nitrite, which is then reduced to nitric oxide (NO), followed by nitrous oxide (N_2O), and finally to nitrogen gas (N_2) (Fig. 2). This pathway mitigates nitrogen accumulation in aquatic and terrestrial ecosystems, thereby preventing eutrophication. Denitrification differs from nitrification in that it is conducted by a wide variety of heterotrophic bacteria rather than specific chemoautotrophs. Denitrifying microorganisms require a source of available carbon. The denitrification process can stall at the N_2O stage if carbon is limited. These heterotrophs are capable of using a diverse array of carbon sources to facilitate denitrification, including organic compounds present in both domestic and industrial wastewater, as well as simpler substances such as methanol, ethanol, acetic acid, and sugars.

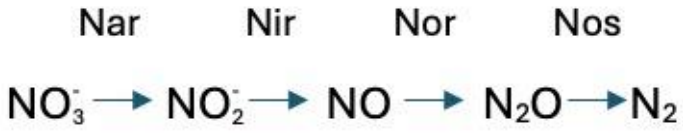

Fig. 2. Complete denitrification diagram. Nar is nitrate reductase, Nir is nitrite reductase, Nor is nitric oxide reductase, and Nos is nitrous oxide reductase.

2.2.1 Nitrifying organisms

As previously discussed, nitrification is a critical biological process within the nitrogen cycle, consisting of two key stages: ammonia oxidation and nitrite oxidation.

2.2.1.1 Ammonia oxidation

The oxidation of ammonia to nitrite is facilitated by:

(1) Ammonia oxidizing bacteria (AOB)

The seminal study that first identified AOB involved the isolation of *Nitrosomonas europaea* from the β-subclass of *proteobacteria*, establishing it as the archetype for AOB in nitrification research (Han et al., 2018; Koops et al., 2006). AOB are divided into two principal phylogenetic classes: *γ-Proteobacteria* and *β-Proteobacteria*. The *γ-Proteobacteria* class, represented by *Nitrosococcus*—a purple sulfur bacterium largely found in marine environments (family Chromatiaceae)—is generally not relevant to wastewater treatment due to its marine nature. In contrast, the *β-Proteobacteria* class, which includes *Nitrosomonas* and *Nitrosospira* (family Nitrosomonadaceae), plays a significant role in wastewater treatment systems due to its effectiveness in such environments (Könneke et al., 2005; Koops et al., 2006).

(2) Ammonia oxidizing archaea (AOA)

Archaeal ammonia monooxygenase (AMO), encoded by all studied AOA, catalyzes the aerobic oxidation of ammonia to hydroxylamine, initiating the nitrification process (Simon & Klotz, 2013). Despite ongoing research, the enzyme responsible for converting hydroxylamine to nitrite, presumably an archaeal hydroxylamine oxidoreductase (HAO), remains unidentified, along with

its cofactors (Radniecki & Lauchnor, 2011). AOA is identified within the mesophilic *Crenarchaeota* and a newly proposed lineage known as *Thaumarchaeota*, spanning two broad phylogenetic groups with origins in aquatic environments and soil/sediment (Tchobanoglous et al., 2014). AOA tend to have a greater affinity for oxygen than AOB, and the concentration of dissolved oxygen (DO) plays a pivotal role in their effectiveness in eutrophic wastewater treatment settings. Studies have shown that marine AOA cultured in isolation can uphold substantial ammonia oxidation activity even when oxygen levels are below 10 μM (Yang et al., 2021). This resilience and efficiency in low-oxygen environments suggest that AOA could play a significant role in improving nitrogen removal processes in various aquatic treatment systems.

(3) Complete ammonia oxidizing bacteria (Comammox)

The recent discovery of the complete ammonia oxidation (Comammox) process has revised the traditional understanding that nitrification requires a rigid two-step sequence (Daims et al., 2015). Comammox involves the transformation of ammonia directly into nitrate by a single organism within the *Nitrospira* genus, known specifically as comammox *Nitrospira* (Lawson & Lücker, 2018). Optimal conditions for the proliferation of comammox *Nitrospira* include environments with low dissolved oxygen (DO) and ammonia levels or those with limited nitrite availability and prolonged solids retention times (SRTs) (Roots et al., 2019). The competitive interactions for ammonia between traditional AOB and Comammox organisms are crucial in promoting nitritation, a key process for streamlined nitrogen removal strategies (Izadi et al., 2021).

Investigations reveal that AOB predominates in wastewater treatment facilities with high ammonia concentrations, whereas environments with low ammonia and limited nutrients tend to favor AOA and comammox bacteria (Kits et al., 2017; M. Zheng et al., 2019).

2.2.1.2 Nitrite oxidation

Following the oxidation of ammonia, the resultant nitrite is further oxidized to nitrate, a step predominantly facilitated by nitrite oxidizing bacteria (NOB). The seven established genera of NOB include *Nitrobacter*, *Nitrotoga*, *Nitrococcus*, *Nitrospira*, *Nitrospina*, *Nitrolancea*, *and Candidatus Nitromaritima*, spanning four bacterial phyla: *Proteobacteria*, *Chloroflexi*, *Nitrospinae*, and *Nitrospirae* (Daims et al., 2016; Watson & Waterbury, 1971). In wastewater treatment plants (WWTPs), the genus *Nitrospira* is the predominant NOB. Additionally, *Nitrotoga* has recently been acknowledged as an important contributor to nitrite oxidation within these engineered systems (Daims et al., 2001; Hüpeden et al., 2016; Kruse et al., 2013). Particularly in industrial WWTPs, NOB activity tends to be unstable. Interruptions in the nitrite oxidation process can result in substantial ecological damage if nitrite unintentionally enters natural waterways.

2.2.2 Denitrifying organisms

Bacteria capable of denitrification include both heterotrophic and autotrophic types. Denitrifying microorganisms consist of bacterial groups such as *Bacillus*, *Enterobacter*, *Micrococcus*, *Pseudomonas*, *Spirillum*, *Proteus*, *Aerobacter*, and *Flavobacterium*. Most of these bacteria are facultative anaerobic chemoheterotrophs, which utilize organic compounds as electron donors and carbon sources, and use nitrate as the terminal electron acceptor (Meng et al., 2014).

2.2.2.1 Heterotrophic denitrifying organisms

Several studies have reported that the common heterotrophic/anoxic denitrifying genera in wastewater treatment include *Acidovorax*, *Thermomonas*, *Defluviimonas*, *Gemmobacter*, *Thauera*, *Zoogloea*, and *Azoarcus* (Holmes et al., 2019). Additionally, the heterotrophic bacterium Paracoccus pantotropha has been extensively studied for its ability to simultaneously oxidize ammonia and reduce nitrate (Tchobanoglous et al., 2014).

2.2.2.2 Autotrophic denitrifying organisms

Autotrophic denitrifiers utilize CO_2 or bicarbonate as their carbon source. Several autotrophic bacteria have been found to reduce nitrate or nitrite and oxidize various electron donors. These include zero-valence iron and Fe (II) by *Paracoccus ferrooxidans*, *Paracoccus denitrificans*, *P. pantotrophus*, and *P. versutus* reduced sulfur compounds by *Thiobacillus denitrificans* and ammonia by *Nitrosomonas eutropha*, *Nitrosomonas europaea*, and *Nitrosolobus multiformis* (Tchobanoglous et al., 2014).

2.3 Nitritation and denitritation

Nitritation refers to the conversion of nitrogen from ammonium into nitrite, facilitated by aerobic AOB. Denitritation is the process where biological reduction converts nitrite into nitrogen gas and other nitrogenous gaseous byproducts. The advancement of nitritation-denitritation processes during the 1990s was propelled by the need to lower energy and chemical consumption in the treatment of high strength sidestream nitrogen (Tchobanoglous et al., 2014).

2.3.1 Achieving nitritation and denitritation

The key to achieving nitritation-denitritation is to control the oxidation of ammonia to nitrite instead of nitrate and to accumulate nitrite. This requires inhibiting or eliminating NOB while allowing AOB to dominate as the primary nitrifying bacteria. Several parameters, such as pH, temperature, dissolved oxygen (DO), sludge retention time (SRT), free ammonia (FA), and free nitrous acid (FNA), have been reported to influence the growth kinetics of AOB and NOB, which are crucial for achieving nitritation (Qian et al., 2016).

