Temperature-controlled partial nitrification for mainstream wastewater deammonification using batch, semi-batch and continuous systems

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Abstract

Aeration is a critical component of the two-step nitrification, the biological conversion of ammonia (NH$_3$) to nitrite (NO$_2$-) and then to nitrate (NO$_3$-), in municipal wastewater treatment plants. Aeration provides the necessary dissolved oxygen for the growth and activity of nitrifying bacteria to Partial nitrification-Anammox (PN-Anammox) is a two-step biological wastewater treatment process, used to remove nitrogen compounds from wastewater efficiently without the need for external carbon. It combines partial nitrification, where ammonia is partially oxidized to nitrite, with anaerobic ammonium oxidation (Anammox) to remove both ammonia and nitrite. About a 60% to 80% of the nitrification energy and 100% of the carbon sources can be saved by PN-Anammox process.

Mainstream partial nitrification, which relies on the inhibition of nitrite oxidizing bacteria (NOB) has drawn considerable attention from researchers and wastewater treatment professionals for several compelling reasons: energy efficiency, enhanced nitrogen removal, reduced carbon footprint and cost savings. 60-80°C to achieve mainstream partial nitrification. In this study, heat treatment was explored as a new approach in continuous and intermittent for achieving mainstream PN.

Sustained continuous heating of 100% MLSS and offline heating of 20% of biomass at 37°C-42°C at solids retention time (SRT) of 7 days, DO of 4 mg/L did not achieve any stable nitrite accumulation. In intermittent heating in sequencing batch reactors (SBR), heating once every 10 days at 45°C-2hr, 50°C-1hr and 55°C-0.5 hr with SRT of 7 days, DO of 4 mg/L showed 12% to 17% of nitrification energy savings with stable nitrite accumulation ratio (NAR) of 60%-80%. Similar NAR of 79% was found in continuous-flow systems using membrane bioreactors (MBRs), however with heating once in 4 days. A modified model, incorporating two Arrhenius temperature correction factors for positive and negative growths of nitrifiers: $\theta_1$=1.05 and 1.03, and $\theta_2$=1.02 and 1.01 respectively for ammonia oxidizing bacteria AOBs and NOBs as a pose to $\theta$=1.072 and $\theta$=1.065 for (AOBs) and NOBs respectively was developed for intermittent heating. This model, which
deviated only 6%-20% from experimental data, is an impactful scientific contribution in PN modeling.

Optimization of temperature and contact time for NOB suppression using batches is not appropriate as NOBs are revived in 10-12 days in long-term studies. The impact of heating on the increase of COD of 45-90 mg/L at 45℃-60℃ compared to control (30 mg/L) and the reduced NOx of 17 mg/L compared to control (25 mg/L) which potentially occurred due to the lysis of heterotrophs and reduced ammonification, needs to be explored further. Any heat treatment higher than 45℃-2hr will solubilize microbial community and any heating higher than 60℃, will need external reactivation of AOBs before application for PN.

**Keywords**

Mainstream Partial Nitrification; Heat Shock; Sequencing Batch Reactor; Membrane Bioreactors; Extended Arrhenius; Temperature Factor θ; Aeration; Activated sludge.
Summary for Lay Audience

Nitrogen is an essential part of environment, comprised of ammonia, nitrate, nitrite-nitrogen, and organically bonded nitrogen. Although nitrogen has several forms, fresh wastewater mainly contains ammonia and organic nitrogen. Ammonia causes direct toxic effect on the aquatic life. Due to high concentration of ammonia, oxygen is reduced, causes eutrophication which leads to excessive growth of blue green algae. Consequently, aquatic lives that are forced to store toxicants in their tissue and blood; eventually die.

Conventionally Ammonia is removed by biological nitrification and denitrification processes. Ammonia oxidation happens in two steps: In first step, ammonia is oxidized to nitrite by a bacteria called, ammonia oxidizing bacteria (AOB) and in second step nitrite is oxidized to nitrates by nitrite oxidizing bacteria (NOB). In denitrification process, nitrate that produced in nitrification process used by denitrification bacteria that are heterotrophic and hence requires external carbon source. However, an alternative process that can reduce energy from nitrification process and carbon source process is called partial nitrification and annamox process. In this process, NOBs in are supressed by the controlled application of the operating conditions and produces nitrite that is oxidized by Annamox bacteria without the requirement of carbon source.

In this Ph.D. thesis, the production of nitrate after the suppression of NOBs was investigated by the direct application of heat to the activated sludge. Batch reactors, sequencing batch reactors (SBRs) and membrane bioreactors (MBRs) were used for heating the activated sludge to achieve mainstream PN. The heat shock at 45°C-2hr, 50°C-1hr, 55°C-0.5hr produced nitrite of 10-14 mg/L in SBRs whereas 55°C-1hr produces same level of nitrite concentration in MBRs. About 80% of stable nitrite accumulation ratio (NAR) was achieved for 30-70 days in SBRs. Heat optimization in SBRs showed that about 20% less energy is required for heating every 10 days compared to complete nitrification. For both systems, SRT of 7 days was optimum for achieving mainstream PN. Heating SBRs every 10 days and heating MBRs every 4 days produce effluent nitrite of 10-14 mg/L with maximum NAR of 80%.
Authorship Statement

The content of this Ph.D. thesis is under review, or in preparation for submission in peer-reviewed journals as listed below:

**Chapter 3:** Effect of Thermal Shock and Sustained Heat Treatment on Mainstream Partial Nitrification and Microbial Community in Sequencing Batch Reactors

**Authors:** Niema Afroze, Mingu Kim, Mohammad M I Chowdhury, Basem Haroun, Mehran Andalib, Arthur Umble and George Nakhla

**Authors contribution:**
Niema Afroze, Mingu Kim, Mohammad M I Chowdhury and Basem Haroun performed experimental analysis and wastewater collection. Mingu kim contributed to the revision of the manuscript. Niema was contributing to the sample collection, experimental analysis, data analysis and writing the draft of the manuscript.

Mehran Andalib, Arthur Umble, Mingu Kim and George Nakhla were involved in the conception, design of the study, data interpretation and critical evaluation of the manuscript.

**Status:** Under review in Environmental Science and Pollution Research

**Chapter 4:** Mainstream partial nitrification using heat shocks.

**Authors:** Niema Afroze, George Nakhla, Mehran Andalib

**Authors contribution:**
Niema Afroze performed all the experimental analysis, data analysis, and interpretation, as well as drafted the manuscript.

George Nakhla and Mehran Andalib were involved in the data interpretation and evaluating the manuscript critically in terms of novelty and scientific contribution.

**Status:** In preparation for submission to Environment International

**Chapter 5:** Effect of partial intermittent heat on suspended nitrifiers on achieving mainstream partial nitrification: A new temperature model approach
**Authors:** Niema Afroze, George Nakhla, Mehran Andalib

**Authors contribution:**

Niema Afroze performed all the activated sludge modeling, data analysis, and interpretation, as well as drafted the manuscript.

George Nakhla and Mehran Andalib were involved in evaluating the model critically for novelty and scientific contribution.

**Status:** In preparation for submission to Water Research

**Chapter 6: Heat assisted mainstream partial nitrification in membrane bioreactors: the combined effect of heat and SRT.**

**Authors:** Niema Afroze, George Nakhla, Mehran Andalib

**Authors contribution:**

Niema Afroze performed the system design, the experimental analysis, data analysis, and interpretation, as well as drafted the manuscript.

George Nakhla and Mehran Andalib were involved in data interpretation and evaluating the manuscript critically for novelty and scientific contribution.

**Status:** In preparation for submission to Science of The Total Environment
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<th>Description</th>
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<tbody>
<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
</tr>
<tr>
<td>BNR</td>
<td>Biological nutrient removal</td>
</tr>
<tr>
<td>PN</td>
<td>Partial nitrification</td>
</tr>
<tr>
<td>PE</td>
<td>Primary Effluent</td>
</tr>
<tr>
<td>SBR</td>
<td>Sequencing batch reactor</td>
</tr>
<tr>
<td>MBR</td>
<td>Membrane Bioreactor</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>SON</td>
<td>Soluble organic nitrogen</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>SCOD</td>
<td>Soluble chemical oxygen demand</td>
</tr>
<tr>
<td>ffCOD</td>
<td>Filtered flocculated COD</td>
</tr>
<tr>
<td>TN</td>
<td>Total Nitrogen</td>
</tr>
<tr>
<td>SN</td>
<td>Soluble Nitrogen</td>
</tr>
<tr>
<td>TKN</td>
<td>Total Kjeldahl Nitrogen</td>
</tr>
<tr>
<td>SON</td>
<td>Soluble organic nitrogen</td>
</tr>
<tr>
<td>rSON</td>
<td>Refractory soluble organic nitrogen</td>
</tr>
<tr>
<td>FA</td>
<td>Free ammonia</td>
</tr>
<tr>
<td>FNA</td>
<td>Free nitrous acid</td>
</tr>
<tr>
<td>TP</td>
<td>Total phosphorus</td>
</tr>
<tr>
<td>SP</td>
<td>Soluble phosphorus</td>
</tr>
<tr>
<td>SRT</td>
<td>Solids retention time</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>AOB</td>
<td>Ammonia oxidizing bacteria</td>
</tr>
<tr>
<td>NOB</td>
<td>Nitrite oxidizing bacteria</td>
</tr>
<tr>
<td>AMX</td>
<td>Anammox bacteria</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solids</td>
</tr>
<tr>
<td>VSS</td>
<td>Volatile suspended solids</td>
</tr>
<tr>
<td>MLSS</td>
<td>Mixed liquor suspended solids</td>
</tr>
<tr>
<td>MLVSS</td>
<td>Mixed liquor volatile suspended solids</td>
</tr>
<tr>
<td>WAS</td>
<td>Waste activated sludge</td>
</tr>
<tr>
<td>NAR</td>
<td>Nitrite accumulation ratio</td>
</tr>
<tr>
<td>AUR</td>
<td>Ammonia uptake rate</td>
</tr>
<tr>
<td>NO3-PR</td>
<td>Nitrate production rate</td>
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<td>NO2-PR</td>
<td>Nitrite production rate</td>
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<tr>
<td>Ko, AOB</td>
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<td>Ko, AOB</td>
<td>Half saturation constant for oxygen for NOB</td>
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Chapter 1

1 Introduction

1.1 Rationale

Conventional biological nitrification process requires 75% and 25% of aeration energy for the conversion of ammonia to nitrite and nitrite to nitrate respectively (Wang et al., 2021). In order to reduce the nitrification energy, it is of great significance to implement partial nitrification, also known as short-cut nitrification, technologies in wastewater treatment plant (Ma et al., 2020). Short cut nitrification which is the elimination of the second step of nitrification i.e., oxidation of nitrite to nitrite can reduce 25% of nitrification aeration and 40% of the carbon required for denitrification (Pollice et al., 2002).

Partial nitrification is successful in high strength reject water from dewatering due to the high concentration of ammonia, low COD/N ratio (Aitken, 1983; Mavinic & Turk, 1987), and high temperature which favours ammonia oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB) (Muszyński-Huhajlo & Miodoński, 2017). Hence, partial nitrification processes such as SHARON (Single Reactor for High Ammonia Removal Over Nitrite)(Mulder et al., 2001; van Kempen et al., 2005) and CANON (Completely Autotrophic Nitrogen Removal over Nitrite), (Li et al., 2012) are successfully implemented in wastewater treatment plants.

However, partial nitrification for mainstream remains challenging due to low and variable ammonia concentrations and low temperatures(Hu et al., 2013; Jeong et al., 2021) that can cause decreased bacterial activity and growth. Moreover, high COD/N ratios in wastewater deter the growth of ammonia oxidizing bacteria and anammox bacteria relative to ordinary heterotrophs, making it difficult to apply selective pressures to promote AOB growth and suppress NOB(Hanaki et al., 1990). Consequently, COD removal is necessary before partial nitrification process, requiring pre-COD removal process like A-B type process. The additional A processes (combination of chemically enhanced primary treatment
(CEPT) and high rate activated sludge process (HRAS)) before process B (PN) are required (Xu et al., 2015). Moreover, free ammonia (FA) and free nitrous acid (FNA) as NOB inhibition control parameters are no longer effective for mainstream wastewater due to the low ammonia concentration. Although warm temperatures suppress NOB, seasonal mainstream temperature changes of temperature especially less than 15°C are not enough for NOB suppression (Yu et al., 2021).

Mainstream PN can be achieved at low temperature conditions if the optimum operational conditions i.e., low DO, and SRT are maintained to favor AOBs over NOB (Liu et al., 2020). However, microbial shift of NOBs could provide complete nitrification if operated at low DO for long term (Cui et al., 2020). An alternative potentially more feasible strategy for suppressing NOB is to apply heat shock to suspended nitrifiers. Previous studies achieved PN by entrapping nitrifiers in carriers or suspended biomass, heated at 60°C-80°C in batch and sequencing batch reactors and continuous system (Chen et al., 2016; Chen et al., 2019; Choi et al., 2021; Isaka et al., 2008).

This study is inspired by the paucity of research on the effect of heat shocks on the mainstream suspended growth partial nitrification using real wastewater without any pretreatment for removing COD. Since, the microbial substrate utilization rates and the growth rates are functions of temperature, this study also explored the modification of the conventional Arrhenius model for temperature correction to account for both increase in nitrification rates in the temperature range of 35°C to 60°C and decrease in rates at temperatures above 45°C using long-term performance data from sequencing batch reactors (SBR). A modelling approach was implemented to include the new temperature correction model. In order to validate the results in the SBR systems, this study also investigated the heat application in continuous systems, with submerged membrane for the purpose of solid liquid separation.

1.2 Research Objectives

- Investigating the effect of temperature in the range of 35°C-60°C at DO >2 mg/L and SRTs of 7 days on mainstream partial nitrification using municipal wastewater
in SBRs and the feasibility of using the heat for achieving mainstream partial nitrification

- Investigating the impact of heat on mainstream partial nitrification at 50°C-60°C using continuous-flow membrane bioreactors in contrast to the cyclic SBR
- Development of a new temperature correction model for nitrification kinetics over a wide range of stimulatory and inhibitory temperatures
- Modeling the heated partial nitrification processes using the new temperature correction model for the range of heating temperature that achieved successful stable partial nitrification.

1.3 Thesis Organization

Chapter 1 This chapter provided a brief summary and objective of the Ph.D. It also provides the significant literature review and knowledge gaps in this field of partial nitrification and demonstrates the need for this research.

Chapter 2 provided comprehensive literature review on partial nitrification achieved by controlling DO, SRTs, C/N ratio, reactor configuration, temperature. It also provides the literature review on the modelling approaches that was done on partial nitrification. A comprehensive literature review on the PN achieved by the application by heat using synthetic and real wastewater in laboratory scales, pilot scale on the treatment of nitrogen in mainstream and sidestream nitrogen removal. This provides the research gap of achieving partial nitrification with the application of heat shock and scope of research in the field of achieving mainstream partial nitrification.

Chapter 3 is a research paper accepted in *Environmental Science and Pollution Research* entitled “Effect of Thermal Shock and Sustained Heat Treatment on Mainstream Partial Nitrification and Microbial Community in Sequencing Batch Reactors”. This study aimed at estimating the time for nitrite oxidizing microorganism (NOB) recovery, the activities of the nitrifiers in different heating condition and microbial response due to heat application of online and offline heating using real municipal wastewater.

Chapter 4 is a paper entitled “Mainstream partial nitrification using heat shocks,” to be submitted to the journal of “*Environment International*”. This study aimed to investigate the mainstream partial nitrification using intermittent heating in different heating
temperature and heating duration in order to achieve stable NOB suppression by the heat using real wastewater. This study also aimed to achieve PN with heat optimization in view to reduce energy consumption in the heating process using real wastewater.

Chapter 5 is a research paper, entitled “Modeling the effect of partial intermittent heat on suspended nitrifiers on achieving mainstream partial nitrification using new temperature dependance coefficients,” Which will be submitted to Water Research. This chapter aimed at modeling stable of stable nitritation process Modeling of PN in SBR system “. This model aimed to modify the conventional Arrhenius temperature model to double Arrhenius model and integrating the model performance.

Chapter 6 is a research paper entitled “Mainstream partial nitrification using submerged membrane and application in heat treatment using different SRT conditions” which will be submitted to Science of The Total Environment. This study investigated the effect of SRT on heat treated partial nitrification, the stable partial nitrification using a specific heat and the heat optimization.

Chapter 7 summarizes the major contributions of this research and includes recommendations for future work.

1.4 Thesis Format

The chapters in this thesis were written in an integrated article format, required by the School of Graduate and Postdoctoral Studies (SGPS), Western University. Chapter 3 is accepted in Environmental Science and Pollution Research. Chapter 4 will be submitted to Environment International. Chapter 5 will be submitted to Water Research. Chapter 6 will be submitted to Science of the Total Environment.
References


Chapter 2

2 Literature review

2.1 Nitrogen and the Environment

Nitrogen is an essential part of the environment, comprised of ammonia-nitrogen (NH₃-N), nitrate-nitrogen (NO₃-N), nitrite-nitrogen (NO₂-N), and organically bonded nitrogen. Although nitrogen has several forms, raw municipal wastewater mainly contains ammonia and organic nitrogen, with the latter slowly converting to ammonia by natural biochemical processes. Ammonia is the form of nitrogen which is predominantly utilized as a nutrient by microorganisms in the biological treatment processes (Hanson & Lee, 1971). Unlike other forms of nitrogen that can cause indirect toxic effects on aquatic life, ammonia causes direct toxicity. Due to the high concentrations of ammonia, oxygen is reduced, causing eutrophication, which leads to excessive growth of blue green algae. Consequently, aquatic lives that are forced to store toxicants in their tissue and blood; eventually die (USEPA, 2013). While the main source of ammonia is the natural environment, a prominent source could be the excreta of humans and animals. Sources of ammonia could also be commercial (e.g., fertilizers) and/or industrial, and could travel to the natural waters either by direct or indirect pathways. Discharges of municipal wastewater, human and animal excretion, and agricultural runoff are some examples of these pathways. Scarcity of fresh water is increasing with the increasing population and clean water demand in the world. Although nonpoint sources are difficult to control, point sources of ammonia such as wastewater treatment plants (WWTPs) can easily be controlled.

2.2 Nitrogen in Municipal wastewater

Figure 2-1 shows the nitrogen constituents in municipal wastewater. Total Kjeldahl nitrogen, includes organic nitrogen and ammonia. Within TKN, 60%-70% is ammonia which contributes to the bacterial synthesis and aerobic nitrification (Metcalf & Eddy,
Organic nitrogen constitutes both biodegradable and non biodegradable portions, both of which comprise soluble and particulate fractions. The particulate portion needs longer time to biodegrade as it has to be hydrolyzed first. The soluble non biodegradable nitrogen concentration in wastewater ranges from 1 to 2 mg/L as N (Parkin & McCarty, 1981).

**Figure 2-1. Nitrogen constituents in municipal wastewater (Metcalf & Eddy, 2003)**

### 2.3 Physicochemical process of nitrogen removal

Nitrogen removal from wastewater can be achieved by physicochemical or biological processes. However, biological processes are more economical and sustainable than physicochemical processes and are thus widely used in municipal WWTPs (Atkins & Scherger, 1975). Use of physicochemical processes for nitrogen removal essentially depends on economical viability. Physicochemical treatment processes are divided into two categories:
2.3.1 Processes for Dissolved nitrogen (ammonia, nitrates, and dissolved organic nitrogen)

Physicochemical processes like ion exchange or ammonia stripping and breakpoint chlorination are not usually used in municipal wastewater treatment due to high operational and capital costs. Ammonia stripping could be an economical option for the treatment of supernatant from anaerobic digestion where pH and temperature are naturally favourable (Maroneze et al. 2014, Borea 2016). Breakpoint chlorination is typically used as an emergency measure due to the high chlorine cost and the formation of higher chlorine compounds. Selective ions exchange synthetic or natural resins (zeolite) are used for removing nitrogen from wastewater. However, all the physiochemical processes are too expensive for mainstream nitrogen removal from municipal wastewaters and are used where higher efficiency is required (Capodaglio et al., 2015; EPA, 1974).

2.3.2 Processes for Particulate/Colloidal nitrogen

Removal of particulate nitrogen (pN) is done mainly by coagulation-flocculation and filtration processes. Solids with pN could be removed using deep bed denitrification filters, (Zheng et al., 2018). Tertiary filtration i.e. Ultrafiltration (UF), Nanofiltration (NF) and Reverse osmosis (RO) are used for capturing pN from secondary effluents. Although continuously backwashed filters and deep bed filters are used for pN removal from secondary effluent, RO is used as a final polishing process (Mortula et al., 2022). Actiflo® process (compact coagulation, flocculation, and decantation technology, uses micro sand and polymer for coagulation and settling) which was previously used primarily in drinking water treatment, is recently becoming popular for polishing secondary effluents and wet weather flows (Howe et al., 2012). Figure 2-2 shows a schematic of Actiflo.
2.4. Biological nitrogen removal process

Biological nitrogen removal processes are economical, sustainable, and effective, carried out by microorganisms that already exist in wastewater environment. However, these processes went through numerous modifications due to the evolution of operational and economical challenges (Matsuo et al., 2001).

2.4.1 Principles of conventional nitrogen removal process

Conventional nitrogen removal process consists of two steps: autotrophic nitrification and heterotrophic denitrification. Autotrophic ammonia oxidizing bacteria (AOBs) oxidizes ammonia to nitrite (NO\textsuperscript{2–}) through hydroxylamine (NH\textsubscript{2}OH) pathway. Nitrite (NO\textsuperscript{2–}), thus formed, is then oxidized to nitrate (NO\textsuperscript{3–}) by autotrophic nitrite oxidizing bacteria (NOBs). Finally, anoxic denitrifiers reduce nitrate and nitrite to nitrogen gas in the presence of various electron donors (methanol, acetate and other organic substances (Ward et al., 2011).

Nitrification reactions:

\[
\begin{align*}
\text{NH}_3 & + \text{O}_2 + 2[\text{H}] \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O} \\
\text{HN}_2\text{OH} + 0.5\text{O}_2 & \rightarrow \text{NHO}_2 + 2\text{H}^+ + 2\text{e}^- \\
\text{NO}_2^- & + 0.5\text{O}_2 \rightarrow \text{NO}_3^-
\end{align*}
\]
Denitrification reactions:

\[
2\text{NO}_3^- + 10\text{H}^+ + 10\text{e}^- \rightarrow \text{N}_2 + 2\text{OH}^- + 4\text{H}_2\text{O} \\
2\text{NO}_2^- + 6\text{H}^+ + 6\text{e}^- \rightarrow \text{N}_2 + 2\text{OH}^- + 2\text{H}_2\text{O}
\]

2.4.2 Conventional biological nitrogen removal processes

2.4.2.1 Predenitrification

In this process, nitrifying MLSS and formed nitrates are recycled from the aerobic zone to the anoxic zone, where denitrification occurs; for example Modified Ludzack Ettinger Process (MLE). A schematic of MLE process is shown in Figure 2-3. This process contains consecutive anoxic and oxic zones. Predenitrification has two advantages. First, the organic carbon in the wastewater serves as an electron donor and is removed, thus reducing the oxygen demand in the aerobic zone. Second, better nitrification in the following nitrification process requires less aeration time due to higher activities of nitrifiers and reduction of organics. However, overall nitrogen removal efficiency is controlled by the mixed liquor recirculation rate.

![Schematic of Modified Ludzack Ettinger Process](image)

**Figure 2-3. Modified Ludzack Ettinger Process (Yorko & Momoh, 2019)**
2.4.2.2 Post denitrification

In this process, denitrification units (anoxic zone) are located downstream of the nitrification (aerobic zone). Most of the carbon source is taken by the aerobic heterotrophs in the aerobic zone and consequently the only carbon source that is available for the downstream denitrification is carbon from endogenous respiration, which significantly limits the process kinetics. Hence, to gain fast denitrification in the downstream, external carbon is needed in the downstream anoxic zone such as the Wuhrmann Process. A schematic of post denitrification process in shown Figure 2-4.

![Figure 2-4. Wuhrman Single-Stage Process (Post Denitrification)(Yorkor & Momoh, 2019)](image)

2.4.2.3 Bardenpho process

This process is a series of anoxic and aerobic processes. In the first stage of this process, MLSS from the aerobic zone and RAS from the clarifier are returned to the predenitrification reactor, The successive second anoxic zone performs the denitrification of the remaining nitrate with the help of additional external carbon. The last aerobic zone is considered as physical process since the air helps the detachment of nitrogen gas from MLSS to avert sludge flotation in the clarifier. A schematic of the 4-stage Bardenpho process is shown in Figure 2-5.
2.4.2.4 Bio-Denitro

This process utilizes the alternation between nitrification or denitrification. This process can overcome the limitation of predenitrification process where high concentrations of nitrate are recycled. The flow is continuous, but nitrification and denitrification happen in a semi batch manner. Figure 2-6 shows a schematic of Bio-Denitro process.

Figure 2-6. A typical Bio-Denitro process (Carstensen, 1996)
2.4.2.5 Sequencing batch reactor (SBR)

SBRs are operated in four basic steps: Feeding, reacting, settle and decanting. This system can use different aeration and anoxic period to achieve TN removal as much as MLE or 4 stage Bardenpho. SBRs are operated 4 to 6 cycles per day to treat the wastewater. Figure 2-7 shows the typical phases of a SBR for removing nitrogen.

Figure 2-7. Sequencing batch reactor for nitrogen removal (EPA, 1999)

2.4.2.6 Oxidation Ditch (OD)

The schematic of oxidation tank is shown in following Figure 2-8. The basin is aerated by surface aeration and water moves circularly until complete nitrification occurs, following which the nitrified wastewater flows through an anoxic zone for denitrification.

Figure 2-8. Schematic diagram of oxidation ditch (B. J. Ni et al., 2017)
2.4.2.7 Step feed process

The step feed system (Figure 2-9) can be considered as modified predenitrification process where carbon sources are distributed along several ports of denitrifying reactors instead of applying the wastewater to the first anoxic reactor. In this system, the use of external carbon sources is optimized. The zones in the Figure 2-9 are: (1) Influent tank; (2) pre-anoxic zone (PAN); (3) anaerobic zone (ANA); (4) first oxic zone (N1); (5) second anoxic zone (DN2); (6) second oxic zone (N2); (7) third anoxic zone (DN3); (8) third oxic zone (N3); (9) settler; (10) effluent; (11) influent pump; (12) stirrer; (13) sand bubble diffuser; (14) valve; (15) gas flowmeter; (16) air compressor; (17) sludge return pump; (18) waste sludge (Cao et al., 2013).

Figure 2-9. Schematic diagram of the modified four step-feed process

The advantages, disadvantages, and limitations of the conventional activated sludge process of achieving nitrogen removal are summarized in Table 2-1.
Table 2- 1. Conventional process for nitrogen removal

<table>
<thead>
<tr>
<th>Process</th>
<th>Design Criteria</th>
<th>Scale of operation</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Pre denitrification      | TN concentration< 10 mg/L | Full scale         | • TN range of 5-8 mg/L achievable  
• Adaptable to the existing facility  
• Reparation of Alkalinity | • Nitrogen removal depends on recycle.  
• DO control is required before recycling | (D. Zhang et al., 2020; Q. Zhang et al., 2021) |
| Post denitrification     | TN concentration < 3 mg/L | Full scale         | • Recycle does not contain nitrate | • Higher cost due to addition of carbon source  
• Required C-dosage control.  
• All organics are utilized in aerobic zone | (Winkler et al., 2011) |
<p>| 4-Satge Bardenpho        | TN concentration &lt; 3 mg/L | Full scale         | • Low nitrogen concentration can be achieved. | • Require Large tank. | (Esfahani et al., 2018) |</p>
<table>
<thead>
<tr>
<th>System</th>
<th>Outflow</th>
<th>Scale</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing batch reactor system (SBR)</td>
<td>&lt; 10 mg/L</td>
<td>Full Scale</td>
<td>- Polishing of the effluent</td>
<td>- Second anoxic zone has less efficiency</td>
<td>(Munch et al., 1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Can use discrete water volume and mimic other systems (MLE or Bardenohor).</td>
<td>- System is not continuous.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Flexible process and easy to design.</td>
<td>- Effluent quality depends on decanting quality.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Quiescent settling provided better MLSS concentration</td>
<td>- Complex operation</td>
<td></td>
</tr>
<tr>
<td>Bio-denitro</td>
<td>TN range of 5-8 mg/L</td>
<td>Full Scale</td>
<td>- Capable to shock load</td>
<td></td>
<td>(Irizar et al., 2003; Isaacs S., 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Internal recirculation is not required</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidation ditch</td>
<td>TN&lt;4 mg/L</td>
<td>Full Scale</td>
<td>- Simple operation</td>
<td>- Sludge bulking due to low F/M</td>
<td>(EPA, 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- No Settling tank.</td>
<td>- Large footprint</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Low sludge production and economical</td>
<td>- Nitrogen removal capability depends on the skilled</td>
<td></td>
</tr>
</tbody>
</table>

18
| Step feeding | TN < 5 mg/L | Full Scale | • Easily adaptable to existing process | • Flow split control.  
• DO control | (G. Cao et al., 2013) |

- Treat toxic or shock load without effecting the effluent quality.
- Highly reliable control of the system.
2.5 Development of novel nitrification denitrification processes

As different microorganisms and conditions are involved in conventional nitrification and denitrification, processes should be operated in different time sequences or separate spaces (Lee et al., 2001) requiring long retention time or large reactor volume. Consequently, a high demand of oxygen needs to be supplied for aerobic nitrification. At the same time, anoxic denitrifiers require enough readily biodegradable carbon sources to perform denitrification when there exists high concentration of nitrogen (low C/N ratio) in the wastewater to be treated. Hence, a substantial amount of external carbon sources needs to be added for operating denitrification process when denitrifiers are facing high concentration of nitrogen i.e., low C/N ratio (Tam et al., 1992) resulting in increased operational costs. While WWTPs are seeking cost cut strategies, high cost of nitrification denitrification is a challenge. To address these limitations, modifications to be done on the conventional nitrification denitrification process were necessary, called simultaneous nitrification denitrification (SND) process which is operated in a single reactor (Collivignarelli & Bertanza, 1999).

SND follows two mechanisms in the nitrogen removal process: physical mechanism and biological mechanism. The oxygen on the surface of the floc is high enough (≥2 mg/L) (Stenstrom & Poduska, 1980) to perform nitrification by the nitrifiers, however the concentration of oxygen can be as low as 0.5 mg/L (Xu et al., 2013) in the inner surface of the floc or biofilm, so denitrification happens due the diffusion limitation of oxygen to the inner surface of the floc or biofilm. Figure 2-10 shows the physical mechanism of SND processes.
The biological mechanisms are complex and do not follow the conventional concept of autotrophic nitrification and heterotrophic denitrification. Some heterotrophs can perform nitrification whereas some autotrophs can perform denitrification (Joong et al., 2005; Song et al., 2021). Carbon and nitrogen removal in SND process is highly affected by the operational and physio chemical parameters. The SND process was tested with various reactor configurations e.g., fixing bacteria in polymeric gel in moving bed biofilm reactor (MBBR), formation of biofilm in the activated sludge reactor on the moving disk, or in a moving bed bioreactor (Table 2-2). But process operation revealed that fixed bacteria is unstable, could be detached and hinder process performance, resulting in very low nitrification and denitrification rates, and carbon limitations for denitrification (Meyer et al., 2005).
<table>
<thead>
<tr>
<th>Reactor Configuration</th>
<th>Wastewater Used</th>
<th>Scale</th>
<th>DO (mg/L)</th>
<th>Temp (℃)</th>
<th>pH</th>
<th>SRT (days)</th>
<th>HRT (h)</th>
<th>C/N ratio</th>
<th>COD removal (%)</th>
<th>TN removal (%)</th>
<th>SND efficiency (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBR</td>
<td>SWW</td>
<td>Lab</td>
<td>0.35-0.8</td>
<td>23</td>
<td>7.0-7.8</td>
<td>20</td>
<td>12</td>
<td>8</td>
<td>93.5</td>
<td>90.4</td>
<td>--</td>
<td>(Kong et al., 2016)</td>
</tr>
<tr>
<td>SBBR</td>
<td>SWW</td>
<td>Lab</td>
<td>3-4</td>
<td>35</td>
<td>7.15-7.62</td>
<td>--</td>
<td>--</td>
<td>7.8</td>
<td>--</td>
<td>85.2</td>
<td>--</td>
<td>(L. Zhang et al., 2009)</td>
</tr>
<tr>
<td>MBR</td>
<td>SWW</td>
<td>Lab</td>
<td>0.8-5</td>
<td>20</td>
<td>7.6</td>
<td>--</td>
<td>6</td>
<td>12.4</td>
<td>92.3-94.7</td>
<td>86.6-54.2</td>
<td>--</td>
<td>(S. bing He et al., 2009)</td>
</tr>
<tr>
<td>MBBR</td>
<td>SWW</td>
<td>Lab</td>
<td>3-4</td>
<td>24</td>
<td>7-8</td>
<td>--</td>
<td>10</td>
<td>4.5</td>
<td>91</td>
<td>--</td>
<td>97.6</td>
<td>(Fu et al., 2009)</td>
</tr>
<tr>
<td>IFAS</td>
<td>SWW</td>
<td>Pilot</td>
<td>1.2</td>
<td>18-26</td>
<td>6.0-8.0</td>
<td>21</td>
<td>28</td>
<td>10</td>
<td>96.01</td>
<td>81.8%</td>
<td>-</td>
<td>(Machat et al., 2019)</td>
</tr>
<tr>
<td>Oxidation ditch</td>
<td>RWW</td>
<td>Pilot</td>
<td>0.2-3</td>
<td>20-24</td>
<td>7.4</td>
<td>5.8</td>
<td>5.3</td>
<td>94%</td>
<td>70%</td>
<td>--</td>
<td>--</td>
<td>(Y. Liu et al., 2010)</td>
</tr>
<tr>
<td>Compacted suspended carrier biofilm</td>
<td>MWW</td>
<td></td>
<td>1.5-2.5</td>
<td>15-22</td>
<td>--</td>
<td>--</td>
<td>4</td>
<td>7</td>
<td>95</td>
<td>78.4%</td>
<td>83%</td>
<td>(Xia et al., 2008)</td>
</tr>
</tbody>
</table>

Note: SWW=Synthetic wastewater, MWW=Municipal Wastewater, SBBR=Sequencing batch biofilm reactor, MBR=Membrane bioreactor, MBBR=Moving bed bioreactor, IFAS=Integrated fixed film activated sludge
All these challenges i.e. low removal efficiency, high oxygen demand, long retention time, addition of external carbon sources, unsatisfactory performance of SND, are the driving force for the development of low-cost nitrification denitrification processes that can completely remove high concentrations of nitrogen (Jetten et al., 2002). These processes are primarily based on eliminating the oxidation of nitrite to nitrate, named as partial nitrification (PN) which is a state-of-the-art process for side-stream high concentration nitrogen removal, that can be integrated with both conventional and other novel denitrification processes, such as the ANAMOX process. (using novel microorganisms e.g., anamox).

2.6 Partial nitrification (PN) process

Since the discovery of the anaerobic ammonia oxidation, ANNAMOX (AMX) process at Delft University of Technology in 1990s (Hauck et al., 2016), PN attracted a great attention in nitrogen removal. For achieving PN, the last step of conventional nitrification process where nitrite is converted to nitrate, is being removed by suppressing NOBs) (Ahn et al., 2011). The schematic of PN process is shown in Figure 2-11.

![Figure 2-11. Schematic of PN/A process (Jaramillo et al., 2018)](image)

PN is an engineered process aimed to save 25% of aeration nitrification energy and 100% of carbon when integrated with Anammox for sustainable nitrogen removal than conventional (Kosgey et al., 2022). The integrated PN/Annamox (PN/A) process does not need supplemental carbon sources; sludge production is very low and nearly no oxygen requirement. Consequently, PN integrated with Annamox can offer the same results as
conventional nitrification-denitrification process with less energy and carbon (Casagrande et al., 2011; S. Q. Ni et al., 2010). A municipal wastewater treatment plant with influent flow of 100000 m$^3$/d, COD of 400 mg/L, NH$_4^+$-N of 40 mg/L, treated with PN/A process integrated with COD recovery system (43% of COD recovery), can convert 57% of ammonia to NO$_2^-$, which saves 43% of aeration energy and removes 89% of nitrogen of the influent (S. Cao et al., 2019). Figure 2-12 showed PN-Anammox process (Flow rate = 100000 m$^3$/d, COD=400 mg/L, NH$_4^+$-N=40 mg/L) with energy savings.

![PN-Anammox Process](image)

**Figure 2-12. PN-based anammox process treating municipal wastewater (Du et al., 2016; Rahman et al., 2019)**

Various operational parameters such as dissolved oxygen (DO), pH, temperature etc. are maintained to hinder NOBs (Chen, Song, and Yuan 2021; Banashri Sinha and Annachhatre 2007). Application of inhibitors, heat shocks are also practised in the suppression process. Although PN had successfully been implemented in full scale WWTPs, for side-stream high concentration nitrogen removal, laboratory scale studies had been put forward to investigate the applicability of mainstream PN (Chen et al. 2019).