2.3.1.1 pH

For optimal nitrification, the pH should be maintained between 7.5 and 8.0, whereas denitrification works best within a pH range of 7 to 9 (Tchobanoglous et al., 2014). When pH drops below 7, nitritation efficiency can decrease by 80-90%. Additives like sodium bicarbonate or lime can be used to keep pH within the ideal range. Pure cultures of AOB grow best within a pH range of 5.8 to 8.5, while NOB cultures thrive between 6.5 and 8.5 (Claros et al., 2013). Nitrification is inhibited at pH levels below 6.5 or above 8.9. The optimal pH ranges for AOB are 7.5 to 8.0 or 7.4 to 7.8, and for NOB, it is 7.6 to 7.8 (Holmes et al., 2019). Thus, solely adjusting pH to eliminate NOB is not feasible. Moreover, pH influences FA and FNA levels, which in turn affect nitritation (Qian et al., 2016).

2.3.1.2 DO

The concentration of dissolved oxygen (DO) plays a pivotal role in the effectiveness of nitritation-denitritation processes in wastewater treatment. AOB have an oxygen half-saturation coefficient of 0.2-0.4 mg/L, whereas NOB have a coefficient of 1.2-1.5 mg/L (Tchobanoglous et

al., 2014). This indicates that AOB possess a higher oxygen affinity, allowing them to dominate over NOB under low DO conditions, which is essential for achieving nitritation. Nitritation has been successfully achieved at DO concentrations of 1.4 mg/L (Ciudad et al., 2005), and DO ranges between 0.5 mg/L and 1.5 mg/L have been found suitable for partial nitrification (Ruiz et al., 2006). Optimal DO levels reported in some studies range from 1.5 to 2.0 mg/L, suggesting that a DO concentration between 0.5 and 2.0 mg/L may be ideal for this process (Ge et al., 2014). Additionally, elevated DO concentrations can hinder denitrification because enzymes such as nitrite reductases (Nir) and nitrous oxide reductases (Nos) are sensitive to DO, causing the buildup of harmful NO and N_2O. Thus, maintaining a low DO concentration (0.5-1 mg/L) is not only beneficial for promoting nitritation but also enhances energy efficiency in WWTPs (Ge et al., 2015).

2.3.1.3 SRT

Sludge retention time (SRT) significantly influences nitritation efficiency. The minimum doubling time for AOB is 7-8 hours, which is shorter than the 10-13 hours required for NOB (Soliman & Eldyasti, 2018). Adjusting the SRT can therefore impact the composition of the microbial community. Research indicates that a short SRT (less than 2 days) can successfully wash out NOB, while also promoting nitrite production (Hellinga et al., 1998). To effectively suppress NOB, the chosen SRT should be longer than their minimum reproduction time, and this should be coupled with specific DO conditions (Reino & Carrera, 2021). However, if the SRT is extremely long or if sludge is not regularly discharged, factors such as DO and temperature may not adequately inhibit NOB, and additional measures may be necessary to control their growth.

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

AOB and NOB activities can be inhibited by high ammonia or nitrite levels, commonly found in aerobic digesters and high-ammonia wastewater treatments like anaerobic digester centrate return and animal feedlots. The inhibitors are un-ionized ammonia (NH_3-N) and un-ionized nitrous acid (HNO_2), with their concentrations depending on reactor pH, temperature, total ammonia nitrogen (TAN) for NH_3-N, and NO_2-N for HNO_2. The concentrations of FA and FNA can be estimated using Equations 6 and 7.

$$\text{FA as } NH_3\text{-N} = \frac{TAN(10^{pH})}{\left(\frac{1}{K_a}\right)+10^{pH}} \qquad \text{(Equation 6)}$$

Where $\frac{1}{K_a} = e^{[\frac{6334}{273+T}]}$

$$\text{FNA as } HNO_2\text{-N} = \frac{NO_2-N}{K_n(10^{pH})} \qquad \text{(Equation 7)}$$

Where $K_n = e^{[-\frac{2300}{273+T}]}$

Inhibiting NOB activity using FNA in wastewater is a well-known method. Research has shown that FNA concentrations of 0.42–1.72 mg HNO_2-N/L can reduce AOB activity by 50%. However, NOB can be inhibited at much lower concentrations of 0.011–0.07 mg HNO_2-N/L and completely inhibited at concentrations of 0.026–0.22 mg HNO_2-N/L (Zhou et al., 2011). FA inhibits the oxidation of ammonium and nitrite ions during nitritation. NOB activity is inhibited by FA at concentrations of 0.1–1.0 mg NH_3-N/L, while AOB activity is inhibited at higher concentrations of 10–150 mg NH_3-N/L (Liu et al., 2019). Therefore, FA concentrations of 1–10 mg NH_3-N/L are considered optimal for nitritation, with studies indicating that concentrations of 5–10 mg NH_3-N/L effectively inhibit NOB without affecting AOB activity (Sui et al., 2016).

2.3.2 Nitritation/denitritation in granular sludge reactors

Aerobic granular sludge (AGS) technology has been recognized for its superior biological nutrient removal (BNR) efficiencies and exceptional resilience to operational shocks, such as load fluctuations (Abdullah et al., 2013; X. Liu et al., 2015; Miyake et al., 2022; Sarma & Tay, 2018). Its dense, granular sludge structure enhances robust biomass retention and supports a diverse microbial community, contributing to significant reductions in energy requirements and operational costs compared to conventional activated sludge (CAS) systems (Chen et al., 2024; Hussain et al., 2024). This innovative technology promotes the growth of ammonia oxidizing bacteria (AOB) and denitrifiers, facilitating the concurrent reduction of nitrogen and COD. The unique properties of granular sludge make it an ideal and efficient solution for nitritation/denitritation in a single reactor, optimizing nitrogen removal. This innovation reduces the land footprint and capital costs for WWTPs by eliminating the need for separate settling tanks and significantly boosts the performance and reliability of industrial wastewater treatment operations.

2.4 Technologies for high ammonia and high COD wastewater treatment

Current strategies for treating high ammonia and high COD wastewater primarily employ advanced technologies such as membrane bioreactors (MBR), moving bed biofilm reactors (MBBR), and simultaneous nitrification and denitrification (SND). These methods are highly effective and widely used in various wastewater treatment applications.

2.4.1 Membrane bioreactor (MBR)

Membrane bioreactor (MBR) technology revolutionizes wastewater treatment by merging the activated sludge process with advanced membrane filtration. Utilizing microfiltration (MF) or ultrafiltration (UF) membranes with pore sizes between 0.05 and 0.4 μm, MBRs effectively retain almost all suspended solids and biomass within the bioreactor, ensuring superior solid-liquid separation and enhancing overall treatment efficiency. The MBR has been reported to effectively handle high ammonium nitrogen (NH_4^+-N) concentrations up to 1000 mg/L, with COD levels increasing from 0 to 2000 mg/L and a C/N ratio of up to 2. As the C/N ratio increased, the relative abundances of both AOB and nitrite-oxidizing bacteria NOB involved in the nitrification process gradually declined (Xu et al., 2021).

2.4.2 Moving bed biofilm reactors (MBBR)

Due to early challenges with biofilm reactors, such as hydraulic instability and uneven biofilm distribution, moving bed biofilm reactor (MBBR) technology was developed (Di Biase et al., 2019). The MBBR system features an aeration tank with special plastic carriers that provide a surface for biofilm growth. These carriers come in various shapes and sizes, each with unique benefits and drawbacks. A large internal surface area is essential for effective biofilm formation as it enhances contact with water, air, bacteria, and nutrients (Mazioti et al., 2017). The aeration system mixes the carriers in the tank, ensuring good interaction between the wastewater substrate and the biomass on the carriers. The MBBR has been demonstrated to effectively manage NH_4^+-N concentrations ranging from 500 to 1,000 mg/L, with COD levels increasing from 4,000 to 8,000 mg/L and a C/N ratio reaching up to 8, with a hydraulic retention time (HRT) of 5 days.

Microbial analysis revealed that *Acinetobacter*, *Pseudomonas*, and *Paracoccus* were the primary bacteria involved in nitrogen removal.

2.4.3 Simultaneous nitrification and denitrification (SND)

Simultaneous nitrification-denitrification (SND) enables complete nitrogen removal within a single bioreactor under certain operating conditions. This method contrasts with the conventional process of sequential nitrification and denitrification, which are usually conducted in separate bioreactors at WWTPs (Di Capua et al., 2022). SND serves as an effective and promising alternative to pre-denitrification in WWTPs for simultaneously removing carbon and nitrogen, offering several benefits: (1) it reduces carbon demand and sludge production by over 30% (Ma et al., 2017) (2) denitrification provides alkalinity, maintaining a neutral pH, (3) it eliminates the need for nitrate recirculation, (4) it requires less energy for aeration and (5) it necessitates a smaller footprint (Zinatizadeh & Ghaytooli, 2015). SND has proven to effectively handle NH_4^+-N concentrations between 2000 and 2500 mg/L, with COD levels ranging from 10,000 to 15,000 mg/L and a C/N ratio of up to 7, operating with an HRT of 8 days. A consortium of artificial microbial consortia, with *Bacillus haynesii*, *Bacillus subtilis*, and *Sphingobacterium sp*. as the core members, was developed to exhibit excellent simultaneous nitrification and denitrification capabilities (Ke et al., 2024).

2.5 Operation strategy for ATAD centrate wastewater

2.5.1 Characteristics of ATAD centrate wastewater

Autothermal Thermophilic Aerobic Digestion (ATAD) is a high-temperature sludge treatment technology that operates between 55°C and 65°C, using heat generated from microbial

metabolism. This process rapidly degrades organic matter, reduces sludge volume, and inactivates pathogens, making the treated sludge safer. ATAD is recognized for its simplicity and cost-effectiveness in stabilizing various types of organic waste. (Martín et al., 2018; Tashiro et al., 2018). ATAD sludge centrate exhibits several distinct characteristics which are critical for both operational efficiency:

1. Elevated temperature: The centrate typically maintains high temperatures, generally between 50°C to 70°C, due to the thermophilic conditions inherent in the ATAD process.

2. Pathogen reduction: The elevated temperatures in ATAD effectively reduce pathogen levels, resulting in a centrate with significantly lower concentrations of harmful microorganisms, enhancing its safety for handling and disposal.

3. Organic matter degradation: ATAD is highly efficient in degrading organic compounds, leading to a centrate with reduced concentrations of organic matter. This reduction improves the stability of the centrate and decreases its potential environmental impact.

4. Ammonia concentration: The centrate from ATAD processes often contains elevated levels of ammonia. This is a result of the thermophilic degradation of nitrogenous organic matter, which releases ammonia as a byproduct.

5. Nutrient content: The nutrient profile of ATAD sludge centrate includes significant concentrations of nitrogen and phosphorus. While these nutrients can be beneficial for

agricultural reuse, they may also necessitate further treatment to prevent environmental contamination if discharged into water bodies (Liu et al., 2013).

6. Odor control: One of the advantages of the ATAD process is the significant reduction in odorous compounds, attributed to the effective breakdown of volatile organic substances under thermophilic conditions (Martín et al., 2018).

7. Solids content: The centrate may still contain dissolved and suspended solids that need to be managed through additional treatment or filtration processes to meet regulatory discharge standards (Liu et al., 2011).

When the ATAD system is not performing effectively, the centrate can have extremely high COD (Chemical Oxygen Demand) levels, leading to wastewater that is exceedingly difficult to treat due to the combined presence of high ammonia and COD concentrations. This presents significant challenges for wastewater treatment facilities and may require advanced treatment methods to meet discharge regulations.

2.5.2 Impact of C/N ratios

Previous studies have indicated that the presence of high levels of COD can adversely impact the autotrophic nitrification process. This is primarily due to the competition for oxygen between autotrophic nitrifying bacteria and heterotrophic microorganisms. High COD levels provide a substrate for heterotrophs, which can outcompete autotrophs for oxygen, thereby inhibiting the efficiency of the nitrification process (Zhao et al., 2013). The C/N ratio is a key factor affecting granule formation, size, and stability (Wang et al., 2020). Studies have used a high C/N ratio (~8)

to cultivate granular sludge but this can negatively impact nitrogen removal (Hamza et al., 2019; L. Wang et al., 2020). Conversely, low C/N ratios, such as 1, have been linked to granule disintegration (Luo et al., 2014).

2.5.3 Two-stage MBBR-AGS configuration

The MBBR has proven effective for treating wastewater with COD levels from 1500 to 8000 mg/L, achieving efficient treatment within HRT ranging from 7 hours to 5 days (Phan et al., 2022; Zhang et al., 2020). By initially using an MBBR to reduce biodegradable COD, this configuration stabilizes the C/N ratio, enhancing the subsequent performance of the AGS in nitritation/denitritation processes and optimizing the treatment of high COD and high ammonia wastewater (Ahmadi et al., 2023; Kamilya et al., 2023).

Chapter 3 – Methodology

3.1 Experimental set-up

3.1.1 Process design

The study was conducted in a two-stage configuration. Raw wastewater was fed into a 6 L MBBR reactor. This reactor was operated under continuous aerating as pre-treatment to accomplish the removal of COD. Then the effluent of the MBBR reactor was fed into a 4.5L AGS reactor (diameter: 11cm, height: 63cm). This reactor was operated under aerobic/anoxic conditions to achieve nitrogen removal via nitritation/denitritation process. The system was operated for more than 200 days at 20 °C to monitor its performance on a long-term basis.

3.1.2 Wastewater collection and characteristics

Autothermal thermophilic aerobic digestion (ATAD) centrate wastewater used in this study was regularly sampled from a full-scale wastewater treatment plant (WWTP) in Canada, with three distinct batches employed. These batches were collected onsite and preserved in a cold room at 4° C to reduce microbial activity before bioreactor treatment. The basic characteristics of the three different batches of wastewater are shown in Table 1.

Table 1. The physicochemical characteristics of three different batches of wastewater.

	pH	NH_4^+-N (mg/L)	COD (mg/L)	COD/ NH_4^+-N ratios	Alkalinity (mg/L)	TN (mg/L)
Batch 1	7.5	805-850	5000-5800	5.9-7.2	1995-2010	1050-1080
Batch 2	7.5	780-810	4400-4600	5.5-5.9	1700-1770	990-1010
Batch 3	8.3	725-740	3200-3400	4.4-4.7	1980-2010	975-995

3.1.3 Operation of the MBBR reactor

The MBBR was filled with polyethylene biocarriers, occupying 50% of the reactor's apparent volume. These carriers are cylindrical, with dimensions of 15mm in diameter and 9 mm in height and offer an effective specific surface area of approximately 463 m^2/m^3. During phase 1 (days 1-49), a 6-hour HRT was applied to cultivate biofilm on the carriers with wastewater from batch 1. In phases 2 and 3, covering days 50 to 130 and 131 to 200 respectively, a reduced 4-hour HRT was employed for the wastewater from batches 2 and 3. Air was pumped and diffused from the bottom of the reactor to supply oxygen to the reactor. An airflow meter was used to control the airflow precisely. During the reaction, the dissolved oxygen (DO) in the reactor was maintained at 2.2-2.5 mg/L by an air flowmeter. A ribbon impeller was installed to guarantee the effective mixing of carriers in the reactor.

3.1.4 Operation of the AGS reactor

The seeding sludge of the AGS reactor was obtained from the waste sludge of a lab-scale AGS cultivation reactor treating ammonia rich wastewater for a fast reactor startup (Zou, Gao, et al., 2024). The initial seed sludge concentration was ~7.0 g/L mixed liquor volatile suspended solids (MLVSS). During process optimization, the operational cycle of the AGS reactor was gradually shortened from 12 h to 8 h to 6 h to 4 h. The system was controlled using timers. During the reactor operation, the feeding and discharging lasted 10 min. Settling times were 30 min for Stages 1-3 and reduced to 10 min for Stage 4. The anoxic period was reduced from 6 hours to 1 hour, and the aerobic period was reduced from 7 hours to 2.5 hours. In the start-up phase, raw water was used as the external carbon source, and from Stage 1 to Stage 4, methanol was used as the carbon source. The influent pH was adjusted to between 7.5 and 8.0 by adding $NaHCO_3$.