### 2.6.1 Microbiology of nitrogen removal process

Conventionally, in nitrogen removal AOB and NOB that are combinedly responsible for nitrification of ammonium, consist of *Nitrosomonas, Nitrosopira, Nitrosococcus, Nitrosovibrio,* and *Nitrobacter, Nitrosolobus* respectively (Ge et al., 2015).
The principal genera in activated sludge, *Nitrosomonas* and *Nitrobacter*, are gram-negative, coexist in wastewater and perform the nitrification process in the presence of dissolved oxygen. Although many factors might affect this microbial community, variation of seasonal temperature affect their abundance significantly (Siripong & Rittmann, 2007). AOBs and NOBs, as autotrophs, use carbon dioxide for their anabolic process where a net input of energy is needed for the conversion of carbon dioxide, resulting in lower biomass yields and growth rates (Metcalf & Eddy, 2004). Moreover, nitrifiers have low reproduction rate, even under optimal conditions, their reproduction is minimal. Hence, maintenance of high mean cell retention time (MCRT/SRT) is used (Basu, 2013) in the reactors. Only 3% to 10% of the microbes in the activated sludge are nitrifiers while 90% to 97% are denitrifying heterotrophs (Elmerich, 2002; Xie et al., 2012). Due to the rapid growth of heterotrophs, the slow growing small population of AOBs and NOBs can easily be diluted in the activated sludge reactor and washed out.

Although *Nitrosomonas*, *Nitrospira*, and *Nitrobacter* are considered key nitrifiers for nitrification in activated sludge processes (Feng et al., 2021; Zhou et al., 2021), *Thauera*, *Nitrospira*, *Dechloromonas*, and *Ignavibacterium* are the most abundant microbial genera in the anaerobic/anoxic/aerobic sludge (Kim et al., 2013; Xiang et al., 2021). *Truepera*, *Paracoccus*, and *Denitratisoma* were found to primarily carry out denitrification (Wang et al., 2019; Wang & He, 2020). Conventionally, autotrophic microorganisms perform nitrification and heterotrophic microorganism perform denitrification. However, recent studies showed that denitrification enzymes Nir and Nor were identified in the genes of *Nitrosomonas Europaea*, *Nitrosospira lineage* which are considered as strong ammonia oxidizing bacteria (Chain et al., 2003; Shaw et al., 2006). As such, many heterotrophic bacteria such as *Bacillus badius*, *Burkholderia cepacia*, convert NO$_2$- to NO$_3$- in aerobic environments (Matsuzaka et al., 2003; Sakai et al., 2000). Moreover, recent studies showed that not only bacteria but also archaea and Eukarya perform nitrification and denitrification (Aldossari & Ishii, 2021; Könneke et al., 2005). Nitrite is used as an electron acceptor and CO$_2$ is the main carbon source for anammox bacteria. The overall annamox reaction is shown below that reflects no use of oxygen in this process.
\[
\text{NH}_4^+ + 1.31\text{NO}_2^- + 0.066\text{HCO}_3^- + 1.3\text{H}^+ \\
\rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}
\]

Two anaerobic ammonium oxidizers *Brocadia anammoxidans* (van Niftrik & Jetten, 2012) *Kuenenia stuttgartiensi* (Speth et al., 2012) are primary ammonia oxidizers in this process. Three main advantages of annamox process over conventional nitrogen removal processes: annamox process does not need carbon sources; sludge production is very low and nearly no oxygen requirement. One study stoichiometrically showed that the yield of 0.066 mol VSS/mol NH$_4^+$ (Casagrande et al., 2011) leads to low sludge production and consequently low cost for sludge treatment and disposal (Ni et al., 2010).

COMMAMOX, a single microorganism community, could change the historical concept of nitrogen cycle. COMMAMOX, a genus of NOBs, oxidize ammonia directly to nitrate unlike conventional nitrification by AOBs and NOBs (Daims et al., 2016). Ideally AOB community is expected to be more dominant than NOB in PN process, however their numbers in favourable environmental condition e.g., low temperature in main stream could change. One study showed 2.9% of AOB and 8.6% of NOB (Harms et al., 2003) existed in mainstream nitrification processes. As PN process is entirely based on the accumulation of nitrite, abundance of COMMAMOX could affect PN process significantly due to accumulation of nitrate.

### 2.6.2 Factors affecting PN process

One of the most common strategies that researchers or WWTPs are using for achieving partial nitrification is controlling operating parameters to suppress NOBs, while keeping the AOBs alive. Operating parameters are controlled in such a way that AOBs are favoured. Control Parameters for achieving partial nitrification are dissolved oxygen (DO), pH, temperature, sludge retention time (SRT) (Liu et al., 2017a).

#### 2.6.2.1 DO control.

The primary goal of DO control is maintaining selective half saturation constant of oxygen (Ks) for AOBs. As per Monod model, if half saturation constant (Ks) is lower, the affinity to oxygen is higher (Metcalf & Eddy, 2004). Several studies showed that Ks, AOB is lower
than $K_{s,NOB}$ indicating the higher affinity of AOBs, to low DO while NOBs are suppressed (Law et al., 2018; Wu et al., 2017a). Conventionally, studies used low DO strategy for PN as $K_o$ for NOB is higher than AOB (Sliekers et al., 2005; L. Zhang et al., 2022). However, long term low DO operation caused microbial shift from *Nitroacter*-like NOB to *Nitrospira*-like NOB resulting in complete nitrification (G Liu & Wang, 2013). Liu et al. (2020) explained that bacteria like *Nitroacter* can thrive at high substake concentrations which are called r- strategies and others like *Nitrospira* that thrive at low concentrations are called K-strategists (Liu et al., 2020). However, a model showed more than 80% nitrite accumulation when $K_o, AOB$ and $K_o, NOB$ of 1.0 mgO$_2$/L and 0.35 mg O$_2$/L respectively were maintained with $SRT=10$ days and $pH=8.2$ at temperature 25°C (Wu et al., 2015). Low DO can achieve complete nitrification and increasing to high DO can cause PN (Bao et al., 2017). Partial nitrification can be achieved at DO concentration of 2-4 mg/L when low temperature is maintained at 11°C-25°C(Gu et al., 2012a; Guo et al., 2010). Nonetheless, previous investigations were conducted at a temperature range of 20°C- 35°C and no investigation of $K_o, AOB$ and $K_o, NOB$ had been done at temperature higher than 35°C.

### 2.6.2.2 SRT and HRT

Due to the higher maximum growth rate and yield, and lower oxygen saturation coefficients, AOBs can be retained in the reactors while NOBs would be washed out. Meanwhile, increasing the flowrate i.e., decreasing HRT can reduce the oxidation time of NOBs. Hence, $SRT=7$ days and $HRT=3$ hours were maintained for quick start up and stable partial nitrification (Regmi et al., 2014; Wett et al., 2013). The SHARON process maintains 1.5 days of SRT and HRT for achieving partial nitrification (Hellinga et al., 1998). However, short SRT and HRT can affect the growth environment in semi-continuous or continuous-flow reactors, and ammonia oxidation would be at risk due to fluctuations in wastewater flows. Hence, longer SRT of 7-13 days are maintained in these systems(Yang et al., 2007).
2.6.2.3 pH, free ammonia (FA) and free nitrous acids (FNA)

pH plays a vital role in nitrification process as the presence of the fractions of free ammonia (NH$_3$) and ammonium (NH$_4^+$), nitrite (NO$_2^-$) and free nitrous acid (HNO$_2$) are pH dependant (Claros et al., 2013). pH along with temperature produces free ammonia (FA) and free nitrous acid (FNA) in activated sludge processes according to the following deterministic models (Anthonisen et al., 1976):

\[
FA\left(\frac{mg}{L}\right) = \frac{17}{14} \times \frac{NH_4^+ - N \times 10^{PH}}{10^{PH+ex} (6344/(273+T))} \quad (2-1)
\]

\[
FNA\left(\frac{mg}{L}\right) = \frac{46}{14} \times \frac{NO_2^- - N}{10^{PH} \times exp (-230/(273+T))} \quad (2-2)
\]

where, NH$_4^+$-N, NO$_2^-$-N, and NO$_3^-$-N the effluent ammonia, nitrite, and nitrate respectively.

The inhibition of nitrifiers mainly NOBs by the higher concentration is already established (Turk, 1989; Turk & Mavinic, 1989; Yun & Kim, 2003). Higher free ammonia can inhibit NOBs as well as AOBs, hence the free ammonia level needs to be kept in a way that AOBs don’t inhibit (Kim et al., 2006). NOBs and AOBs perform differently in various pH ranges in nitrification process. Table 2-3 shows the reactors with various operational conditions for achieving PN.
Table 2-3. Operation of reactors in literature for achieving PN

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Temp. (℃)</th>
<th>DO mg/L</th>
<th>pH</th>
<th>NAR (%)</th>
<th>Key message</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respirometer</td>
<td>25</td>
<td>2</td>
<td>7.5</td>
<td>-</td>
<td>- Inhibitor, sodium azite was used</td>
<td>(Guisasola et al., 2005)</td>
</tr>
<tr>
<td>Biofilm air-lift suspension reactor</td>
<td>30</td>
<td>1-2</td>
<td>7</td>
<td>50%</td>
<td>50% nitrate and 50% nitrite were produced from oxidation of ammonia at DO of 1-2 mg/l with no significant impact of pH</td>
<td>(Picioreanu et al., 1997),</td>
</tr>
<tr>
<td>Continuous-flow reactors</td>
<td>19 &amp; 23</td>
<td>7.6</td>
<td></td>
<td></td>
<td>The most stable operation was at DO of 0.3 mg/L resulting nitritation capacity of 0.25 kg Nm$^{-3}$d$^{-1}$ but only about 70% conversion of ammonium</td>
<td>(Blackburne et al., 2008b)</td>
</tr>
<tr>
<td>SBR</td>
<td>25</td>
<td>1-3</td>
<td>7</td>
<td></td>
<td>This study simulated the effect of floc diameter on half saturation constant of nitrifiers. The measured values of $K_o$, AOB and $K_o$,NOB were low under small floc diameter. However, the measured $K_o$,NOB only increased slightly under the same diameter</td>
<td>(Wu et al., 2017b)</td>
</tr>
<tr>
<td>Mathematical model</td>
<td>25</td>
<td>0.4 - 5</td>
<td>7.7-8.4</td>
<td>90 - 100</td>
<td>(Park et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>----</td>
<td>---------</td>
<td>---------</td>
<td>----------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Upflow column reactor</td>
<td>25</td>
<td>5</td>
<td>8.3</td>
<td>93</td>
<td>Nitritation was stable for 120 days with SRT=13 days and HRT= 24 hours, the nitrogen loading rates was 1 kgNm⁻³d⁻¹.</td>
<td>Yamamoto et al. (2008)</td>
</tr>
<tr>
<td>SBR model</td>
<td>22</td>
<td></td>
<td>8.2-7.9</td>
<td></td>
<td>NOBs are more sensitive to pH change compared to AOBs</td>
<td>Park et al., 2007</td>
</tr>
<tr>
<td>Modified UCT</td>
<td>20</td>
<td></td>
<td>8-8.75</td>
<td></td>
<td>NOBs stopped activity at less than pH less than 6.5 but high pH value does not affect NOB activity. The NOB growth rate does not depend on FNA concentration for values below 0.0032 mg/L</td>
<td>Jiménez et al., 2011</td>
</tr>
<tr>
<td>Batch</td>
<td>13.8-24.9</td>
<td></td>
<td>7.5-8.5</td>
<td></td>
<td>Higher influent pH values had a higher AOB abundance in relative to NOBs</td>
<td>Y. He et al., 2012</td>
</tr>
<tr>
<td>MABR</td>
<td>&gt;3</td>
<td></td>
<td>5.6-6.7</td>
<td>&gt; 80%</td>
<td>AOB activity was not limited by FNA, however nitrite oxidation rates declined with increasing FNA. Higher FA of 8–120 mg/L is required AOB inhibition however overall FA ranged from 0.16 to 11.7 mg/L</td>
<td>Pourbavarsad et al., 2022</td>
</tr>
<tr>
<td>SBR</td>
<td>33 ± 2</td>
<td>&gt; 3</td>
<td>6.2-8.5</td>
<td>78%</td>
<td>Higher NAR were obtained at FNA range of 0.08–0.37 mg/L. However, NAR was quickly lost once the inhibitory conditions for NOB</td>
<td>Belmonte et al., 2017</td>
</tr>
</tbody>
</table>
were suppressed at SRT=15–20ys and DO >3 mg/L. NOB inhibition by FA was not observed

| SBR   | 22.0–23.0°C | 1.5-2 | 90% | Using SRT 15 of days, HRT of 24 hr, NAR reduced from 88% to 5.3% in 7 days when FNA treatment was withdrawn. However, the FA treatment produced NAR of over 90% in 60 days. | (Duan et al., 2019) |
2.6.2.4 Application of heat

AOBs and NOBs both survive and operate in different temperature zones. AOBs are dominant at temperature range of 25 to 35 °C (Gabarró et al., 2012) while NOBs outcompete AOBs at low temperature at 5 to 15°C (Yao & Peng, 2017). Raising sludge temperature from 25°C to 35°C enhanced the ammonia oxidation activity and decreased nitrite oxidation as confirmed by Balmelle et al. (Balmelle et al. 1992). As shown in Figure 2-13, at a sludge age of 3.5 to 6 days and temperature range of 5 to 15 °C, NOBs could outcompete AOBs while at sludge age of 1.5 to 3.5 days and temperature of 25 to 30 °C, AOBs outcompete NOBs (Peng & Zhu, 2008).

Figure 2-13. Activities of AOBs and NOBs at different temperature and SRT (Y. Peng & Zhu, 2006)
However, only four studies have been done to investigate the effect of high temperature and contact time on AOB and NOB, with three of them using entrapped nitrifiers. Isaka et al. (Isaka et al., 2008) reported that no NOB activity existed at temperature higher than 60 °C for 20 min while AOB could exist even at 80 °C after 1 h contact time. Chen et al.(G. Chen et al., 2016) reported that NOB can be completely deactivated at temperatures above at 60˚C after 10 min contact time, whereas AOB continued to exhibit some activity. In a PN-ANAMMOX system(Choi et al., 2021) treating pre-coagulated municipal wastewater, employing pre-heated entrapped inoculum in the PN reactor in polyvinyl alcohol (PVA) and sodium alginate, achieved PN. The optimum heating strategy for achieving PN using the entrapped inoculum was 60 °C for 20 min once a day. The PN reactor were operated at DO concentration from 0.7 mg/L to 2.3 mg/L. The operation continued for 35 days and achieved a nitritation efficiency of 50.8±4.3%, a nitrite accumulation ratio (NAR) of 0.86±0.1 and the ratio of nitrite to ammonia in the effluent 1.2±0.4. Similar to the previous two studies, this study considered both DO concentration of 0.7 mg/L to 2.3 mg/L along with temperature shock.

The fourth study used the heat shock on thickened side stream suspended sludge (Chen et al., 2019) and used the treated thickened sludge to treat pre coagulated real sewage so that the effect of COD on nitrification can be removed. First, they conducted batch tests with 25 °C, 40 °C, 45 °C, 50 °C, 60 °C, 80 °C and adopted the suitable heat shock temperature of 50 °C and 60 °C for SBR operation. The authors took 50% of the mixed sludge from the SBR (side stream), heated it at 60 C for 40 min and returned it to the SBR with the feed real sewage. After providing the heat-shock treatment four times, the effluent nitrate decreased from 22.5 to 3.2 mg/L. The nitritation was stable for 91 days with the ammonium removal efficiency of 80.6% and nitrite accumulation rate of 91.2%. However, these studies either achieved PN by the combined effect of DO limitation and heat shock or used pretreatment for COD removal.

2.6.2.5 Inhibitors

Apart from the above factors, NOBs could be suppressed by adding some organic and inorganic substances e.g. sulfide, hydroxylamine, salt, heavy metals, chlorate, cyanate,
halide, azide, hydrazine, and organic chemicals that are known as chemical inhibitors (Sinha & Annachhatre, 2007).

2.7 Partial nitrification in side stream

2.7.1 Single reactor high activity ammonia removal over nitrite, SHARON

SHARON is a cost effective, compact, and sustainable biological PN process of reject water from sludge dewatering (van Kempen et al., 2005) that achieves nitrite accumulation through controlling operational parameters mainly temperature. SHARON is the first PN technology developed for integrating in WWTPs and specific to CSTR (Continuously stirred tank reactor) without solid liquid separation and no biomass recirculation. Although a number of studies had been done on operational parameters, a temperature range of (30-40 °C), pH of 6.5-8 are typically maintained in SHARON process to achieve 90% ammonium removal in Dokhaven WWTP (Mulder et al., 2001). A schematic of the SHARON process is shown in Figure 2-14.

![Figure 2-14. Schematic of Sharon-Annamox process for removing ammonia from sludge digestion (Jetten et al. 2002)](image-url)
2.7.2 Completely Autotrophic Nitrogen Removal over Nitrite, (CANON)

In the CANON process, aerobic AOB (Nitrosomonas-like aerobic bacteria) and anaerobic AOB (Planctomycete-like anaerobic ammonium oxidising bacteria) interact simultaneously under oxygen-limited conditions (DO < 0.5 mg/L), resulting in a complete conversion of ammonium to nitrogen gas in a single reactor (Figueroa et al., 2012; Liu et al., 2016; Zhang et al., 2017). In CANON, $\text{NH}_4^+$ and $\text{NO}_2^-$ are converted to nitrogen gas. $\text{NO}_2^-$ is the electron donor and accumulated from shortcut nitrification by aerobic AOB. The overall reaction for CANON process is as follows (Third et al. 2001):

$$\text{NH}_4^+ + 0.85\text{O}_2 \rightarrow 0.435\text{N}_2 + 0.13\text{NO}_3^- + 1.3\text{H}_2\text{O} + 1.4\text{H}^+$$

CANON needs 63% less oxygen than conventional nitrogen removal processes (Sliekers et al., 2002). Although AMMAMOX process coupled with PN is operating in full scale, CANON process is still operating in lab scale with some studies on full scale application (Li et al., 2017). The schematic of CANON process is shown in Figure 2-15:

Figure 2-15. Schematic of CANON process (Huynh et al. 2019)
2.7.3 Oxygen-Limited Autotrophic Nitrification/-Denitrification, OLAND

Nitrogen removal can also be accomplished in another single-step process, OLAND process, in which AOB oxidize a portion of ammonium to nitrite with oxygen as the electron acceptor and then reduce nitrite to nitrogen with the other portion of ammonium as the electron donor. The overall reaction of OLAND process is given below:

\[
2\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{N}_2 + 3\text{H}_2\text{O} + 2\text{H}^+
\]

OLAND and CANON processes are quite similar, only difference is, under oxygen limited condition OLAND is achieved by aerobic AOB while CANON is carried out by both aerobic AOB and anammox. OLAND does not require additional alkalinity and shows a good tolerance of ammonium and nitrite shocks (Windey, De Bo, and Verstraete 2005). A schematic of OLAND process is shown in Figure 2-16.

![Figure 2-16. schematic of OLAND process (Philips et al., 2002)](image-url)
2.7.4 Single stage nitrogen removal using anammox and partial nitrification, SNAP

SNAP (Figure 2-17) is an economical nitrogen removal process for nitrogen rich wastewater. A novel hydrophilic acrylic fibre biomass carrier carries both AOB and ANAMOX (Furukawa et al., 2006a). The novelty of SNAP is its integrated hydrophilic net type gel carrier which has a high biomass retention capacity, thus eliminating the need for clarification (Thi Hai, Nguyen, Tho Bach, Leu 2005).

![Figure 2-17. Schematic diagram of SNAP reactor (Furukawa et al., 2006)](image)

2.7.5 Comparative Assessment of Sidestream N removal Technologies

PN processes evolved with time and are operating in full scale for sidestream nitrogen removal. A high-level comparison of various PN processes is provided in Table 2-4 (Zhu et al. 2008).
Table 2-4: High Level comparison on PN processes

<table>
<thead>
<tr>
<th>Processes Technology</th>
<th>Conventional</th>
<th>SND</th>
<th>SHARON</th>
<th>Aerobic DeAmmon</th>
<th>CANON</th>
<th>OLAND</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of reactors</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Operating conditions</td>
<td>Aerobic, Anoxic</td>
<td>Aerobic</td>
<td>Aerobic, Anoxic</td>
<td>Aerobic</td>
<td>Oxygen limited</td>
<td>Oxygen Limited</td>
<td></td>
</tr>
<tr>
<td>Oxygen requirements (mg/L)</td>
<td>$\geq 2$</td>
<td>$\geq 2$</td>
<td>0.4</td>
<td>0.1-0.4</td>
<td>0.5</td>
<td>0.1-0.2</td>
<td>(Blackburne et al., 2008b) (X. Li et al., 2004)</td>
</tr>
<tr>
<td>HRT (h)</td>
<td></td>
<td>2.5</td>
<td></td>
<td>6-16</td>
<td></td>
<td></td>
<td>U.S. EPA, 2007, (Huynh et al., 2019)</td>
</tr>
<tr>
<td>SRT (d)</td>
<td></td>
<td>2.5</td>
<td></td>
<td>$\geq 15$</td>
<td></td>
<td></td>
<td>(Figueroa et al., 2012)</td>
</tr>
<tr>
<td>Biomass retention</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<td>-----</td>
<td>-----</td>
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<td></td>
</tr>
<tr>
<td>COD Requirements</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Total-N removal efficiency</td>
<td>95%</td>
<td>100%</td>
<td>90%</td>
<td>60%</td>
<td>75%</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>Sludge Production</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Max N Loading (kg N/ m3.d)</td>
<td>2-8</td>
<td>1-1.35</td>
<td>0.5-1.5</td>
<td>1-2</td>
<td>2-3</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Optimum Temperature (°C)</td>
<td>12-35</td>
<td>20-30</td>
<td>Above 25</td>
<td>Unknown</td>
<td>30-40</td>
<td>30-40</td>
<td></td>
</tr>
<tr>
<td>Common reactor configuration</td>
<td>AS, BF</td>
<td>OD, SBR</td>
<td>AS, BF</td>
<td>RBC, GLF, FBR</td>
<td>FB, FBR, SBR</td>
<td>FB, FBR, SBR</td>
<td></td>
</tr>
<tr>
<td>Suspension or Biofilm</td>
<td>SP</td>
<td>BFM/SP</td>
<td>BFM/SP</td>
<td>BFM/SP</td>
<td>BFM/SP</td>
<td>BFM/SP</td>
<td></td>
</tr>
<tr>
<td>Application</td>
<td>Established</td>
<td>Lab</td>
<td>Full Scale</td>
<td>Lab</td>
<td>lab</td>
<td>lab</td>
<td></td>
</tr>
</tbody>
</table>

Note: AS= Activated Sludge, BF=Fixed Bed Reactor, SBR=Sequencing batch reactor, FBR=Fluidized bed reactor, RBC=Rotating Biological Contactor, GLR=Gas Lift Reactor, SP=Suspension, BFM=Biofilm
2.8 Aeration Control Strategy

Direct and indirect control strategies are used in wastewater treatment to control the partial nitrification. The direct online control strategy is based on the direct measurement of the nitrogen concentration (NH4+–N, NO2–N and/or NO3–N) by the sensors. The robustness of probes for nitrogen measurement is still challenging (Zanetti et al., 2012) The indirect real time control strategies measure the general water quality parameters instead of direct measurements of nitrogen species. The indirect method is more reliable and cost effective. The indirect water quality parameter probes are pH, DO, ORP, oxygen utilization rate (OUR) and blower frequency (BF). These probes are dependable and cost effective and most frequently used in partial nitrification research (Gu et al., 2012b; P. H. Lee et al., 2013; Z. Yuan et al., 2008).

The gradient of ammonia concentration is associated with the general water quality parameter and indicative of ammonia concentration in the reactor and therefore can be used in aeration control. pH variation highly depends on ammonia oxidation and CO2 stripping (Volcke et al., 2006). During nitrification pH goes down. The first order gradient of the pH curve where pH less than 0.1 is considered the point of stopping the aeration (Liu et al., 2020) for SBRs and membrane biofilm reactors (Lee et al. 2013; Yuan et al. 2008). Oxygen uptake rate (OUR) was used as a control strategy with a threshold value of 15 to 25 mgO2L−1h−1 until the ammonia concentration reaches 1 mg/L (Blackburne et al., 2008a). Blower frequency was used to estimate the end point of nitrification and aeration was stopped when the blower frequency goes to -1. However, the above-mentioned strategies are reliable when the infection points can be detected, which sometimes could not be detectable with one parameter sensors like pH, ORP, OUR (Casellas et al., 2006). Therefore, combination of two parameters such as DO, and pH were used to detect the end point of nitrification (Peng et al. 2004).

2.9 Mainstream PN

A two stage mainstream partial nitrification-annamox (PN/A) in an SBR was investigated with intermittent aeration (from 30 min on/30 min off to 30 min on/15 min off); nitrite
increased from 19.96 to 38.62 mg/L together with 0.97 of ammonia and 2.52 mg/L of total inorganic nitrogen in the SBR effluent (Y. Chen et al., 2020). Temperature transition from 25°C to 15°C provided overall nitrogen removal rate of 330 mg N/L⁻¹·d⁻¹ in one stage PN/A reactor at DO concentration 0.2 μmol O₂/L (Akaboci et al., 2018). Coupling denitrification with one stage partial nitrification annamox process caused NO₃⁻ reduction from 24 mg/L to 5 mg/L at 20°C-25°C and DO from 0.5 to 1 mg/L with total nitrogen removal efficiency of 83.5% (Yuan et al., 2020). Investigation of PN with the mixture of nitrifying activated sludge and granular sludge operated at 25°C and DO of 4-8 mg/L showed more than 95% nitrite accumulation respectively during the treatment of domestic sewage (Liang et al., 2015). In a simultaneous nitrification annamox denitrification (SNAD) reactor, it was also suggested that applying a ratio of 1:25 of annamox sludge and nitrification sludge removed 80.3% of TN at temperature 22.6 to 28.6°C and DO range of 0.5 to 2 mg/L (Miao et al., 2020). Investigating the impact of C/N ratio on partial nitrification, a study showed that if SBR aeration changed from 5-40 min at DO of 0.8 to 1.2 mg/L, nitrogen removal increased from 30.8% to 77.3% at C/N of 2.5, which is contradictory to another study reporting that PN occurs if C/N ratio can be maintained only to 0.5 (Daigger, 2014). A novel partial nitrification-synchronous anammox and endogenous partial denitrification (PN-SAEPD) process was tested with pretreated domestic sewage at 15.4°C and C/N ratio 1.7 and resulted in 66.1% nitrite accumulation and 1.1 mg/L of nitrate in the effluent in SBR reactors (Ji et al., 2020). A modelling study proposed a C/N ratio of 0.2-0.6, DO of 0.2-0.4 mg/L and granular biomass radius of 300-600 micrometer to maintain effluent total nitrogen concentration less than 5 mg/L in aerobic granular sludge systems (Liu et al., 2017). In investigation of the impact of FA on PN, a continuous flocculent sludge reactor operated with side stream FA of 65.2–261.1 mgNH₄-N/L at temperatures of 7°C-31°C and DO of 0.2 ±0.1 mg/L, showed that the nitrite accumulation rate (NAR) was twice as the NAR of control (Yu et al., 2020). The application of FA also showed a stable inhibition of NOBs in mainstream uplift airflow reactor (An et al., 2020). PN was also investigated with fixed film systems. A nitrification-annamox biofilter where annamox bacteria was immobilized on a volcanic carrier resulted in partial nitrification in a DO-pH controlled system (2.5±0.5 mg/L, 7.5±0.5), with NAR at 90% and ammonium less than 5 mg/L at 25±1°C (Jiang et al., 2018). Also, a SNAD process operated in
integrated fixed film activated sludge (IFAS) reactor at temperature 25±2°C, DO of 0.4±1, pH of 7.2±0.2, achieved average nitrogen and COD removal of 72±2% and 88% respectively (Liu, et al., 2018). Another fixed film mainstream activated sludge system, operated at a DO of 0.5 mg/L and reduced anoxic time from 40 to 20 min led to nitrogen removal efficiency of 80-89% (Xu et al., 2020).

2.10 Modeling of Partial Nitrification in SBRs

PN was mathematically modelled using SBRs (Liu et al., 2017; V., Pambrun, E. Paul, M., 1996). Liu. 2020 (Liu, 2020a) compared three mathematical models considering different substrates of for AOBs and NOBs in order to determine the minimum DO requirement for the PN and found that using ammonium and nitrite as true substrates, adjusting the SRT of (10-30d), pH of (7-8.5) and temperature of (10-35°C), the model predicted consistent minimum DO for NOB washout with literature data than other two models. Optimizing SRT (10-15 days) and DO (0.6-7.5 mg/L) at pH 8.2 and nitrogen loading rate 0.325 kg NH4-N/m3. d provided 80% nitrite accumulation (Wu et al., 2015). Another dynamic model for achieving PN by rotating disc biofilm reactor, found 68% to 88% nitrite accumulation at the optimal operational NLRs of 8.5 and 11.5gN/m3.d, pH > 8 and dissolved oxygen concentration <3 mg/L at 20°C (Huiliñir et al., 2010).

Kinetics based models with increasing complexities had been proposed for activated sludge (ASM) evolving from ASM1, ASM2 and ASM3(Henze et al., 2015). ASM models had been widely used in carbon, nitrogen, and phosphorus removal in different treatment processes(Hauduc et al., 2013). ASM models consider nitrification process as a single step process where conversion of nitrite to nitrate is not considered, and consequently are not suitable for PN modeling (Béline et al., 2007). However, a modified ASM3 had been used by Hoang et al. (Hoang et al., 2012), where nitrite was an intermediate substrate, resulted in maximum growth rates of 0.61 d⁻¹ and 0.108 d⁻¹, half velocity constant of 1.37 and 1.59 mgO₂/L for AOB and NOB respectively in pilot scale SBR system. Gao et al. (2010) developed a mathematical model and used DO of 3.5 mg/L at 26°C, the observed yield and maximum growth for AOB of 0.26 mgSS/ mg NH4, and 0.46 d⁻¹ respectively which resulted in more than 95% of nitrite accumulation in SBR systems(Gao et al., 2010). However,
previous models did not consider temperatures higher than 35°C and were predominantly applied in side-stream N removal. A mathematical model was developed using FA and FNA as true substrates, solids retention time of 10-30 days, pH of 7-8.5 and temperature range of 10-35°C to predict minimum dissolved oxygen and found that predicted minimum DO for AOB was increased if FA was used as true substrate. The input maximum growth rates, half saturation constants for oxygen, inhibition constants for FA and FNA were 0.74 d\(^{-1}\) and 0.65d\(^{-1}\), 0.5 mgO\(_2\)/L and 0.59 mgO\(_2\)/L, 7 mgFA/L and 0.95 FAmg/L, 0.55 FNA mg/L and 0.06 FNA mg/L for AOB and NOB respectively (Liu, 2020). A study using zeolite biological aerated filter with synthetic wastewater found 98% stable nitrite accumulation due to the FA inhibition of NOB, and kinetic analysis showed that production of nitrite followed the zero order kinetics (Yang et al., 2017). Park et al. (2010) used a mathematical model considering the simultaneous effect of pH, oxygen, and FA and FNA inhibition using maximum specific substrate utilization rates, half saturation constants for oxygen, FA and FNA inhibition. Using specific substrate utilization rates of 3.1 gN/mgVSS-d and 13 gN/mgVSS-d, half saturation constants of 0.5 mgDO/L and 0.68 mgDO/L, concentrations of FA of 10 mgFA/L and 0.75 mgFA/L, concentrations of FNA of 0.5 mgFNA/L and 0.1 mgFNA/L respectively for AOBs and NOB, found that FA inhibition increases with pH, but FNA inhibition increases as pH is decreased (Park et al., 2010).

2.11 Synopsis of literature review

Currently, mainstream wastewater nitrogen removal is done by conventional nitrification denitrification processes, which is not only energy intensive and requires carbon supplementation but also produces substantial quantities of sludge, thus increasing treatment costs. PN can cut down the nitrification oxygen and energy by around 60%, eliminate exogenous carbon addition and reduce sludge production.

There are many strategies that are used for achieving partial nitrification such as DO, pH, SRT, aeration mode. As the accumulation of nitrite is the main goal of PN, the conditions that favour AOB that should be maintained. Side-stream conditions are completely favourable for AOB and detrimental to NOBs due to the inhibition of NOB by FA and FNA at high temperature (35°C) and high ammonia (500-1000 mg/L). Hence, side-stream partial
nitrification has been implemented in full scale plants, with various suspended growth and attached growth technologies. Since side-stream contains only 30% of total nitrogen of the wastewater (Lacroix, Mentzer, and Pagilla 2020), mainstream PN is attracting the interest of both researchers and practitioners. However, due to high COD/N ratio, no FA/FNA inhibition and low temperature, mainstream PN is challenging to achieve. To decouple the effect of low ammonium concentration and low temperature in mainstream, the heat shock strategy is considered in this study to achieve PN. To date, only four studies have been conducted to investigate the heat shock effect on NOB suppression, two of which used encapsulated biomass. However, only one study had been conducted on the heat shock in mainstream suspended growth systems.

Modeling and simulation were done predominantly for investigating the optimum operating conditions for successful PN in a temperature range of 10°C - 35°C. Hence, no model included the extended Arrhenius equation for temperature greater than 35°C, and in the range inhibitory to both AOB and NOB.

2.12 Research Gaps

In achieving mainstream PN, optimum pH, low DO and intermittent aeration along with tight process control (Jiang et al., 2018) to suppress NOB (Erdirenceli & Koyuncu, 2017) are used. However, these studies are mostly focused on operating temperature ranging from 25°C - 35°C.

Only four studies used heat shock, three of them used DO limitation and heat to achieve partial nitrification (Chen et al., 2016; Choi et al., 2021; Isaka et al., 2008) with synthetic wastewater in biofilm processes. The fourth study used intermittent heating and seeding in a suspended growth system with real municipal wastewater. However, none of the studies used direct heat to the MLSS to achieve PN. Additionally, none of the study modelled the temperature coefficient for microbial substrate utilization and growth at higher temperature
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Chapter 3

3 Effect of Thermal Shock and Sustained Heat Treatment on Mainstream Partial Nitrification and Microbial Community in Sequencing Batch Reactors

3.1 Introduction

Second-generation biological nutrient removal (BNR), such as partial nitrification-denitrification (PN-d) and PN-anaerobic ammonia oxidation (ANAMMOX) processes have been gaining wide acceptance due to lower energy consumption (Peng & Zhu, 2006; van Dongen et al., 2001). Various operational parameters, including dissolved oxygen (DO) concentration, solids retention time (SRT), pH, free ammonia (FA), and free nitrous acid (FNA) concentrations, are controlled to hinder the growth of nitrite oxidizing bacteria (NOBs) (Liu et al., 2020). By maintaining a low DO concentration that favors ammonium-oxidizing bacteria (AOBs) over NOBs, the growth of NOBs can be suppressed (Law et al., 2018). Liu et al. (2020) reported that FA and FNA concentrations of 10 to 605 mg/L and 0.1 to 5 respectively inhibited AOBs whereas an FNA concentration of 0.011 mg/L was adequate for NOBs inhibition. The range of mainstream ammonia concentration typically varies from 15 to 50 mg/L (Negulescu, 1986) which produces FA and FNA in the range of 0.01-0.05 mg/L and 0.001-0.005 mg/L, respectively (Anthonisen et al., 1976) that are well below the thresholds of NOB inhibition. Optimum pH levels have also been suggested for achieving PN (He et al., 2012; Park et al., 2007). However, pH is variable in real wastewater and controlling pH is uneconomical (Xu et al., 2015).

Side stream anaerobic digester liquor temperature of >30°C favors AOBs over NOBs as the high ammonia concentration (300-900 mg/L) produces FA and FNA which inhibit NOBs (He et al., 2012; Xu et al., 2015). Therefore, PN has been successfully implemented in full scale wastewater treatment plants (WWTPs) for side stream nitrogen removal (Hauck et al., 2016). However, mainstream PN faces numerous challenges mainly due to low ammonia and low temperature (Ma et al., 2020). Many studies showed the effect of
temperature on the process parameters within the range of 20°C-35°C (Bougard et al., 2006; Kim et al., 2008). To the best of the author’s knowledge, only four studies have investigated the effect of high heat shock and contact time on the inhibition of AOBs and NOBs.

For instance, Isaka et al. (2008) investigated the impact of heat shock in the temperature range of 50°C to 90°C and contact time ranging from 20 to 120 minutes on NOB suppression in batch tests using encapsulated gel carriers. This study found that AOBs were present up to 90°C, and no NOBs were detected after the heating temperature reached at 60°C. Chen et al. (2016) utilized encapsulated heated nitrifying sludge at 60°C and 70°C for 20 minutes in a continuous flow reactor fed with synthetic influent and achieved a nitrite accumulation ratio (NAR) of 90%. However, the unheated control reactor of these two studies exhibited rapid nitritation, which limits the validity of achieving PN solely through the heat shock to the carriers. Similarly, a study by Choi et al. (2021) who operated a PN-ANAMMOX system employing a pre-heated encapsulated inoculum, reported that the optimum heating was at 60°C for 20 min once a day, produced a NAR of 0.86±0.1. Similar to the previous two studies, this study considered the application of variations of DO (0.7 mg/L to 2.3 mg/L) along with heat. Chen et al. (2019) demonstrated the feasibility of heating suspended biomass to inhibit NOBs in SBRs at temperature 50°C and 60°C with NARs of 94.5% and 75.6% respectively.

However, only one of the four aforementioned studies using heat shock for PN confirmed that the nitritation can be achieved only by the application of heat to the suspended inoculum and none reported the soluble COD (SCOD) and soluble organic nitrogen (SON) increase due to solubilization of particulate matter in the heated sludge (Penaud et al., 2000; Stark, J, 2016). Moreover, elevated refractory soluble organic nitrogen (rSON) in the process due to thermal treatment of the sludge can cause challenges with stringent effluent TN criteria (Pagilla et al., 2006).