3.1.5 MBBR-AGS system operation conditions

Detailed in Table 2 is a compilation of the OLR for the MBBR, the nitrogen loading rate (NLR) for the AGS, and the ratio of bCOD to NH_4^+-N in the influent, categorized by various operational stages. Throughout the stages, the system experienced an overall decrease in OLR from an initial 22.4±0.69 kg COD/m³/d during startup to 19.9±0.40 kg COD/m³/d by Stage 4. Conversely, the NLR increased from 0.67±0.014 kg N/m³/d to 1.77±0.0065 kg N/m³/d, reflecting an optimization of nitrogen removal. The bCOD/ NH_4^+-N ratio saw a declining trend, starting at 5.03±0.39 and ending at 2.86±0.14.

Table 2. MBBR-AGS operation conditions.

Stages	Time (days)	OLR of MBBR (kg COD/m^3/d)	NLR of AGS (kg N/m^3/d)	bCOD/ NH_4^+-N ratios
Start-up	1-49	22.4±0.69	0.67±0.014	5.03±0.39
Stage 1	50-85	27.3±0.33	0.63±0.0064	4.41±0.11
Stage 2	86-119	27.4±0.42	0.94±0.0088	4.44±0.10
Stage 3	120-150	22.5±3.51	1.20±0.036	3.24±0.06
Stage 4	151-200	19.9±0.40	1.77±0.0065	2.86±0.14

3.2 Analytical procedures

Samples of the MBBR and AGS reactors effluent were collected daily. NH_4^+-N, NO_2^--N, NO_3^--N alkalinity, and COD concentrations were measured to determine the process performance. The concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the AGS reactor were measured once a week. The analysis of NH_4^+-N, NO_2^--N, NO_3^--N and alkalinity was conducted using HACH kits and read by a DR 3900 spectrometer

(DR3900, HACH, Germany). The COD, MLSS, MLVSS, and sludge volume index (SVI) were carried out according to standard methods (American public health association et al., 2012).

3.3 Cycle tests

Cycle tests were conducted at the end of each of the five stages in the AGS reactor to understand nitrogen transformations, informing subsequent HRT adjustments and cycle optimization. Samples of mixed liquor were collected, filtered through 0.45 μm filters, and determined for NH_4^+-N, NO_2^--N, and NO_3^--N concentrations in each sample.

3.4 Microbial activity tests

Batch assays were conducted to assess the microbial activities of ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), denitritation and denitrification. The specific microbial activities represent the maximum removal performance of the sludge under each operation stage. The biomass concentration was maintained constant at 4 g VSS/L in each batch test. All the batch assays were replicated in triplicate using sludge samples collected at steady state during the aerobic phase from the AGS reactor at 20 °C. The microbial activities were calculated and expressed as g N/g VSS/d.

AOB and NOB activity tests were performed in 160 mL serum bottles with 30 mL mixed liquor. The initial substrate concentration for the AOB activity test was 400 mg/L NH_4^+-N and 2800 mg/L $CaCO_3$ alkalinity. For the NOB activity, the initial substrate concentration was 200 mg/L

NO_2^--N and 1400 mg/L $CaCO_3$ alkalinity. To ensure the best performance, the pH of the mixed liquor was adjusted to 7.5-7.8 before sealing the serum bottles with rubber stoppers and aluminum caps. The serum bottles were then shaken at 160 rpm for an hour. Every 15 minutes, liquid samples were collected and immediately passed through 0.45 μm filters for the measurements of NH_4-N removal to assess AOB activity and NO_3^--N production for NOB activity evaluation.

Denitritation and denitrification activities were conducted in 120 mL serum bottles with 80 mL mixed liquor. In the denitritation activity test, the mixed liquor contained 200 mg/L NO_2^--N and 1400 mg/L $CaCO_3$ alkalinity. For the denitrification activity test, the mixed liquor contained 100 mg/L NO_3^--N and 1400 mg $CaCO_3$/L alkalinity. Each bottle was flushed with N_2 gas for 5 min to provide an anoxic condition before being sealed with a rubber stopper and an aluminum cap and the initial pH was adjusted to 7.5-7.8. Then methanol was injected into the sealed bottles as a carbon source to provide a C/N ratio of 10. The serum bottles were then shaken at 180 rpm for 40 min. Every 10 minutes, liquid samples were collected and immediately passed through 0.45 μm filters for the measurements of removal of NO_2^--N to assess denitritation activity and NO_3^--N removal for denitrification evaluation.

3.5 DNA extraction and microbial analysis

Biomass samples of biofilm and flocs were collected during Phase 3 of the MBBR reactor operation. Samples of granular sludge were collected from the AGS reactor during each stable operational stage. Flocs and granular sludge samples were collected by centrifuging at 4000 rpm

for 5 min and extracting the DNA from the pellet. The biofilm sample was washed by PBS three times before being cut into small pieces to be directly used in the DNA extraction. Then duplicate samples were extracted following the protocol using DNeasy PowerSoil® DNA Isolation Kits (QIAGEN, Hilden, Germany). NanoDrop One (ThermoFisher, Waltham, MA) was used to check the DNA concentration and quality. The extracted DNA was stored at −20 ℃ before downstream analysis. 16S rRNA genes in the sludge were amplified using the universal primer pairs 515F and 806R, then sent for sequencing on the Illumina Miseq platform at the McGill University and Génome Québec Innovation Centre (Montréal, QC, Canada). Qiime2 DADA pipeline and the Silva 138 database with 99 % similarity were used to analyze raw data. R version 4.3.1 was used for data analysis (Bolyen et al., 2019; Callahan et al., 2016; Yilmaz et al., 2014).

3.6. Statistical analysis

Statistical analysis utilizing the T-test within Microsoft® Excel® (version 2023) established the significance of the results, with a P-value under 0.05 denoting a significant difference.

Chapter 4 - Results and Discussion

4.1 Performance of the MBBR reactor

Fig. 3a. shows the variations in the C/N ratios across different phases of the MBBR system operation. During Phase 1, the system acclimatized to the first batch of wastewater, which started with a high C/N ratio of 6.8. After 50 days, this ratio stabilized to approximately 3.5. Transitioning to Phase 2, the system processed the second batch with an initial effluent C/N ratio of 5.8. With the hydraulic retention time (HRT) reduced from 6 to 4 hours during this phase, the effluent C/N ratio subsequently averaged 3.3. In Phase 3, the system handled the third batch of wastewater, starting with an influent C/N ratio of 4.5 and maintaining the HRT. This phase achieved a notable decrease in the effluent C/N ratio to an average of 2.7, demonstrating the system's adaptability and efficiency in managing nutrient ratios under varied operational conditions.

Short solids retention time (SRT) and HRT created conditions that were insufficient for optimal ammonia-oxidizing bacteria (AOB) proliferation (Pedrouso et al., 2023). Over 200 days of operation, the average ammonia removal efficiency was 5.56% (Fig. 1b.), demonstrating proficient removal of bCOD removal while maintaining a consistent concentration of ammonia nitrogen.

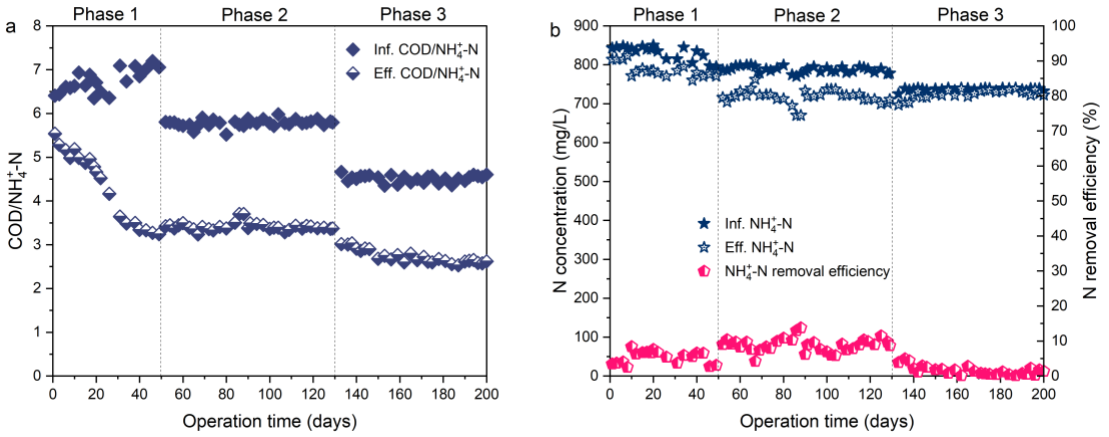

Fig. 3. Performance of MBBR reactor across varied influent wastewater batches: (a) Influent and effluent COD/ NH_4^+-N ratio; (b) Influent and effluent NH_4^+-N concentrations and NH_4^+-N removal efficiencies.