Heat shock NOB suppression may have its merit in treatment plants that already have thermal processes, such as thermal hydrolysis (THP). Heating a portion of the returned mixed liquor via heat exchanger after the thermal hydrolysis unit could have some benefits for the nitrification process in the wastewater treatment plant. According to González et al.
(2021), increasing the temperature of the mixed liquor from 42 to 47°C could enhance the activity and growth of ammonia-oxidizing archaea (AOA), which are more thermophilic than ammonia-oxidizing bacteria (AOB) (González et al., 2021). AOA are also more resistant to inhibition by free ammonia and nitrous acid, which are common in high-strength wastewaters. Therefore, heating a portion of the mixed liquor could improve the ammonia removal efficiency and stability of the nitrification process. However, this approach also has some implications, such as higher energy consumption, potential inhibition of nitrite-oxidizing bacteria (NOB), and possible interference with denitrification and phosphorus removal (Chuang Shun-Hsing et al., 1996). Therefore, the optimal temperature and proportion of the heated mixed liquor should be carefully determined based on the characteristics of the wastewater and the performance of the treatment plant.

Given the paucity of information on the impact of heat shock on mainstream nitrification using suspended microorganisms, the main objective of this work is to assess the feasibility of achieving mainstream PN using offline heating of a portion of the system biomass, as well as the sustained in-line heating of the entire reactor, at moderate offline and inline temperatures (37°C to 47°C) in contrast to the 50°C-80°C used in the literature. This study also correlated and evaluated the microbial communities in heated reactors with temperatures and reactor performance. Effluent refractory soluble organic nitrogen that is a key parameter impacting the achievability of stringent TN effluent criteria, was also investigated. The temperature and duration of offline heating was selected based on the results of the batch reactors of this study. Despite impracticality, the sustained heat at 37°C and 42°C were explored to investigate the effect of continuous heat on mainstream PN and the possible revivability of NOBs.

3.2 Materials and Methods

3.2.1 Municipal wastewater characteristics

Four SBRs: R1, R2, R3 and R4, with a working volume of 2L each were operated with primary effluent (PE) from the Greenway Wastewater Treatment plant (WWTP), London, Ontario, Canada. The primary effluent was stored at 4°C before use. Wastewater was
characterized twice a week. The reactors were inoculated with RAS from the Greenway WWTP (TSS of 5.9 g/L and VSS of 4.1 g/L) diluted to an initial MLVSS concentration of 2 g/L in the reactors. PE characteristics are shown in Table 3-1.

Table 3-1. Composition of primary effluent ((Mean ± Standard Deviation (No. of samples))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total suspended solids (TSS)</td>
<td>88±32(18)</td>
</tr>
<tr>
<td>Volatile suspended solids (VSS)</td>
<td>68±21(18)</td>
</tr>
<tr>
<td>Total Chemical oxygen demand (TCOD)</td>
<td>164±56(19)</td>
</tr>
<tr>
<td>Soluble Chemical Oxygen demand (SCOD)</td>
<td>81±26(19)</td>
</tr>
<tr>
<td>Ammonia-N (NH$_3$-N)</td>
<td>24±3.8(18)</td>
</tr>
<tr>
<td>Nitrate-N (NO$_3$-N)</td>
<td>0.6±0.5(17)</td>
</tr>
<tr>
<td>Nitrite-N (NO$_2$-N)</td>
<td>0.2±0.3(17)</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>37±7.3(17)</td>
</tr>
<tr>
<td>Soluble nitrogen (SN)</td>
<td>28±5.7(17)</td>
</tr>
<tr>
<td>Total Phosphorus (TP)</td>
<td>4.6±1.5(17)</td>
</tr>
<tr>
<td>Soluble Phosphorus (SP)</td>
<td>2.6±0.4(18)</td>
</tr>
<tr>
<td>pH</td>
<td>8.2±0.4(15)</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO$_3$/L)</td>
<td>330±32(15)</td>
</tr>
</tbody>
</table>

3.2.2 Offline batch tests

Specific nitrification rate (SNR) tests were performed in batch reactors with a working volume of 1L to determine the activity of AOBs and NOBs, using the settled sludge from a pilot scale nitrifying/denitrifying SBR at Greenway (MLVSS of 4.1-6.5 g/L) operated two cycles per day, at a fill ratio of 50%, DO of 1.5±0.5 mg/L and SRT of 10 days (Ahmed et al., 2021)(Ahmed et al., 2021). A control reactor at ambient temperature (22°C) and the heat-treated reactors (at 40°C, 45°C, 50°C, 55°C) were simultaneously operated for contact times of 2 hours. The SNR tests were conducted for 4 hours, and samples were collected from the start to the end of the operation. After completion, all the heated reactors were cooled down to room temperature, and an additional SNR test was conducted after cooling for 4 hours. Prior to starting the tests during the heating phase and after cooling, the reactors were fed with ammonia at a concentration of 20 mg/L, alkalinity of 160 mg/L, phosphorus of 4 mg/L, and trace elements, including MgSO$_4$ (69.6 mg/L), CuSO$_4$.5H$_2$O (0.06 mg/L),
MnCl$_2$·4H$_2$O (0.24 mg/L), CoCl$_2$·6H$_2$O (0.24 mg/L), and ZnCl$_3$ (0.3 mg/L) (Zaman et al., 2019). An air pump (Marina, 75 Air Pump) was used to maintain the DO concentration in the batches at 3-4 mg/L.

3.2.3 Operation of SBRs

Reactor R1 was operated as the baseline reactor at 22°C (room temperature) and reactor R2 was operated at room temperature, with offline intermittent heating of 20% of the MLSS to 42°C/47°C using a water bath (VWR® Heated Circulating Baths). For offline heating, 286 mL (20% of the MLSS of R2) was placed in an 800 mL jacketed reactor. Wasting occurred before the 1-hr idle phase started. The jacketed reactor was heated by the water bath to approx. 42°C/47°C for 2 hours. Two continuously heated reactors at 37°C (R3) and 42°C (R4) were operated to assess long-term acclimatization and revivability of NOBs, after potential suppression by heat. These reactors were covered with aluminium paper insulation and temperature was confirmed by the daily measurements with a special application instrument thermometer (Thermco®, Quebec, Canada). The experimental set up of the SBRs is shown in Figure 3-1.

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Figure 3-1. Schematic of the SBRs with inline heating
The 2-L working volume SBRs were operated at an SRT of 7 days, with 2 and 4 cycles per day, with a volumetric nitrogen loading rate of 0.04 gN/L·d and 0.08 gN/L·d, using diffused aeration for 8 hours and 4 hours. As the ammonia concentration in all reactors was zero after 4 hours of aeration, the aeration time was reduced to 4 hours to increase the nitrogen loading to the reactors. The air flow rate was 0.4 L/min, which provided DO of 4 mg/L in the reactors. The decanting (0.5 hour), feeding (0.5 hour), and settling (1 hour) periods were anoxic. The pH was not controlled in the reactors and was measured between 8.0 to 8.6 in the influent. All the reactors were operated in 3 stages (S1, S2 & S3) for 28, 19 and 20 days respectively. S1 was operated with 2 cycles per day while S2 and S3 were operated with 4 cycles per day. The stages were defined to demonstrate the duration of NOB inhibition and NOB revival. The duration of operation was selected to maintain approximately 2.5 to 3 SRT turnovers to reach steady state (SS). Details of the operation are provided in Table 3-2.

Table 3-2. Operational conditions of the sequencing batch reactors (SBRs)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R1 (22°C)</th>
<th>R2 (42°C/47°C)</th>
<th>R3 (37°C)</th>
<th>R4 (42°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2 hours, off-line</td>
<td>Continuous in-</td>
<td>Continuous in-</td>
</tr>
<tr>
<td>Stages (S)</td>
<td>S1</td>
<td>S2&amp;S3</td>
<td>S1</td>
<td>S2&amp;S3</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>S2&amp;S3</td>
<td>S1</td>
<td>S2&amp;S3</td>
</tr>
<tr>
<td>pH (Avg)</td>
<td>8.3</td>
<td>8.3</td>
<td>8.3</td>
<td>8.3</td>
</tr>
<tr>
<td>Operation (days)</td>
<td>28</td>
<td>19&amp;20</td>
<td>28</td>
<td>19&amp;20</td>
</tr>
<tr>
<td>Fill ratio</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7(^a)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cycles (per day)</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Aeration (min)</td>
<td>480</td>
<td>240</td>
<td>480</td>
<td>240</td>
</tr>
</tbody>
</table>

\(^a\) R2 was filled with 1L of influent and 0.34L of heated MLSS before aeration.

### 3.2.4 Sampling and monitoring

Filtered and unfiltered influent and effluent samples were collected and analyzed for TSS, VSS, TCOD, SCOD, TN, SN, NH₃-N, NO₃--N, NO₂--N, alkalinity, and pH twice a week.
MLSS and MLVSS during the aeration were analyzed once a week. The SRT in the reactors was controlled at 7 days by wasting 286 mL (approx.) of the MLSS daily at the end of the aeration period in one cycle. The volume of the waste activated sludge (WAS) was calculated by using the actual working volume in the reactors (mL MLSS in the reactors), effluent VSS concentration and the influent and effluent flow rates. WAS was analyzed for TSS, VSS, TN and TCOD once a week.

3.2.5 Cyclic SNR tests

During the aeration period, filtered samples for SNR tests were collected from all the reactors for analyzing COD, NH\textsubscript{3}-N, NO\textsubscript{3}--N and NO\textsubscript{2}--N, alkalinity, and pH. MLSS and MLVSS were collected at the start and at the end of the aeration period during the SNR test. SNR samples were collected from all reactors during the same cycle of reactor R2 when 20% of heated MLSS were returned to R2. The maximum specific ammonia uptake rates, specific nitrite, nitrate, and NO\textsubscript{x} production rates were calculated using Equation (3-1).

\[
K = \frac{\Delta C}{\Delta t \cdot MLVSS} \quad (3-1)
\]

Where K is the maximum specific uptake or production rates. ΔC is the change of nitrogen at time interval \(\Delta t\), MLVSS is initial concentration of mixed liquor volatile suspended solids.

3.2.6 Wastewater parameters

Primary effluent (PE) was stored at 4°C for a week and characterized before use. Total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, and pH of the samples were measured by Standard Methods (APHA): 2540D, 2540E, 2320B and 4500-H+ B respectively (Federation & Association, 2005). Total and Soluble COD (TCOD and SCOD), total and soluble nitrogen (TN and SN), ammonia nitrogen (NH\textsubscript{3}-N), nitrite nitrogen (NO\textsubscript{2}--N), and nitrate nitrogen (NO\textsubscript{3}--N) were measured by HACH methods. Sterile 0.45 µm membrane filter papers were used to obtain soluble fractions of the samples. Hach methods i.e., chemical oxygen demand (COD, 200-15,000 mg/L, method 8000), soluble nitrogen,
ammonia (0.4-50 mg/L, method 10031), and nitrogen (10-150 mg/L, method 10071) were used. All the parameters were analyzed twice a week.

### 3.2.7 Microbial community analysis

On day 67, MLSS samples were collected from all reactors and were sent to Institut de recherche et de développement en agroenvironnement (IRDA) to investigate the microbial community. All the samples were analyzed for the diversity (quantification) and abundance of microorganisms at the phylum and genus levels to identify the bacteria respectively. For the microbial test, 2mL homogenous samples of MLSS were prepared from the aforementioned reactors. Then DNA was extracted using the FastDNA Spin Kit for Soil Extraction Kit (MP Biomedicals, Solon, OH, USA). The quantity and quality of the extracted genomic DNAs were determined using spectrophotometric technique with absorbance measurements at 260 nm and 280 nm and the A260 / A280 ratio. Total archaea/bacteria were detected by molecular detection with quantitative PCR (qPCR) with primers eub338 / eub518 (Fierer et al., 2005). The detections were conducted using two replicates on a CFX96 (Biorad, Hercules, CA, USA) with a SYBR green qPCR mix (Qiagen, Toronto, ON, Canada). The detection system was developed on a detection range of 4 LOGs (Efficiency-system total bacteria: 89.1% $r^2 = 0.99$). The archaeal and bacterial diversity was evaluated by using metagenomic analysis. Amplifications of the V4 regions of archaeal and bacterial 16S rRNA were performed using the primer sequences specific to those regions (Apprill et al., 2015), employing a two-step dual-indexed PCR approach (PCR) designed specifically for the analysis with Illumina MiSeq sequencing platform. The MIDAS 3.0 database was used for taxonomic identification of prokaryotes.

### 3.3 Results and Discussion

#### 3.3.1 Activities of AOBs and NOBs in batch reactors

For investigating the activities of the nitrifiers and the recoverability of NOBs, SNR tests were conducted at room temperature (22°C), 40°C, 45°C, 50°C and 55°C during heating and after cooling to room temperature. The activities of AOBs and NOBs were monitored by the maximum specific nitrogen oxidation rates. The maximum specific NH$_3$-N uptake
rate (AUR) and the maximum specific NO\textsubscript{2}--N, NO\textsubscript{3}--N and NOx--N production/uptake rates (PR/UR), estimated using the slope of the best linear fit of nitrogen versus time, are shown in Table 3-3.

**Table 3-3. Activities of AOBs and NOBs in the batch reactors**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>22℃</th>
<th>40℃</th>
<th>45℃</th>
<th>50℃</th>
<th>55℃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT\textsuperscript{b}</td>
<td>HT\textsuperscript{c}</td>
<td>HT\textsuperscript{c}</td>
<td>AC\textsuperscript{d}</td>
<td>HT\textsuperscript{c}</td>
</tr>
<tr>
<td>No of SNR tests</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>AUR (mgN/gVSS.hr)</td>
<td>3.80</td>
<td>2.49</td>
<td>1.19</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>NO\textsubscript{3}-PR (mgN/gVSS.hr)</td>
<td>3.50</td>
<td>2.05</td>
<td>0.02</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>NO\textsubscript{2}-PR (mgN/gVSS.hr)</td>
<td>0.8</td>
<td>0.44</td>
<td>1.17</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>NOx-PR (mgN/gVSS.hr)</td>
<td>4.3</td>
<td>2.49</td>
<td>1.22</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>NO\textsubscript{3}-PR/NO\textsubscript{x}-PR</td>
<td>0.80</td>
<td>0.82</td>
<td>~0</td>
<td>-</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Note: AUR = NH\textsubscript{4}-N uptake rate, NO\textsubscript{3}-PR = NO\textsubscript{3}-N production rate, NO\textsubscript{2}-PR = NO\textsubscript{2}-N production rate, NO\textsubscript{x}-PR = NOx production rate, \textsuperscript{b}=Room Temperature, \textsuperscript{c}=Heat Treatment, \textsuperscript{d}=After Cooling

At 22℃, AOBs and NOBs showed similar kinetics with maximum specific AUR and NO\textsubscript{3}-PR rates of 3.8 mgN/gVSS·hr and 3.5 mgN/gVSS·hr, respectively, indicating that AOBs and NOBs had similar ammonia and nitrite assimilation capacity at the control temperature. At 40℃, the maximum specific AUR was 2.49 mgN/gVSS·hr with a maximum specific NO\textsubscript{3}-PR rate of 2.05 mgN/gVSS·hr indicating that at 40℃, the activity of AOBs and NOBs decreased, by 35%, and 42%, compared to the control. However, at 22℃ and 40℃, NO\textsubscript{3}-PR/NO\textsubscript{x}-PR were 0.80 and 0.82, respectively, indicating essentially identical activities of AOBs and NOBs at these temperatures. At 45℃ -2-hour heating, two duplicates were run to confirm the specific nitrogen uptake or production rates. In first duplicate, the AUR, NO\textsubscript{2}-PR and NO\textsubscript{3}-PR were 1.19 mgN/gVSS·hr, 1.17 mgN/gVSS·hr and 0.02 mgN/gVSS·hr respectively while in second duplicate the rates were 1.39 mgN/gVSS·hr, 1.43 mgN/gVSS·hr, and 0.02 mgN/gVSS·hr respectively, confirming the suppression of NOBs. These results indicate that although NOBs were completely suppressed, AOBs were still active with a lower specific AUR rate (1.19/1.39 mgN/gVSS·hr) compared to control (3.80 mgN/gVSS·hr at 22℃).

However, the rates decreased significantly after cooling from 45℃ to room temperature at 22℃. When exposed to temperatures of 50℃ and 55℃ for a heating time of 2 hours, no significant activities of AOBs and NOBs were observed during heating and after cooling, indicating a complete suppression of AOBs and NOBs. Nonetheless, no NOBs were
recovered after cooling at 45°C-55°C in batch tests. Based on these experimental outcomes, the starting temperature of the offline heating in SBR was selected as 42°C for 2 hours.

3.3.2 COD and SON

The SCOD, filtered flocculated COD (ffCOD), and soluble organic nitrogen (SON) were analyzed after heat treatment of 2 hours, and are shown in Table 3-4.

Table 3-4. Summary of sludge characteristics after heat treatment for 2 hours

<table>
<thead>
<tr>
<th>Sample</th>
<th>SCOD (mg/L)</th>
<th>ffCOD (mg/L)</th>
<th>SON (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control at 22°C</td>
<td>33</td>
<td>26</td>
<td>1.3</td>
</tr>
<tr>
<td>Heated at 40°C</td>
<td>36</td>
<td>-</td>
<td>3.1</td>
</tr>
<tr>
<td>Heated at 45°C</td>
<td>39</td>
<td>-</td>
<td>3.2</td>
</tr>
<tr>
<td>Heated at 50°C</td>
<td>91</td>
<td>69</td>
<td>7.2</td>
</tr>
<tr>
<td>Heated at 55°C</td>
<td>118</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

It is evident that both SCOD and SON increased with the increase in temperature. It is also apparent that significant solubilization of particulate organic matter occurred at temperatures 50°C and above. The overall ffCOD and SON increased by 43 mg/L and 5.9 mg/L respectively for pre-heated sludges at 50°C compared to control. While the typical non-biodegradable soluble organic nitrogen in domestic wastewater of 1-2 mg/L (Parkin & McCarty, 1981), preheating at 40°C-45°C, increased the SON by a factor 2.5 relative to the control, and preheating at 50°C or above, significantly increased SON by close to an order-of-magnitude. Such elevated SON in the effluent of heat-treated biomass may impact compliance with stringent nitrogen limits of below 5 mg/L, without tertiary treatment.

3.3.3 SBR Operation

3.3.3.1 Performance of SBRs

The PE from Greenway WWTP is a low strength wastewater with COD:N ratio, COD:P ratio and VSS:TSS ratio of 4.5, 33 and 0.76 respectively. The overall soluble fractions of PE COD, N, and P were 50%, 76%, and 60%, respectively. The overall ranges of removal
of TSS, VSS, TCOD, SCOD, and TKN removal ranged from 72%-85%, 76%-85%, 70%-83%, 48%-78%, and 83%-95% respectively. The overall removals at steady state by the reactors are shown in Table 3-5.

Table 3-5. Percent removal TSS, VSS, COD and TKN at steady state (Average ± Standard Deviation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R1 (22°C)</th>
<th>R2 (42°C/47°C)</th>
<th>R3 (37°C)</th>
<th>R4 (42°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 hours, off-line</td>
<td>Continuous in-line</td>
<td>Continuous in-line</td>
<td></td>
</tr>
<tr>
<td>Stages (S)</td>
<td>S1</td>
<td>S2&amp;S3</td>
<td>S1</td>
<td>S2&amp;S3</td>
</tr>
<tr>
<td>TSS</td>
<td>72±10</td>
<td>79±11</td>
<td>83±7</td>
<td>75±10</td>
</tr>
<tr>
<td>VSS</td>
<td>77±5.9</td>
<td>82±10</td>
<td>81±10</td>
<td>77±9.7</td>
</tr>
<tr>
<td>TCOD</td>
<td>81±2.34</td>
<td>79±2.8</td>
<td>81.5±3.7</td>
<td>74±9.2</td>
</tr>
<tr>
<td>SCOD</td>
<td>62±5.8</td>
<td>78±1.8</td>
<td>60±15</td>
<td>76±2.9</td>
</tr>
<tr>
<td>TKN</td>
<td>84±4.32</td>
<td>94±3.9</td>
<td>83±9.7</td>
<td>85.1±9.2</td>
</tr>
</tbody>
</table>

3.3.3.2 Correlation of SBR performance with heat and nitrogen loading rates

Effluent TCOD, SCOD, and TSS ranged from 21-34 mg/L, 19-32mg/L and 8.7-29 mg/L (Table 3-6), respectively in the control reactor as compared to 21-34 mg/L, 19-32 mg/L and 11-29 mg/L, respectively in the heated reactors. Further investigation was conducted to determine the impact of loading rates in the control and heated SBRs which is shown in Figure 3-2.
Table 3-6. Effluent quality at steady state (Average ± Standard Deviation)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (22°C)</th>
<th>R2 (42°C/47°C) 2 hours, off-line</th>
<th>R3 (37°C) Continuous in-line</th>
<th>R4 (42°C) Continuous in-line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stages (S)</td>
<td>S1</td>
<td>S2&amp;S3</td>
<td>S1</td>
<td>S2&amp;S3</td>
</tr>
<tr>
<td>TCOD</td>
<td>24±2.9</td>
<td>24±3.4</td>
<td>23±4.7</td>
<td>29.6±11.4</td>
</tr>
<tr>
<td>SCOD</td>
<td>21±1.4</td>
<td>22±4.8</td>
<td>21±6.6</td>
<td>25±6.1</td>
</tr>
<tr>
<td>TN</td>
<td>25±3.7</td>
<td>29±1.9</td>
<td>27±2.1</td>
<td>28±4.7</td>
</tr>
<tr>
<td>SN</td>
<td>22±4.9</td>
<td>23.5±4.5</td>
<td>23±5.2</td>
<td>20±0.84</td>
</tr>
<tr>
<td>TP</td>
<td>2.1±0.4</td>
<td>3.1±0.5</td>
<td>2.3±0.4</td>
<td>4.1±1.1</td>
</tr>
<tr>
<td>SP</td>
<td>1.24±0.3</td>
<td>1.42±0.6</td>
<td>1.74±0.3</td>
<td>2.3±0.91</td>
</tr>
<tr>
<td>TSS</td>
<td>20±3.26</td>
<td>8.7±2.63</td>
<td>12.6±2.5</td>
<td>14.8±4.8</td>
</tr>
<tr>
<td>VSS</td>
<td>10.7±2.1</td>
<td>7.3±1.24</td>
<td>7.3±2.9</td>
<td>12±0.81</td>
</tr>
</tbody>
</table>

![Figure 3-2. COD, TKN, and TP removal efficiencies in the SBRs](image)

The overall COD, TKN and TP removal were 81%, 84% and 46% in the control reactor compared to 76-83%, 83%-89% and 42%-48% respectively in the heated at loading rates.
of 0.04 gN/L·d. At loading rate of 0.08 gN/L·d the COD, TKN and TP removals were 80%, 94% and 49% compared to heated reactors with COD, TKN and TP ranges of 73% to 75%, 85%-95% and 44-55%. It is conspicuous from Figure 3-2 that the performance of the control reactor was generally more stable than the heated reactors, as reflected by the low standard deviations. Although at the higher loading rate, COD removal in the heated reactors was generally lower than the control reactor, both TKN removal and TP removal were considerably higher. The effluent quality depicted in Table 6 indicates that the effluent VSS from the heated reactors were generally higher than the control, thus rationalizing the lower COD removal efficiency. As mentioned above at the low loading rate, the biomass in the SBRs was in the decay phase for about 4 hours per cycle, which coupled with higher decay rates in the heated reactors results in solubilization of cellular organics, nitrogen, and phosphorous. Conversely, at the high loadings, the aerobic reaction time of 6 hours was just sufficient for the biological reactions, with minimal endogenous decay.

3.3.3.3 Effluent nitrogen species

Nitrogen species in the SBR effluents and the NAR are shown in Figure 3-3.
The overall effluent NH$_3$-N concentration was at 0.5 mg NH$_3$-N/L in the reactors at all temperature conditions. As depicted in Figure 3, most of the NO$_x$ in the effluent from the reactors were predominantly NO$_3$--N except in S2 in R2 and R3 and S1 in R4. In S2 at 47°C offline heating (R2), the effluent NO$_2$--N concentrations were 5.02 to 8.37 mg/L, compared to 8.9 to 14.9 mg/L and 10.1 to 20.7 mg/L in S2 (R3) and S1 (R4), respectively, at inline continuous temperatures of 37°C and 42°C respectively.

Figure 3.3. Concentration of nitrogen species in the SBRs effluents and the nitrite accumulation ratio (NAR)
The NAR was calculated by dividing the effluent nitrite concentration by the effluent NOx concentration. The achievability of PN in the reactors was checked whether the NAR was equal or greater than 0.78 (Ma et al., 2020). At 22°C, the NAR ranged from 0.01 to 0.05. However, in the heated reactors, NAR values were significantly higher: 0.30-0.47 for R2 (offline heating at 47°C for 2 hours), 0.37-0.59 for continuous heating at 37°C (R3), and 0.63-0.79 for continuous heating at 42°C (R4). This indicates that higher temperatures and longer durations led to higher NOB inhibition.

Since the operational conditions i.e., DO, SRT and pH did not change in the reactors, and the concentrations of FA/FNA were well below the AOB/NOB inhibition threshold (discussed below), the reason for the accumulation of NO$_2$--N in the reactors is hypothesized to be the suppression of NOBs via the heat treatment. The inhibition rate increased with temperature, as confirmed by the more rapid NO$_2$--N production in R4 than R2 and R3, shown in Figure 3. However, although NOBs inhibition persisted only for 10 to 12 days in all SBRs, NOB recovered in both the intermittent and continuously heated SBRs. In comparison, NOBs were not revived upon cooling after heating at 45°C in the batch reactors operating with 4 hours of aeration (see section 3.1), indicating that the revival of NOBs takes time, as discussed later.

3.3.3.4 Ammonia accumulation and lost in mainstream PN dynamics.

In the heated reactors at 37°C -continuous, 42°C continuous and 47°C-2 hours heating, nitrite accumulation was stable for 10-12 days and then declined to complete nitrification. Conventionally, mainstream wastewater with high C/N will not provide the FA and FNA concentration that can achieve PN at low temperature. However, elevated temperatures along with the pH in the wastewater can be a cursor to produce FA and FNA in the system that may exceed inhibition threshold of AOBs and NOBs limiting the hypothesis of achieving mainstream PN solely with the application of the heat. Therefore, FA and FNA were estimated according to the following equations:

$$\text{FA (mg L}^{-1}) = \frac{17}{14} \times \frac{\text{NH}_2^+ - N \times 10^{-\text{pH}}}{10^{-\text{pH}} + \exp (6344/(273+T))} \quad (3-2)$$
where, \( \text{NH}_4^+ - N \), \( \text{NO}_2^- - N \), and \( \text{NO}_3^- - N \) are the effluent ammonia, nitrite, and nitrate respectively. pH in the equation refers to pH of the effluent. The estimated FA and FNA concentrations with the inhibition thresholds are shown in Figure 3-4.

\[
\text{FNA} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{46}{14} \times \frac{\text{NO}_2^- - N}{10^{\text{pH}} \times \exp (-23)/(273+T)} \quad (3-3)
\]

Figure 3-4. FA and FNA in control and heated SBRs
The FA and FNA concentrations vary in the ranges of 0-0.08 mg/L and 0 to 0.005 mg/L respectively in the reactors. These FA concentrations are way below the minimum AOB and NOB inhibition threshold reported by Liu et al. (2020) (minimum concentration of AOB and NOB inhibition by FA=10 mg/L and 0.1 mg/L respectively). Similarly, FNA concentrations were well below the inhibition threshold of AOBs and NOBs confirming that nitrite accumulation was not achieved by FA or FNA.

In this study all other operational parameters were kept fixed while temperature was applied to the reactors. Is it obvious that no operational parameters as well as FA and FNA concentrations were responsible for nitrite accumulation in the system other than heat. Studies reported that high temperature shock has profound effect on the structural and physiological properties of bacteria (Ratkowsky et al., 1983; Russell, 2003). Therefore, the accumulation of nitrite is the result of faster recovery of AOBs than NOBs. However, nitrite accumulation was lost after 10-12 days, and complete nitrification occurred. This can be due to the fact that nitrifiers were acclimatized in routine intermittent/sustained high temperature and could perform complete nitrification (Lopez-Vazquez et al., 2014).

3.3.3.5 Revivability of NOBs

The nitrite accumulation in the heated reactors is shown in Figure 3-5.

![Figure 3-5. Nitrite accumulation in the heated SBRs](image)
It is apparent that at 42℃ continuous heating, the activities of NOBs decreased after one day of operation whereas at 37℃, it required 37 days for inhibition to start. At 47℃ offline heating for 2 hours, the NOBs started to experience suppression from day 28. Furthermore, after the lag, the rates of nitrite accumulation were highest in R4 (at 42℃) and lowest in R2 (offline heating of 20% at 47℃), indicating the pronounced impact of continuous heating on NOB suppression, despite the lower sustained temperature compared to intermittent heating. This indicates that both the proportion of heated biomass and temperature impact NOB suppression. The nitrite accumulation rate in the reactor at 47℃ offline heating indicates that there may be a potential to achieve higher nitrite accumulation ratio at the same temperature with a higher proportion of heated biomass than 20%. At 42℃ and 37℃ continuous and 47℃ offline heating, nitrite accumulation was stable for 10 days, 10 days and 12 days, respectively, indicating that these temperatures inhibited the NOBs for the same period (about 1.5 turnovers of the mean SRT) regardless of the heating conditions.

Chowdhury & Nakhla, (2021) operated a continuous stirred-tank reactor (CSTR) with synthetic wastewater containing 37.8 ± 0.5 mg NH₃-N/L, 50.2 ± 1.2 mg NO₂--N/L and 2.5 ± 0.9 mg NO₃--N/L at temperature 37℃ and HRT of 2 days with gel-entrapped anammox in sodium alginate and sodium silicate beads and found that the SRT of the entrapped microorganisms was 66 days. The studies of Isaka et al. (2008), Chen et al. (2016), and Choi et al. (2021) who employed gel-encapsulated biomass preheated at 60℃-80℃ reported loss of PN after 77, 65, and 95 days, respectively, but the SRT values were not reported. Thus, using the 66-d SRT reported for gel-encapsulated systems by Chowdhury and Nakhla (2021), PN was lost after 1-1.5 SRT turnover. The suspended growth system used by Chen et al. (2019) reported that they needed to heat 50% of the process biomass every two weeks to maintain PN over 5 months. This data suggests that regardless of temperature or type of biological system (suspended or attached), the NOBs are revived in 1-1.5 SRT turnovers. The results of this study as well as the 4 other literature studies point to the reversibility of NOB inhibition within a narrow range of SRT turnovers. The effective microbial growth rate in any biological system is inversely proportional to the SRT and NOBs are no exception. Thus, although these data suggest an advantage of attached-growth systems over suspended-growth systems with respect to the absolute duration of stable PN,
both systems lose PN within 1-1.5 SRT turnovers, implying that NOB revival occurs, irrespective of the initial inhibition levels as result of different heat shock conditions. Based on the bacterial exponential growth model, at 1.5-SRT turnover, the NOB population is approximately 4.5 folds the initial population (which is inhibited by heat shocks). Conversely, heat shocks thus appear to completely inactivate about 80% of the initial NOB. The primary reason why the NOB activity as reflected by nitrate production rates at room temperature, after the heat shock, was almost negligible (Table 3) is the impact of the heat shock on the enzymatic activity of NOBs. Jurburg et al. (2020) who examined the impact of a heat shock (65℃ for only 90 seconds) on soil nitrifiers reported that AOB, *Nitrobacter*, and *Nitrospira* genes continued to decline for 15 days after the first shock and 10 days after the second shock. Thus, frequent heat shocks rather than sustained heat application are required irrespective of the treatment process, as NOBs appear to recover and adapt to continuous heating.

The results of the SBR operation discussed above clearly indicate that within the temperature range of 37°C-42°C operational temperature, PN can be achieved albeit lost after 1-1.5 SRT turnover. The higher the sustained temperature, the faster is the initiation of PN, and its subsequent loss. For example, In SBR 4, where a temperature of 42°C was maintained, PN occurred within the first 10 days (Fig. 3-5), and similarly in SBR2 where only 20% of the biomass was heated at 42/47°C, PN was initiated after one month. Thus, although the above-mentioned literature showed that both AOBs and NOBs can be adversely impacted by the heat, the results of this work show that the sensitivity of AOBs to heat shocks at or above 42°C is less than NOBs. As apparent from the SNR batch tests (Table 3-3), at temperatures of 45°C-55°C, the ammonia uptake rate was higher than the nitrate production rate.

### 3.3.3.6 Kinetics of nitrogen uptake and production rates in the reactors

The SNRs i.e., AUR, NO₂-PR NO₃-PR and NOₓ-PR in SBR operation were calculated as described in batch operation section and shown in Figure 3-6. As expected, no nitrite production was observed in R1. For R2, NO₂-PR was observed in S2 and ranged from 0.8
to 2.9 mg N/gVSS·hr while AUR ranged from 3.5 to 12.7 mg N/gVSS·hr. In S3, NO₂-PR had been completely lost in R2. For R3, AUR and NO₂-PR ranged from 8.1 to 11.6 mg N/gVSS·hr and 1.7 to 4.3 mg N/gVSS·hr respectively in S2 and S3 and NO₂-PR was lost in a week. For R4, PN had been observed in S1 resulting in NO₂-PR of 1.4 to 5.8 mg N/gVSS·hr with an AUR range of 4.4 to 8.5 mg N/gVSS·hr but was lost in S2 and in S3.
3.3.3.7 Nitrogen and alkalinity balances

To better understand the fate of nitrogen in the SBRs, steady-state (SS) mass balances for nitrogen and alkalinity were performed. The nitrogen mass balance was determined from the influent nitrogen to the SBRs, the effluent nitrogen from the SBR and the WAS nitrogen. The nonbiodegradable particulate nitrogen mass was estimated based on the ratio of non biodegradable fraction in particulate nitrogen of 0.14 mg non biodegradable N/particulate-N, within the 10%-15% used in ASM2 model (Henze et al., 1995). Inert particulate nitrogen fractions were 0.03, 0.02, 0.04 in S1, S2 and S3, respectively reflecting minimal variability. In the alkalinity balance, net alkalinity from nitrification, denitrification and ammonification was calculated based on stoichiometric alkalinity consumption of 7.14 mg CaCO$_3$/mg N oxidized, alkalinity production of 3.57 mg CaCO$_3$/mg NO$_3$--N denitrified, and 3.57 mg CaCO$_3$/mg organic nitrogen ammonified (Haandel et al., 2012) and then compared with the change in measured consumed alkalinity at SS. The nitrogen and alkalinity balances are provided in SI (Sections 1 and 2). The nitrogen balance closures were 84% to 102% in the reactors S1 (Table S1). SBR effluent nitrogen accounted for 43%-82% of the influent nitrogen, while denitrification accounted for only 6%-32% of the influent, despite the lack of a dedicated anoxic period. The WAS contained 7% to 36% of the influent nitrogen. To confirm the nitrogen balance, alkalinity balance was also performed as shown in SI (Table S2). The alkalinity balance ranged from 81% to 112% confirming the nitrogen distributions in the reactors.

Figure 3-6. Maximum specific nitrification rates in the SBRs
3.3.3.8 Effluent SCOD and rSON

The overall effluent SCOD was analyzed and the rSON was estimated by deducting NH$_3$-N, NO$_3$--N and NO$_2$--N from SN concentrations in the effluents of the reactors. The overall effluent SCOD and rSON concentrations are illustrated in Figure 3-7.

The overall average effluent SCOD in the effluents were 23 mg/L, 30 mg/L and 33 mg/L and 32 mg/L in R1, R2, R3, and R4, respectively, indicating increased SCOD in the heated reactors. Although soluble effluent BOD was not measured, it is postulated that the increased effluent SCOD in the heated reactors is nonbiodegradable. If the additional 10 mgSCOD/L in the heated SBRs effluent is biodegradable, it would correspond to about 6 mg SBOD$_5$/L, which may negatively impact nitrification. The study of Figueroa and Silverstein (1992) established a negative linear correlation of ammonia removal with soluble and particulate BOD, at concentrations as low as 10 mg/L (Figueroa & Silverstein, 1992). Thus, since complete ammonia removal was observed in the SBRs, the incremental effluent SCOD observed in the heated SBRs, is expected to be nonbiodegradable, which may still impact plants with COD rather than BOD effluent limits. The overall nitrogen in R1, R2, R3 and R4 were 2.1 mg/L, 2.2 mg/L, 3.8 mg/L and 3.9 mg/L indicating an increase of up to 1.8 mg rSON/L in the heated reactors compared to the control. For plants that have to meet stringent TN criteria of < 5 mg/L, the 1.8 mg/L increase in rSON may have profound implications, with respect to required nitrification and denitrification efficiencies, since municipal wastewaters already contain about 1-2 mg rSON/L (Kim & Nakhla, 2010).

Figure 3-7. SCOD and rSON in the SBRs
3.4 Energy consumption and feasibility of heat assisted PN in PN-Anammox process

Nitrification energy estimates for a real 1000 m$^3$/d WWTP equipped with diffused aeration system in 4-m deep aeration tanks were evaluated to assess the energy savings arising from PN. The operational conditions, the effluent ammonia and nitrite concentrations were selected based on the effluent ammonia and nitrite concentrations from PN reactors of this study and Chen et al. (2019b) are shown in Table S3. The heating temperature and duration were considered from the range 47℃ -2hr (this study) to 60℃ -0.67hr (Chen et al., 2019b) as the latter showed 81% stable NAR. For heat optimization, the heating frequency of once in 10 days was selected based on the stability of PN of 10-12 days in this study and all the aforementioned studies that used heat to suppress NOBs. The oxygen transfer efficiency and mechanical efficiency were taken as 10% and 70% respectively. Two scenarios were evaluated with respect to aeration energy required for nitrification only: a- complete nitrification, and b-PN-Anammox using influent flow splitting, based on the anammox stoichiometry, and the observed stable effluent nitrites and ammonia. The detailed calculation of flow split is shown in Table S3 to provide a molar ammonia to nitrite ratio of 0.76. It is estimated that, for heating at 47℃ -2hr, 81% of the flow should go to PN reactor i.e., 19% should bypass PN flow straight to the successive annamox reactor. It must be asserted that in calculating the split ratio, the impact of bypassed COD on anammox was disregarded.