4.2 Performance of AGS

The AGS was fed by the treated effluent from the MBBR with sodium bicarbonate added to maintain the influent pH between 7.5 and 8.0. Its nutrient removal performance was monitored over 200 days (Fig. 4). Fig. 4a. reveals that after treatment with MBBR and AGS, the effluent still presented up to 1200 mg/L of nonbiodegradable COD. After Phase 1, the influent to the AGS system achieved a stable bCOD/ NH_4^+-N ratio of 1.0 to 1.3, markedly less than the initial ratio of 5. This lower ratio significantly improved conditions for autotrophic AOB in comparison to heterotrophic bacteria, thereby enhancing the nitritation processes. The integrated MBBR-AGS treatment system achieved a COD removal efficiency of up to 70.2%, indicating a robust performance in organic load reduction.

Fig. 4b. shows the influent NH_4^+-N and effluent NH_4^+-N, NO_2^--N, and NO_3^--N concentrations at different AGS reactor operation stages. The average NH_4^+-N removal efficiencies were 95.1%, 98.2%, 96.3% and 98.9% for Stages 1 to 4, respectively. During the start-up phase, the

inoculated sludge progressively acclimated to the systemic environment and the nitritation process improved. Almost all of the ammonia was oxidized to nitrite at the superficial air up-flow velocity of 1.1 cm/s. The accumulated NO_2^--N accounted for 90 % of the oxidized NH_4^+-N, suggesting that NOB inhibition was successful. To enhance process efficiency, the HRT was systematically decreased from 30 h to 10 h. Despite varied NLR, the system consistently accomplished efficient NH_4^+-N removal, illustrating its capacity to achieve nitrogen elimination with elevated NLR.

As shown in Fig. 4c., the average TIN removal efficiencies were 77.1%, 91.7%, 88.5% and 81.1% for Stages 1 to 4, respectively. Carbon source plays an important role in the denitritation process. In most cases, denitrifying bacteria, which are heterotrophic, depend on an organic carbon source to facilitate cell growth and the conversion of nitrite and nitrate. The choice of carbon source greatly influences the efficacy of denitrification. Methanol is typically the preferred carbon source, as it appears to maximize the rate of denitrification (Tchobanoglous et al., 2014). In practical applications, internal carbon sources such as those from raw wastewater and endogenous processes are considered secondary importance as carbon sources (Christensen & Harremoës, 2013; Water Environment Federation & Environmental and Water Resources Institute (U.S.), 2006). In the start-up phase, without the addition of an external carbon source, raw wastewater was introduced as the carbon source during the anoxic phase. To provide an optimal environment for AOB, the bulk of the biodegradable COD is removed before entering the AGS reactor. Accordingly, from Stage 1 to Stage 4, methanol is administered as an external carbon source in the anoxic phase to maintain a bCOD/N ratio of 2.5, thus promoting the growth of denitrifying bacteria. In the stable phase (Stages 1-4), TIN removal efficiency reached 84.1%,

with effluent NO_2^--N and NO_3^--N averaging 35 mg N/L and 5 mg N/L, respectively. These results indicate that the addition of easily biodegradable external carbon can achieve higher TIN removal efficiency as previously reported (Wang et al., 2022; Zhang et al., 2020). In Stage 4, the reduction of the anoxic phase to 60 minutes resulted in the TIN removal efficiency drop to 57.8% in the first week. With system stabilization, the efficiency of TIN removal steadily increased, ultimately exceeding an average of 81.1%.

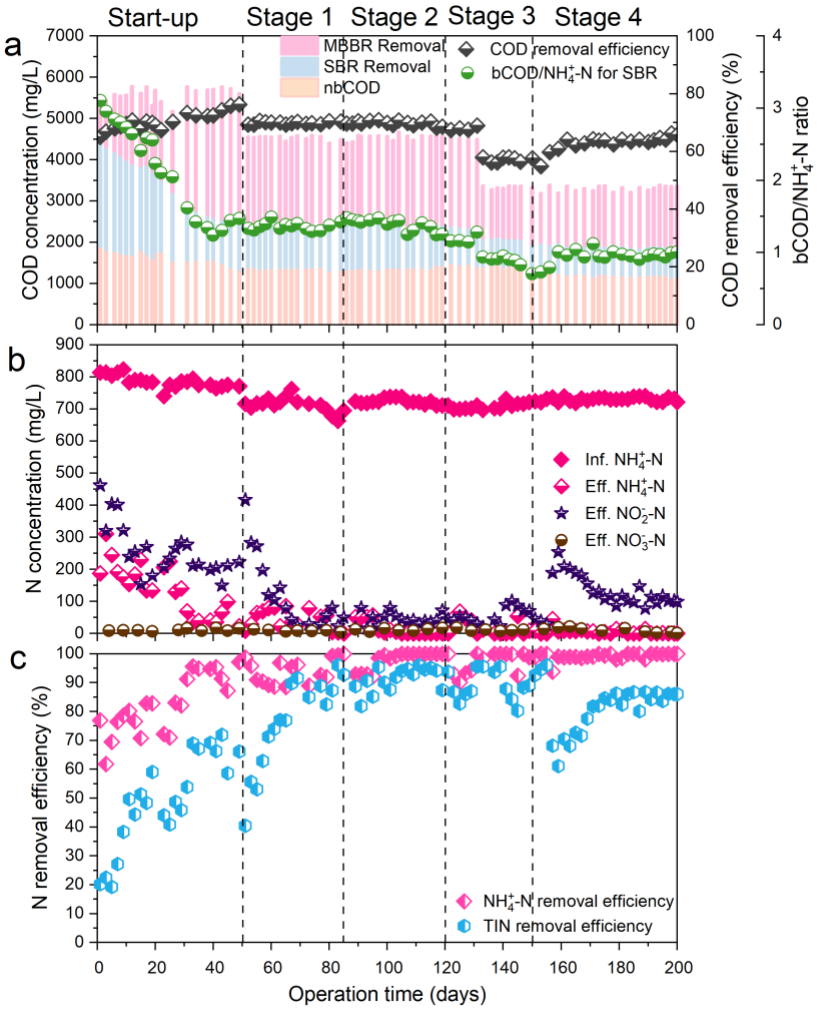

Fig. 4. AGS reactor performance following MBBR reactor treatment: (a) COD Removal by MBBR and AGS, nonbiodegradable COD, COD removal efficiency, and bCOD/ NH_4^+-N Ratio; (b) Influent and effluent nitrogen concentration; (c) N removal efficiencies.

4.3 Sludge properties in the AGS reactor

Fig. 5a. shows the MLSS and MLVSS throughout start-up to stage 4. Initial MLSS and MLVSS concentrations were 8.1 and 6.8 g/L, respectively. During the first 20 days, MLSS and MLVSS continuously increased to 9.1 g/L and 7.6 g/L, resulting in increased microbial mass. Between days 21 and 40, there was a noted decrease in the levels of MLSS and MLVSS concentrations due to the sludge loss caused by foaming in the aerobic phase. Afterwards, as the bCOD/N ratio stabilized, both MLSS and MLVSS gradually increased, and reached 9.3 g/L and 7.4 g/L at the end of the Start-up stage, respectively. Following the decrease of the influent COD in the AGS reactor, a significant increase in biomass concentration (MLSS and MLVSS) was observed within the first week of Stage 1. The increase in biomass concentration in the AGS system is attributed to a substantial reduction in COD, which likely mitigated foaming issues and improved biomass retention. Towards the end of Stage 3, a decrease in biodegradable COD occurred with the shift to batch 3 raw wastewater on day 130, coinciding with significant biomass loss and foaming. These changes suggest biomass washout and increased cell death, potentially due to a reduced C/N ratio, disrupting microbial balance and promoting filamentous bacteria growth, notably with *Microthrix* only detected in Stage 3 with a relative abundance of less than 0.1%, leading to foam formation. Upon increasing the methanol dosage appropriately, the foaming issue was resolved starting from Stage 4. Throughout this stage, reducing the settling time from 30 minutes to 10 minutes had negligible effects on the MLSS and MLVSS concentrations. After 200 days of operation, the concentrations of MLSS and MLVSS had stabilized at 12.7 and 9.3 g/L, respectively.