In order to estimate total energy consumption in the PN reactors, the aeration and heating energy were calculated using the following formula for adiabatic compression (Metcalf & Eddy, 2004) and specific heat capacity (Manahan, 2008) respectively.

\[
\text{Aeration Energy (kWh/d)} = \frac{Q_{\text{air}} \times \frac{1}{0.0224} \times \frac{1}{\text{mol}} \times RT \left[ \frac{P_d}{P_{\text{inlet}}} - 1 \right] \times 2.78 \times 10^{-7} \text{ kWh/J}}{0.283 \times \eta}
\]

\[
\text{Heat Energy (kWh/d)} = m \times C_p \times (T_r - T_r)
\]
Where $Q_{air} =$ air flow in $m^3/d$, $R =$ universal gas constant for air, 8.314 J/mole.K, $n =$0.283 for dry air, $T =$ temperature in the system in °K, $P_{inlet} =$ atmospheric pressure, 0.21 atm, $P_d =$ Calculated discharge pressure in atm, $Ƞ =$ mechanical efficiency of the diffuser, 70%, $m =$ mass of the heated sludge in kg/d, $C_p =$ specific heat capacity of water, 4.186 KJ/Kg°C, $T_t =$ Temperature to be heated the RAS, in °K, $T_r =$ room temperature in °K. Detailed calculations are shown in in SI, Table S4 & Section 2.

As evident from SI, Table S4, the aeration energy required for achieving complete nitrification was 73 kWh/d at 22°C (room temperature). The aeration energy required for the maximum NAR of 0.37 was 61 kWh/d whereas the heating energy required was 229 kWh/d at 47°C -2hr intermittent heating, providing the total energy requirements of 290 kWh/d, 4 times the complete nitrification energy of 73 kWh/d. Thus, this heating strategy is not economically feasible to be applied in PN-A process. Since it was previously revealed that NOBs recovered in 1-1.5 turnover of SRT (7 days), heating the reactors once every 10 days at 47°C-2hr was used for energy estimation. The total energy required for 1 day heating in 10 days at 47°C-2hr was 92 kWh/d which are greater than energy required for complete nitrification of 73 kWh/d, indicating that although NOBs were revived in 10 days, this heating temperature and heating frequency was not also economically feasible.

However, since this study and Chen et al.(2019b) showed that heating at 47°C -2hr and 60°C -0.67hr respectively can produce nitrite accumulation, we have further estimated the energy requirement for heating every 10 days for the temperature range of 50 to 60°C. The flow split for the PN and annamox process are 79% and 21% ($50°C$ -1hr), 90% and 10% ($55°C$ -0.67hr), 89% and 11% ($60°C$ -0.67hr) respectively. The total energy requirements are shown in Figure 3-8.
Figure 3-8. Energy consumption for a 1 MLD plant with heat shock induced PN coupled with anammox

The total energy requirement at 50°C-1hr, 55°C-0.75hr and 60°C-0.67hr heating once in 10 days were 63 kWh/d, 59 kWh/d and 57 kWh/d compared to 73 kWh/d for complete nitrification leading to 14%, 19% and 23% energy saving, indicating that this heat strategy could be economically feasible for PN-A system.

3.5 Microbial analysis

3.5.1 Diversity and relative abundance of archaeal and bacterial group in the reactors

Samples were collected at the end (day 67) of the operation to investigate the microbial community in the heated SBRs. The total number of prokaryotes were $2.92 \times 10^9$ AU/mL, $1.08 \times 10^9$ AU/mL, $1.72 \times 10^9$ AU/mL, and $2.47 \times 10^9$ AU/mL in R1, R2, R3 and R4, respectively. The most abundant phyla in all SBRs were Proteobacteria (49% -65%) and Bacteroidetes (6%-24%) as they can adapt and thrive at very high or very low temperatures (Zhang et al., 2020). The abundance of phyla Acidobacteria, Actinobacteria, Chlorobi, Gemmatimonadetes, Nitrospirae, and Planctomycetes varied from 1%-12% in all reactors. Phylum Proteobacteria was dominated by *Tauera* (3%-51%), *Nannocyctis* (0.5% to 45%) and *zoogloea* (7%-25%). The relative abundances of the species are shown in Fig.3- 9. The
diversity of the microbial community was calculated by using Shannon diversity index (Table S5). The Shannon diversity index for R1 (22°C), R2 (47°C-2hr intermittent heating), R3 (37°C continuous heating), R4 (42°C continuous heating) were 4.31, 4.26, 4.03, and 3.46, respectively, indicating that the diversity of microbes decreased with the percent of MLSS heated, temperature and contact time.

![Graph showing relative abundance of microbial community in the SBRs]

**Figure 3-9. Relative abundance of microbial community in the SBRs**

### 3.5.2 Main Functional groups of bacteria in the SBR biomass

The major functional bacterial group that are significant in the activated sludge process are aerobic heterotrophs, nitrifiers (AOB and NOB), polyphosphate-accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). The relative abundances of the main functional group microorganisms are shown in Figure 3-9.

90% of the detected significant functional groups were heterotrophs. The most abundant group was *Thauera* (Ren et al., 2021) with relative abundances of 3%, 5%, 20%, and 52%
in the control, R2, R3, and R4, respectively, showed the increase of relative abundances with percent of MLSS heated and contact time. The ranges of relative abundance of *Nannocystis*, *Zoogloea*, *Haliangium*, *Flavobacterium*, *Thiothrix*, *Dechloromonas*, *Lewinella*, *Terrimonas*, *Phaeodactylibacter*, *Novosphingobium*, *Hydrogenophaga*, *Bryobacter* were 1-51%, 8-25%, 1-20%, 1-25%, 11-15%, 1-17%, 0-6%, 1-6%, 0-6%, 0-3%, 2.5-3%, 3-3.5%, and 0-3%, respectively in the reactors. Interestingly, amongst the 14 genera of the bacteria, only 5 genera: *Thauera*, *Levinella*, *Terrimonas*, *Bryobacter*, *Dechloromonas* were detected in R4 (operated at 42°C).

The relative abundance of *Accumulibacter* was 7.8% and 2% in R1 and R2 reactors, respectively. However, *Accumulibacter* was not identified in reactor R3 and R4 at temperature 37°C and 42°C due to the low thermostolerance of *Accumulibacter* when temperature increases from 20°C to 35.5°C (Panswad et al., 2003). The abundance of *Halomonas*, a potential PAO (Hien et al., 2012), ranged from 2.5% -3% in R1 and R2, respectively, and was 0.5% in R3 and R4, indicating 80% to 83% reduction at 37°C and 42°C, respectively. The relatively higher abundance of *Halomonas* in R1 and R2 compared to the continuously heated reactors (R3 and R4) is due to its low thermostolerance. The only GAO detected in the reactors was *Competibacter*, with abundances of 7%, 3%, 3.1% and 7% in R1, R2, R3 and R4 respectively. The ratio of PAO (*Accumulibacter*) : GAO (*Competibacter*) were 0.9:1, 0.7:1, 0, and 0, respectively, indicating a potential microbial shift from PAO to GAO due to the increase in heating time and temperature (Acevedo et al., 2012).

Two dominant nitrifiers i.e., *Nitrosomonas* and *Nitrospira* were present in all reactors throughout the entire operation. Although *Nitrobacter* is a dominant nitrite oxidizing bacteria, it was not found in the MLSS samples since *Nitrobacter* was outcompeted by *Nitrospira* at temperatures higher than 25°C, and *Nitrospira* showed adaptability at high temperature from 29°C to 30°C (Huang et al., 2010). The relative abundance of *Nitrosomonas* was 0.5% to 5% while *Nitrospira* was 1% to 20%.

*Nitrospira* was more dominant than *Nitrosomonas* in all reactors, with ratios of 1.4:1, 2.4:1, 2.4:1, and 3.7:1 for the control SBR (22°C), 47°C-2hr offline heating SBR, 37°C SBR, and
42°C SBR, respectively when PN was lost. Despite the expected numerical ratio of AOBs: NOBs being 2:1 according to thermodynamics and electron transfer (Winkler, 2012), the relative abundance of *Nitrospira* to *Nitrosomonas* was 2-4 times higher in full-scale wastewater treatment plants (Yao & Peng, 2017). On day 67, complete ammonia oxidation was observed, which was likely performed by Comammox *Nitrospira*. Given the dominance of *Nitrospira*, it is plausible that the complete ammonia oxidation, particularly in the heated SBRs, could be attributed to the presence of both Comammox *Nitrospira* (Hsu et al., 2022; Y. Zhang et al., 2023), and *Thauera* as shown below.

### 3.5.3 Microbial community and performance of the reactors

The mass of each bacteria was calculated by using the relative abundance of each bacteria and the produced biomass in the system. Since the heterotrophs and the nitrifiers were the most abundant microorganism in the MLVSS samples, the produced biomass per day was calculated based on the following Equations 4-7 (Metcalf & Eddy, 2004b). The overall masses in the reactors were calculated by multiplying the mass rates of Equation 5 by the SRT of 7 days. All the values of the parameters used in these equations, are presented in S6.

\[
\text{Heterotrophs} \left( \frac{g_{VSS}}{d} \right) = \left( \frac{Q \times Y_h \times (S_o - S)}{1 + b_n T \times SRT} + f_d \times b_n T \times \frac{Q \times Y_h \times (S_o - S) \times SRT}{1 + b_n T \times SRT} \right) \quad (3 - 6)
\]

\[
\text{Nitrifiers} \left( \frac{g_{VSS}}{d} \right) = \frac{Q \times Y_n \times NO_x}{1 + b_n T \times SRT} + f_d \times b_n T \times \frac{Q \times Y_n \times NO_x \times SRT}{1 + b_n T \times SRT} \quad (3 - 7)
\]

\[
\text{Total biomass} \left( \frac{g_{VSS}}{d} \right) = \text{Heterotrophs} \left( \frac{g_{VSS}}{d} \right) + \text{Nitrifiers} \left( \frac{g_{VSS}}{d} \right) \quad (3 - 8)
\]

where \( bT = b_{20} \theta(T - T_{20}) \) \quad (3 - 9)

In this study, the Pearson correlation was estimated to correlate mass of microorganisms with temperature and reactor performance. The bacterial mass is shown in Table S7 whereas the correlation with temperature and the removal performance by the microorganisms is provided in the Table 3-7 with significant Pearson correlation coefficients presented in bold.
Table 3-7. Pearson correlation coefficients based on bacterial mass in the SBRs

<table>
<thead>
<tr>
<th>Genera of bacteria</th>
<th>Temperature</th>
<th>% COD removal</th>
<th>% TKN removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thauera sp</td>
<td>0.90</td>
<td>0.39</td>
<td>0.78</td>
</tr>
<tr>
<td>Nannocystis</td>
<td>0.40</td>
<td>-0.43</td>
<td>0.08</td>
</tr>
<tr>
<td>Nitrosospira</td>
<td>0.90</td>
<td>0.51</td>
<td>0.89</td>
</tr>
<tr>
<td>Zoogloea</td>
<td>-0.94</td>
<td>-0.42</td>
<td>-0.91</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>-0.71</td>
<td>-0.58</td>
<td>-0.92</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>-0.65</td>
<td>-0.63</td>
<td>-0.92</td>
</tr>
<tr>
<td>Terrimonas</td>
<td>0.93</td>
<td>0.29</td>
<td>0.73</td>
</tr>
<tr>
<td>Ca_Competibacter</td>
<td>-0.16</td>
<td>0.95</td>
<td>0.53</td>
</tr>
<tr>
<td>Nitrosomonas</td>
<td>0.80</td>
<td>0.65</td>
<td>0.99</td>
</tr>
<tr>
<td>Phaeodactylibacter</td>
<td>-0.73</td>
<td>0.45</td>
<td>-0.09</td>
</tr>
<tr>
<td>Novosphingobium</td>
<td>-0.91</td>
<td>-0.40</td>
<td>-0.89</td>
</tr>
<tr>
<td>Bryobacter</td>
<td>0.90</td>
<td>0.03</td>
<td>0.65</td>
</tr>
<tr>
<td>Halomonas</td>
<td>-0.97</td>
<td>0.13</td>
<td>-0.53</td>
</tr>
</tbody>
</table>

Table 3-7 shows that 4 of the 13 microorganisms showed strong positive correlation ($r > 0.8$) with temperature, 3 of the microorganisms showed strong negative correlation ($r < -0.8$) and 6 of them showed moderate to no correlation with temperature. *Nitrosomonas* and *Nitrobas* showed strong positive correlation with temperature ($r = 0.8$ and $r = 0.9$) respectively as both the nitrifiers survived in the temperature range from 20°C to 42°C. *Thauera* which is a thermophilic heterotrophic bacteria, that thrives at 37°C-50°C, had strong positive correlation with temperature (Yang et al., 2018). *Terrimonas* and *Bryobacter* also showed strong positive correlation with temperature. *Terrimonas* which is a strict aerobe can grow in the temperature range of 10°C -37°C, at pH 7-8 in bulking sludge from municipal wastewater (Jin et al., 2013). *Zoogloea, Novosphingobium, Halomonas* showed strong negative correlation with temperature, indicating that the decay rates of these bacteria are high at 42°C.

There is a strong correlation between COD removal and *Competibacter* that compete with the PAO *Accumulibater* for the carbon source in the reactors during the anaerobic feed period. However, *Accumulibater* had zero mass and was outcompeted by *Competibacter* in R3 ($2.3 \times 10^{-2}$ g) and R4 ($7.3 \times 10^{-2}$ g), at temperatures 37°C and 42°C (Lopez-Vazquez et
al., 2008) as the activities of PAO deteriorate at temperature > 20°C. Although Zoogloea is a strong oxidizer of organic carbon (Dugan et al., 2006), it did not show a strong correlation with COD removal which is due to the fact that Zoogloea has strong negative correlation with temperature, indicating increased decay rate with the increase of temperature. Nannocystis and Phaeodactylibacter had moderate positive correlation with COD removal.

There was a strong correlation between Nitrosomonas and Nitrospira and TKN removal, with the correlation factors of 0.99 and 0.89, respectively. At room temperature, only Nitrosomonas was responsible for removing TKN in the reactors, with a Nitrosomonas to Nitrospira ratio of 1:1.4. However, in heated reactors, the higher abundance of Nitrospira compared to Nitrosomonas (2.4:1, 2.4:1, and 3.7:1) suggests that the correlation between TKN levels and Nitrospira may be attributed to the potential role of Comammox Nitrospira in these heated systems.

*Thauera* sp. strain SND5 have been shown to uptake nitrite under anoxic conditions at C/N of 10:1, DO of 1 mg/L, and temperature of 30°C with a nitrite uptake rate of 10.24 mg N/L·h. It was also shown to uptake nitrite at a rate of 1.96 mgN/L·h and ammonia at a rate of a 2.39 mgN/L·h under aerobic conditions in batch reactors fed with 80 mg/L of nitrate and ammonia, respectively (Wang & He, 2020). Considering the high relative abundances of *Thauera* at 37°C (R3) and 42°C (R4), an investigation of its potential contribution to nitrogen removal was undertaken based on comparing the maximum experimental and theoretical ammonia and nitrite uptake rates in R3 and R4. The detailed calculations are provided in Table S8. It is evident from Table S8 that the maximum theoretical AUR based on only Nitrosomonas was lower than the measured experimental, with the differences higher in R4 than R3. For example, the maximum theoretical and experimental AUR were 125, and 151 mg/L·d in R3 while 121 and 225 mg/L·d, respectively in R4. This indicates that Thauera sp. may have removed ammonia in the aerobic period in the continuously heated reactors. Figure 3-10 depicts the correlation between the ratio of experimental and theoretical ammonia uptake rate and relative abundance of Thauera sp.
Moreover, the positive correlation between Thauera sp and TKN removal of 0.78 supports the above finding. However, the experimental nitrite uptake rates were lower than the theoretical based on Nitrospira for R3 and R4, indicating the lack of contribution of Thauera sp. to nitrite oxidation.

### 3.6. Summary and Conclusions

This work emphatically refutes the validity of using short-term batch tests to optimize the temperature and time of the heat shock needed to achieve partial nitrification, as NOB were revived after 1-1.5 turnovers of the mean SRT. From an energy perspective, operating mainstream secondary treatment at temperatures above ambient to achieve PN may not be practical and thus future research should focus on optimizing offline partial heating, with respect to frequency, temperature, time, and proportion of system biomass. However, using intermittent heat shocks at 50°C-60°C for about 1 hour could sustain stable mainstream PN, which when coupled with anammox will reduce energy by 14%-23%, relative to full nitrification.

The major findings of the work are:

- At 45°C in short-term batch tests, all the NOB were temporarily deactivated and no change of activities of NOB and AOB after cooling was observed. At temperature >45°C, no activities of AOB and NOB were found. Long-term operation of SBRs
at 42°C and 37°C also caused the acclimatization of NOB with time. In batches and SBRs, the solubilization of particulate organic matter occurred. In batch reactors, the-overall ffCOD increased by 43 mg/L during heating at 50°C for 2 hours. The SON was increased by a factor of 2.5 and by close to an order of magnitude at 40 °C - 45 °C, and ≥ 50°C, respectively.

- *Nitrospira* was the dominant NOB in all temperatures, with highest ratio of *Nitrospira* to *Nitrosomonas* of 3.7 at 42°C. Interestingly, the thermotolerant heterotroph *Thaurea* contributed to ammonia oxidation but not to nitrite oxidation in the two continuously heated reactors at 37°C and 42°C. Furthermore, the atypically high ratio of *Nitrospira*-to *Nitrosomonas*, observed in the heated reactors may indicate the increasing presence of Comammox *Nitrospira* with increased temperature.
References


Hien Thi Thu Nguyen, Jeppe Lund Nielsen, P. H. N. (2012). ’*Candidatus Halomonas Phosphatis’*, a novel polyphosphate-accumulation organism in full-scale enhanced


Chapter 4

4 Mainstream partial nitrification using heat shocks.

4.1 Introduction

Conventionally, nitrogen removal has been carried out by nitrification/denitrification. This process requires external carbon sources and has a higher operating cost (Isaka et al., 2008). In contrast to the conventional nitrification denitrification process, the nitrite that was produced in the first stage of the nitrification process, can be utilized by the anaerobic ammonium oxidation bacteria (anammox) in partial nitrification (PN)-anammox process which can produce high rate of nitrogen removal, up to 11.5 kg N m\(^{-3}\) d\(^{-1}\) (Isaka et al., 2007). A nitritation-anammox process can decrease aeration by 60%, carbon by 100% and sludge production by 90%, leading to savings in chemical cost and sludge disposal (Kuenen, 2008; Sliekers et al., 2002). The ammonium-rich digester reject water, which contains approximately 10-20% of the nitrogen load of the raw influent and has a low carbon-to-nitrogen (C/N) ratio, is thus expensive to treat with conventional nitrification/denitrification (Fux et al., 2002). However, the temperature, pH and high ammonia concentration of the reject water, create favourable condition for AOBs over NOBs due to the higher tolerance of AOB to free ammonia and free nitrous acid. This makes PN a significant process for treating reject water in real wastewater treatment plants, with PN-based commercial technologies such as SHARON® (van Dongen et al., 2001), DEMON® (Wett et al., 2007) and ANITA™ Mox (Veuillet et al., 2014).

Mainstream PN can be achieved at low temperatures if the optimum operational conditions such as pH, DO, free ammonia (FA), and free nitrous acid (FNA) are maintained to favor AOBs over NOBs (X. Liu et al., 2020). pH impacts both FA (Qian et al., 2017; Q. Wang et al., 2014) and FNA (Hellinga C, Schellen A.A.J.C., 1998), can influence nitrite accumulation along with DO (Gilbert et al., 2014a; Li et al., 2015) and temperature. However, it is recommended that pH for nitritation should be in the range of 7.4-8.5 and the fluctuation of pH from 8.0 ± 0.1 to 7.5 ± 0.2 can decrease the ammonia oxidation and thus nitrite accumulation (Feng et al., 2017; Tang & Chen, 2015). Controlling pH in mainstream is not economically viable is (Kinh et al., 2017; Tao et al., 2012). Furthermore,
the concentration of ammonia in mainstream ranges from 15 to 50 mg/L (Negulescu, 1986) producing FA and FNA ranges of 0.01-0.05 mg/L and 0.001-0.005 mg/L, respectively, which are lower than NOB inhibition threshold (Anthonisen et al., 1976). Additionally, the optimal temperature of wastewater for NOB suppression should be higher than 25°C (Pedrouso et al., 2021).

The application of low DO concentration to inhibit NOB, has been well established (Aslan et al., 2009; Blackburne et al., 2008; C. Chen et al., 2021). However, in the long run even at low DO, NOBs shifted to species that can outcompete AOBs, resulting in complete nitrification (Liu & Wang, 2013), confirmed by a study who found gradual conversion of partial nitrification to complete nitrification at a DO concentration of 0.5 mg/L (Zhou et al., 2018). Conversely, it was found that switching from low DO to high DO condition, complete nitrification resulted in partial nitrification system (Bao et al., 2017). Consequently, the conventional low DO operation for influencing partial nitrification remains unclear and can be misleading.

The application of intermittent heat as a strategy to suppress NOBs has shown promising results in several studies that used synthetic wastewater (Chen et al., 2016; Choi et al., 2021; Isaka et al., 2008). However, these studies used encapsulated nitrifiers which may not be feasible for suspended growth systems. A study (Chen et al., 2019) applied offline heat to thickened waste activated sludge (TWAS) and primary sludge (PS) without the encapsulation of the nitrifiers. They applied temperature of 60°C for 40 min every 2 weeks, followed by washing and recovery of the sludge in another SBR, which was then used for achieving nitrite accumulation treating real wastewater with COD of 350mg/L, NH3-N of 50 mg/L. They achieved NAR of 81.8%-91.2% for up to 91 days. While Isaka et al. (2008) and Chen et al. (2016) used encapsulated nitrifiers and potentially achieving PN with the combined effect of heat shock and oxygen diffusion limitations, Choi et al. (2021) used heat with variable DO conflicting with the hypothesis of achieving PN by the application of the heat only. Chen et al. (2019) did not report the DO concentration while using heat shock to the suspended nitrifiers that also created the speculation of achieving PN with only by the heat shock while literatures show that using only various DO concentrations, PN can be achieved. They heated, washed, and reactivated the nitrifiers outside of the nitrifying reactors, however the effect of heat directly on the nitrifying bacteria such as:
death or inhibition and recovery were not explored. The effluent quality that can be affected by the solubilized microorganism (soluble COD and N) as well as the behaviour of all other microbes (heterotrophs, ammonifying bacteria, nitrifying bacteria etc.) in the heated environment were not investigated. While SRT along with heat shock is a significant factor for the growth of nitrifiers, which is a function of substrate utilization rates, none of the studies investigated the nitrogen uptake or production rates in heated reactors. In regard to microbial analysis, although Chen et al. (2016) and Choi et al. (2021) investigated the nitrifiers in the heated reactors, none of the studies investigated the complete behaviour of the microbial community due to heat and did not statistically correlate the active microbial community with the substrate utilization.

Therefore, while intermittent heat to the nitrifiers showed potential for achieving NOB suppression in mainstream PN, further research is required to completely understand the effects of heat shock on entire wastewater treatment process. In this study, we have used intermittent heat in the range of 35°C-60°C to the MLSS of the SBRs during the aeration stage to achieve nitrite accumulation. The detailed objectives of this study are:

- Application of fixed DO concentration and SRT, sufficient to ensure full nitrification, for the entire operation to ensure the nitrite accumulation occurred due to the heat treatment only
- Investigation of specific substrate utilization rates (ammonia uptake rates, nitrate or nitrite production rates) of the nitrifiers
- Estimation of the inhibition and recovery of nitrifiers due to heat and their effect on ammonia and nitrite accumulation in the heated reactors
- Investigation of the effluent quality due to the solubilization of microorganisms
- Analysis of the comprehensive microbial community structure and their statistical correlation with substrate utilization.
- Heat optimization assays (recovery tests of nitrifiers) at optimum PN temperature conditions were explored.
Estimation of heat energy, inclusive of heat losses, for everyday heating and optimized heating for achieving PN along with sensitivity analysis considering the real wastewater conditions was performed.

4.2 Materials and methods

4.2.1 Wastewater and inoculum

The control and heated sequencing batch reactors (SBRs) were operated with primary effluent from the nitrifying Greenway wastewater treatment plant, London, ON, Canada. The SBRs were inoculated with nitrifying MLSS at 2 g/L. Wastewater was collected once a week and characterized for chemical oxygen demand (Total and soluble COD), nitrogen (N) (total and soluble N), ammonia (NH₃-N), nitrate (NO₃-N), nitrite (NO₂-N), phosphorus (total and soluble P), alkalinity, pH, total suspended solids (TSS), volatile suspended solids (VSS) before storage at 4°C. Samples from influent, effluent and mixed liquor were collected twice a week during the operation. Soluble parameters were measured in samples filtered through, sterile 0.45 µm membrane filters. The main characteristics of the PE were: TCOD of 230±78 mg/L, SCOD of 109±36 mg/L, ammonia nitrogen of 23.8±3.2 mg N/L, nitrate nitrogen of 0.5±0.3 mg N/L, nitrite nitrogen of 0.2±0.2 mg N/L, alkalinity of 335±39 mg CaCO₃/L, and pH of 8.5±0.2.

4.2.2 Analytical methods

Both wastewater and effluent were characterized twice a week. Samples were analyzed for total and soluble COD, total and soluble N, NH₃-N, NO₃-N, NO₂-N, total and soluble P, alkalinity, pH, TSS and VSS. The solid samples (TSS and VSS) were characterized for wastewater, effluent, and mixed liquor twice a week. Cyclic tests were also done once a week and samples were collected for analyzing NH₃-N, NO₃-N, NO₂-N. TSS and VSS Samples from reactors at the start and end of the aeration to calculate specific nitrogen uptake or production rates. The complete characteristics of PE and the analytical methods are shown in Table 4-1.
Table 4-1. Influent characteristics ((Mean ± Standard Deviation (No. of samples))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration (mg/L)</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total suspended solids (TSS)</td>
<td>116±25(49)</td>
<td>2540D(American Public Health Association, 2005, 2005)</td>
</tr>
<tr>
<td>Volatile suspended solids (VSS)</td>
<td>88±24(49)</td>
<td>2540E(American Public Health Association, 2005, 2005)</td>
</tr>
<tr>
<td>Total Chemical oxygen demand (TCOD)</td>
<td>230±78(49)</td>
<td>COD, 200-15,000 mg/L, method 8000</td>
</tr>
<tr>
<td>Soluble Chemical Oxygen demand (SCOD)</td>
<td>109±36(49)</td>
<td>200-15,000 mg/L, method 8000</td>
</tr>
<tr>
<td>Ammonia-N (NH$_3$-N)</td>
<td>24±3.8(49)</td>
<td>0.4-50 mg/L, method 10031</td>
</tr>
<tr>
<td>Nitrate- (NO$_3$-N)</td>
<td>0.5±0.3(49)</td>
<td>0.2-30 mg/L, Method 10020</td>
</tr>
<tr>
<td>Nitrite-N (NO$_2$-N)</td>
<td>0.2±0.2(49)</td>
<td>0.003-0.500, Method 10019</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>35±6.1(49)</td>
<td>10-150 mg/L, method 10071</td>
</tr>
<tr>
<td>Soluble nitrogen (SN)</td>
<td>34±6.1(49)</td>
<td>0.4-50 mg/L, method 10031</td>
</tr>
<tr>
<td>Total Phosphorus (TP)</td>
<td>4.0±1.3(49)</td>
<td>1-100 mg PO$_4^3$-L, method 10121</td>
</tr>
<tr>
<td>Soluble Phosphorus (SP)</td>
<td>2.1±0.6(49)</td>
<td>1-100 mg PO$_4^3$-L, method 10121</td>
</tr>
<tr>
<td>pH</td>
<td>8.5±0.2(49)</td>
<td>4500-H$^+$/B(Federation &amp; Association, 2005)</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO$_3$/L)</td>
<td>335±39(49)</td>
<td>2320B(Federation &amp; Association, 2005)</td>
</tr>
</tbody>
</table>

4.2.3 Reactor configuration and operation

Three 2L-SBRs were operated in parallel for 3 cycles per day with a nitrogen loading rate of 0.05 kgNL$^{-1}$d$^{-1}$. The hydraulic retention time (HRT) and SRT were 16 hr and 7 days respectively. The SBR cycle constituted the following phases: 0.5 hr anoxic fill, 6 hr
aeration and mixing, 1 hr settle, and 0.5 hr decanting. The wasting was done 10 minutes prior to the end of the reaction period to ensure that no biodegradable components were present in the effluent. Reactors were operated for a duration of 2.5-3 turnovers of SRT to reach steady state.

The volume fill ratio was 50%. Air pumps (Marina, 75 Air Pump) were set to 0.4 L/min to maintain a DO concentration of 4±0.02 mg/L during the aeration periods. 286 mL (14% of the MLSS) was wasted from each reactor 10 minutes prior to the start of the settling to maintain the SRT of 7 days. The MLSS concentration ranged from 1400-2000 mg/L in the reactors. pH was not controlled. A control SBR was operated as a baseline at room temperature (23℃) to compare the performance with the heated SBR. Two other SBRs were operated at temperature 35℃ to 60℃ based on the temperature used by Lopez-Vasques et al. (2014). The lower heating boundary was selected based on the temperature that favors AOBs over NOBs in side stream PN process, whereas the upper boundary of 60℃ was identified because at this temperature, both AOBs and NOBs were suppressed. The heating times of 0.5hr, 1hr, 2hr and 3hr were selected based on three considerations: nitrite accumulation, effluent quality and minimizing heat energy (Manahan, 2008). The combination of temperature, heating time and operation duration were selected as follows: 35℃-1hr (21 days), 35℃-2hr (20 days), 35℃-3hr (22 days), 45℃-1hr (43 days), 40℃-2hr (19 days), 40℃-3hr (22 days), 45℃-1hr (25 days), 45℃-2h (35 days), 50℃-1hr (68 days), 55-0.5hr (55 days) and 60℃-0.5 hr (21 days). The selection of higher temperatures and shorter heating durations were confirmed by a study of Yu et al (2021) which reported that longer treatment time and higher treatment temperature effectively inhibited the nitrite oxidoreductase (NOB) activity (Yu et al., 2021). The reactors were heated using a water bath (VWR® Heated Circulating Baths) and insulated by covering them with aluminum paper insulator. The heating was started at the beginning of the aeration period and continued for the specific heating time. The temperatures inside the reactors were taken with a special application instrument thermometer (Thermco®, Quebec, Canada) inserted to the reactor. The diffused aeration and heating duration were operated with automated power control. The reactor configuration is shown in Figure 4-1.
4.2.4 Nitrogen oxidation, specific nitrogen uptake/production rates and percent removal

The percent ammonia oxidation efficiency (AOE) and percent nitrate oxidation efficiency (NOE) were calculated from analysis results of influent and effluent ammonia, nitrate, and nitrite concentrations, shown in Equations (4-1) and (4-2). The overall removal efficiency of COD, N and P was calculated using the Equation (4-3).

\[
\text{AOE} (\%) = \left( 1 - \frac{(\text{NH}_3-N)_{\text{eff}}}{(\text{NH}_3-N)_{\text{eff}} + (\text{NO}_3-N)_{\text{eff}} + (\text{NO}_2-N)_{\text{eff}}} \right) \times 100 \quad (4 - 1)
\]

\[
\text{NOE} (\%) = \left( 1 - \frac{(\text{NO}_2-N)_{\text{eff}}}{(\text{NO}_3-N)_{\text{eff}} + (\text{NO}_2-N)_{\text{eff}}} \right) \times 100 \quad (4 - 2)
\]

Figure 4-1. Experimental set up of the heated reactors
Removal (%) = \(1 - \left(\frac{\text{Effluent Concentration}}{\text{Influent concentration}}\right)\) \times 100 \hspace{1cm} (4 - 3)

Where \((\text{NH}_3-N)_{\text{eff}}, (\text{NO}_3-N)_{\text{eff}}\) and \((\text{NO}_2-N)_{\text{eff}}\) are the effluent ammonia, nitrate and nitrite respectively. Effluent concentration and influent concentration refer the effluent and influent concentration of \(\text{C}, \text{N}\) and \(\text{P}\), respectively.

For calculating specific nitrogen uptake/ production rates (SNR) such as ammonia uptake rates, nitrate and nitrite accumulation rates from cyclic tests, overall MLVSS samples in the reactors during the cyclic tests were used. The SNR was calculated using Equation (4-4).

\[
\text{SNR} = \frac{\Delta C}{\Delta T \times \text{MLVSS}} \hspace{1cm} (4 - 4)
\]

Where, \(\Delta C\) is the concentration difference of nitrogen species for a time interval \(\Delta T\), MLVSS is the average mixed liquor suspended solids concentration in the reactor during aeration period.

4.2.5 Full nitrification recovery tests

This test was conducted for temperature 55°C-0.5hr and 60°C-0.5hr. At the beginning of the heating, both AOB and NOB were inhibited at temperatures 55°C-0.5hr and 60°C-0.5hr. In order to investigate the recovery of AOBs and NOBs, the heating of SBRs at 55°C-0.5hr and 60°C-0.5hr were stopped after 55 and 21 days for PN operation, respectively. These SBRs were then operated at room temperature (23°C) until the effluent ammonia gradually reached to zero and nitrate concentration goes to as high as influent ammonia. The recovery time AOBs and NOBs were estimated from the graph of concentration Vs time.

4.2.6 Microbial Analysis

The first batch of MLSS samples were collected from the SBRs at 23°C (control), 45°C-2hr, 50°C-1hr. The collection was done on day 34 of the 45°C-2hr and 50°C-1hr operations. On day 34, the heated SBRs were at steady state and were producing nitrite. Another batch of samples from reactor at 55°C-0.5hr for three conditions were collected: day 51 (heating and nitrite accumulation), day 68 (no heating and nitrite accumulation lost) and day 75 (one
turnover of SRT after nitrite accumulation was lost) for microbial detection. The samples were sent to IRDA for PCR analysis. All the samples were analyzed for the diversity (quantification) and abundance test of microorganisms at the phylum and genus levels to identify the bacteria and their activities in MLSS, respectively. For the microbial test, 2mL of homogenous samples of MLSS were prepared from the aforementioned reactors. Then DNA was extracted using the FastDNA Spin Kit for Soil Extraction Kit (MP Biomedicals, Solon, OH, USA). The quantity and quality of the extracted genomic DNAs were determined using spectrophotometric technique with absorbance measurements at 260 nm and 280 nm and the A260 / A280 ratio. Total archaea/bacteria were detected by molecular detection with quantitative PCR (qPCR) with primers eub338 / eub518 (Fierer et al., 2005). The detections were conducted using two replicates on a CFX96 (Biorad, Hercules, CA, USA) with a SYBR green qPCR mix (Qiagen, Toronto, ON, Canada). The detection system was developed on a detection range of 4 LOGs (Efficiency-system total bacteria: 89.1% r² = 0.99). The archaeal and bacterial diversity was evaluated by using metagenomic analysis. Amplifications of the V4 regions of archaeal and bacterial 16S rRNA were performed using the primer sequences of the specific to those regions (Apprill et al., 2015), employing a two-step dual-indexed PCR approach (PCR) designed specifically for the analysis with Illumina MiSeq sequencing platform. The MIDAS 3.0 database was used for taxonomic identification of prokaryotes.

4.3 Results and Discussion

4.3.1 Reactor performance and heat application

The reactors were fed with real wastewater to demonstrate the feasibility and evaluate the practicality of application of heat in real wastewater treatment plants. Three SBRs: one control and two heated were operated simultaneously for a total of 352 days. The range of MLSS concentration in the reactors was 1400-2000 mg/L. The Greenway wastewater treatment plant operates with medium strength wastewater with COD/N, COD/P and VSS/TSS ratios ranging from 5 to 9, 44 to 76, and 0.6 to 0.9 respectively. The alkalinity/TKN ratio in the PE ranged from 7.17 to 12.2 which was adequate for the complete oxidation of influent TKN in nitrifying environment at room temperature (23°C). The SBRs were started up with a TKN of 34.3 mgN/L and HRT of 16hr, corresponding to
a nitrogen loading rate of 0.05 kgN/d.m$^3$. The influent and effluent ammonia, nitrate and nitrite that were analyzed twice a week, were used to estimate the nitrogen oxidation rates and performance of the reactors.

4.3.1.1 Thermotolerance of nitrifiers and nitrogen oxidation

Figure 4-2 showed the percentages ammonia and nitrite oxidation along with effluent nitrogen species in the SBRs. The control reactor and heated reactors (35℃-1hr to 45℃-1hr) that did not achieve PN, was not shown in the Figure 4-2(a,b,c,d).

![Graph showing nitrogen species and oxidation efficiency](image)

a) Nitrogen species and nitrogen oxidation from reactor heated at 45℃-2hr.
b) Nitrogen species and nitrogen oxidation from reactor heated at 50°C

c) Nitrogen species and nitrogen oxidation from reactor heated at 55°C-0.5hr
d) Nitrogen species and nitrogen oxidation from reactor heated at 60℃-0.5hr

**Figure 4-2. Nitrogen species and nitrogen oxidation in heated reactors**

Table 4-2 shows the summary of the nitrogen oxidation and corresponding nitrogen uptake/production rates in all reactors.
Table 4-2. Percent ammonia and nitrite removal efficiency due to heating

<table>
<thead>
<tr>
<th>Heating Temperature</th>
<th>Heating duration</th>
<th>Ammonia oxidation efficiency (AOE,%)</th>
<th>Nitrite oxidation efficiency (NOE,%)</th>
<th>Specific ammonia uptake rate (mgN/gVSS.hr)</th>
<th>Specific nitrate production rate (mgN/gVSS.hr)</th>
<th>Specific nitrite production rate (mgN/gVSS.hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22℃ - 98.8-99.6%</td>
<td>-</td>
<td>98.5-99.7%</td>
<td>4.2±0.4 (3)</td>
<td>4.3±0.1(3)</td>
<td>0.2±0.1(3)</td>
<td></td>
</tr>
<tr>
<td>35℃ 1hr 95.1-100%</td>
<td>1 hr</td>
<td>97.2-99.8%</td>
<td>3.7±0.8(3)</td>
<td>3.6±0.5(3)</td>
<td>0.4±0.1(3)</td>
<td></td>
</tr>
<tr>
<td>35℃ 2hr 97.1-97.7%</td>
<td>2 hr</td>
<td>99.1-99.5%</td>
<td>4.2±0.5(3)</td>
<td>4.2±0.6(3)</td>
<td>0.1±0.01(3)</td>
<td></td>
</tr>
<tr>
<td>35℃ 3hr 95.9-100%</td>
<td>3 hr</td>
<td>99.5-99.7%</td>
<td>4.2±0.3(3)</td>
<td>4.3±0.0(3)</td>
<td>0.08±0.01(3)</td>
<td></td>
</tr>
<tr>
<td>40℃ 1hr 96.1%-99.7%</td>
<td>1 hr</td>
<td>95.1%-99.6%</td>
<td>3.1±0.6(4)</td>
<td>3±0.6(4)</td>
<td>0.2±0.1(4)</td>
<td></td>
</tr>
<tr>
<td>40℃ 2hr 96.1%-99.7%</td>
<td>2 hr</td>
<td>95.1%-99.9%</td>
<td>3.9±0.2(4)</td>
<td>4.1±0.2(4)</td>
<td>0.1±0.01(4)</td>
<td></td>
</tr>
<tr>
<td>40℃ 3hr 96.1%-98.7%</td>
<td>3 hr</td>
<td>95.1%-97.7%</td>
<td>4.5±0.3(4)</td>
<td>4.9±0.4(4)</td>
<td>0.2±0.1(4)</td>
<td></td>
</tr>
<tr>
<td>45℃ 1hr 97.1%-97%</td>
<td>1 hr</td>
<td>96.8%-99.5%</td>
<td>3.6±0.4(3)</td>
<td>3.7±0.5(3)</td>
<td>0.3±0.3(4)</td>
<td></td>
</tr>
<tr>
<td>22℃ - 98.8-99.5%</td>
<td>-</td>
<td>99.1-99.7%</td>
<td>5.2±0.6(4)</td>
<td>5.2±0.9(4)</td>
<td>0.3±0.3(4)</td>
<td></td>
</tr>
<tr>
<td>45℃ 2hr 97.7-99.4%</td>
<td>2 hr</td>
<td>41.4-45.6%</td>
<td>5.0±0.5(4)</td>
<td>2.2±0.6 (4)</td>
<td>1.72±0.4(4)</td>
<td></td>
</tr>
<tr>
<td>50℃ 1hr 98.2-99%</td>
<td>1 hr</td>
<td>38.5-42.9%</td>
<td>5.2±0.5(4)</td>
<td>1.4±0.1(3)</td>
<td>3.0±0.3(3)</td>
<td></td>
</tr>
<tr>
<td>22℃ - 98.7-99.6%</td>
<td>-</td>
<td>98-99.8%</td>
<td>3.4±0.6 (4)</td>
<td>3.6±0.3(4)</td>
<td>0.3±0.1 (4)</td>
<td></td>
</tr>
<tr>
<td>55℃ 0.5hr 56.4-77.4%</td>
<td>0.5 hr</td>
<td>40- 41.4%</td>
<td>1.6±0.2(4)</td>
<td>0.4±0.3(4)</td>
<td>1.9±0.6(4)</td>
<td></td>
</tr>
<tr>
<td>60℃ 0.5hr 5.2-6.6%</td>
<td>0.5 hr</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Note: The specific uptake/production rates in control reactor were calculated all together to compare the specific uptake/production rates from 35℃ to 45℃-1hr as no PN was observed in the heated reactors. However, the rates of controls with each heated reactor that produced PN, starting from to 45℃-2hr to 55℃-0.5hr were included.
At 22°C, the oxidation of ammonia and nitrite was 98-99% indicating that 1%-2% of the ammonia and nitrite nitrogen were used for the cell synthesis of the microorganisms in the system the rest was oxidized. The ammonia oxidation at temperatures of 35°C (1hr, 2hr and 3hr), 40°C (1hr and 2hr), 45°C (1hr and 2 hr) and 50°C-1hr, are slightly less ammonia oxidation 95-97% which is 2% to 3% less than control reactor at 22°C. This indicates a slight decrease in ammonia oxidation that was not significant compared to control as confirmed by the effluent ammonia concentration range of 0.1-0.5 mg/L in these heated reactors and the number of AOBs were sufficient to oxidize all influent ammonia at these heating temperatures. Although it is well-established that, complete nitrification occurs within the range of 5-20°C, study has observed that both AOBs and NOBs can survive and perform complete nitrification at temperature as high as 45°C (Hellinga et al., 1998). However, our findings, indicate that AOBs can sustain intermittent heating of 50°C-1hr. In the case of nitrite oxidation, 98-99% of the nitrite was completely oxidized at temperatures of 35°C (1hr, 2hr and 3hr), 40°C (1hr and 2hr) and 45°C-1hr in the heating scheme, indicating the survival of NOBs in the suspended growth MLSS up to 45°C-1hr. However, the percentage of nitrite oxidation decreased from 45°C-2hr and higher temperatures. This scenario of ammonia and nitrite oxidation indicates that the thermotolerance of AOBs is higher than NOBs.

According to the stoichiometric rule of complete nitrification, the rates of ammonia oxidation and nitrite oxidation should be same at room temperature (20°C)(Peng et al., 2015), as demonstrated by the influent ammonia being equal to the effluent nitrate which was observed in this study up to the temperature of 45°C-1hr. However, at 45°C-2hr, the percentage of nitrite oxidation efficiency (NOE) declined from ammonia oxidation efficiency (AOE). At 45°C-2hr, AOE and NOE ranged from 97.7-99.4% and 41.4-45.6% respectively which indicates that NOBs were significantly affected compared to AOBs. The oxidation of ammonia and nitrite at temperature 50°C-1hr were 98.2-99% and 38.5-42.9% indicates the similar outcomes at 45°C-2hr in terms of nitrite accumulation, confirmed by the similar effluent nitrite concentration of 10-11 mg/L at steady state indicating about 50% of the overall influent ammonia (24mg/L) was converted to nitrite
and not oxidized to nitrate. The effluent nitrate concentrations at 45°C-2hr and 50°C-1hr were 7 mg/L and 6 mg/L (Figure 4-2a and 4-2b) respectively and indicating the similar inhibition of NOBs. Although, 45°C-2hr and 50°C-1hr were producing same level of nitrite accumulation, the selection of these two temperatures would be based on the effluent quality (performance overall of COD, N and P removal) and the required heat energy to achieve the nitrite accumulation.

It is interesting to note that although at 50°C-1hr, the ammonia oxidizing community were sufficient to oxidize all influent ammonia, with just a 5°C increase in temperature to 55°C-0.5hr (Figure 4-2c), the AOB inhibited resulting the oxidation of ammonia of 56.4-77.4%, which corresponds the overall effluent ammonia of 7.4 mg/L. The nitrite oxidation at this temperature was 40-41.4% which is 1-2% lower than the oxidation rates at 45°C-2hr and 50°C-1hr as confirmed by the overall effluent nitrite concentration of 14 mg/L. At 60°C-0.5hr, only 5.2% to 6.6% of ammonia was oxidized for the entire operation of 21 days indicating that all AOBs are completely inhibited (Figure 4-2d) for the entire period. The oxidation of nitrite is zero confirmed the NOB inhibition as well. Chen et al. (2019) who heated the combined thickened waste activated sludge (TWAS) and primary sludge (PS) at 60°C-40 minutes and reported inhibited both AOBs and NOBs during heating which is similar to this study. Nonetheless, the inactivation of NOB due to the application of the heating from 45°C-2hr and higher temperature, is likely due to the destruction DNA and RNA and protein denaturation in both AOB and NOB cells (Russell, 2003).

4.3.1.2 Nitrogen species, nitrite accumulation ratio (NAR) at heating conditions

In heated reactors until heating at 45°C-1hr, influent ammonia ranged from 18-30 mg/L, effluent ammonia ranged from 0.1-1 mg/L, effluent nitrite ranged from 0.1-0.3 mg/L and effluent nitrate ranged from 17-27 mg/L (not shown in the figure) which is similar to control indicating no significant effect of temperature on the nitrification in these temperature and heating duration. From Figure 4-2(a), (b), (c) and (d) it is apparent that at 45°C-2hr, the effluent ammonia concentration was 0.1-0.7 mg/L over the entire operational period of 36 days. On day 1, the effluent nitrite and nitrite concentrations were 0.003 mg/L
and 18mg/L respectively indicating complete nitrification; however, shifted towards partial nitrification from day 7, with an overall nitrite concentration of 10 mg/L, and was stable for 29 days thereafter. This indicated that, at this temperature and heating time, the NOBs were not impacted directly but after 7 days of the operation or one turnover of the mean SRT. At the start of the heating at 50℃-1hr and 55℃-0.5hr, the effluent ammonia varied from 12 to 20 mg/L. However, after one SRT turnover, the overall effluent ammonia concentrations were reduced to 0 mg/L and 7.4 mg/L respectively and became stable. This indicates that although at 50℃-1hr heating, AOBs recovered completely, heating at 55℃-0.5hr complete recovery of AOB was hindered at steady state. The overall steady state effluent nitrite concentrations were 10 mg/L, 10 mg/L and 14 mg/L and the nitrate concentrations were in the range of 7-8 mg/L, 6-7 mg/L and 4-5 mg/L at 45℃-2hr 50℃-1hr, 55℃-0.5hr indicating the inhibition of NOBs increased with the increased heating range from 45℃-2hr to 55℃-0.5hr. At 60℃-0.5 hours, the AOBs and NOBs were not recovered with the ammonia and nitrite concentration similar to the influent.

NAR was calculated by dividing the effluent nitrite by the effluent NOx concentrations. Figure 4-3 shows the overall NAR in the control reactors and heated reactors. Nitrite accumulation ratios of the control reactor (22℃) were averaged for entire operation. Figure 3 showed that, the heated reactors until 45-1hr, achieved complete nitrification as in control reactor. In control reactors, NAR ranged from 0.001 to 0.03 whereas the NARs at 35℃ for all duration, 40℃ for all duration and 45℃-1hr were 0.001-0.004, 0.003-0.005 and 0.004 respectively. However, the NARs at 45℃-2hr, 50℃-1hr and 55℃-0.5hr were 0.58±0.1, 0.61±0.2 and 0.72±0.1 respectively. Although at 60℃-0.5hr, there is a NAR of 0.001, it is due to the fact that all AOBs were inhibited, and consequently no nitrite accumulation occurs. Since at this temperature all nitrifiers were inhibited, no further temperature increase was evaluated in this research.
Nitrite accumulation can occur either by partial nitrification (PN) or partial denitrification (PDN) due to the presence of aerobic and anoxic conditions in the SBR operational cycle. However, PN or PDN can happen only by the impact of heat as the complete nitrification happened in control SBR. The feeding in the SBRs was anoxic, however the mixed concentrations of ammonia, nitrate, and nitrite at the start of the cycle (half of the concentrations of influent ammonia and effluent nitrate and nitrite), confirms that no nitrification or denitrification happened during feeding. During the aeration period, the dissolved oxygen was always around 4 mg/L which is not conducive for denitrification. The occurrence of denitrification can happen at low DO (<0.5 mg/L), during the settling period. However, no mixing was provided during this period to ensure quiescent settling. Moreover, the nitrite concentration at the end of aerobic period and in the effluent (end of the anoxic period) were similar. This indicates that the accumulation of nitrite in the heated reactors was achieved by PN, not by PDN. The nitrogen species at different times of SBR cycles are shown in Table 4-3 indicating that nitrite accumulation happened due to PN.

![Figure 4-3. Nitrite accumulation ratios in control and heated reactors](image)

Nitrite accumulation ratio: NO₂⁻/NOₓ⁻

Temperature (°C)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Nitrite Accumulation Ratio</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35°C - 1hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35°C - 3hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40°C - 1hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40°C - 2hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40°C - 3hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°C - 1hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°C - 3hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50°C - 1hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55°C - 0.5hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60°C - 0.5hr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4-3: Nitrogen Species during different phases of SBRs

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Ammonia</th>
<th>Nitrite</th>
<th>Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (23℃)</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>45℃-2hr</td>
<td>9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>50℃-1hr</td>
<td>9</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>55℃-0.5hr</td>
<td>4</td>
<td>7</td>
<td>7.5</td>
</tr>
</tbody>
</table>

A=start of aeration, B=End of aeration, C=Effluent

### 4.3.1.3 Substrate utilization or accumulation rates in the reactors

At 23℃, the specific ammonia uptake rate, nitrate production rate and nitrite production rates were 4.2±0.4, 4.3±0.1 and 0.2±0.1 mgN/gVSS.hr indicating that AOBs and NOBs have the similar kinetics and complete nitrification was achieved with no nitrite accumulation (substrate utilization or production rates are shown in Table 4-2). In the heated reactors at 35℃ (1-3hr), 40℃(1-3hr), 45℃ (1hr), the overall ammonia uptake rates ranged from 3.1-4.5 mgN/gVSS.hr while nitrate production rates ranged from 3.6-4.9 mgN/gVSS.hr and the specific nitrite production rate ranged from 0.1-0.4 mgN/gVSS.hr indicating similar results to the control reactor. The activity of AOB was similar to a study (Lopez-Vazquez et al., 2014) particularly at 35℃ where the ammonia uptake was 3.3 mgN/gVSS.hr in a batch reactor operated at 35℃ with initial ammonia concentration of 20 mg/L. At 45℃-2hr and 50℃-1hr the ammonia uptake rates are 5.0 and 5.2 mgN/gVSS.hr, and nitrate production rate of 2.2 mgN/gVSS.hr and 1.4 mgN/gVSS.hr respectively at 45℃-2hr and 50℃-1hr leading to nitrite production rates of 2 mgN/gVSS.hr and 3 mgN/gVSS.hr respectively. At 55℃-0.5 h, the overall ammonia uptake rates, nitrate
production rate and nitrite production rates were 1.7, 0.5 and 1.6 mgN/gVSS.hr respectively reflecting the reduction of NOB activities compared to 45°C-2hr and 50°C-1hr.

4.3.1.4 Inhibition of nitrifiers

In order to correlate the ammonia and nitrite accumulation ratio with the percent inhibition of AOBs and NOBs, heating temperatures at 45°C-2hr, 50°C-1hr and 55°C-0.5hr have been considered. The ammonia accumulation ratio (AAR), nitrite accumulation ratio (NAR) and inhibition of nitrifiers were calculated by the following equations:

\[
\% \text{AAR} = \frac{(\text{NH}_3-N)_{\text{eff}}}{(\text{NH}_3-N)_{\text{eff}} + (\text{NO}_3-N)_{\text{eff}} + (\text{NO}_2-N)_{\text{eff}}} \times 100
\]  \hspace{1cm} (4 - 5)

\[
\% \text{NAR} = \frac{(\text{NO}_2-N)_{\text{eff}}}{(\text{NO}_3-N)_{\text{eff}} + (\text{NO}_2-N)_{\text{eff}}} \times 100
\]  \hspace{1cm} (4 - 6)

\[
\% \text{AOB inhibition} = \frac{\text{Spc. AUR control} - \text{Spc. AUR heated}}{\text{Spc. AUR control}} \times 100
\]  \hspace{1cm} (4 - 7)

\[
\% \text{NOB inhibition} = \frac{\text{Spc. AUR control} - \text{Spc. NO}_3 - \text{PR heated}}{\text{Spc. AUR control}} \times 100
\]  \hspace{1cm} (4 - 8)

Where Spc.AUR-control, Spc.AUR_heated and Spc.NO\textsubscript{3} – PR\textsubscript{heated} are maximum specific ammonia uptake rates in control reactor, maximum specific ammonia uptake rates in heated reactors and maximum specific nitrate production rates in heated reactors respectively. The %AAR, %NAR and inhibitions are shown in Figure 4-4 for heating at 45°C-2hr, 50°C-1hr and 55°C-0.5hr. Figure 4-4(a) & (b), Figure 4-4 (c) & (d), Figure 4-4 (e) & (f) showed the % AAR/ inhibitions and % NAR/inhibition respectively at 45°C-2hr, 50°C-1hr and 55°C-0.5hr.
Nitrite production ratio/NOB inhibition (%)
b) Days of operation
NAR %NOB inhibition

Ammonia accumulation ratio/AOB inhibition (%)
a) Days of operation
AAR %AOB inhibition

Nitrite accumulation ratio/NOB inhibition (%)
c) Days of operation
AAR %AOB inhibition

Ammonia accumulation ratio/AOB inhibition (%)
d) Days of operation
NAR %NOB inhibition

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Figure 4-4. Ammonia and nitrite accumulation and inhibition of nitrifiers
From Figure 4-4 (a), the AAR varied from 0 to 3.8% corresponding the AOB inhibition of 0.7 to 8.7% which is insignificant at temperature 45°C for heating 2 hours. However, NAR ranged from 56% to 61% with percent NOB inhibition of 34 to 63% during stable nitrite accumulation. This result clearly showed about 50% more inhibition of NOB compared to AOBs. At 50°C-1-hour complete AOB and NOB were inhibited until day 8 with about 100% AAR and 0% NAR (Figure 4-4 (c) &(d)). From day 8 to day 20, there was a sharp decrease of AAR from 100% to 0% and the AAR continued to 0% until the end of the operation. Interestingly, AOB inhibition also followed a sharp change and reached 20% at the end of the operation. However, the NAR became stable with the range of 50-68% from day 8 to the end of the entire operation period. The NOB inhibition became stable at 80% till the end of the operation period. At 55°C-0.5hr of heating (Figure 4-4(e) &(f)), after day 4, AOB and NOB inhibitions became stable at a level of about 60% and 80% respectively. However, the AAR and NAR ranged from 22-45% and 62-82% respectively. These tends of AAR and AOB inhibition, the NAR and NOB inhibition, indicate that at the steady state condition the AOB recovery and NOB inhibition meets a point when the AAR and NAR is steady leading to complete/partial ammonia removal and nitrite accumulation at intermittent heating temperatures.

At 45°C-2hr where AOB inhibition is insignificant, the NOB inhibition was stabilized on day 8 follows a range of 1-1.7 SRT turnover. At 55°C-0.5hr and 50°C-1hr, after the 1-1.7 SRT turnover, the AAR and NAR were proportional to the AOB and NOB inhibition respectively. The inhibition and recovery of AOBs and NOBs after the application of heat shock was achieved due to the denaturation and renaturation of DNA/RNA during the duration of heat shock and the time interval of heating stopped and heating start respectively (Geiduschek, 1962)

4.3.1.4 Optimum temperature for achieving mainstream PN

Alt 45°C-2hr and 50°C-1, no significant effluent ammonia concentration was observed. However, considering the successive annamox process, external ammonia needs to be provided if all influent ammonia would be consumed in PN reactors at 45°C-2hr and 50°C-
1hr. However, at 55℃-0.5hr heating, the overall ratio of nitrite and ammonia is approximately 1.32 which follows the annamox stoichiometry (Yao et al., 2015). Moreover, the produced overall NAR at 55℃-0.5hr was 72% (maximum NAR was 82%) which is greater than 58% and 61% at 45℃-2hr and 50℃-1hr respectively. Therefore, we consider 55℃-0.5hr is the optimum temperature for PN-A process for operating the SBRs at NLR of 0.05 kgN/m³.d with SRT of 7 days and HRT of 16 days for nitrite accumulation.

The effluent nitrite concentration was observed at 45℃-2hr, 50℃-1 and 55℃-0.5hr while effluent ammonia concentration was observed at 55℃-0.5hr while both nitrifiers were completely inhibited at 60℃-0.5hr. indicating that NOB inhibition occurred through 15℃ temperature changed starting from 45℃-2hr to 60℃-0.5hr whereas AOB inhibition occurred through 10℃ of temperature change from 50℃-1hr to 60℃-0.5hr. The slower inhibition of NOBs compared to AOBs can be described by Yu et al. (2021) who performed enzymatic activity tests for AOBs and NOBs from 25℃ to 40℃. This study reported that the overall AMO (ammonia monoxygenase) activities remain steady at 35℃ and the activity of HAO (Hydroxylamine oxidoreductase) increased up to 31% compared to control but decreased at 40℃. However, the activity of NOR (nitrite oxidoreductase) decreased over the whole experimental thermal treatment process to different degrees from 2.85% to 100% at temperature range 25℃ to 40℃ (Yu et al., 2021).

4.3.1.5 Impact of other operating parameters on PN

In this study, the dissolved oxygen (DO) was maintained at 4 mg/L in both the control and heated SBRs throughout the entire 352 days operation period. This ensured sufficient DO levels for both AOBs and NOBs to thrive, resulting in complete nitrification in the control reactor. However, to investigate the effect of FA (Free Ammonia) and FNA (Free Nitrous Acid), which are functions of pH, temperature, and effluent ammonia and nitrite concentrations, it is important to understand their potential for the inhibition of NOBs in the heated reactors. The FA and FNA were calculated Based on the following Equations (4-9) & (4-10) (Liu et al., 2020).

\[
FA \left( \frac{mg}{L} \right) = \frac{17}{14} \times \frac{NH_3^- - N \times 10^{PH}}{10^{PH+ex} \times \frac{6.34}{273+T}}
\]  

(4-9)
where, $\text{NH}_4^+\text{-N}, \text{NO}_2^-\text{-N}, \text{and NO}_3^-\text{-N}$ the effluent ammonia, nitrite, and nitrate respectively. For estimating FA and FNA from PN reactors at 45°C-2hr, 50°C-1hr and 55°C-0.5hr, nitrite data points after steady nitrite accumulation were considered. For 45°C-2hr, data points from day 18 to day 36, for 50-1hr, data points from day 48 to day 68, for 55°C-0.5hr, data points from day 31 to day 55 were considered for estimating FA and FNA. Figure 4-5 showed the FA and FNA concentrations.

![Figure 4-5. FA and FNA concentrations in control and heated reactors](image_url)

Figure 4-5. FA and FNA concentrations in control and heated reactors

At 22°C (control), 45°C-2hr, 50°C-1hr and at 55°C-0.5hr, the FNA concentrations were 0, 0.01±0.003, 0.002±0.0003 and 0.01±0.002 mg/L which are well below the NOB inhibition threshold of 0.026-0.22mg/L (Yu et al., 2021)) and hence there was no impact of FNA on NOB suppression in the heated reactors. The FA concentrations at 22°C (control), 45°C-2hr, 50°C-1hr and at 55°C-0.5hr, were 0.01±0.009, 0.01±0.005, 0.02±0.003 and 0.22±0.06 mg/L indicating that out of the three heated reactors, only the SBR at 55°C-0.5hr, have FA concentrations that can inhibit NOBs which is in the range 0.1-1 mg/L (Yu et al., 2021) or in the range of 0.1-5 mg/L (Liu et al., 2020) However the overall pH in this heated reactor
was 6.8 which was close to control (pH=7) and other heated reactors. The effluent FA concentration in the control reactor was 0.01±0.009 mg/L which is below FA threshold of NOB. Therefore, we can say that although the FA concentration at 55°C-0.5hr was above the inhibition threshold of NOBs (0.22±0.06 mg/L), the produced FA concentrations occurred due to the effluent ammonia concentration, caused by the inhibition of AOBs due to the application of heat.

### 4.3.1.6 Impact of heating on overall effluent quality

The heating was performed in two reactors simultaneously. Heating at 35°C-1hr, 35°C-2hr, 35°C-3hr, 45°C-1hr, 40°C-2hr, 40°C-3hr was performed in one reactor and heating at 45°C-1hr, 45°C-2h, 50°C-1hr, 55-0.5hr and 60°C-0.5 hr was performed in another reactor. In order to show the comparison of performance, results from two reactors are integrated together to capture the change of effluent quality that is are shown in Figure 4-6. Table 4-4 shows the summary of effluent quality from the control and heated reactors at steady state.
a) Days of operation

- COD-Influent
- sCOD-Influent
- COD-Control
- sCOD-Control
- COD-Heated reactors
- sCOD-Heated reactors

b) Days of operation

- TKN-Influent
- TKN-Control
- TKN-Heated reactors
Figure 4-6. Influent and effluent quality in control and heated reactors
Table 4-4. Overall effluent characteristics from reactors at steady state (Mean ± standard deviation (No. of samples))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>23°C</th>
<th>35°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1hr</td>
<td>2hr</td>
<td>3hr</td>
</tr>
<tr>
<td>COD</td>
<td>40±12(4)</td>
<td>37±6(4)</td>
<td>44.5±10.8(5)</td>
</tr>
<tr>
<td>SCOD</td>
<td>31±6.1(4)</td>
<td>29±5.4(5)</td>
<td>28.5±4.5(5)</td>
</tr>
<tr>
<td>TSS</td>
<td>12±0.4(3)</td>
<td>12±0.5(3)</td>
<td>22±8.9(3)</td>
</tr>
<tr>
<td>VSS</td>
<td>8±0(3)</td>
<td>7±0.1(3)</td>
<td>11±3.2(3)</td>
</tr>
<tr>
<td>TKN</td>
<td>3±0.5(5)</td>
<td>1.9±1.2(5)</td>
<td>2.4±1.8(5)</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>0.32±0.22(4)</td>
<td>0.52±0.2(3)</td>
<td>0.52±0.2(3)</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>24±0.9(4)</td>
<td>28±4.4(3)</td>
<td>27±4.4(3)</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>0.05±0.3(4)</td>
<td>0.06±0.08(3)</td>
<td>0.08±0.04(3)</td>
</tr>
<tr>
<td>NOx</td>
<td>29±3.2(4)</td>
<td>28±4.5(5)</td>
<td>28±1.6(4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>45°C</th>
<th>50°C-1hr</th>
<th>55°C-0.5hr</th>
<th>60°C-0.5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1hr</td>
<td>2hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COD</td>
<td>65±20(4)</td>
<td>62.4±9.2(4)</td>
<td>85.5±14.3(4)</td>
<td>108±17.3(4)</td>
</tr>
<tr>
<td>SCOD</td>
<td>44±14(4)</td>
<td>50.7±9.3(4)</td>
<td>57.4±6.3(4)</td>
<td>64.3±10.3(4)</td>
</tr>
<tr>
<td>TSS</td>
<td>18±1.6(3)</td>
<td>15±3.3(5)</td>
<td>15±4.1(3)</td>
<td>11±2.1(3)</td>
</tr>
<tr>
<td>VSS</td>
<td>11±1.9(3)</td>
<td>8.6±0.9(3)</td>
<td>8±1.6(3)</td>
<td>8±1(3)</td>
</tr>
<tr>
<td>TKN</td>
<td>4.3±1.4(5)</td>
<td>5.6±2.1(5)</td>
<td>9.7±3.1(5)</td>
<td>11±0.7(5)</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>0.4±0.3(3)</td>
<td>0.3±0.2(4)</td>
<td>0.5±0.1(3)</td>
<td>6.5±0.4(3)</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>18±1.1(3)</td>
<td>7.3±0.3(4)</td>
<td>6.4±0.3(3)</td>
<td>6.4±1.9(3)</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>0.06±0.3</td>
<td>10.3±0.2(4)</td>
<td>10.2±0.07(3)</td>
<td>14.9±1.8(3)</td>
</tr>
<tr>
<td>NOx</td>
<td>20±3.8(4)</td>
<td>17.6±0.4(4)</td>
<td>16±1.8(4)</td>
<td>18.7±3.6(4)</td>
</tr>
</tbody>
</table>
From Figure 4-6 (a), it is obvious that, from 35℃-1hr to 40℃-3hr the overall concentrations of COD and sCOD ranged from 32 to 48 mg/L and 26 to 29 mg/L respectively indicating no significant change in effluent COD concentration in these reactors compared to control at 23℃ until temperature 40℃-3hr. However, at 45℃-1hr and 45℃-2hr, the overall COD and sCOD increased by 25 mg/L and 18 mg/L, and 25 mg/L and 24.3 mg/L respectively, compared to control. The COD at 50℃-1hr, 55℃-0.5hr and 60℃-0.5hr were 85.5 mg/L, 108 mg/L and 123 mg/L, while sCOD were 57 mg/L, 64 mg/L and 85 mg/L indicating that both COD and sCOD increased drastically with temperature. The reason of increasing effluent COD and sCOD is due to the inhibition, death, fragmentation of microbial cells and solubilization of the cell materials in the reactor environment (Xu et al., 2011) which impacted the overall COD removal in the heated reactors.

The influent TKN ranged from 24 to 50 mg/L in the entire operating period (Figure 4-6 (b)). The effluent TKN concentration in the control reactor was as low as 1.2 mg/L (day 168). The range of TKN in the control effluent is 1.2 to 6.2 mg/L (day 248). At a temperature range of 35℃ to 45℃(2hr), the overall TKN ranged from 0.9-8mg/L indicating that, 86-94% of influent 35 mgTKN/L was removed. The overall ammonia concentration during the stable TKN was 24.5 mg/L (not shown in the Figure) which produced 12 mg/L of ammonia at the start of the reaction (aeration period) in the reactor. After the aeration period the overall concentration of NOx is 17.5 mg/L (Figure 4-6(c)) meaning that only approximately 5.5 mg/L of 10.2 mg/L of TKN came from influent organic nitrogen. All the ammonia (17.5 mg/L) oxidized by the AOBs confirmed by the overall effluent ammonia concentration of around 0.5 mg/L at 45-2hr. Nitrifiers inhibit at day 1 after the application of heating at 50-1hr and continued until day 4 (217-221), with increased TKN concentration of to 26 mg/L on day 217. Considering influent ammonia of 24.5 mg/L and NOx of 16 mg/L, the overall effluent TKN of 4 mg/L was coming from influent TKN (organic nitrogen as ammonium is approximately zero) indicating that with temperature the ammonification is increasing. However, during the stable TKN period at 55℃-0.5 hr, the overall effluent TKN was 12 mg/L. The overall influent ammonia was 21
mg/L during this operation and the NOx concentration was 18.7 mg/L (Table 4-4), providing 8.7 mg/L of TKN was coming from influent, indicating the ammonification is reducing at this temperature due to inactivation of ammonifying bacteria. This can be explained by a study (Milks, 1975) who conducted biological ammonification test in tropical soil to investigate the effect ammonification through the application of heat from 20℃-60℃. This study observed that until temperature of 50℃, the ammonium accumulation occurs in tropical soil at a rate of 2.8µgN/g.d and then declined at temperature 60℃. Since, overall effluent ammonia od 7.4 mg/L was observed at this temperature, the AOBs were also inhibited. The overall TKN at 60℃-0.5hr was 30 mg/L with NOx of 1 mg/L, which is mostly inhibition of ammonifying bacteria and all nitrifiers. Although, most of the studies (Gilbert et al., 2014b; Guo et al., 2010; J. Wang et al., 2023) on PN were conducted at room temperature and only four studies (Chen et al., 2016; J. Chen et al., 2019; Choi et al., 2021; Isaka et al., 2008) were done on PN using intermittent heating, no studies provided information about the impact of temperature on aerobic ammonification and the potential for inhibition. However, in this study, we revealed that the activity of ammonifying bacteria increased from 45℃-2hr to 50℃-1hr and then declined which follows the same trend as AOBs.

The range of influent TSS and VSS were 70-150 mg/L and 40-120 mg/L. The effluent TSS and VSS at 22℃, 35 ℃-1hr, 2hr and 3hr, 40 ℃-1hr, 2hr and 3hr, 45℃-1hr and 2hr, 50℃-1hr were in the range of 7-22mg/L, overall, which did not change in entire operational period 352 days (Figure 4-6(d)). The overall phosphorus in the heated reactor were same as the control reactor for the entire operating period ranging from 0.8 mg/L to 3.2 mg/L indicating that the heating does not have any significant effect on phosphorus removal (Figure 4-6(e)).

4.3.1.2 COD, TKN and TP Removal Efficiencies

The overall COD, TKN and TP removal reduced due to increased temperatures in the reactors are shown in Figure 4-7. It is clear that the removal efficiency of all reactors decreased with the increase of temperature with COD removal of 90% to 50% (Figure 4-7(a), TKN from 90% to 13% (Figure 4-7(a)) and Figure 4-7(b) in the SBRs.
However, the phosphorus removal of 77% to 22% (Figure 4-7 (c)) in this system occurred due to biomass synthesis, not due to biological phosphorus removal as the environment in the reactor was completely aerobic (DO of 4 mg/L). Moreover, at high temperature (35°C-60°C) phosphorus accumulating organisms could not survive (Brdjanovic et al., 1997) which was confirmed by the microbial analysis of the study where no PAOs were found in the MLSS samples.

4.4 Recovery of nitrifiers and heat optimization

In order to estimate the recovery of NOBs and AOBs, the temperatures of 60°C-0.5hr (Figure 4-8(a)) and 55°C-0.5hr (Figure 4-8 (b)) were considered as both AOBs and NOBs were inhibited at these temperatures. At 55°C-0.5 hr and 60-0.5 hr, the reactivation time
was estimated by stopping the heating at the end of the operation of PN tests (at 55 days and 21 days respectively). Effluent ammonia, nitrites, and nitrates after stopping heating is shown in Figure 4-8.

![Figure 4-8. AOB and NOB recovery after heating stopped](image)

After complete inactivation at 60°C-0.5hr, when heating stopped, the concentration of ammonia decreased from 21 mg/L to 0.5 mg/L in 11 days indicating complete activation of AOBs. Nitrate concentration went up from 0 to 27 mg/L in 13 days for the NOBs to fully recovered. This indicates that AOBs recovered 3 days earlier than NOBs which is approximately 0.4 SRT turnovers. At 55°C-0.5hr, AOBs needed 10 days to recover completely, while NOB needed 13 days, with the difference again being 3 days or 0.4 SRT turnovers. Based on this recovery time, in order to optimize heat energy, heat was applied once every 10 days at 50°C-1hr and 55°C -0.5hr. Effluent ammonia, nitrites, and nitrates at 50°C-1hr and 55°C -0.5hr are depicted in Figure 4-9(a) and Figure 4-9(b). Before implementing these optimized heating strategies, both reactors were achieving complete nitrification when the influent ammonia was oxidized to nitrate (data not shown in the figure).
Figure 4-9(a) shows that heating at 50℃-1hr on day 1, increased effluent mgNO$_2$-N/L from 0 to 12 with overall effluent mgNH$_4$-N/L and mgNO$_3$-N/L at 11 and 6 respectively until day 9 and decreased to 8 at day 10. However, the second heating on day 11 maintained effluent mgNO$_2$-N/L, mgNH$_4$-N/L, and mgNO$_3$-N/L of 11, 0.5 and 7 respectively until day 16. It is thus evident that PN could be maintained by heating once every 10 days at 50℃-1hr and 55℃-0.5hr.

**Figure 4-9. Stability of nitrite accumulation for heating once in 10 days in SBRs**

Figure 4-9(a) shows that heating at 50℃-1hr on day 1, increased effluent mgNO$_2$-N/L from 0 to 12 with overall effluent mgNH$_4$-N/L and mgNO$_3$-N/L at 11 and 6 respectively until day 9 and decreased to 8 at day 10. However, the second heating on day 11 maintained effluent mgNO$_2$-N/L, mgNH$_4$-N/L, and mgNO$_3$-N/L of 11, 0.5 and 7 respectively until day 16. Figure 4-9 (b) showed that heating at 55℃-0.5hr at day 1, increased the average effluent mgNO$_2$-N/L to 10 until day 8 while maintaining overall mgNH$_4$-N/L and mgNO$_3$-N/L at 12 and 5 respectively and decreased to 7 at day 10. However, second heating at day 10 increased the mgNO$_2$-N/L to 11 until day 16. It is thus evident that PN could be maintained by heating once every 10 days at 50℃-1hr and 55℃-0.5hr.
4.5 Energy consumption

Nitrification energy estimates for a real 1000 m$^3$/d WWTP equipped with diffused aeration system having an aeration tank of 4 m depth were evaluated to assess the energy calculation in PN. The heating energy was calculated for 50°C-1hr and 55°C -0.5hr for once everyday and once in 10 days, were selected based on the heating strategy shown in Figure 4-9. The oxygen transfer rate and mechanical efficiency were taken as 10% and 70% respectively. Two scenarios were evaluated with respect to aeration energy required for nitrification only: a- complete nitrification, and b-PN-Anammox using influent flow splitting, based on the anammox stoichiometry, and the observed stable effluent nitrites and ammonia. The detailed calculation of flow split is shown in supplementary information in Table 1 and example calculation to provide a molar ammonia to nitrite ratio of 0.76. According to flow split, 89% flow will go to PN reactor and 11% flow will go to Anammox reactor (SI, Table 1).