Decreases in the sludge volume indexes SVI_5 and SVI_{30} are shown in Fig. 5b., the SVI is the indicator of settling and compact granule formation. Beginning with SVI_5 at 127.3 mL/g and SVI_{30} at 100.9 mL/g, the sludge was characterized as flocculent. By the end of Stage 4, these indices reduced to 56.7 and 44.3 mL/g, respectively. During the stable operational phase (Stage 1- Stage 4), the sludge showcased optimal settleability, reflected in SVI_{30} of 45-80 mL/g, with a gradual convergence of SVI_{30} towards SVI_5. The low SVI values indicate that the granular sludge cultivated in this study has excellent settleability.

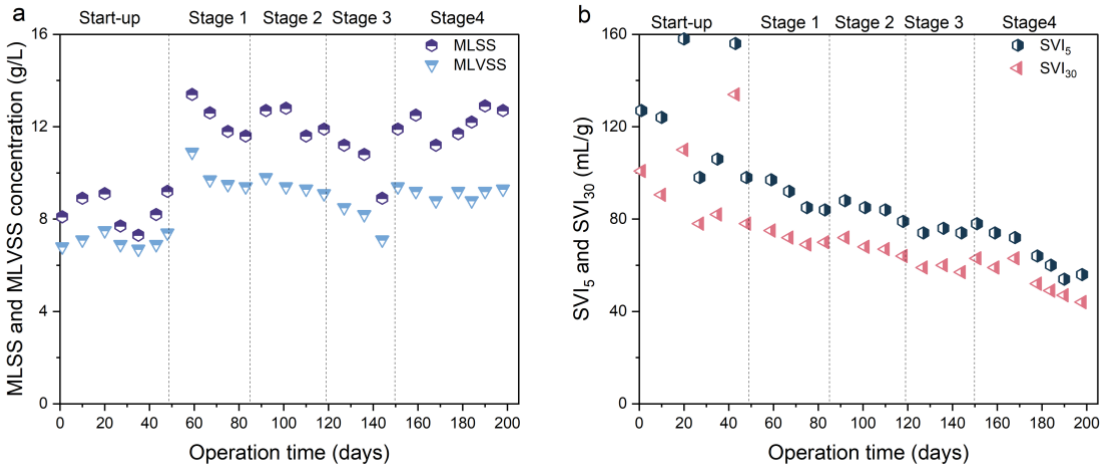

Fig. 5. Sludge properties: (a) Concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS); (b) Sludge volume index (SVI) values at 5 min and 30 min.

4.4 Specific microbial activities in the AGS reactor

Fig. 6a. demonstrates that the AOB activity remains consistently higher than the NOB activity. The peak activity of AOB observed in Stage 4 at 0.57 ± 0.02 g N/g VSS/d aligns with the high end of the previously reported range of 0.07 to 0.64 g N/g VSS/d (Chen et al., 2022; Zou et al., 2022, 2023; Gao, et al., 2024; Zhang, et al., 2024). In contrast, the activity of NOB steadily decreases from Stage 1 to Stage 4, indicating the successful suppression of NOB throughout 200 days of operation. In contrast to the NOB activity (0.14 ± 0.02 g N/g VSS/d) in the start-up phase, a significant reduction ($p < 0.05$) of the NOB activity was observed in Stage 1, 2, 3, and 4: 0.06 ± 0.013 g N/g VSS/d, 0.05 ± 0.008 g N/g VSS/d, 0.04 ± 0.009 g N/g VSS/d, and 0.057 ± 0.006 g N/g VSS/d, respectively. This observed decrease may be associated with the diminished residual nitrite, following the enhanced denitritation capacity.

Fig. 6b. illustrates that, during the start-up phase, denitritation and denitrification activities were 0.26 ± 0.02 and 0.16 ± 0.04 g N/g VSS/d, respectively. In Stage 1, a significantly higher reduction rate of NO_3^--N and NO_3^--N ($p < 0.05$) was observed, which can be attributed to methanol serving as an external carbon source. The NO_2^--N reduction rate in Stage 1 (0.96 ± 0.03 g N/g VSS/d) was significantly higher ($p < 0.05$) than the NO_3^--N reduction rate in Stage 1 (0.45 ± 0.01 g N/g VSS/d). Consistent trends were observed from Stage 2 through Stage 4. The highest denitritation activity was achieved in Stage 3 with 1.27 ± 0.01 g N/g VSS/d. The observed decline in denitritation activity to 1.08 ± 0.02 g N/g VSS/d in Stage 4 is likely attributable to the shortened duration of the anoxic phase.

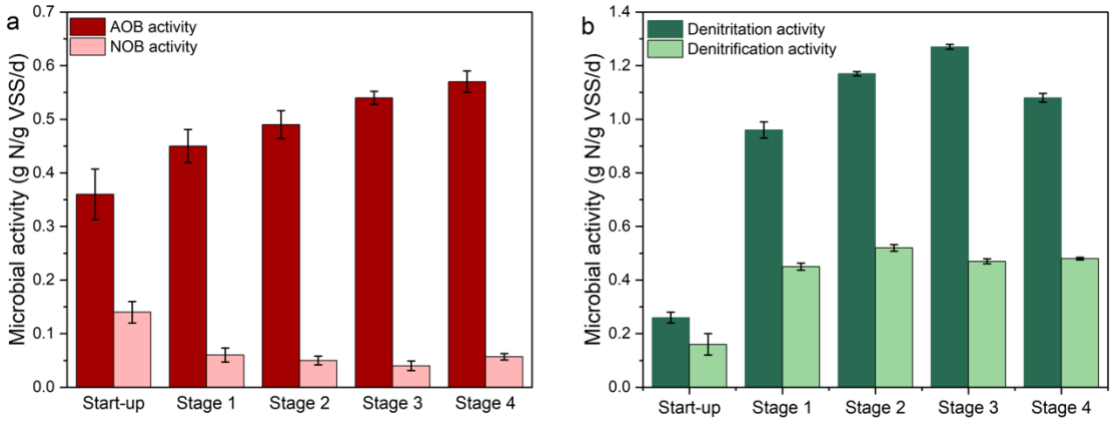

Fig. 6. Specific microbial activity tests. (a) Activity of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB); (b) Activity of denitritation and denitrification.

4.5 Nitrogen removal on typical AGS cycle

Fig. 7a-d. illustrates the variations in concentrations of NH_4^+-N, NO_2^--N and NO_3^--N throughout the respective stages of the typical cycle. By the end of each treatment cycle, NH_4^+-N concentrations were reduced to undetectable levels, and TIN exhibited consistently high removal efficiencies across all stages. These results highlight the effectiveness of the two-stage AGS system in nitrogen removal, demonstrating its adaptability to various NLRs.