In order to estimate total energy consumption in the PN reactors, the aeration and heating energy were calculated using the equation for adiabatic compression (Metcalf & Eddy, 2004) and specific heat capacity (Manahan, 2008) respectively, shown in Equations (4-11) & (4-12).

\[
\text{Aeration Energy} (\frac{\text{kWh}}{\text{d}}) = \frac{Q_{\text{air}} \times \frac{1}{0.0224} \times RT \times \left(\frac{P_d}{P_{\text{inlet}}}\right)^0.283 \times 2.78 \times 10^{-7}}{0.283 \times \eta} \quad (4 - 11)
\]

\[
\text{Heat Energy} (\frac{\text{kWh}}{\text{d}}) = m \times C_p \times (T_t - T_r) \quad (4 - 12)
\]

Where $Q_{\text{air}}$= air flow in m$^3$/d, $R$= universal gas constant for air, 8.314 J/mole.K, n=0.283 for dry air, $T$= temperature in the system in °K, $P_{\text{inlet}}$= atmospheric pressure, 0.21 atm, $P_d$ =Calculated discharge pressure in atm , $\eta$ = mechanical efficiency of the diffuser, 70%, $m$=mass of the heated sludge in kg/d, $C_p$=specific heat capacity of water, 4.186 KJ/Kg°C, $T_t$= Temperature to be heated the RAS, in °K, $T_r$=room temperature in °C. Detailed calculations are shown in SI- Table 2 and example calculations.

Figure 4-10 showed the energy requirement for complete nitrification and total energy (aeration and heating) for heating.
The detailed calculation is shown in supplementary information, Table 2. From Figure 4-10 and SI-Table 2 it is evident that the aeration energy required for achieving complete nitrification was 73 kWh/d at 23℃ (room temperature) with influent COD of 230 mg/L and effluent COD of 30 mg/L and influent TKN and effluent ammonia concentration of 37 mg/L and 0.5 mg/L respectively. The overall effluent sCOD at 55℃-0.5hr of heating at steady state was 64 mg/L and ammonia of 7.4 mg/L. The aeration energy required for maximum NAR of 0.79 at 55℃-0.5hr was 53 kWh/d whereas the heating energy required for heating one hour per day was 74.8 kWh/d, which is 75% more energy than complete nitrification without heating. However, heat is applied once every 10 days, the total energy required for intermittent heating is 60.5 KWh/d (53 + 74.8/10), reflecting 17% energy savings saved compared to complete nitrification at 23℃. Similarly, Figure 4-10 shows that the total energy for complete nitrification without heating, heating once a day and heating in once in 10 days required 73 kWh/d, 169 KWh/d and 64 KWh/d at 50℃-1hr respectively (not shown in detailed calculation) indicating that
heating at this temperature once in 10 days can reduce 12.3% of total energy requirement at maximum NA of 0.63 at 50℃-1hr.

4.6 Sensitivity Analysis

Our baseline temperature in the lab (control reactor) was 23℃. However, since the usual temperature of municipal wastewater is 12°C (Sallanko & Pekkala, 2008), a sensitivity analysis considering the WW temperature of 12°C was considered for heating at 50℃-1hr and 55℃-0.5hr. The calculation was shown at the end of the example calculation in supplementary information and the heating requirements are shown in Figure 4-11. The required total heat for heating once in 10 days from 12℃ to 50℃-1hr and 12℃ to 55℃-0.5hr were 68.4 KWh/d and 63 KWh/d indicating 6.3% and 13.7 % less energy than that required for complete nitrification.

Figure 4-11. Sensitivity analysis considering real wastewater temperature (12°C)
4.7 Heat loss from the heated systems

For estimating heat losses from the aeration tank, conductive and convective heat transfer were estimated by Fourier's Law and Newton's law of cooling shown in following equations (Holman, 2008):

Conductive heat loss, \( q_1 = U \times A_1 (t1-t2) \) \hspace{1cm} (4-13)

Convective heat loss: \( q_2 = hA (t1-t2) \) \hspace{1cm} (4-14)

Where \( q_1 = \) heat loss due to conduction (W) through concrete wall, \( U = \) Coefficient of heat transfer for conduction 1.3 (W/(m.K)) for concrete without insulation and 0.08 W/m2.K with insulation (Misri et al., 2018; Yun et al., 2014), \( A_1 = \) total area of the concrete wall=384 m2, \( q_2 = \) Heat loss due to convection (W), \( A_2 = \) Surface area of the open surface=170 m2, \( h = \) convection heat transfer coefficient = 4.5 W/m2.K (Halman, 2008) for temperature change of 30 °C for free convection, \( t1-t2 = \) temperature gradient. The overall temperature of air and soil was considered as 20 °C (Alam et al., 2015).

Since the effective reactor volume was 667 m³ (~700 m³), the height of the tank=4m, length=17m and width=10 m was considered which produced area of the side wall of 384 m² and the area of the top slab=170 m². The thickness of the concrete was considered 0.3m. Three scenarios were considered for heat loss calculations: Scenario 1. Sidewalls and top slab were insulated (conduction); Scenario 2: Sidewalls and top slab were not insulated (conduction) Scenario 3: Side walls were not insulated, and top was open (conduction through side walls plus convection to open air). The heat loss calculations are shown in Table 3 Appendix B.

Considering the heat loss along with nitrification energy, there is still energy savings from 4.8% to 17.5% using this heating Scenarios which is shown in Table 4-5. Depending on the insulation of the aeration tank, the heat requirements need to be calculated.
Table 4-5. Total heat requirements for different Scenarios

<table>
<thead>
<tr>
<th>Energy requirement</th>
<th>Scenario 1</th>
<th>Scenario 2</th>
<th>Scenario 3</th>
<th>Energy Savings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-1hr (kWh)</td>
<td>63.3</td>
<td>69.5</td>
<td>72.9</td>
<td>13.3%, 4.8%, 0%</td>
</tr>
<tr>
<td>55-0.5 hr (kWh)</td>
<td>60.2</td>
<td>63.7</td>
<td>66.1</td>
<td>17.5%, 12.7%, 9.4%</td>
</tr>
<tr>
<td>Energy for complete nitrification</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>(kWh)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8 Feasibility of using the temperature strategy for PN in real wastewater treatment plant

This study presented findings on the effects of heating in wastewater treatment plants to achieve nitrite accumulation and improve nitrification efficiency. According to this study, heating the mixed liquor suspended solids (MLSS) once every 10 days at a temperature of 55°C for 0.5 hours resulted in a maximum nitrite accumulation of 79%. The overall ammonia level in the wastewater was 7.4 mg/L, and the overall nitrite level was 13.9 mg/L which provide nitrite to ammonia ratio of 1.89. However, providing 89% of the flow to the PN reactor gives the ratio 1.32 (SI, table 1) that could be provided the successive anammox reactor. The block diagram in Figure 4-12 illustrates a nitrifying plant with integrated COD removal and anammox process. It is well established that higher heat favored AOBs over NOBs in the side stream partial nitrification. Factors such as FA and FNA were identified as main contributors to NOB suppression. However, NOBs can be shifted to another form to overcome the resistance of FA and FNA thresholds and enable complete nitrification (Duan et al., 2019). Therefore, intermittent high had real potential that may be applied both side stream and mainstream.
Figure 4-12. Block flow diagram of heat treated PN-AMX system in real WWTP.
Chen et al., (2019) used TWAS and PS for heating and achieved PN. Yu et al. (2021) suggested heating the RAS for achieving PN. However, before implementing the heated TWAS or RAS method in real wastewater treatment, additional facilities are required for NOB inhibition, AOB recovery, removing the significantly higher concentration of soluble COD and nitrogen (N) from heating TWAS compared to MLSS. Similarly, the heating of return activated sludge (RAS) before it flows into the nitrifying reactor needs further exploration of the required heating temperature, duration, and frequency for effective NOB inhibition.

In the proposed block flow diagram (Figure 4-12), it was noted that heating conditions above 45°C for 1 hour would increase COD in the system. To achieve stable nitrite accumulation, a COD removal system is necessary to meet the COD criteria after heating the secondary nitrifying sludge. While heating at 45°C for 2 hours and 50°C for 1 hours can provide nitrite accumulation, AOBs remain active in the system. A certain level of AOB inhibition is required for the subsequent anammox process to achieve an ammonia-to-nitrite ratio of 0.76. This can reduce the reliance on external ammonia. As the overall influent ammonia concentration is 24 mg/L, with a flow diversion of 11.4 m$^3$/d directly to the anammox reactor, while 89% to the partial nitrification (PN) reactor would provide the ammonia-to-nitrite ratio of 0.76.

Cost estimation showed that heating at 55°C for 0.5 hours can reduce the total energy cost by 9-17% compared to the cost required for complete nitrification at the control temperature of 23°C. Considering the practical temperature of the wastewater treatment plant at 12°C, the cost reduction can be approximately 13.9%. Therefore, the proposed heating strategy could be applicable in real wastewater treatment plants. Adding aerobic COD removal after the PN can consume another 8% (73*0.11) of aeration energy, however, still saves 4-9% of the aeration energy compared to conventional nitrification denitrification process.
4.9 Dynamics of bacterial community in nitrifying reactors

4.9.1 Microbial diversity in the reactors

Samples from all nitrifying SBRs were taken in three batches and were sent to Institut de recherche et de développement en agroenvironnement (IRDA) for microbial analysis. The first batch was collected from reactors at 23°C (Control), 45°C-2hr, and 50°C-1hr on day 34 when PN occurred. The second batch was taken from the optimum reactor at 55°C-0.5hr at different operation times: day 51 (heating and nitrite accumulation present), day 68 (no heating and nitrite accumulation lost), and day 75 (one turnover of SRT after nitrite accumulation was lost). The third batch of samples was taken from the control reactor at 23°C and 60°C-0.5hr on day 12 after the heating was stopped to compare the microbial samples in control at 23°C and reactor being heated at 60°C-0.5hr, operated at 23°C. The sampling schedule, operational taxonomic units (OTUs), indices and coverage are shown in Table 4-6.

Table 4-6. Sampling details of microbial analysis

<table>
<thead>
<tr>
<th>Samples</th>
<th>PN (Y/N)</th>
<th>No of OTUs</th>
<th>Shannon</th>
<th>Chao1</th>
<th>Evenness</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First Batch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22°C (Control)</td>
<td>N</td>
<td>706</td>
<td>5.78</td>
<td>710.57</td>
<td>0.88</td>
<td>100%</td>
</tr>
<tr>
<td>45°C-2hr</td>
<td>Y</td>
<td>396</td>
<td>4.71</td>
<td>400.71</td>
<td>0.79</td>
<td>100%</td>
</tr>
<tr>
<td>50°C-1hr</td>
<td>Y</td>
<td>280</td>
<td>4.14</td>
<td>280.17</td>
<td>0.73</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Second Batch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55°C-0.5hr</td>
<td>Y</td>
<td>371</td>
<td>4.923</td>
<td>379.261</td>
<td>0.832</td>
<td>100%</td>
</tr>
<tr>
<td>55°C-0.5hr</td>
<td>N (right after PN lost)</td>
<td>415</td>
<td>5.103</td>
<td>415.455</td>
<td>0.847</td>
<td>100%</td>
</tr>
<tr>
<td>55°C-0.5hr</td>
<td>N (1 SRT turnover after PN lost)</td>
<td>404</td>
<td>4.847</td>
<td>410.107</td>
<td>0.808</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Third Batch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22°C (Control)</td>
<td>N</td>
<td>421</td>
<td>5.17</td>
<td>427.563</td>
<td>0.856</td>
<td>100%</td>
</tr>
<tr>
<td>60°C-0.5hr</td>
<td>N (After PN lost)</td>
<td>364</td>
<td>4.981</td>
<td>366.154</td>
<td>0.845</td>
<td>100%</td>
</tr>
</tbody>
</table>

The coverage of all prokaryotes was 100% indicating that the sequencing reliably captured the full range of microbial taxa in the samples. This confirms the reliability of the RNA
sequencing. The Shannon evenness index for reactor at 23°C was 5.78 in the first batch and 5.17 in the third batch, respectively which are higher than the indices in heated reactors on these batches indicating that the evenness in the heated reactors decreased due to increased temperatures. The decreasing trend of Shannon index with the increase of temperature indicating that higher temperatures had negative impact on the evenness of the microbial community. The richness index of bacteria measured by Chao1 index, followed the same trend as Shannon index. This means that increasing temperature had negative impact on the bacterial community, resulting in decrease in species richness. Figure 4-13, 4-14 and 4-15 showed the relative abundances of phylum in the batches.

![Relative abundance of Phylum in Batch 1]

Figure 4-13. Relative abundance of Phylum in Batch 1
Figure 4-14. Relative abundance of Phylum in Batch 2
A total of 23, 26 and 24 phyla were detected in Batch 1, while Batch 2 and Batch 3 respectively. In Batch, higher relative abundances were observed for Proteobacteria (43.4%, 41.4%, and 57.8%), Bacteroidota (21.7%, 21.1%, and 19.3%), and Actinobacteriota (4.4%, 5.9%, and 3.6%) at 23°C, 45°C-2hr, and 50°C-1hr, respectively. Proteobacteria showed a proportional relationship with temperature, increasing from 43.4% at 23°C to 57.8% at 50°C-1hr in Batch 1, and from 37.6% at 23°C to 52.1% at 65°C-0.5hr in Batch 3. Bacteroidota did not exhibit significant changes, with a relative abundance of 21.7% decreasing to 19.3% in Batch 1 and 21.5% at 23°C remaining relatively stable in Batch 3. In Batch 2, Proteobacteria (50%) and Bacteroidota (27%). Myxococcota (14.5%) appeared to be dominant in the control reactors.

Table 4-6 and Table 4-7 present the bacterial community structure (genus level) in batches 1, 2 & 3 of the nitrifying reactors. These tables include the top 100 species with a relative abundance of at least 4% in one of the reactors. In order to accurately reflect their true abundances in real wastewater treatment plants and for analytical purposes, \textit{Nitrospira} and
Nitrosomonas have been included in the tables, despite not meeting the specified set criteria.

Table 4-7. Relative abundance of significant bacterial species (>4% at any temperature) in 100 ranked microorganisms in Batch 1

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Species</th>
<th>Control (23℃)</th>
<th>45℃-2hr</th>
<th>50℃-1hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling condition</td>
<td></td>
<td>Room temperature</td>
<td>Partial Nitrification</td>
<td>Partial Nitrification</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Ahniella</td>
<td>2.06</td>
<td>9.29</td>
<td>13.18</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Thauera</td>
<td>0.28</td>
<td>0.51</td>
<td>6.19</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Arenimonas</td>
<td>4.02</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Nitrosomonas</td>
<td>2.24</td>
<td>0.47</td>
<td>0.56</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Lewinella</td>
<td>0.53</td>
<td>9.27</td>
<td>0.08</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Terrimonas</td>
<td>0.27</td>
<td>2.32</td>
<td>6.27</td>
</tr>
<tr>
<td>Nitrospirota</td>
<td>Nitrospira</td>
<td>1.09</td>
<td>0.14</td>
<td>0.00</td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td>10.48</td>
<td>22.04</td>
<td>26.28</td>
</tr>
</tbody>
</table>
Table 4-8. Relative abundance of significant species (>4% in any temperature) in 100 ranked bacterial community in the reactors Batch 2&3

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Species</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Control (23℃)</th>
<th>23℃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>55℃-0.5hr</td>
<td>55℃-0.5hr</td>
<td>55℃-0.5hr</td>
<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Arenimonas</td>
<td>0.4</td>
<td>0.4</td>
<td>5.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Dechloromonas</td>
<td>1.7</td>
<td>1.6</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Nitrosomonas</td>
<td>1.2</td>
<td>1.6</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Pseudomonas</td>
<td>0.5</td>
<td>1.0</td>
<td>4.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Thermomonas</td>
<td>2.1</td>
<td>1.1</td>
<td>7.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Zoogloea</td>
<td>4.6</td>
<td>5.1</td>
<td>3.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Ca_Epiflobacter</td>
<td>1.0</td>
<td>11.5</td>
<td>2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Flavobacterium</td>
<td>6.0</td>
<td>0.7</td>
<td>6.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Hassallia</td>
<td>0.0</td>
<td>0.0</td>
<td>4.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Bacteroidota</td>
<td>Ignavibacterium</td>
<td>4.0</td>
<td>1.2</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Trichococcus</td>
<td>4.6</td>
<td>1.7</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Myxococcota</td>
<td>Haliangium</td>
<td>0.0</td>
<td>0.2</td>
<td>1.4</td>
<td>6.3</td>
</tr>
<tr>
<td>Nitrospirota</td>
<td>Nitrospira</td>
<td>0.2</td>
<td>1.3</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>SUM</td>
<td></td>
<td>26.4</td>
<td>27.3</td>
<td>42.9</td>
<td>23.8</td>
</tr>
</tbody>
</table>

From Table 4-6, in Batch 1, Ahniella, Thauera, and Terrimonas exhibit the positive impact of temperature, as they thrive more with an increment in temperature from 23℃ to 50℃-1hr. Their relative abundances increased from 2.06% to 13.18%, 0.28% to 6.19%, and 0.27% to 6.27% respectively. On the other hand, Arenimonas, Nitrosomonas, and Nitrospira showed the negative impact on temperature increase from 23℃ to 50℃-1hr. However, the ratios of Nitrosomonas and Nitrospira at 23℃ and 45℃-2hr, and at 23℃ and 50℃-1hr, were 2.2:1 and 3.35:1 respectively. This implies that although both nitrifiers did not thrive with the increase of temperature, the abundance of Nitrosomonas was significant enough to oxidize all influent ammonia, as confirmed by the effluent ammonia concentration being zero in this temperature range. However, the abundances of Nitrospira
were not sufficiently high to oxidize all of the nitrite, resulting in a nitrite accumulation of 10 mg/L.

Batch 2 was analyzed to compare three conditions at 55°C-1hr: samples taken during partial nitrification, immediately after partial nitrification was lost (no heating, complete nitrification resumed), and 7 days after partial nitrification was lost. It is evident from Table 4-6 that the majority of bacteria, including *Arenimonas, Dechloromonas, Thermomonas, Zoogloea, Hassallia,* and *Haliangium* (which account for 46% of the significant bacteria), showed insignificant changes right after the loss of partial nitrification. *Trichococcus* decreased from 4.6% to 1.7%, while Ca *Epiflobacter* increased from 1% to 11.5%. However, the total abundances of significant bacteria in these two conditions were 26.4% and 27.3% respectively, suggesting only a 1% change in significant bacteria in the reactor. This could be due to the fact that the reactor was still approaching steady state conditions right after the PN was lost. This was further confirmed by the next condition: at the first turnover of SRT (7 days) after the loss of partial nitrification. In this condition, most bacteria increased, resulting in a 14% overall increment in the significant bacteria in the reactors. The significant growth of these bacteria occurred after the reactor reached the steady state condition at the new temperature of 23°C (on 21 days). *Nitrosomonas* increased only by 0.4% in the time interval of heating stopped and PN lost while 0.2% in the time interval of PN lost and 1-SRT turnover after PN lost respectively. However, *Nitrospira* increased from 0.2 to 1.3% indicating that *Nitrospira* was completely revived at these conditions, which was confirmed by the nitrite concentration of 0 mg/L right after PN lost and 1 SRT turnover of PN lost.

In batch 3, the overall relative abundance of bacteria increased by approximately 10% from the control reactor to the control condition (from 60°C-0.5 hr to 23°C). The most abundant heterotrophs observed at 23°C were *Zoogloea* (5.5%) and *Haliangium* (6.3%), while in the control condition reactor, the most abundant heterotrophs were *Arenimonas* (5.5%), *Dechloromonas* (5.6%), *Pseudomonas* (8.7%), and *Flavobacterium* (7%). In both of these conditions, complete nitrification was achieved, with a *Nitrosomonas* to *Nitrospira* ratio of 0.38:1 and 0.74:1 respectively. This indicates that complete nitrification can be achieved
even when the diversity of *Nitrosomonas* is approximately one third of the diversity of *Nitrospira*.

### 4.9.2 Microbial community and performance of the reactors

The mass of nitrifiers was calculated by using relative abundance of each bacterium and the produced biomass in the system. The produced nitrifiers per day was calculated based on the following Equations (4-13) and (4-14).

\[
\text{Nitrifiers (gVSS/d)} = \frac{Q \times Y_n \times \text{NH}_3-N}{1 + b_n T \times \text{SRT}} + f_d \times b_n T \times \frac{Q \times Y_n \times \text{NH}_3-N \times \text{SRT}}{1 + b_n T \times \text{SRT}} \tag{4 - 15}
\]

Where \( b_n = b_{20} \theta(T - T_{20}) \) \tag{4 - 16}

where \( Y_n \), NOx, \( b_n \), and \( \theta \) are the true yield of nitrifiers (0.12gVSS/gN), concentration of average nitrified \( \text{NH}_3-N \) (25 mg/L), endogenous decay coefficient for nitrifiers (0.08 gVSS/gVSS.d) at 20°C and Arrhenius coefficient for endogenous decay (1.04) respectively. All other terms are as previously defined.

In this study, Pearson correlation (\( r \)) was estimated to correlate two variables linearly which is shown in Figure 4-16.
Figure 4-16. Correlation with nitrifiers with temperature, substrate utilization rates and overall TKN removal
From Figure 4-16 (a), it is clear that, both *Nitrosomonas* and *Nitrospira* had negative correlation with temperature with Pearson correlation $r=-0.95$ ($R^2=0.90$) and $r=-0.96$ ($R^2=0.93$) indicating that both nitrifiers are moderate thermophiles and can grow until temperature 50°C (Lebedeva et al., 2005).

Since we correlated up to 55°C-0.5hr, the growth of nitrifiers decreased. Although *Nitrosomonas* and *Nitrospira* showed similar correlation with temperature, lower numbers of *Nitrosomonas* could completely oxidize ammonia as shown in the control reactor (Batch 3 samples), with the ratio of *Nitrosomonas* and *Nitrospira* of 0.38:1. This is confirmed by the fact that at 50°C-1hr heating, complete ammonia oxidation was occurred, resulting in an overall effluent concentration of 0.5 mg/L. However, it is essential for *Nitrospira* to be present in at least 60% abundance compared to *Nitrosomonas*.

Figure 4-16 (b) and 4-16 (c) showed that both *Nitrosomonas* and *Nitrospira* had positive correlation with ammonia uptake and nitrate production rates. The specific ammonia uptake at 23°C, 45°C-1hr, 50°C-1hr and 55°C-0.5hr were 4.2 mgN/L.hr.VSS, 4.4 mgN/L.hr.VSS, 4.5 mgN/L.hr.VSS and 1.7 mgN/L.hr.VSS while the specific nitrate production rates were 4.3 mgN/L.hr.VSS, 1.6 mgN/L.hr.VSS, 1.4 mgN/L.hr.VSS and 0.5 mgN/L.hr.VSS respectively. The VSS concentration of at the specific day were 1100 mg/L, 900 mg/L, 750 mg/L and 800 mg/L respectively. The ammonia uptake rates, and nitrate production rated were calculated by multiplying the specific uptake/production rates by VSS concentrations in the reactors. The Pearson correlation $r=0.57$ ($R^2=0.33$) and $r=0.99$ ($R^2=0.95$) respectively showing moderate to strong correlation with *Nitrosomonas* and *Nitrospira* respectively. However, the ammonia and nitrate production showed strong negative correction with $r=-0.8$ and $r=-0.99$ for both *Nitrosomonas* and *Nitrospira* meaning that the substrate utilization rates decreased with increase of temperature. The negative effect of increasing temperature on the growth of nitrifiers and substrate utilization led to the observed outcomes. The ammonia uptake rates exhibited a moderate correlation ($r=0.3$) with *Nitrosomonas*, while the nitrate uptake rate showed a strong correlation with *Nitrospira*. This disparity is attributed to the drastic inhibition of *Nitrosomonas* in the temperature range of 5°C (encompassing 55°C for 0.5 hours and 60°C for 0.5 hours), whereas *Nitrospira* demonstrated gradual inhibition in the temperature range of 10°C.
(encompassing 45°C for 2 hours and 55°C for 0.5 hours). Consequently, the relationship between temperature and TKN (Total Kjeldahl Nitrogen) removal exhibited a negative correlation, with an r-value of -0.89 (R²=0.94) as shown in Figure 4-16 (d).

4.10 Conclusions

Partial nitrification can be achieved by heating the mixed liquor suspended solids without any preprocessing. However, stable inhibition of nitrite-oxidizing bacteria (NOBs) requires at least one turnover of sludge retention time (SRT) for the inhibition to become established. Therefore, the selection of heat and heating duration using short-term batch tests is invalid. It is important to note that any heating temperature and time exceeding 45°C for 1 hour will increase effluent COD, necessitating COD removal to meet the COD guidelines. The maximum nitrite accumulation ratio of 80% can be achieved by heating the mixed liquor at 55°C for 0.5 hours. Temperatures higher than 55°C for 0.5 would require AOB reactivation in a separate reactivation system before implementing partial nitrification. In the case of real wastewater, heating it once every 10 days at 55°C for 0.5 hours can result in a 7.9% reduction in total energy consumption. Both *Nitrosomonas* and *Nitrospira* are negatively affected by heat application, with *Nitrospira* being more sensitive to the heat treatment.
References


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Chapter 5

5 Modeling the effect of partial intermittent heat on suspended nitrifiers on achieving mainstream partial nitrification using new temperature dependance coefficients.

5.1 Introduction

Mainstream partial nitrification can provide economical treatment of nitrogen from municipal wastewater. Due to requirements of oxygen, alkalinity for nitrifiers and external carbon source for denitrifies, conventional nitrification denitrification is cost and resource extensive (Kirim et al., 2022). The optimized partial nitrification with successive denitrification process can reduce oxygen and carbon sources by 60% and 100% (Miao et al., 2016), and 25% and 40% (Daigger, 2014) respectively compared to conventional nitrification denitrification. To achieve partial nitrification, conventional approaches target the growth and kinetic differences of AOBs, and NOBs by controlling operating conditions such as low or high DO, pH and OPR, aerobic SRT and aeration (Liu et al., 2020) in the Arrhenius temperature range where substrate utilization exhibits positive exponential behavior (Metcalf & Eddy, 2004). However, studies showed that, intermittent high heat can also differentiate the substrate utilization dynamics between AOBs, and NOBs and partial nitrification can be achieved, leading to the achievement of partial nitrification (Chen et al., 2016; Chen et al., 2019; Choi et al., 2021; Isaka et al., 2008), with the kinetics of nitrifiers exhibiting negative growth behavior from temperature 50°C-55°C (Lopez-Vazquez et al., 2014a). Although, mainstream PN using aforementioned operating conditions is possible, however shifted to complete nitrification in long term operation.

Mathematical models are employed to simulate the mainstream conditions by calibrating the operational parameters and kinetics to gain better understanding of the complex dynamics of mainstream PN, however exhibiting challenges (Agrawal et al., 2018). Moreover, models for the PN achieved through high intermittent heat to mixed liquor were not attempted. All conventional mathematical models rely on the Arrhenius single-coefficient temperature correction factor to represent growth kinetics (Pérez et al., 2014).
To implement nitrite shunt (partial nitrification) a two step nitrification model is necessary which was not implemented in activated sludge models (ASM)(Henze et al., 2015). However, lab scale SBR results achieved PN by controlling the oxygen mass transfer resistance model developed in MATLAB -6.5 (Blackburne et al., 2008b), applying intermittent aeration by defining an objective function that provide required aeration for PN (Bournazou et al., 2013), using sensitivity analysis of DO and COD/N ratio and identifying the appropriate DO and COD/N ratio in SUMO™ in MBBR and IFAS process (Tao & Hamouda, 2019), mathematical model using high ammonia concentration in synthetic wastewater at 30°C-(Pambrun et al., 2006) were exercised.

The model parameters used in various modeling approaches and the factors that are provided in the models are given in following Table 5-1.
<table>
<thead>
<tr>
<th>Reactor configuration</th>
<th>Ks,AOB (mg/L)</th>
<th>Ks,NOB (mg/L)</th>
<th>Ko, AOB (mg/L)</th>
<th>bAOB; bNOB (mg/L)</th>
<th>SRT (day)</th>
<th>V or SA (L or m2/m3)*</th>
<th>YAOB (gVSS gN⁻¹)</th>
<th>YNOB (gVSS gN⁻¹)</th>
<th>µAOB (day⁻¹)</th>
<th>µNOB (day⁻¹)</th>
<th>Temp (℃)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory SBR</td>
<td>0.75</td>
<td>0.15</td>
<td>0.03</td>
<td>0.4</td>
<td>0.05</td>
<td>8</td>
<td>10</td>
<td>0.17</td>
<td>0.1</td>
<td>0.65</td>
<td>0.65</td>
<td>18-25℃</td>
</tr>
<tr>
<td>Lab SBR</td>
<td>2.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.05</td>
<td>1</td>
<td>12</td>
<td>0.18</td>
<td>0.08</td>
<td>0.30</td>
<td>0.34</td>
<td>15.5 ± 1.0℃</td>
</tr>
<tr>
<td>MBBR and IFAS</td>
<td>0.7</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>0.1</td>
<td>0.85</td>
<td>0.65</td>
<td>22-30℃</td>
<td>(Tao &amp; Hamouda, 2019)</td>
</tr>
<tr>
<td>MBBR and IFAS</td>
<td>1.1</td>
<td>0.5</td>
<td>0.12</td>
<td>0.06</td>
<td>0.014;0.020</td>
<td>5-20</td>
<td>300</td>
<td>0.18</td>
<td>0.08</td>
<td>0.28</td>
<td>0.40</td>
<td>10℃</td>
</tr>
<tr>
<td>Lab-scale SBR</td>
<td>2.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>0.08</td>
<td>0.30</td>
<td>0.34</td>
<td>-</td>
</tr>
<tr>
<td>Activated sludge-pilot scale</td>
<td>0.7</td>
<td>0.05</td>
<td>0.4</td>
<td>0.14</td>
<td>0.17;0.17</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
<td>0.09</td>
<td>0.9</td>
<td>0.7</td>
<td>-</td>
</tr>
<tr>
<td>Biofilm model</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.14</td>
<td>0.14</td>
<td>0.18</td>
<td>0.06</td>
<td>0.74</td>
<td>0.65</td>
<td>18℃</td>
<td>(Liu et al., 2020)</td>
</tr>
</tbody>
</table>

Note: None of these modeling approaches were based on temperature correction due to high heat. SSA= ratio of area in reactor volume
The temperature dependency of a microbial growth system is usually described by van’t Hoff–Arrhenius equation and the growth kinetic has a significant effect on the reaction rates. The van’t Hoff–Arrhenius equation for describing the reaction rate is shown in Equation (5-1) (Metcalf & Eddy, 2004; Myszograj, 2018):

$$k(T) = A \exp \left( \frac{-E_a}{R \cdot T} \right)$$  \hspace{1cm} (5-1)

Where $k(T)$ is the rate constant as a function of temperature, $A$ is the pre-exponential factor, $E_a$ is the activation energy in J/mol, $R$ is the gas constant $= 8.314 \text{ J/(mol} \cdot \text{K})$ and $T$ is the absolute temperature in Kelvin.

In wastewater treatment systems, the Arrhenius equation has been modified in following form for the convenience of calculation of the reaction rates as the temperature is in degree Celsius and the temperature factor can be measured (Water, 2012) by Equation (5-2).

$$k_T = k_{20} \cdot \theta^{(T-20)}$$  \hspace{1cm} (5-2)

$T$ is the operating temperature in °C, $k_T$ is the reaction rate at temperature $T$, $k_{20}$ is the reaction rate at ambient temperature (20°C), $\theta$ is the temperature coefficient.

The BNR process can successfully achieve complete nitrification in the temperature range of 10- 42°C (Lopez-Vazquez et al., 2014b). This range of temperature is optimal for complete nitrification and the growth of nitrifiers does not hinder this range. Therefore, conventional Arrhenius temperature dependency can be utilized to estimate the growth rates of this range of temperature (Milks, 1975; Németh et al., 2023; Taylor et al., 2017; Zheng et al., 2017). However, recent studies showed that the kinetics of NOB is lower than AOBs and temperature greater than the optimal range resulted in the suppression of NOBs (G. Chen et al., 2016; J. Chen et al., 2019; Choi et al., 2021; Isaka et al., 2008). These studies showed that NOB suppression i.e. nitrite accumulation can be achieved by heating the nitrifiers at the temperature range of 60-80°C using synthetic or real wastewater. However, none of these studies had explored the kinetics (substrate utilization rates, yield or growth rates, oxygen uptake rates) of AOBs and NOBs that produced the partial nitrification. Furthermore, the decline in rates at these temperature ranges does not follow the conventional Arrhenius equation to estimate the specific substrate utilization rates of AOBs and NOBs.
Replacing the $k_T$ with growth rate $r$ and $E_a$ by a quantity $\mu$, the conventional Arrhenius equation forms the following Equation (5-3).

$$r = A \exp \left( \frac{-\mu}{RT} \right)$$ (5-3)

Although $\mu$ should be a constant, however plotting $\ln r$ against reciprocal of $T$ showed that the data does fit in downward curve like with the increase of reciprocal $T$, indicating that $\mu$ is not a constant quantity rather decreasing with the reciprocal of $T$ (Ratkowsky et al., 1982). Although conventionally the plot showed the downward straight line, using the temperature data in this study showed the nonlinear curve which takes the following form:

$$\sqrt{r} = b (T - T_o)$$ (5-4)

Where $b$ is the regression coefficient, $T_o$ is the conceptual temperature with no metabolism, and $T$ is the operating temperature. However, at higher temperature beyond the optimum, the growth rate would decrease due to protein denaturation (Geiduschek, 1962). Thus, the temperature dependency was suggested in the following Equation (5-5) (Ratkowsky,’ et al., 1983).

$$\sqrt{r} = b(T - T_{min}) \{1 - \exp[c(T - T_{max})]\}$$ (5-5)

Where $T_{min}$ and $T_{max}$ are the temperature where microbial growth is zero, $c$ is the constant for the curved line from the optimum temperature to the $T_{max}$. The limitation of this equation is that the $T_{min}$ is going below the freezing point where no microbial activities will be occurring. However, this hypothesis does not follow the range of temperate of optimum nitrification temperature range of 10-42°C.

The nonlinearity was applied from temperature range of 10-55°C in some studies (Lopez-Vazquez et al., 2007, 2014b, 2014a) which does not require the hypothesis of $T_{min}$ that can go below freezing point and inactivate nitrification. In our study we used the range of 23°C-60°C to investigate the impact of heat starting from ambient temperature (23°C) to a maximum temperature (60°C) when nitrification was completely hindered. The nitrifiers can survive the temperature of 50°C and then decline and the suppression behavior follows the temperature dependence in Equation (5-6) below(Lopez-Vazquez et al., 2014a).

$$r_T = r_{T_o} \theta_1^{(T-T_o)} \left[1 - \theta_2^{(T-T_{max})}\right]$$ (5-6)

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where \( r_T \) is the specific substrate utilization at temperature \( T \), \( r_{T_0} \) is the specific substrate utilization rate at ambient temperature \( T_0 \), \( T_{\text{max}} \) is the maximum temperature when activities of nitrifiers are completely hindered. In this study we have used Equation (5-6) to implement the temperature dependency of nitrifiers and instigate the impact on the achievement of nitrite accumulation.

5.2 Materials and methods

5.2.1 Lab scale SBR operation

A total of 3 SBRs were run simultaneously, one as a control at room temperature (23°C) and two other reactors at each of the investigated temperatures from 35°C-60°C. Reactor 1 represents the control reactor, Heated reactor 1 represent the reactor used for 35°C-1,2 and 3 hr, 40°C-1,2 and 3hr, 45°C-1 and 2hr, Heating reactor 2 represents the reactor used for 50°C-1hr, 55°C-0.5hr and 60°C-0.5 hour in Figure 5.1. The heated reactors were operated with a duration of 1,2 and 3 hours in order to achieve partial nitrification.
Definitions:

<table>
<thead>
<tr>
<th>Control reactor</th>
<th>Heated reactor 1</th>
<th>Heated reactor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Temperature</td>
<td>Temperature</td>
</tr>
<tr>
<td>=23°C</td>
<td>35°C-45°C</td>
<td>50°C-55°C</td>
</tr>
</tbody>
</table>

The SBR cycle constituted the following phases: 0.5 hr anoxic fill, 6 hr aeration and mixing (diffused aeration, DO >2mg/L), 1 hr settle, and 0.5 hr decanting with an SRT of 7 days. The automated heating was applied by the water bath for the specified duration. Heated reactors were covered with aluminum paper insulation to reduce the heat loss from the reactors and digital thermometers were inserted into the mixed liquor to register the temperature. Samples from the influent and effluent from the reactors were collected and analyzed weekly. Two SBRs were run parallelly to investigate the effect of heating on nitrifiers for a specified time. For the modeling approach, reactor at 45°C-2hr, 50°C-1hr, 55°C-0.5hr and 60°C-0.5hr were operated for 37 days, 66 days, 55 days and 21 days respectively. The operation details of the reactors were provided in Table 5-2.

Figure 5-1. Reactor systems for heat application
Table 5- 2. Operational parameters of SBRs

<table>
<thead>
<tr>
<th>Operational parameters</th>
<th>Control reactor</th>
<th>Heated reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles (cycles/day)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Number of cycles per day heat was applied</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Flow rate (L/day)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Reactor volume (L)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SRT (day)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>HRT (hour)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Components of SBR cycle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fill period (hour)</td>
<td>0.5 (anoxic)</td>
<td>0.5 (anoxic)</td>
</tr>
<tr>
<td>Reaction Period (hour)</td>
<td>6 (Aerobic)</td>
<td>6 (Aerobic)</td>
</tr>
<tr>
<td>Settling (hour)</td>
<td>1 (anoxic)</td>
<td>1 (anoxic)</td>
</tr>
<tr>
<td>Decanting (hour)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

5.2.2 Wastewater Characterization

Chemical oxygen demand (COD), Nitrogen (total N and soluble N), phosphorus (total P and soluble P) was measured using Hack Methods. Total suspended solids (TSS) and volatile suspended solids were measured using standard method (American Public Health Association, 2005). Before measuring soluble fraction of COD, nitrogen and phosphorus, samples were filtered through sterile 0.45-μm membrane filter papers (VWR International, Canada).

5.2.3 Cyclic test and specific substrate utilization/production rates

In these tests, the ammonia nitrate and nitrite concentrations were measured during the reaction period at 0hr, 0.25hr, 0.5hr, 1hr, 2hr and 4hr until the ammonia was completely utilized by nitrifiers. Although the reaction period was 6 hours, the ammonia was utilized in 4 hours. Therefore, cyclic test for estimating the specific substrate utilization or
production rates were performed for 4 hours. The specific substrate utilization or production rates were found dividing the rates by the average MLVSS concentrations.

In Figure 5-2 (a, b, c and d), it is shown that the ammonia uptake rate, nitrate production rate, nitrite production rate and NOx production rates are 3.26 mgN/L.hr, 0.97 mgN/L.hr, 1.73 mgN/L.hr and 2.69 mgN/L.hr at steady state. The concentration of MLVSS in this reactor was 680 mg/L which produced specific ammonia production rate of 4.9 mgN/gVSS.hr, nitrate production rate of 1.4 mgN/gVSS.hr, nitrite production rate of 2.5 mgN/gVSS.hr and NOx production rate of 4 mgN/gVSS.hr. The specific uptake and production rates were estimated as described here for all other temperatures providing partial nitrification at 45°C-2hr, 50°C-1hr, 55°C-0.5hr. Although 60°C-0.5hr were

Figure 5-2. Ammonia uptake rate (a), nitrate production rate (b), nitrite production rate (c) and NOx-N production rate at 50°C-1hr heating

In Figure 5-2 (a, b, c and d), it is shown that the ammonia uptake rate, nitrate production rate, nitrite production rate and NOx production rates are 3.26 mgN/L.hr, 0.97 mgN/L.hr, 1.73 mgN/L.hr and 2.69 mgN/L.hr at steady state. The concentration of MLVSS in this reactor was 680 mg/L which produced specific ammonia production rate of 4.9 mgN/gVSS.hr, nitrate production rate of 1.4 mgN/gVSS.hr, nitrite production rate of 2.5 mgN/gVSS.hr and NOx production rate of 4 mgN/gVSS.hr. The specific uptake and production rates were estimated as described here for all other temperatures providing partial nitrification at 45°C-2hr, 50°C-1hr, 55°C-0.5hr. Although 60°C-0.5hr were
investigated for achieving PN, the heating scheme inhibited all nitrifiers and no nitrification happened and therefore is not reported in this modeling study. All specific rates are provided in the appendix C, Figure S1.

5.2.4 Estimation of temperature factors with extended Arrhenius model for model calibration

In biological treatment two models are being used to represent the growth rates of the nitrifiers, one considering freezing temperature to maximum temperature when growth rates are zero (Ratkowsky et al., 1982) and the another one was used when temperature range of 10-55℃ (Lopez-Vazquez et al., 2007, 2008, 2014b) with the later used for thermophilic nitrogen removal from oil refinery wastewater (Lopez-Vazquez et al., 2014b). The aforementioned study showed that thermophilic nitrogen removal can happen until 50℃ and nitrification inhibited at 55℃ which is closely describing the thermal nitrification occurring in this study. Therefore, following extended double Arrhenius equation (Eq. 5-6) had been used for estimating the growth rate.

5.3 SBR layout in SUMO simulator, Model set up

5.3.1 Modification of conventional SBR model

SBR was typically used for the simulation of the two-step nitrification that produces complete nitrification. In order to implement the extended Arrhenius function and the temperature factors, the conventional SBR model was modified to integrate the intermittent heating facilities to the MLSS. The modified SBR unit can simulate the filling, reaction, settling and decanting phases. The operational DO concentration (2 mg/L) was set with the application of diffused aeration model along with heat application.

5.3.2 Modified Sumo2_dArrhenius model for heat shock and temperature control

The Sumo2 model file was modified with growth rates and new temperature factors for the temperature range of 35℃-60℃. To implement the intermittent heating, dynamic heating profile was provided to the SBRs during the aeration instead of built in time based on/off
to ensure the nonlinearity of heating profile be applied to the SBR runs. The process unit (PU) of the heat based PN SBR is shown in Figure 5-3.

![Figure 5- 3. Layout of heated reactor with intermittent heating controller](image)

### 5.3 3 Synchronization of modified Sumo2_dArrhenius model with modified SBR

The single Arrhenius exponential equation for converting the growth rates of the nitrifiers was replaced by double Arrhenius model in Sumo model function for both AOBs and NOBs and the modified SBR was connected to the model. By applying this extended function both the negative and positive effects of intermittent heat on the growth of the nitrifiers were implemented.

### 5.4 Input of influent constituents in dynamic SBR run

#### 5.4.1 Influent fractions

The SUMO converts all the input constituents in fractions and simulate the fractions to calculate all the influent, effluent, PU variables as the display on the GUI of the model output. Although SUMO has the default fractions for all the influent elements in municipal wastewater, the fractions were modified for the characteristics of the primary effluent from Greenway Wastewater Treatment Plant (GWWTP). The model default fractions of
constituents in the primary effluent in SUMO were slightly different than constituents in
the primary effluent from GWWTP. The built-in default fractions of influent and fractions
of PE from greenway wastewater treatment plant are shown in Table 5-3.

Table 5-3 Comparison of fractions of primary elements with default and Greenway
WWTP

<table>
<thead>
<tr>
<th>PE substances</th>
<th>Model default</th>
<th>Greenway WWTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSS/TSS</td>
<td>0.85</td>
<td>0.79</td>
</tr>
<tr>
<td>Particulate COD/VSS</td>
<td>1.60</td>
<td>1.42</td>
</tr>
<tr>
<td>Filtered COD fraction</td>
<td>0.46</td>
<td>0.5</td>
</tr>
<tr>
<td>VFA fraction of filtered COD</td>
<td>10-20</td>
<td>15</td>
</tr>
<tr>
<td>NH4 fraction of TKN</td>
<td>0.73</td>
<td>70</td>
</tr>
<tr>
<td>PO4 fraction of TP</td>
<td>0.67</td>
<td>0.60</td>
</tr>
</tbody>
</table>

5.4.2 The balance of COD, TKN and TP in SUMO

The balance of the constituent in SUMO was conceptualized based on the biggest fraction
of TCOD, TN or TP. The biggest fraction is typically the particulate biodegradable fraction
in municipal wastewater. The balances for COD, TKN, TP were performed as particulate
biodegradable fraction should be greater than zero. Equation 5-7 showed an example of
COD balancing of Greenway PE.

COD Balance: Particulate slowly biodegradable substrate = COD\textsubscript{infl} – COD in
bacteria-COD in stored PHA and GLY-COD in heterotrophs (OHO)- Endogenous decay
products >0                                                                  (5-7)

5.5 SBR hydraulics in SUMO

The plant HRT in the SBRs calculated was done based on the reactor effective volume V
and the influent flow rate, Q and is expressed as the following Equation (5-8)

\[ \text{HRT} = \frac{V}{Q} \] (5-8)
The apparent flow rate of influent and wastage from SBRs were calculated as the following Equations (5-8) and (5-9).

\[
\text{Waste (L/d)} = \frac{\text{Waste volume per day (L)}}{\text{Number of cycles} \times \text{wasting time (h)}} \times \frac{24h}{d} \tag{5-8}
\]

\[
\text{Flow (L/d)} = \frac{\text{Volume of PE (L)}}{\text{Filling time (h)}} \times \frac{24h}{d} \tag{5-9}
\]

5.6 Model kinetic and output dynamics

The growth and decay kinetics of AOBs and NOBs are shown in a matrix (Table 5-4) which is known as Gujer matrix (Grady et al., 2011). where the apparent growth of AOBs and NOBs: \( \mu_{AOB} \) and \( \mu_{NOB} \), are modified by the extended Arrhenius temperature functions.
Table 5-4. A simplistic form of Stoichiometry and kinetics for Sumo2

<table>
<thead>
<tr>
<th>Component, i</th>
<th>$X_B$</th>
<th>$X_{AOB} / X_{NOB}$</th>
<th>$S_{NHx}$</th>
<th>$S_{NO2}$</th>
<th>$S_{O2}$</th>
<th>$X_E$</th>
<th>Process Rate, $\rho_i$ [ML$^{-3}$T$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>j</td>
<td>Process</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Aerobic growth of AOBs</td>
<td>1</td>
<td>$-1/Y_{AOB}$</td>
<td>$1/Y_{AOB}$</td>
<td>$-(EEQ_{NO2}^{-}\text{i}_N,\text{BIO})$</td>
<td>$Y_{AOB}/Y_{AOB}$</td>
<td>$\mu_A\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_O}{K_O+S_O}\right)$</td>
</tr>
<tr>
<td>2</td>
<td>Aerobic growth of NOB</td>
<td>1</td>
<td>$-i_{N,\text{BIO}}$</td>
<td>$-1/Y_{NOB}$</td>
<td>$-(EEQ_{NO2, NO3}^{-}\text{i}_N,\text{NOB})$</td>
<td>$Y_{NOB}/Y_{NOB}$</td>
<td>$\mu_A\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\left(\frac{S_O}{K_O+S_O}\right)$</td>
</tr>
<tr>
<td>3</td>
<td>Decay of AOB</td>
<td>$1-f_E$</td>
<td>$-1$</td>
<td>$-f_E$</td>
<td>$f_E$</td>
<td>$f_E$</td>
<td>$b_{AOB}X_{AOB}$</td>
</tr>
<tr>
<td>4</td>
<td>Decay of NOB</td>
<td>$1-f_E$</td>
<td>$-1$</td>
<td>$-f_E$</td>
<td>$f_E$</td>
<td>$f_E$</td>
<td>$b_{NOB}X_{NOB}$</td>
</tr>
</tbody>
</table>

$i_{N,\text{BIO}}$ = Nitrogen content in microorganism, $i_{N,\text{XE}}$ = nitrogen content in endogenous products, $f_E$ = fraction of endogenous product, $b_{AOB}$ and $b_{NOB}$ are the decay rates of AOBs and NOBs.
5. 7 Results and discussion

5.7.1 Temperature calibration for Intermittent heating

Due to dynamics of cycles, intermittent heating cannot be provided to the SBRs ahead of aeration cycle. Therefore, water bath should be automatically turned on at the start of the aeration period. However, increase of temperature is not instantaneous. In order to automate the heating, heating calibration (rate of heat increase and decrease) were estimated to apply heat for specified time of 0.5, 1, 2 or 3 hours for a specified temperature of water bath. For 45℃-2-hour heating, for 50℃ for 1 hour heating and 55℃ for 0.5-hour heating, the water bath was set to 70, 80 and 90℃ respectively. However, the rate of temperature increases, and decrease was different for different bath temperatures. Before the heating assay, since the nitrifiers are sensitive to heat, the SBR with 2L of water was used for temperature calibration and rate calculation by using the aforementioned water bath temperature for SBR temperature of 45℃, 50℃ and 55℃ which is shown in Figure 5-4. The rate of increase of temperature is 0.17℃/min, however, the rate of decrease of temperature 0.07 ℃/min for heating at 45℃-2hr. Based on this rate, the automated temperature for 2 hours every day was set with water bath to apply steady heat every day to the reactor. The heat calibration in other reactors were also automated calibrating rate of temperature increase and decrease. The room temperature affects the temperature increase or decrease but not more than ±1℃ inside the reactors.
From Figure 5-4 it is clear that, SRT and DO was constants at 7 days and 2 mg/L respectively in the entire operation while temperature profile varied nonlinearly, after the specified heating times.

5.7.2 Estimation of temperature factors θ1 and θ2 for AOBs and NOBs

In the modeling purpose, the overall maximum specific ammonia uptake rates for AOBs and maximum specific nitrate production rates for NOBs at steady state were considered for estimating temperature dependency factors of the nitrifiers. The estimation considered temperature range of 35°C-55°C for AOBs and NOBs. The activities of AOBs started to
decline at 45°C whereas the activities of NOBs started to decline at 40°C as shown in Figure 5-5 (a) and Figure 5-5 (b) respectively. The overall maximum specific ammonia uptake rates were 4.7-4.9 mgN/gVSS.hr at 35°C- 45°C for AOBs whereas maximum specific nitrate production rates of 3.6 -3.9 mgN/gVSS.hr for NOBs until 40°C. The overall maximum specific ammonia uptake rates for AOBs and maximum specific nitrate production rates for NOBs were 4.6 to 3 mgN/gVSS.hr and 4.2 to 2.7 mgN/gVSS.hr from 45 to 55°C and 40-55°C respectively. This indicates the higher substrate utilization rates of AOBs than the NOBs in the heated SBRs which is consistent with other studies (Hellinga et al., 1999). Lopez-Vazquez at al. (2014) found that in oil refinery wastewater, the nitrifiers can survive until the optimum temperature of 50°C. However, the aforementioned study did not explore the possibility of nitrite accumulatio by heat shock. The optimum temperature was reported at 50°C and the nitrifiers were inhibited at 55°C. Conversely, we found the optimum temperature of 45°C for AOBs and 40°C for NOBs and both the nitrifiers can survive until 55°C-0.5hr with reduced growth rates following a double Arrhenius temperature dependence model.

The data for the quantification of temperature factors in this study were taken from the overall steady state uptake/ production rate data. The data fitting (Solver, Microsoft Excel 365) is shown in the following Figures 5-5 (a) and 5-5 (b) with R²=0.99 and R²=0.98 for AOB and NOB respectively.
5.7.3 Calibration and performance of heated SBRs

The overall COD, TKN and TP in the primary effluent in the greenway treatment plant varied 175-200 mg/L, 25-35 and 3-5 mg/L and the overall the overall , COD, TKN and TP removal in the SBRs varied in the range of 48% to 90% in the control reactor , and 48% to
66% in the heated reactors at 45°C-2hr, 50°C-1hr and 55°C-0.5hr. TKN removal varied from 68%-94%, TP removal was 59% to 43% in heated reactors. To compare the performance of reactors with predicted values, the reactors were modelled using all default kinetics in two step nitrification model (Sumo2) in SUMO™ graphic user interface (GUI). The control reactor was operated with the conventional Arrhenius temperature coefficients of 1.072 and 1.060 respectively for AOBS and NOBs while the heated reactors were modelled based on temperature factor shown in Table 5-5. The default maximum specific growth rates used in Sumo2 for AOBs, and NOBs are 1 d⁻¹, 0.65 d⁻¹ respectively. The default half saturation constants of O2 for AOBs and NOBs are 0.25 g O2/m3 for both the nitrifiers. Half saturation of AOB and NOBs for NHx and NO2 are 0.7 g N/m3 and 0.1 g N/m3 respectively in the default setup. The modified parameters in the Sumo2_dArrhenius model is shown in Table 5-5.

**Table 5-5. Key kinetic parameters used in Sumo2_dArrhenius model.**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>μAOB</td>
<td>Maximum specific growth rate of AOBs</td>
<td>0.9</td>
<td>1/d</td>
</tr>
<tr>
<td>μNOB</td>
<td>Maximum specific growth rate of NOBs</td>
<td>0.74</td>
<td>1/d</td>
</tr>
<tr>
<td>θ1μ, AOB</td>
<td>Arrhenius coefficient for AOB growth</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>θ2μ, AOB</td>
<td>Arrhenius coefficient for AOB growth from temperature 45°C-60°C</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>θ1μ, NOB</td>
<td>Arrhenius coefficient for NOB growth</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>θ2μ, NOB</td>
<td>Arrhenius coefficient for NOB growth from temperature 40°C to 60°C</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

The control SBR1 was modelled with the data of 180 days in order to confirm the complete nitrification in the entire operation period. The range of predicted effluent concentrations of NOx and NO2-N were 25-30 mg/L and 0-0.1 mg/L respectively confirming the complete nitrification. The graphs of complete nitrification in control reactor are shown in Appendix C, Figure S2. Measurement of suspended solid in mixed liquor is significant in process control (food/microorganism). The SBRs at 45°C-2h, 50°C-1hr and 55°C-0.5 hr were operated at least 3-turnover of SRT to reach steady state. The overall influent COD
during the operation at 45°C-2h, 50°C-1hr and 55°C-0.5 hr were 175-200 mg/L. The overall measured and predicted MLSS and MLVSS for the heated reactors are shown in Appendix C, Figure S3. However, the fluctuation of MLSS and MLVSS is mainly due organic loading rates in the SBRs.

Although the primary focus on this modeling is to model nitrite accumulation due to heat application, all other parameters other than nitrite were also predicted to identify the difference between prediction with experimental data that may happened due to heat. All the predicted and experimental data along with standard deviation are shown in Table 5.6.

**Table 5-6. Comparison of effluent model values with experimental values (Average±Std Deviation (No. of samples))**

<table>
<thead>
<tr>
<th>Elements</th>
<th>Prediction/Experiment</th>
<th>Unit</th>
<th>Temperature and duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>22°C</td>
</tr>
<tr>
<td>MLSS</td>
<td>Model</td>
<td>mg/L</td>
<td>1500±312 (57)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>1476±301 (64)</td>
</tr>
<tr>
<td>MLVSS</td>
<td>Model</td>
<td>mg/L</td>
<td>1200±200 (63)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>1040±205 (63)</td>
</tr>
<tr>
<td>COD</td>
<td>Model</td>
<td>mg/L</td>
<td>44±2.14 (60)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>43±3.26 (60)</td>
</tr>
<tr>
<td>TKN</td>
<td>Model</td>
<td>mg/L</td>
<td>3.12±1.1 (40)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>3.33±0.99 (40)</td>
</tr>
<tr>
<td>NH3-N</td>
<td>Model</td>
<td>mg/L</td>
<td>1.2±0.22 (40)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>0.7±0.66 (40)</td>
</tr>
<tr>
<td>NO2-N</td>
<td>Model</td>
<td>mg/L</td>
<td>0.07±0.06 (150)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>0.08±0.09 (150)</td>
</tr>
<tr>
<td>NOX-N</td>
<td>Model</td>
<td>mg/L</td>
<td>27±3.6 (45)</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>mg/L</td>
<td>25±4.4 (45)</td>
</tr>
<tr>
<td>TP</td>
<td>Model value</td>
<td>mg/L</td>
<td>1.63±0.22 (40)</td>
</tr>
<tr>
<td></td>
<td>Experimental value</td>
<td>mg/L</td>
<td>1.83±0.21 (40)</td>
</tr>
</tbody>
</table>

Note: Two step Sumo2 model at temperature 22°C was used for the prediction of solids and effluent quality with temperature coefficient of 1.072 and 1.065 for AOBs and NOB respectively. The elements in heated reactor from 45-55°C were simulated with the modified double Arrhenius model.
5.7.4 Predicted and experimental COD and TP

The effluent total COD prediction is shown in appendix C, Figure S4. and summarized in Table 5-6. The total COD in Sumo2 is calculated by the summation of soluble and particulate COD. Although particulate COD fractions are comprised of the summation of the soluble COD in all heterotrophs and autotrophs in aerobic systems, slowly biodegradable substrate, particulate unbiodegradable organics, stored polyhydroxyalkanoates, stored glycogen, and endogenous decay product, the pCOD prediction is mainly dominated by heterotrophs and nitrifiers in Sumo model.

The total phosphorus was calculated by total phosphorus content in the biomass, organic and inorganic phosphorus content and soluble phosphate in the wastewater. However, enhanced biological phosphorus removal was not integrated in Sumo2 model as this model is defined by COD and nitrogen removal. The effluent phosphorus concentrations in Sumo model are basically due to the synthesis by heterotrophs and autotrophs. The prediction of TP is shown in Appendix C, Figure S5.

From Table 5-6, the simulated results of COD were 44 mg/L, 49 mg/L, 56 mg/L and 49 mg/L in control, at 45°C-2hr, 50°C-1hr and 55°C-0.5hr respectively indicating the similar CODs in the heated reactors and insignificant change compared to control. The experimental data showed that the effluent COD were 43 mg/L, 64 mg/L, 86 mg/L and 100 mg/L respectively indicating the microbial inability to degrade COD or the lysis of the microorganisms releasing unbiodegradable COD (Islam et al., 2017). The underpredicted COD results is due to the fact that the temperature dependency of heterotrophs was not explored and was not applied in the Sumo2_dArrhenius model. Similarly, the increase of the effluent phosphorus concentration by 1 mg/L at temperature 55°C-0.5hr occurred due to the microbial lysis.
5.7.5 Prediction of Nitrite accumulation PN SBRs

The reactors were maintained with nitrifying environment as shown in Figure 5-4, with the specified heat. The nitrite accumulation predicted by the model and the experimental results are shown in the Figure 5-6 (a), (b) and (c). The initial nitrite accumulation for about one SRT turnover were over predicted by the model as the temperature factors were considered only for the steady state. Therefore, after about 1 SRT turnover, the prediction becomes steady. The overall nitrite accumulation in Table 5-6 showed that the predicted nitrite were 0.07±0.06, 10.7±2.9, 10.2±1.1, 10.1±0.68 compared to experimental nitrite of 0.08±0.09, 10.3±0.21, 10.2±0.11 and 13.5±2.64 respectively at 22°C, 45°C-2hr, 50°C-1hr and 55°C-0.5hr respectively.

Figure 5-6. Predicted effluent nitrite and experimental effluent nitrite
To date, only one study used extended Arrhenius function to model temperature dependence for nitrification and denitrification at temperature range of 35°C-55°C in short term batch reactors using the oil refinery wastewater. This study estimated temperature factor $\theta$ of 1.03 for optimum nitrification and 1.02 for optimum denitrification (Lopez-Vazquez et al., 2014). However, the temperature dependence of NOBs was not explored.

The predicted and experimental results of other nitrogen components were also compared in Table 5-6. From Table 5-6, it is obvious that ammonia prediction was slightly higher than the experimental however not significant until 50°C-1hr. However, at 55°C-0.5hr, the predicted ammonia was 4 mg/L which is 2 mg/L less than the experimental. The TKN concentration in the reactors increased with the increase of temperature, however, was maximum at 55°C-0.5hr. These results showed that the ammonia and TKN concentrations increased with the increase of temperature. At 50°C-1hr and 55°C-0.5hr, the same TKN level of 5 mg/L was predicted. However, the ammonia was completely oxidized at 50°C-1hr. Contrarily, at 55°C-0.5hr, the predicted ammonia was comparable to TKN indicating the occurrence of lysis of nitrifiers as well as heterotrophs. The higher concentrations of experimental TKN than predicted in heated reactors, coupled with overprediction of ammonia at 45°C and 50°C SBRs i.e., may potentially indicate the inhibition of ammonification in the heated SBRs compared to the control. Due to this reduction of ammonification, NOx was overpredicted in all heated reactors.

5.8 Error analysis between predicted and experimental data

SUMO models produce continuous data points whereas the experimental data of this study is discrete data points. The experiments were operated for 36 days, 66 days and 55 days for the nitrite accumulating at 45°C-2hr, 50°C-1hr and 55°C-0.5 hr respectively. The error calculation was done from the start of the steady state, which is 21 days (3 SRT turnover) after the operation start, providing small set of data after steady state. Therefore, estimating error matrix considering data dependant error such as root mean squared error (RMSE) or relative percent error (RPE) which significantly depend on number of datapoints, are not applicable for the case of this study (Kim et al., 2017). Although standard error (standard deviation-data spread from the mean) was shown in Table 5-6 for all parameters, the overall
percent error between predicted and experimental data was further estimated for better clarification and showed in Table 5-7-

Table 5- 7 Estimation of overall percent errors in heated reactors

<table>
<thead>
<tr>
<th>Nitrogen Species</th>
<th>22°C</th>
<th>45°C-2hr</th>
<th>50°C-1hr</th>
<th>55°C-0.5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN</td>
<td>6%</td>
<td>9%</td>
<td>27%</td>
<td>50%</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>7%</td>
<td>50%</td>
<td>53%</td>
<td>50%</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>5%</td>
<td>4.4%</td>
<td>1%</td>
<td>16%</td>
</tr>
<tr>
<td>NOx-N</td>
<td>8%</td>
<td>11%</td>
<td>18%</td>
<td>12%</td>
</tr>
</tbody>
</table>

Error of 5% and 10% are usually acceptable in error estimation, however higher error of even 24% can be acceptable based on judgement of the results and number of data points (Jouyban et al., 2002). As discussed earlier, the TKN in heated reactors increased with increased temperature and consequently the model under predicted the results at 50°C-1hr and 55°C-0.5hr. Although model ammonia concentrations seem off from experimental however predicted overall concentration of 1 mg/L compared to experimental of 0.5 mg/L showed precision. The percent error in nitrite and NOx showed good precision between model and experimental data from 1 to 18% for the small data set.

5.9 Summary and conclusion

The double temperature dependencies of the nitrifiers were included in this conventional Sumo2 model. The conversion of state variables that contributed to the growth of microorganism other than nitrifiers was disregarded in this modeling approach. Although both positive and negative response to nitrifiers were identified at different temperature ranges, the substrate utilization and growth rates of other microorganisms, especially for aerobic heterotrophs needs to be explored. From this modified modelling approach the following conclusions can be drawn.
- The first temperature coefficient of θ₁ (1.05 for AOBs and 1.02 for NOBs) was valid until temperature 45°C-2 for AOB, and 40°C for any duration of NOBs. After these temperature ranges, AOBs and NOBs used 1.03 and 1.01 for temperature factor with declined their growth rates.

- The prediction of effluent COD heated reactors was 49,56 and 49 mg/L at 45°C-2hr, 50°C-1hr and 55°C-0.5hr respectively showing insignificant change compared to control (43 mg/L). As no impact of temperature on heterotrophs was modeled, all heated SBRs were acting as control in the model. However, the experimental data that showed 50% to 100% increase in COD, potentially caused by the death and lysis of heterotrophs in the aerobic system which needs to be included in future work.

- The model also predicted TP which is comparable with experimental results until 50°C-1hr, however 1 mg/L of increment was observed at 55°C-0.5hr compared to control.

- The predicted ammonia by using sumo2 and modified sumo2_dArrhenius model overestimated the ammonia in the control and heated reactors however, the concentration varies from 0.5 to 2 mg/L

- Although all heating from 45°C to 55°C showed inhibition of ammonification activities, however at 55°C-0.5hr, both the ammonification and ammonia oxidation were reduced as confirmed by overall 6.6 mg/L of ammonia in the experimental results.

- The NOx concentration was 17-19 mg/L compared to 25 mg/L in the control reactors, indicating the inhibition of ammonification.

- The sumo2_dArrhenius model was efficient in predicting effluent nitrite of 10-14 mg/L which are comparable with experimental results from the heated reactors.
References


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Water, =. (2012). *On modifying the Arrhenius equation to compensate for temperature changes for reactions within biological systems* STRENDÁ View project Filtration View project On modifying the Arrhenius equation to compensate for temperature changes for reactions within biological systems. 38(1). https://doi.org/10.4314/wsa.v38i1.18

Chapter 6

6 Heat assisted mainstream partial nitrification in membrane bioreactors: the combined effect of heat and SRT

6.1 Introduction

Partial nitrification (PN) is a cost-effective process that can reduce the cost of energy, external carbon, and sludge handling, while providing lower footprint (Miao et al., 2018). The PN process using intermittent heat treatment used the mechanism of reduced growth rate of nitrifiers as well as the death and lysis of the nitrifiers to achieve mainstream PN while keeping the dissolved oxygen concentration greater than 2 mg/L (Liu et al., 2020; Willers et al., 1998).

In side stream nitrogen removal using PN with anammox (PN/A) process, 60% less aeration, less than 75% of sludge production can be achieved along with no organic carbon requirement (Kartal et al., 2010; Ma et al., 2016). The sludge handling cost in a conventional nitrification denitrification plant itself can cost 40-60% of the total operating cost of a wastewater treatment plant (Domini et al., 2022). Due to this advantage of PN/A process, approximately 120 wastewater treatment plants globally are using PN/A for treating supernatant from the anaerobically digested sludge containing 500-1500 mg N/L at temperature > 25°C and low COD/N ratio (less than 1gCOD/gN) (Cao et al., 2017; Lackner et al., 2014; Sheng et al., 2020). However, mainstream PN/A process is yet to be implemented in full-scale mainly due to the low wastewater temperature and absence of free ammonia and free nitrous acid inhibition of NOBs, high COD/N ratio which fosters denitrification over anammox (Wang, Liu, et al., 2018b).

In previous studies with high heat shocks, they either used combined effect of heat shock and limitations of oxygen diffusion in batch and continuous system (G. Chen et al., 2016; Isaka et al., 2008) or used only heat shock in semi batch system (Chen et al., 2019). No studies used heat shock using real wastewater or only the effect of heat shock in continuous system. In the previous stages of this study all the heat shock experiments were performed in batch and semi batch systems using real municipal wastewater. The systems experienced...
ammonia concentration gradients which may impact growth rates, potentially providing an advantage to AOBs due to the high initial ammonia. In continuous-flow completely mixed system, there is no ammonia gradient, which is a better representation of full-scale systems. In order to investigate the effect of intermittent heat shock on nitrification, in this study MBRs were used to provide continuous flow and heat was applied in the reactor when complete nitrification was achieved. At this stage, we have investigated the combined effect of heat treatment and SRT on the suppression of NOBs in mainstream nitrogen removal. The long SRT of 40 days was maintained to assist the microorganisms to grow so that high MLSS concentrations can be maintained in the main reactor to avert the need to heat concentrated biomass for deactivation of NOBs outside the reactor that was done in previous studies. Heating MLSS is not an economically viable approach to achieve PN. Therefore, the response of nitrifiers to inline heat at the higher concentration of mixed liquor (resembling RAS) was investigated. Furthermore, the reproducibility of the NOB suppression was investigated so that the pattern of NOB suppression can be clarified and be used for process control.

6.2 Materials and methods

6.2.1 Inoculum and wastewater
The RAS from secondary clarifier from Greenway Wastewater treatment plant was collected as inoculum. The initial TSS concentration of the RAS that was introduced to the reactors was 4000 mg/L. Primary effluent (PE) from the Greenway wastewater treatment plant was used for the continuous operation of the reactors. The overall average characteristics of the PE were: TSS=120 mg/L, VSS = 95, COD= 250 mg/L, SCOD= 100, TN= 40 mg/L, TP=5 mg/L, pH=8.5 and alkalinity =400 mg/L as CaCO₃.

6.2.2 Reactor set up
Figure 6-1 shows the membrane system for control and heating reactors.
Figure 6-1. Control reactors at 23°C (left hand side) and heated reactors at 50°C-60°C (right hand side) for achieving mainstream partial nitrification.
Two reactors with a 2L working volume, mixed with magnetic stirrers with 400 RPM (Corning, PC-620D), were operated simultaneously. One reactor was operated as the control reactor at 23℃ (MBR1) while another one as the heated reactor (MBR2) where all heating strategies (50-60℃) were applied to achieve the partial nitrification. The test MBR was heated with water bath (VWR® Heated Circulating Baths) and was covered with aluminum paper to prevent the heat loss. A digital thermometer was inserted into the reactor to register the temperature inside the reactor. The membrane was a microfiltration membrane made with polypropylene with pore size of 0.1µm, and surface area of 1000 cm² (Zena Membrane, 2023)

### 6.2.3 Membrane operation

Both membranes were operated at room temperature (23℃) for 100 days (2.5 SRT turnovers of 40 days SRT) before applying the heat to the MBR. The DO in both reactors were 7 mg/L throughout the operation at SRT of 40 days, 35 days, and 7 days, maintained by daily wasting of 50mL, 60 mL and 286 mL respectively. The membranes were fed continuously with 3.6L of PE per day. The influent and effluent pumps were synchronized to maintain the correct influent and effluent flow rates. The membrane was set with the effluent pump (Masterflex®, VWR-77200-62). The water level of the reactors was 2±0.1 L and was checked every day. The reactors were filled with the same volume of effluent after wasting to maintain a constant working level of 2L. The membranes were clogged every 10-15 days and were backwashed with water outside of the reactor. Multiple membranes were used so that membranes can be replaced immediately while the backwashing of the fouled membrane can be done without creating lag in the continuous flow system. Although the design flux of the membrane was 121 Lmh @ 1 bar at 15℃ for clean water, it showed that membrane fouled in 3-4 days at MLSS of 3000-3500mg/L. Hence, the flux in the membrane was deliberately maintained as 1.5 LMH to reduce the membrane fouling and backwash frequency for smooth operation of the continues system.
6.2.4 Heating Strategies

The heating strategy was selected based on the nitrite accumulation in the heating system. The heating strategy is provided in Table 6-1. In this research using SBR, it was found that the optimum nitrite accumulation occurred at 50°C-60°C. In SBR operation at 50°C for one hour of heating daily, 50% of the nitrite oxidation happened with 100% oxidation of ammonia. At 55°C for one hour of heating daily, about 60% of the ammonia was oxidized while 40% was unoxidized, at 60°C for 0.5 hour of heating daily, all nitrifiers were suppressed and no oxidation of ammonia occurred. Based on these heating outcomes in SBRs, the heating strategies were chosen for heating of MBR2.
Table 6-1. Heating Strategies in the continuous heating system for achieving PN.

<table>
<thead>
<tr>
<th>Heating, SRT=40</th>
<th>Heating interval (days)</th>
<th>Temperature (°C)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day1-7</td>
<td>6</td>
<td>55°C-1hr</td>
<td>Heated on day1 and no heat was applied until after day 7</td>
</tr>
<tr>
<td>Day 8-13</td>
<td>5</td>
<td>55°C-1hr</td>
<td>Heated on day 8 and no heat was applied until after day 13</td>
</tr>
<tr>
<td>Day 14-24</td>
<td>Everyday</td>
<td>55°C-1hr</td>
<td>Continuously heated from day 14-30</td>
</tr>
<tr>
<td>Day 25-30</td>
<td>5</td>
<td>No heating</td>
<td>Reactors cooled down to attain complete nitrification</td>
</tr>
<tr>
<td>Day 31-53</td>
<td>Everyday</td>
<td>60°C-1hr</td>
<td>Continuously heated from day 31-55</td>
</tr>
<tr>
<td>Day 55-62</td>
<td>8</td>
<td>No heating</td>
<td>Reactors stopped, cooled down to attain complete nitrification</td>
</tr>
<tr>
<td>Day 63-66</td>
<td>Every other day</td>
<td>55°C-1hr</td>
<td>Heated every other day</td>
</tr>
<tr>
<td>Day 67-69</td>
<td>3</td>
<td>No heating</td>
<td>Heating stopped for achieving complete nitrification</td>
</tr>
<tr>
<td>Day 70-75</td>
<td>Every other day</td>
<td>55°C-1hr</td>
<td>Reproduction of results of Day 63-66</td>
</tr>
<tr>
<td>Day 76-77</td>
<td>2</td>
<td>No heating</td>
<td>Heating stopped for achieving complete nitrification</td>
</tr>
<tr>
<td>Day 78-82, SRT=35</td>
<td>5</td>
<td>55°C-1hr</td>
<td>Investigation the change of 5 days of SRT would improve PN</td>
</tr>
<tr>
<td>Day 83-84, SRT=7</td>
<td>Everyday</td>
<td>55°C-1hr</td>
<td>Starting of SRT of 7 days with this heat</td>
</tr>
<tr>
<td>Day 84-87</td>
<td>3</td>
<td>50°C-0.5hr</td>
<td>To confirm lower the temperature can produce same nitrite</td>
</tr>
<tr>
<td>Day 87-96</td>
<td>7</td>
<td>50°C-0.5hr</td>
<td>To confirm lower the temperature can produce same nitrite</td>
</tr>
<tr>
<td>Day 97-103</td>
<td>Everyday</td>
<td>55°C-1hr</td>
<td>To investigate the continuous heating at SRT of 7 days</td>
</tr>
</tbody>
</table>
6.3 Results and discussion

6.3.1 Performance of MBRs at SRT 40 days at 55-60°C

The reactors were operated for 100 days before starting the heating. Table 6-2 shows characteristics of overall influent and effluent from MBR1 and MBR2 before heating. The overall ratios of TCOD and SCOD, SN and TN, ammonia and TN were: 40%, 70% and 60% respectively. The overall MLSS and MLVSS in the reactors were 3300 mg/L and 2700 mg/L respectively in the reactors which is less than theoretical MLSS and MLVSS of 5782 mg/L and 4000 mg/L, because longer SRT enhances endogenous respiration and reduce sludge production (Li & Wu, 2014).

Table 6-2. Characteristics of influent and effluent in MBR operation at steady state before heating ((Average± Standard deviation (sample number))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Effluent MBR1</th>
<th>Effluent MBR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS (mg/L)</td>
<td>120±7.5(3)</td>
<td>3330±240 (3)</td>
<td>3385±593(3)</td>
</tr>
<tr>
<td>MLVSS (mg/L)</td>
<td>95±12.9(3)</td>
<td>2600±28.2(3)</td>
<td>2905±141(3)</td>
</tr>
<tr>
<td>TCOD (mg/L)</td>
<td>250±5.8(3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>100±0.8(3)</td>
<td>33±0 (3)</td>
<td>30±5(3)</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>40.3±0.6(3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SN (mg/L)</td>
<td>29±2.1(3)</td>
<td>32±2.12 (3)</td>
<td>33 ± 3(3)</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>23.8±1.03(3)</td>
<td>0.5±0.4</td>
<td>0.6±0.1(3)</td>
</tr>
<tr>
<td>NO₂⁻-N (mg/L)</td>
<td>0.5±0.1(3)</td>
<td>32±2.5(3)</td>
<td>30±1.4(3)</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/L)</td>
<td>0.003±0.001</td>
<td>0.20±0.15</td>
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6.3.1.1 Heat Shocks and Sustained Heating at 55°C

Heat shock at 55°C for one hour was applied to the MBR2 on day 1 to investigate if heat can be optimized as found in SBR systems. The same heating strategy was provided on day 8 again to investigate if the nitrite accumulation can be reproduced. It showed that heating
On day 1 and day 8 provided 64% and 55% of NAR, however lost to complete nitrification. Since one day heating showed nitrite accumulation and complete nitrification, heating at 55°C for 1 hour was applied daily from day 14-24 to investigate if stable nitrite accumulation can be achieved by applying daily intermittent heating at SRT of 40 days. Figure 6-2(a) and 6-2(b) showed the influent ammonia concentration and effluent nitrogen species in control and heated reactors respectively.