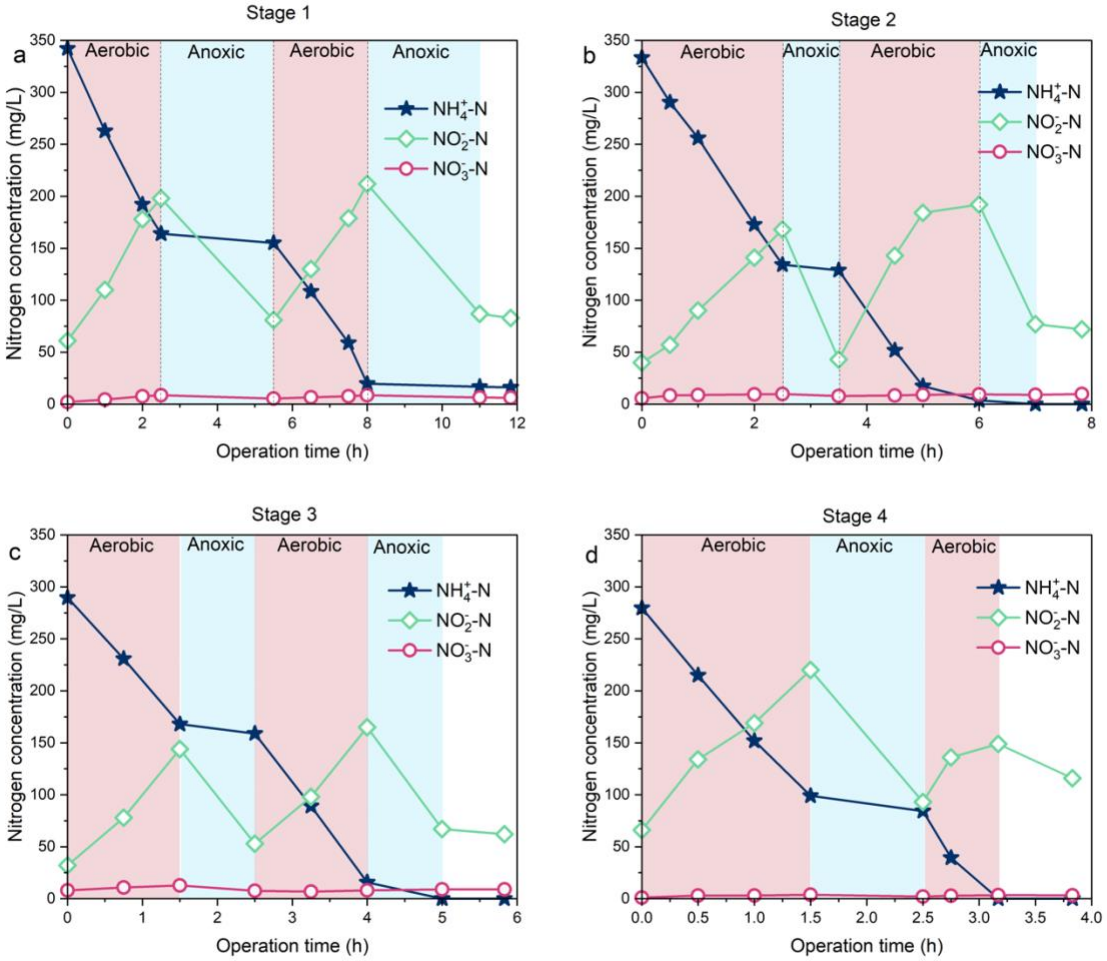

Fig. 7. Variations in NH_4^+-N, NO_2^--N and NO_3^--N concentrations in the typical cycle tests: (a) Stage 1 (Cycle = 12), (b) Stage 2 (Cycle = 8 h), (c) Stage 3 (Cycle = 6 h), (d) Stage 4 (Cycle = 4 h).

4.6 Microbial Community Dynamics

4.6.1 Alpha diversity of the microbial community

Alpha diversity analysis was conducted on seven sludge samples drawn from the MBBR's biofilm and flocs during Phase 3, and from the AGS reactor at the end of each operational stage. Table 3 details species richness and diversity estimates, utilizing indices such as Richness,

Shannon, Simpson, Chao1, and ACE. With coverage indices exceeding 0.99 for all samples, the sequencing results are confirmed to reflect the actual sample conditions accurately. In the MBBR, flocs demonstrated greater diversity (Shannon index 5.45, Simpson index 0.95) than biofilms, indicating a more complex ecosystem within the free-floating particles. Both biofilm and flocs showed high richness levels (243 and 335, respectively), suggesting a diverse species range within the reactor. A comparison between Stage 1 and the seed in the AGS reactor revealed a pronounced decrease in Chao1, ACE, Shannon, and Simpson indices, hinting at inhibitory influences from certain system compounds on microbial proliferation. However, the diversity and richness levels gradually increased from Stage 2 to Stage 4. This indicates a potential gradual acclimation of the microbial community to the treatment environment.

Table 3. Indices of microbial variety in the MBBR and AGS reactors.

Reactor	Sample	Richness	Shannon	Simpson	Chao1	ACE
MBBR	Biofilm	243	3.96	0.81	243	243
	Flocs	335	5.45	0.95	335	335
AGS	Seed	195	4.79	0.92	195	195
	Stage 1	134	4.69	0.93	134	134
	Stage 2	237	4.89	0.94	237	237
	Stage 3	314	5.36	0.95	314	314
	Stage 4	354	5.46	0.95	334	335

4.6.2 MBBR microbial community dynamics

Fig. 8. illustrates distinct microbial community profiles at the phylum level between biofilm and floc samples within the MBBR system. The biofilm is characterized by a substantial proportion

of Actinobacteriota, accounting for 41.7% of its microbial composition. This dominance may be associated with its capability for extracellular polymeric substances (EPS) secretion. The negatively charged functional groups in EPS have strong capabilities to adsorb organic pollutants, potentially playing a critical role in COD removal (Jena et al., 2016). Conversely, the flocs are predominantly composed of Proteobacteria, at 37.6%, and Bacteroidota, at 22.9%. These phyla are integral to the decomposition of complex organics, which is essential in COD reduction processe. Chloroflexi, filamentous bacteria conducive to microbiological structure formation (Godzieba et al., 2022), are more abundant in flocs (10.7%) than in biofilm (1.7%). This abundance, along with their structural adaptation for better substrate access and overall greater microbial diversity, indicates that flocs are highly suited to the high COD wastewater environment, enhancing the treatment process.

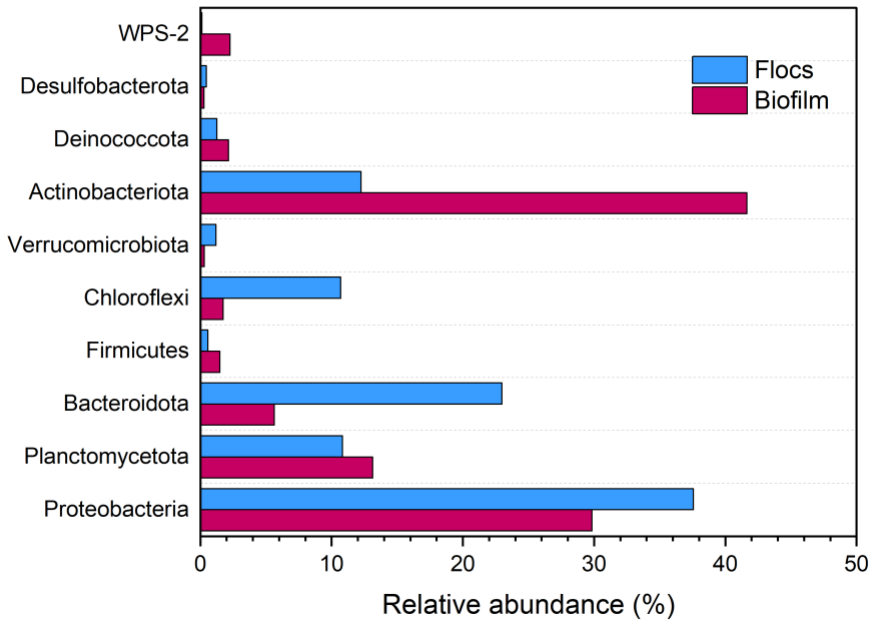

Fig. 8. Relative abundance of the microbial community from the MBBR reactor at the phylum level.

4.6.3 AGS microbial community dynamics

Fig. 9. illustrates the phylum-level shifts in microbial community composition across various stages of the AGS reactor. Proteobacteria predominated in the microbial community across the operation period, with its relative abundance surging from 42.9% in the seed sludge to 66.8% in Stage 4 sludge. The dominance of Proteobacteria has been reported in previous AGS studies (Li et al., 2019; Yuan et al., 2020). Bacteroidota reached the highest relative abundance zenith at Stage 2, accounting for 32% of the total microbial consortium, but witnessed a decline to 13.7% by Stage 4. These phyla are integral to wastewater treatment, with established roles in organic matter degradation and denitrification (Lu et al., 2014). Actinobacteriota displayed a modest reduction in its relative abundance, from 15.9% in the Seed to 6.6% in Stage 4. Actinobacteriota is known for generating substantial secondary metabolites instrumental in organic matter breakdown (Yang et al., 2023). Chloroflexi and Firmicutes were consistently present in lower abundances throughout the treatment stages, with Firmicutes not surpassing a 1.5% relative abundance. In contrast, Planctomycetota exhibited a declining trend, decreasing from 8.6% in the seed to 1.8% by Stage 4, suggesting a diminished role in the reactor's conditions over time.

Figure. 10. illustrates the distribution of microbial genera in the AGS reactor. The relative abundance of *Nitrosomonas*, a key genus of AOB, (Mobarry et al., 1996; S. Zheng et al., 2023) was observed at 0.74% in the seed sludge. From Stage 1 to Stage 4, a consistent increase in relative abundance was noted, reaching 6.34% by Stage 4, which may be attributed to the elevated NLR. Microorganisms associated with NOB, such as *Nitrobacter* and *Nitrospira,* which are commonly reported in wastewater treatment systems, were undetectable in this study (Lin et al., 2023). In Stage 1, *Pseudomonas* was detected at a relative abundance of 6.5%, while in Stage

4, it accounted for 16.7% of the microbial community, functioning as aerobic denitrifying bacteria. *Thauera*, an anaerobic and facultative bacterium essential for denitrification, was present at 11.9% in the Seed sludge (Li et al., 2018; Zhou et al., 2018). The abundance of *Thauera*, indicative of its significant role in the denitrification process, peaked at 18.2% during Stage 3. By Stage 4, its relative abundance stabilized at 15.4%, which suggests a sustained denitrification activity throughout the stages of the operation. *Paracoccus*, capable of nitrate removal under anoxic conditions, registered an initial relative abundance of 1.4% in Stage 1, and it achieved a peak of 2.5% in Stage 3, confirming its continuous role in the denitrification process. Despite a subsequent gradual decrease, by Stage 4, *Paracoccus* still maintained a noteworthy presence with a relative abundance of 1.7% (Li et al., 2023; Yuan et al., 2021).

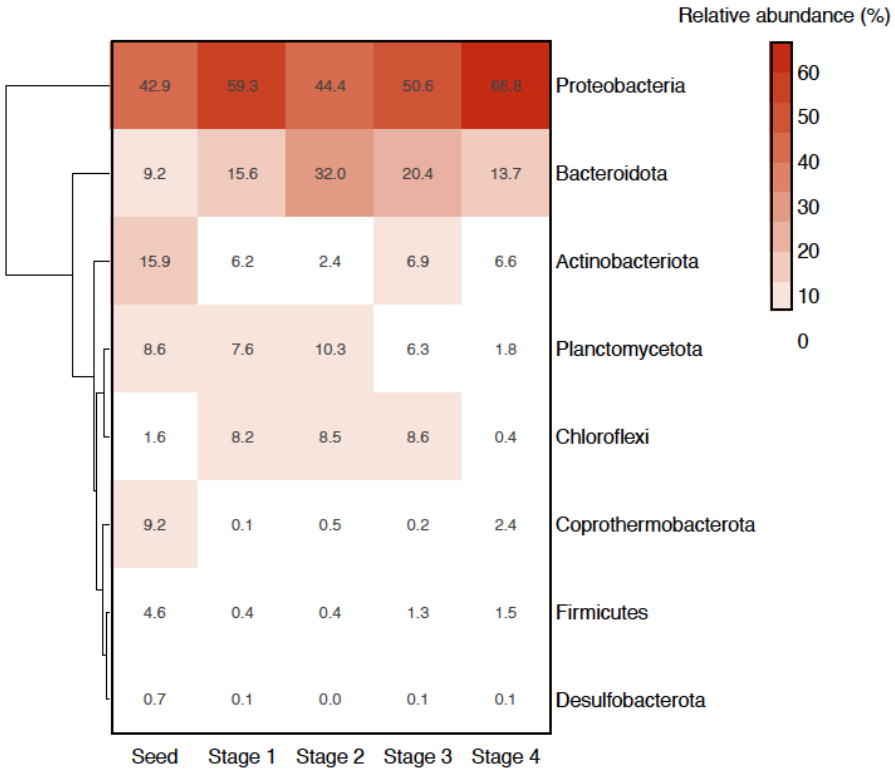

Fig. 9. Microbial community of the granular sludge in the AGS reactor at the phylum.

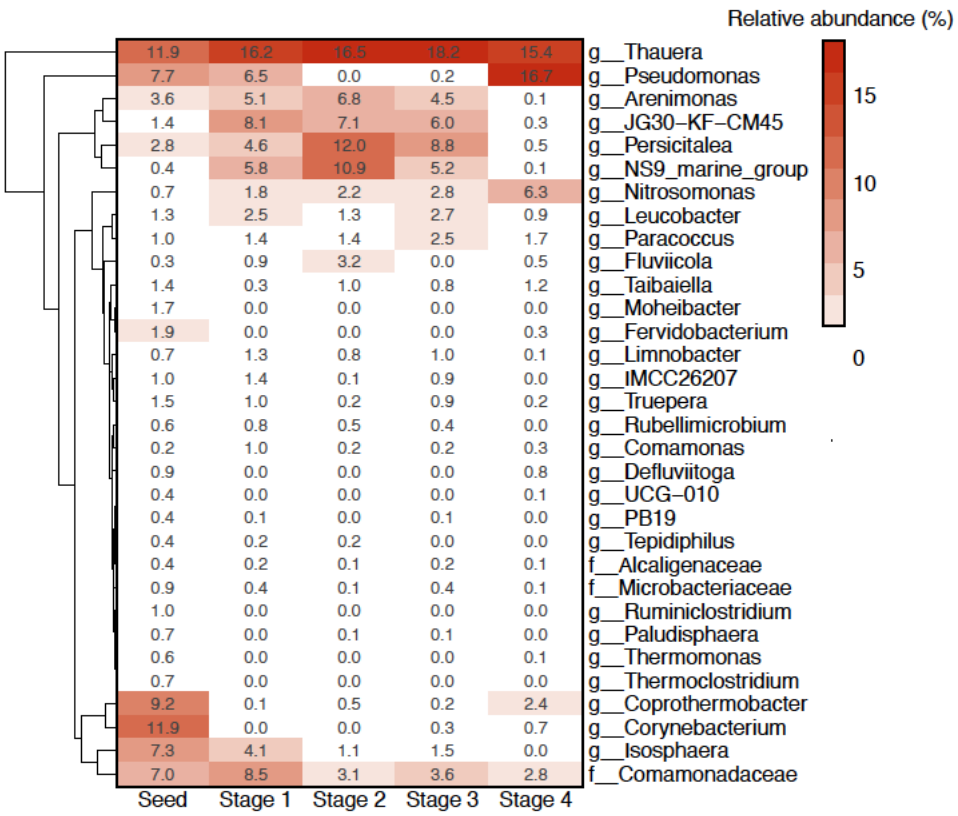

Fig. 10. Microbial community of the granular sludge in the AGS reactor at the genus level.

Chapter 5 – Conclusions and Directions for Future Work

5.1 Conclusions

This investigation substantiates the efficacy of a two-stage AGS system for the treatment of autothermal thermophilic aerobic digestion (ATAD) centrate wastewater. Over an operational duration of 200 days, the system demonstrated robust capacities for simultaneous COD and nitrogen removal. Optimal removal efficiencies of 70.2% for COD, 98.9% for NH_4^+-N, and 91.7% for TIN were achieved, under HRT of 4 hours for the MBBR and 10 hours for the AGS. Additionally, the system achieved organic loading rates (OLRs) exceeding 20 kg/m³/day and demonstrated a nitrogen treatment capacity of 1.77 kg N/m³/day. The microbial consortium, dominated by the phyla Proteobacteria, Bacteroidetes, and Actinobacteriota, along with critical genera from *Nitrosomonas* and *Thauera*, underpinned the system's performance. These findings advocate for the AGS system's applicability to high ammonia, high COD wastewater streams and warrant further investigation on its scalability and operational dynamics.

5.2 Future work

This study confirms the effectiveness of a two-stage AGS system for treating autothermal thermophilic aerobic digestion (ATAD) centrate wastewater. Future research should focus on the following areas to further understand and optimize this system:

1. Investigate the scalability of the two-stage AGS system for industrial applications, focusing on cost analysis. The implementation of a two-stage system may involve higher operational and capital costs compared to single-reactor systems. A detailed economic assessment is necessary to determine the feasibility and cost-effectiveness of large-scale deployment.

2. Explore the potential of enriching heterotrophic nitrifying bacteria to handle higher C/N ratios. If heterotrophic nitrifying bacteria can efficiently manage high C/N ratios through an anoxic-then-aerobic process, it could be possible to achieve effective nitrogen removal in a single reactor system, thereby reducing complexity and cost.

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