Figure 6-2. Nitrogen species in control (a) and in the heated reactor (b) at 55°C-1hour
From Figure 6-2(a), the influent ammonia was completely oxidized to nitrate at the room temperature (23°C), with effluent nitrite concentrations as low as 0.001 mg/L and nitrate concentrations as high as 36 mg/L. The higher effluent nitrate concentration (2-10 mg/L) than the influent ammonia was due the ammonification of the organic nitrogen in the aerobic environment with the ambient DO concentration of 7 mg/L (Wang et al., 2018). The effluent ammonia of 0.1 to 0.7 mg/L coupled with 0.001 to 0.01 mg/L of nitrite confirmed the complete oxidation of ammonia and nitrite respectively at 23°C. In Figure 6-2(b), the first heating was provided on day 1 at 55°C-1hr and no heating was provided until day 7. After heating on day 1, the nitrite gradually accumulated and peaked on day 5 and gradually decreased to complete nitrification on day 7. The maximum nitrite concentration of 11 mg/L happened on day 5. Same heating at 55°C for 1 hour applied on day 8 showed that the maximum nitrite accumulation of 9 mg/L happened on day 11 and complete nitrification on day 13. The heating on day 1 and day 8 showed the same trend of nitrite accumulation followed by complete nitrification. The gradual accumulation of nitrite is due to the gradual revival of AOBs from day 1 faster than NOBs. However, the pattern of AOB and NOB suppression and revival is reproducible for heating only one hour a day. Since this strategy did not provide stable nitrite accumulation, a heating strategy of heating 55°C for 1 hour daily was adopted from day 16 to 24. The overall nitrite concentration was 2 mg/L while the overall influent ammonia was 12 mg/L indicating that these heating strategies does not work for nitrite accumulation at SRT of 40 days. Since, the nitrite accumulation was not sufficient for this heating strategy, a higher heat of 60°C for 1 hour had been applied every day in order to investigate the effect of higher heat than 55°C-1hour. Figure 6-3(a) and Figure 6-3(b) show the nitrogen species in the reactors for heating 60°C for 1 hour every day.
In MBR1, complete nitrification was observed. However, in the heated MBR at 60°C for 1 hour, the overall influent ammonia was 15 mg/L and the effluent ammonia concentration ranged from 10-15 mg/L meaning that this heating strategy suppressed most of the AOBs in the system. The overall nitrate concentration until the continuous heating was 3 mg/L which is identical as heating continuously at 55°C for 1 hour (Figure 6-2(b), days 16-24). The nitrite accumulation was overall 2 mg/L for 24 days (day 31 to 55). From day 55 to
day 62, the heating was stopped. The reactor was kept unheated until day 62 to revive the nitrifiers prior to applying the new heat strategy since heating at 60°C-1hr did not provide satisfactory result. However, on day 60 (no heating), the maximum NAR of about 44% (nitrite concentration of 6 mg/L) was observed which followed the same trend of heating at 55°C for one hour on day 1 and day 8.

6.3.1.2 Nitrite accumulation ratio (NAR) and ammonia concentrations in the effluent

The maximum nitrite accumulation ratio and effluent ammonia are shown in Figure 6-4.

For heating at 55°C for one hour on day, produced 64% of NAR on day 5 while effluent ammonia concentration was 10 mg/L. Another duplicate heating was provided on day 8 that produced 60% of NAR with 11 mg/L of ammonia indicating that nitrite accumulation was reproduced. The everyday heating at 55°C for one hour from day 15-24, showed overall 30% of NAR with overall ammonia concentration of 7 mg/L. From day 31-55, at 60°C for an hour heating everyday showed maximum of NAR of 14-50%, however, the ammonia concentration was very high ranging from 10-18 mg/L indicating that heating continuous system at 60°C for an hour every day at SRT of 40 days, was not producing better nitrite accumulation, which combined with high ammonia concentration indicates loss of nitrification. While heating stopped from day 56, it is observed that 44% of maximum NAR can be produced while ammonia concentration was zero on day 60 in the effluent indicating the complete AOB recovery in 4 days.
6.3.1.3 Reproducibility of nitrite accumulation by heating on alternate days

Since the dynamics of AOBs and NOBs in the heated MBRs were suppression and revival, in this assay the reproducibility of nitrite accumulation by heating alternate days and the investigation of a pattern of nitrite accumulation can be found. Figure 6-5(a) and 6-5(b) show the nitrogen species in the control and heated reactors at 55 for 1 hour.
Figure 6-5. Nitrogen species in control reactor (a) and heated reactors (b) for heating alternate days at 55°C for 1 hour
The alternative day heating started from day 63 and heating stopped on day 64. This heating pattern was provided until day 68 (3-time heating on and off) and revealed that 30% to 59% NAR can be achieved from 63 to 68 days. Heating was not provided on day 69 to achieve complete nitrification. On day 70, the heating and no heating strategy started again and showed the same pattern of NAR of 30-55% and complete nitrification occurring on day 76. However, this strategy provided high ammonia on heating days. Nonetheless, PN/A process requires ammonia, PN with complete ammonia oxidation may require external ammonia to be added to the A process. On day 78, heating was provided at SRT of 35 days at 55°C for 1 hour and showed the same pattern until day 82 heating at 55°C for 1 hour.

The reproducibility of nitrite trend using higher SRT is significant information regarding controlling heat treated PN systems based on the effluent ammonia/nitrate concentration. However, net growth rate of microorganisms is mechanistically reciprocal to SRT. Higher SRT facilitates higher endogenous decay (Metcalf & Eddy, 2004). The simultaneous effect of high SRT of 40 days along with the thermal death of the nitrifiers (Willers et al., 1998), caused detrimental effect on nitrite accumulation. The accumulation of nitrite happened due to the combined effect of AOB and NOB recovery after heating. The recovery of nitrifiers is the function of unheated operational time with faster recovery of AOBs than NOBs which was observed in this study. Therefore, after heating just a day on day 1 and day 8 at 55°C for 1 hour heating, the nitrite accumulation occurred due to the faster recovery of AOBs relative to NOB. However complete nitrification happened when both the nitrifiers recovered completely after days of non-heating (room temperature) operation. During everyday heating at 40/35 days SRT, however, both AOBs and NOBs recovered during the non-heating operation time of the system and the surplus recovery (after reduced growth and heat) of AOBs provided 2 mg/L of stable nitrite which was not significant.

6.3.2 Performance of MBRs at SRT 7 days at 55°C

The application of SRT of 35-40 days were not providing the expected nitrite accumulation, the next experiment had been conducted with SRT of 7 days with same DO of 7 mg/L at 55°C for 1 hour heating with the MLSS concentration approximately 2000 mg/L. Figure 6-6 (a) and 6-6(b) show the concentration of influent ammonia and effluent nitrogen species.
Nitrite accumulation ratio during stable nitrification is shown in Figure 6-6(b). It showed that using this heating strategy, maximum NAR of 0.76 can be achieved which is close to
the NAR achieved in SBR systems. The heating was applied on day 83 at 55°C for 1 hour when effluent ammonia concentration was the same as the influent ammonia concentration due to the instantaneous suppression of AOBs at the start of the heating. No heating was applied until day 86. However, since nitrite concentration decreased to 5 mg/L on day 87, heating was applied on this day at 50°C-0.5hr to suppress NOBs only. The heating temperature was lowered to 50°C in order to avoid the inhibition of AOBs that happened at 55°C for 1 hour of heating, leading to no ammonia removal. In the SBR operation, it was revealed that, heating at 50°C for 1 hour, does not suppress AOBs but suppresses NOBs producing approximately 50% of NAR. Based on the aforementioned result, the second heating at 50°C for 0.5hr was applied on day 92 when nitrite concentration went down to 8 mg/L. The produced overall nitrite concentration was 12 mg/L with no significant increment of effluent ammonia concentration. It is noticeable that from day 93-97, ammonia concentration goes to zero and overall nitrite concentration was around 10 mg/L, meaning that during this period although AOBs were completely acclimatized, 50% of the nitrite oxidation was stopped, which was found in SBR system mentioned above. On day 98, the continuous heating at 55°C for one hour daily just to investigate the nitrite accumulation by this heat as performed in SBR system and revealed that approximately 76% of maximum NAR was achieved that was comparable with PN in SBR.

6.4 Conclusion
This study showed that the heat application is viable to achieve partial nitrification in mainstream wastewater treatment in continuous-flow systems. The optimum heat and duration for achieving 76% of NAR can be achieved at 55°C for heating 1 hour every day. The inhibition and recovery of AOBs and NOBs follows a pattern where recovery AOB is faster than NOBs which is the trigger for partial nitrification by heat. The optimum recovery time for NOB is approximately 0.5times SRT turnover of 7 days and heat can be applied to this operation interval for energy optimization. Since the application of the same heating strategy can be reproduced in duplicates or triplicates and followed the same pattern PN by heating can be controlled by controlled strategy such as ammonia-based control.
References


Chapter 7

7 Conclusions and recommendations for future work.

7.1 Conclusions

Experiments were performed to investigate the impact of offline intermittent heating and online continuous heating to suspended growth nitrifying microorganisms in SBR systems. The heated reactors were heated at 47°C-2hr, 37°C continuous and 42°C continuous heating. The heating of 47°C-2hr was applied to 20% of the MLSS offline, whereas 37°C and 42°C were applied to 100% of the MLSS in the reactors. Batch experiments for investigating the effect of heat shock on the activities of nitrifiers revealed that short term batch experiments should not be used to select the heating temperature and contact time to be applied to SBRs since the recovery time of the NOBs ranges between 10 and 12 days compared to short term batch experiment of 4 hours. The maximum NAR in the SBRs was 0.79 at 42°C continuous heating. *Nitrospira* was dominant in all reactors and increased with increased heating duration, with ratios of *Nitrospira* to *Nitrosomonas* of 1.4:1, 2.4:1, 2.4:1, and 3.7:1 respectively at 22°C, 37°C offline heating, 37°C continuous heating and 42°C continuous heating. Although *Nitrospira* was dominant in the heated reactors, the nitrification process may have been facilitated by Comammox *Nitrospira* which consumes ammonia to convert to nitrite and then nitrate. Although this heating strategy showed nitrite accumulation, PN was never stable and the total energy for achieving the maximum NAR of 0.79 exceeded the aeration energy required for complete nitrification.

SBRs were used to evaluate PN in temperature range of 35°C-60°C applied to 100% of MLSS at 1-3 hours. The stable nitrite accumulation happened with maximum NAR of 79% and was stable for 30 to 60 days. AOB and NOB populations were differently affected in the temperature range of 45°C-2hr to 60°C-0.5hr and 55°C-0.5 hr to 60°C-0.5 hour respectively. At 60°C-0.5 hr both nitrifiers were inhibited and nitrification was stopped completely. It is interesting that after the heating stopped at 55°C-0.5 and 60°C-0.5 hr, both
nitrifiers started to recover with faster recovery of AOBs than NOBs and nitrite accumulation happened for about 1.2-1.5 SRT turnover. The heat optimization assay was performed to investigate the stability of nitrite accumulation after one heating and found that heat be provided every 10 days, at 50°C-1hr and 55°C- 0.5hr can reduce 12%-17% of the total nitrification energy (excluding heat losses) compared to aeration energy required for complete nitrification. Both Nitrosomonas and Nitrospira were negatively affected by heat application, with Nitrospira being more sensitive to the heat treatment. It was revealed that any heat shock using temperature greater than 45°C-1 hour, would require further COD removal to meet stringent COD removal criteria, as heat shock higher than this temperature and duration caused increased effluent COD.

The cyclic test data was used to fit the double Arrhenius exponential model, which showed that the estimated temperature factors in the range of 35°C-60°C factor were 1.05 and 1.02 and 1.03 and 1.01 for AOBs and NOBs respectively compared to conventional Arrhenius factors of 1.072 and 1.065. The model was modified for the temperature of 45°C-2hr, 50°C-1hr and 55°C-0.5hr to simulate the nitrogen effluent nitrogen concentration. The maximum specific ammonia uptake and maximum specific nitrite uptake rates decreased by 50% at temperature 45°C-2hr for NOBs and 55°C-0.5hr for AOBs. Although the new temperature model predicted most of the nitrogen species, the 55°C-0.5hr showed higher error in predicted data than experimental.

Two submerged membrane bioreactors were operated as continuous-flow systems to investigate the impact of heat shock on PN, one as a control at room temperature, and the other heated to various temperatures. The heated reactor produced the same level of partial nitrification with NAR of 79% at DO of greater than 2 mg/L, SRT of 7 days, as the SBR, despite needing higher temperatures and contact times. For example, in the MBR, 55°C-1hr achieved the same NAR as SBRs at temperatures 45-2hr, 50-1hr and 55-0.5hr. The nitrite accumulation was reproducible twice or thrice indicating that nitrite accumulation can be controlled by the frequency of heat application. Stable nitrite accumulation can be achieved by heating every 3-4 days.
7.2 Recommendations for future research

- The range of temperature achieving nitrite accumulation in SBR systems would be 45°C-55°C in the laboratory scale for the duration of 1-2 hours, however the temperature and duration of achieving mainstream PN need further investigation for continuous flow systems.
- The investigation of energy optimization can be done for extended period of time for better heat optimization in continuous-flow systems.
- The impact of heat on the percent of biomass of 30%-90% and corresponding energy optimization need further investigation.
- Further investigation is required for utilization rates of COD generated from biomass lysis due to heating and incorporation in simulation models is needed to better match the effluent COD.
- Long-term operation of continuous-flow systems using heat shock needs further investigation.
- Detailed microbial analysis in continuous-flow systems, mainly nitrifiers in the continuous heating systems needs further investigation.
Appendices

Appendix A: Supplementary information for chapter 3

SECTION 1: Nitrogen Mass balance: The nitrogen mass balance at steady state was calculated by the following Equations: 2-6 (Zaman et al., 2019)(Metcalf & Eddy, 2014)

\[
N_{\text{OUT}} = N_{\text{Eff}} + N_{\text{WAS}} + N_{\text{DN}} \tag{2}
\]

\[
N_{\text{Eff}} = Q \times (SN_{\text{Eff}} + PNNB_{\text{Eff}} + fn \times VSS_{\text{Eff}}) \tag{3}
\]

\[
N_{\text{WAS}} = \left( \frac{MLVSS \times Vr}{\theta_c} - Q \times VSS_{\text{Eff}} \right) \times fn \tag{4}
\]

\[
N_{\text{DN}} = Q \times (TN_{\text{IN}} - PNNB_{\text{IN}} - SN_{\text{Eff}} - N_{\text{Syn}}) \tag{5}
\]

where, \( fn = \frac{(TN- SN)}{MLVSS} \times 100\% \) \( \tag{6} \)

Where \( N_{\text{OUT}}, N_{\text{EFF}}, N_{\text{WAS}}, N_{\text{DN}}, \) are the mass rates of total nitrogen outflow, effluent nitrogen, WAS nitrogen, and denitrified nitrogen, respectively. \( Q, SN_{\text{EFF}}, PNNB_{\text{EFF}}, fn, VSS_{\text{EFF}} \) are the effluent flow rate (3 L/d), effluent soluble nitrogen, effluent non-biodegradable particulate nitrogen, ratio of particulate nitrogen to VSS in WAS, and concentration of effluent volatile suspended solids (mg/L) respectively. \( Vr, \theta_c \) are the working volume of the reactors (2L), solids retention time (SRT=7days) respectively, and \( TN_{\text{IN}}, PNNB_{\text{IN}}, N_{\text{Syn}} \) are the concentrations of influent total nitrogen, influent particulate non-biodegradable nitrogen and nitrogen used for cell synthesis respectively. All nitrogen mass rates in Equations 3-6 have units of mgN/d. TN, SN and MLVSS are concentrations
of total nitrogen, soluble nitrogen and mixed liquor suspended solids in the WAS respectively. The overall fn in the sludge was 10%.
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**SECTION 2:** The following Equations: 7-10 were used to estimate alkalinity balance.

\[
\text{Alk}_{\text{Consumed}} = N_{\text{Nitrified}} \times 7.14 \text{mgCaCO}_3/\text{L} \quad (7)
\]

\[
\text{Alk}_{\text{Produced}} = (\text{Alk}_{\text{DN}} + \text{Alk}_{\text{Ammonification}}) \times 3.57\text{mgCaCO}_3/\text{L} \quad (8)
\]

\[
\text{Net Alk}_{\text{change}} = \text{Alk}_{\text{Consumed}} - \text{Alk}_{\text{Produced}} \quad (9)
\]

\[
\text{Change in Measured Alk} = \text{Alk}_{\text{Eff}} - \text{Alk}_{\text{Inf}} \quad (10)
\]

Where \( \text{Alk}_{\text{Consumed}}, \text{N}_{\text{Nitrified}}, \text{Alk}_{\text{Produced}}, \text{Alk}_{\text{DN}}, \text{Alk}_{\text{Ammonification}}, \text{Alk}_{\text{Eff}} \) and \( \text{Alk}_{\text{Inf}} \) are the alkalinity consumed, nitrogen nitrified (mg/L), alkalinity produced, alkalinity produced due to denitrification, alkalinity produced due to ammonification, alkalinity of effluent and influent respectively. All alkalinity has the unit of mgCaCO\(_3\)/L.
Table S2. Alkalinity balance

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Nitrified-N</td>
<td>26</td>
<td>24</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Alkalinity consumption (7.14xNitrified-N)</td>
<td>mgCaCO3/L</td>
<td>184</td>
<td>171</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from denitrification (3.57x Denitrified-N)</td>
<td>mgCaCO3/L</td>
<td>7.1</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from ammonification (3.57xAmmonified-N)</td>
<td>mgCaCO3/L</td>
<td>0</td>
<td>-7.1</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>1) Net Alkalinity Change</td>
<td>mgCaCO3/L</td>
<td>-177</td>
<td>-171</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2) Measured alkalinity change</td>
<td>mgCaCO3/L</td>
<td>-149</td>
<td>-162</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alkalinity balance closure (2)x100/ (1)</td>
<td>84</td>
<td>95</td>
<td>82</td>
<td>85</td>
</tr>
<tr>
<td>S2</td>
<td>Nitrified-N</td>
<td>27</td>
<td>29</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Alkalinity consumption (7.14xNitrifeid-N)</td>
<td>mgCaCO3/L</td>
<td>196</td>
<td>205</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from denitrification (3.57x Denitrified-N)</td>
<td>mgCaCO3/L</td>
<td>31.5</td>
<td>14.8</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from ammonification (3.57xAmmonified-N)</td>
<td>mgCaCO3/L</td>
<td>8.6</td>
<td>12.2</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>1) Net Alkalinity Change</td>
<td>mgCaCO3/L</td>
<td>-156</td>
<td>-178</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2) Measured alkalinity change</td>
<td>mgCaCO3/L</td>
<td>-175</td>
<td>-183</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alkalinity balance closure (2)x100/ (1)</td>
<td>112</td>
<td>103</td>
<td>98</td>
<td>110</td>
</tr>
<tr>
<td>S3</td>
<td>Nitrified-N</td>
<td>36</td>
<td>35</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Alkalinity consumption (7.14xNitrifeid-N)</td>
<td>mgCaCO3/L</td>
<td>256</td>
<td>251</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from denitrification (3.57x Denitrified-N)</td>
<td>mgCaCO3/L</td>
<td>50.7</td>
<td>11.4</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>Alkalinity production from ammonification (3.57xAmmonified-N)</td>
<td>mgCaCO3/L</td>
<td>22.7</td>
<td>6</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>1) Net Alkalinity Change</td>
<td>mgCaCO3/L</td>
<td>-183</td>
<td>-234</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2) Measured alkalinity change</td>
<td>mgCaCO3/L</td>
<td>-153</td>
<td>-189</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Alkalinity balance closure (2)x100/ (1)</td>
<td>84</td>
<td>81</td>
<td>89</td>
<td>98</td>
</tr>
</tbody>
</table>
## Section 2: Energy Calculations for a 1 MLD plant

Table S3. Flow split for PN-A process

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Temp-Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>47°C-2hr</td>
</tr>
<tr>
<td>Influent _NH3-N (mg/L)</td>
<td>24.5</td>
</tr>
<tr>
<td>PN _NH3-N (mg/L)</td>
<td>0.28</td>
</tr>
<tr>
<td>PN NO2 -N (mg/L)</td>
<td>10.2</td>
</tr>
<tr>
<td>Influent NO2-N (mg/L)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mix _NH3-N (mg/L)</td>
<td>5.97</td>
</tr>
<tr>
<td>Mix NO2 -N (mg/L)</td>
<td>7.85</td>
</tr>
<tr>
<td>Influent Flow (L/d)</td>
<td>235000</td>
</tr>
<tr>
<td>PN flow (L/d)</td>
<td>765000</td>
</tr>
<tr>
<td>Moles of NH3-N</td>
<td>0.43</td>
</tr>
<tr>
<td>Moles of NO2 -N</td>
<td>0.56</td>
</tr>
<tr>
<td>% Flow Influent</td>
<td>23.5</td>
</tr>
<tr>
<td>% Flow PN</td>
<td>76.5</td>
</tr>
<tr>
<td>Molar Ratio</td>
<td>0.76</td>
</tr>
</tbody>
</table>

**Example calculation: R2 (45°C-2hr):**

\[
\text{NH}_3-N \text{ in Anammox reactor (mg/L)} = (\text{Influent NH}_3-N \times \frac{L}{d}) + \text{NH}_3-N \text{ from PN} \times \frac{L}{d} / \text{Total flow} = \frac{24.5 \times 235000 + 0.2 \times 765000}{1000000} = 5.97 \text{ mg/L}
\]

\[
\text{NO}_2-N \text{ in Anammox reactor (mg/L)} = (\text{Influent NO}_2-N \times \frac{L}{d}) + \text{NO}_2-N \text{ from PN} \times \frac{L}{d} / \text{Total flow} = \frac{0.2 \times 235000 + 10.2 \times 765000}{1000000} = 7.85 \text{ mg/L}
\]
$$\text{NH}_3\text{-N to Anammox (mole)} = \frac{\text{mg} \text{L of NH}_3\text{-N to Anammox}}{\text{molecular weight of NH}_3\text{-N}} = \frac{5.7}{14} = 0.43 \text{ mole}$$

$$\text{NO}_2\text{-N to Anammox (mole)} = \frac{\text{mg} \text{L of NO}_2\text{-N to Anammox}}{\text{molecular weight of NO}_2\text{-N}} = \frac{7.85}{14} = 0.56 \text{ mole}$$

$$\frac{\text{NH}_3\text{-N}}{\text{NO}_2\text{-N}} = \frac{0.43}{0.56} = 0.76$$

**Table S4. Energy Calculation**

<table>
<thead>
<tr>
<th>1) Aeration Energy</th>
<th>Complete Nitrification (22°C)</th>
<th>47°C-2hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (L/d)</td>
<td>1000000</td>
<td>812000</td>
</tr>
<tr>
<td>Observed Yield (gVSS/gCOD)</td>
<td>0.36</td>
<td>0.3</td>
</tr>
<tr>
<td>Avg. Effluent Ammonia (mg/L)</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Avg. Influent total COD (mg/L)</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>Avg. Effluent COD (mg/L)</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Avg. Influent TKN (mg/L)</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Biomass synthesis (mgVSS/L)</td>
<td>72</td>
<td>59.4</td>
</tr>
<tr>
<td>Maximum nitrite accumulation ratio (NAR)</td>
<td>0</td>
<td>0.37</td>
</tr>
<tr>
<td>N needed for synthesis (mg/L)</td>
<td>7.2</td>
<td>5.94</td>
</tr>
<tr>
<td>Ammonia Oxidized (mg/L)</td>
<td>29.3</td>
<td>30.86</td>
</tr>
<tr>
<td>Oxygen Saved (mgO(_2)/L)</td>
<td>-</td>
<td>13.05</td>
</tr>
<tr>
<td>Oxygen saved (kgO(_2)/d)</td>
<td>-</td>
<td>10.59</td>
</tr>
<tr>
<td>Density of air (kg/m(^3))</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Oxygen content of air (% by wt.)</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Oxygen transfer rate</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Air flow saved (m(^3)/d)</td>
<td>-</td>
<td>383.80</td>
</tr>
<tr>
<td>Oxygen required for complete nitrification (mgO(_2)/L)</td>
<td>134</td>
<td>141.0</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Oxygen saved for complete nitrification (kgO₂/d)</td>
<td>134</td>
<td>115</td>
</tr>
<tr>
<td>Air flow required for complete nitrification (m³/d)</td>
<td>4851</td>
<td>4149</td>
</tr>
<tr>
<td>Airflow required after PN (m³/d)</td>
<td>-</td>
<td>3765</td>
</tr>
<tr>
<td>Mechanical efficiency of diffuser (%)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Tank depth (m)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>P discharge (atm)</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Aeration Energy (kWh/d)</td>
<td>72.6</td>
<td>61</td>
</tr>
</tbody>
</table>

### 2) Heat Energy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS (mg/L)</td>
<td>-</td>
<td>1410</td>
</tr>
<tr>
<td>MLVSS (mg/L)</td>
<td>-</td>
<td>970</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>-</td>
<td>0.667</td>
</tr>
<tr>
<td>Effective volume of WW in the reactor (m³/d)</td>
<td>-</td>
<td>667</td>
</tr>
<tr>
<td>Biomass heated (MLSS) in the reactor, kg/d</td>
<td>-</td>
<td>940</td>
</tr>
<tr>
<td>RAS concentration (Assumed, 10kg/m³)</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Density of water (kg/m³)</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Heated RAS flow (m³/d)</td>
<td>-</td>
<td>94</td>
</tr>
<tr>
<td>Heat rate Qheat, MJ/d</td>
<td>-</td>
<td>823</td>
</tr>
<tr>
<td>Heat input for heating once in a day (kWh/d)</td>
<td>0</td>
<td>229</td>
</tr>
<tr>
<td>Heat input for once in a 10 day (kWh/d)</td>
<td>0</td>
<td>23</td>
</tr>
</tbody>
</table>

### 3) Total Energy (Aeration+Heat)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>For daily heating (kWh/d)</td>
<td>73</td>
<td>290</td>
</tr>
<tr>
<td>For once in a 10-day heating (kWh/d)</td>
<td>73</td>
<td>84</td>
</tr>
</tbody>
</table>

Note: observed yield had been calculated from the slope of the graph of COD consumed and VSS for the entire period of operation.

Example: **Aeration energy: For 47°C-2hr heating**

Biomass synthesis = Yield*(Influent total COD-effluent soluble COD)  
=0.3*(230-32) = 43 mg/L  
Nitrogen needed for biomass synthesis=10% of synthesis=0.1*43=4.3 mg/L  
Ammonia Oxidized= Influent TKN-Effluent Ammonia-N-synthesis
=37-4.3-0.2=31 mg/L
Oxygen saved (mg/L) =NAR*Ammonia oxidized*4.75*0.25=31*0.37*4.57*0.25=13.05mg/L
Oxygen saved (KgO2/d) = 13.05 mg/L* 921m3/d=10.6 kgO2/d

Air flow, Qair = \frac{Oxygen saved}{Oxygen transfer rate * \% O2 in air * density of air}
= \frac{10.6}{0.1 * 0.23 * 1.2} = 384 m3/d

Oxygen required for complete nitrification= Ammonia oxidized*4.57* 141m3/d=114.5 kgO2/d
Airflow for complete nitrification= 114.5/ (Oxygen transfer rate*0.23*1.2) =4149m3/d
Airflow after Nitrite accumulation= Airflow for complete nitrification-Airflow for PN =4149-338=3769 m3/d

P_{discharge}=(1+0.0968*depth) \times 0.21 atm=0.29 atm

Aeration Energy required =
\frac{Qair \times \frac{1 mol}{0.0224 m3} \times RT \left[ \left( \frac{P_d}{P_{inlet}} \right)^{0.283} - 1 \right] \times 2.78 \times 10^{-7} KWH/J}{0.283 \times 7} = 61 kWh/d

Aeration energy for complete nitrification:
\frac{Qair \times \frac{1 mol}{0.0224 m3} \times RT \left[ \left( \frac{P_d}{P_{inlet}} \right)^{0.283} - 1 \right] \times 2.78 \times 10^{-7} KWH/J}{0.283 \times 7} = 73 kWh/d

Heat Energy: For 47°C-2hr heating
HRT in the system=0.667 days
Volume of wastewater in the reactor =1000\times 0.667=667 m3/d
Biomass in the reactor (MLSS)= 667\times 1410 \times mg/L/1000= 940 kg/d
Considering the RAS concentration 10,000 mgTSS/L =10 kg/m³
The biomass flow=940/10=94 m³/d

Heat energy = 94 m³/d * 1000 kg/m³ × 4.2 Kj/Kg°C × (47-22) * 2/24 = 229 kWh/d

**Heat Optimization calculation for 1 hour in 10 days:**

For 47°C-2hr

The aeration energy would be same, but the heating is provided in every 10 days

Heat Energy = Heat flowrate Qheat (KJ/d) * 2/10/24 = 23 kWh/d

**Section 3: Microbial Analysis**

**Table S5. Shannon diversity index**

<table>
<thead>
<tr>
<th>Kingdom-Phylum</th>
<th>PilnP (R1)</th>
<th>PilnP (R2)</th>
<th>PilnP (R3)</th>
<th>PilnP (R4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archaea-Miscellaneous Euryarchaeotic Group</td>
<td>0</td>
<td>-0.00124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea-Woesearchaeota</td>
<td></td>
<td></td>
<td>-0.00447</td>
<td></td>
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<tr>
<td>Bacteria-Acidobacteria</td>
<td>-0.09262</td>
<td>-0.06362</td>
<td>-0.10086</td>
<td>-0.14946</td>
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<tr>
<td>Bacteria-Actinobacteria</td>
<td>-0.1347</td>
<td>-0.0837</td>
<td>-0.21631</td>
<td>-0.07568</td>
</tr>
<tr>
<td>Bacteria-armatimonadetes</td>
<td>0</td>
<td>0</td>
<td>-0.00201</td>
<td>-0.01208</td>
</tr>
<tr>
<td>Bacteria-Bacteroidetes</td>
<td>-0.99216</td>
<td>-0.92953</td>
<td>-0.46197</td>
<td>-0.44326</td>
</tr>
<tr>
<td>Bacteria-Chlamydiae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacteria-Chlorobi</td>
<td>-0.0642</td>
<td>-0.07239</td>
<td>-0.06855</td>
<td>-0.13322</td>
</tr>
<tr>
<td>Bacteria-Chloroflexi</td>
<td>-0.04616</td>
<td>-0.00929</td>
<td>-0.1559</td>
<td>-0.2231</td>
</tr>
<tr>
<td>Bacteria-Cloacimonetes</td>
<td>-0.00161</td>
<td>-0.01158</td>
<td>-0.00128</td>
<td>-0.00086</td>
</tr>
<tr>
<td>Bacteria-Cyanobacteria</td>
<td>-0.0046</td>
<td>-0.02376</td>
<td>-0.0095</td>
<td>0</td>
</tr>
<tr>
<td>Bacteria-Deinococcus</td>
<td>0</td>
<td>-0.02678</td>
<td>-0.05585</td>
<td>-0.01749</td>
</tr>
<tr>
<td>Bacteria-Dictyoglomi</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>Bacteria-Elusimicrobia</td>
<td>-0.05628</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Bacteria-Fibrobacteres</td>
<td>0</td>
<td>-0.00221</td>
<td>0</td>
<td>-0.00159</td>
</tr>
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<td>Parameters</td>
<td>Description</td>
<td>values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>-------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_h$</td>
<td>True yield of the heterotrophs</td>
<td>0.40 gVSS/gCOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$(S-So)^*$</td>
<td>Average COD removed</td>
<td>149 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_d$</td>
<td>fraction of cell mass as cell debris</td>
<td>0.15 gVSS/gVSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$b_h$</td>
<td>endogenous decay coefficient for heterotrophs</td>
<td>0.12 gVSS/gVSS.d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_n$</td>
<td>True yield of nitrifiers</td>
<td>0.12 gVSS/gN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOx*</td>
<td>Avg. nitrified NH₃-N</td>
<td>27 mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$b_n$</td>
<td>endogenous decay coefficient for nitrifiers</td>
<td>0.08 gVSS/gVSS.d</td>
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<tr>
<td>$\Theta$</td>
<td>Arrhenius coefficient for endogenous decay</td>
<td>1.04</td>
<td></td>
<td></td>
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</tbody>
</table>

Source: *= this study;

Table S6. Kinetic Coefficients for Heterotrophs and Nitrifiers at 20°C - Adapted from Metcalf & Eddy, 2014
<table>
<thead>
<tr>
<th>Genera</th>
<th>R1 x 10^-2 (gVSS)</th>
<th>R2 x 10^-2 (gVSS)</th>
<th>R3 x 10^-2 (gVSS)</th>
<th>R4 x 10^-2 (gVSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thauera</td>
<td>4.3</td>
<td>5.6</td>
<td>19</td>
<td>49</td>
</tr>
<tr>
<td>Nannocystis</td>
<td>1.0</td>
<td>1.0</td>
<td>41</td>
<td>0.0</td>
</tr>
<tr>
<td>Nitrospira</td>
<td>4.3</td>
<td>8.0</td>
<td>9.0</td>
<td>20</td>
</tr>
<tr>
<td>Zoogloea</td>
<td>19</td>
<td>28</td>
<td>6.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Haliangium</td>
<td>27</td>
<td>0.0</td>
<td>3.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>6.4</td>
<td>24</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Thiothrix</td>
<td>2.1</td>
<td>13</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>4.6</td>
<td>20</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Lewinella</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Terrimonas</td>
<td>1.0</td>
<td>1.6</td>
<td>3.1</td>
<td>6.1</td>
</tr>
<tr>
<td>Ca_Competibacter</td>
<td>8.8</td>
<td>3.6</td>
<td>2.8</td>
<td>7.3</td>
</tr>
<tr>
<td>Nitrosomonas</td>
<td>2.9</td>
<td>4.0</td>
<td>3.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Phaeodactylibacter</td>
<td>10</td>
<td>2.1</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Novosporhobium</td>
<td>2.5</td>
<td>4.0</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Hydrogenobacter</td>
<td>5.3</td>
<td>3.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ca_Accumulibacter</td>
<td>1.1</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bryobacter</td>
<td>3.0</td>
<td>0.0</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Halomonas</td>
<td>6.0</td>
<td>4.1</td>
<td>3.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
### Table S8. AOB and NOB Kinetics

**AOB-Theoretical**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_n$ (gVSS/gN)*</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_{max}$ (d$^{-1}$)**</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Theta$ (Temperature coefficient for growth rate)*</td>
<td>1.072</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical biomass (mgVSS/L)</td>
<td></td>
<td>29</td>
<td>26</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td><em>Nitrosomonas/Nitrospira</em> + <em>Nitrospira</em></td>
<td></td>
<td>0.41</td>
<td>0.29</td>
<td>0.30</td>
<td>0.21</td>
</tr>
<tr>
<td>Maximum substrate utilization rate, $K$ (mg/L.d)</td>
<td></td>
<td>74</td>
<td>47</td>
<td>125</td>
<td>121</td>
</tr>
</tbody>
</table>

**AOB-Experimental**

| Specific NH$_3$-N uptake rates (mgN/VSS.h) | 1.6 | 1.4 | 7 | 6.9 |
| MLVSS (g/L) | 1.46 | 0.8 | 0.9 | 1.36 |
| Maximum substrate utilization rate, $K$ (mg/L.d) | 56 | 27 | 151 | 225 |

**NOB-Theoretical**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_n$ (gVSS/gN)*</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_{max}$ (d$^{-1}$)**</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Theta$ (Temperature coefficient for growth rate)*</td>
<td>1.063</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical biomass (mgVSS/L)</td>
<td></td>
<td>29</td>
<td>26</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td><em>Nitrospira/Nitrosomonas</em> + <em>Nitrospira</em></td>
<td></td>
<td>0.59</td>
<td>0.71</td>
<td>0.70</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>93</td>
<td>100</td>
<td>225</td>
<td>328</td>
<td></td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td><strong>NOB-Experimental</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum substrate utilization rate, K (mg/L.d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific NO2-N uptake rates (mgN/gVSS.h)</td>
<td>1.4</td>
<td>1</td>
<td>5.7</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>MLVSS (g/L)</td>
<td>1.46</td>
<td>0.8</td>
<td>0.9</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>Maximum substrate utilization rate, K (mg/L.d)</td>
<td>49</td>
<td>19</td>
<td>123</td>
<td>166</td>
<td></td>
</tr>
</tbody>
</table>

Example Calculation:

For R3 for AOB, theoretical biomass, for Y_n=0.12, $\mu_{max} = 0.74$, $\Theta=1.072$, Theoretical biomass=19, % Nitrosomonas= 21%.

$Maximum\ sustrate\ utilization\ rate, K \left( \frac{mgN}{L.d} \right) = \frac{19 \times 0.21 \times 0.74 + 1.072^{(37-2)}}{0.12}$

For R3 for AOB, Experimental, for Specific NH$_3$-N uptake rates =7, MLVSS=0.9, 

$Maximum\ sustrate\ utilization\ rate, K \left( \frac{mgN}{L.d} \right) = 7 \times 0.9 \times 24 = 151$

REFERENCE:


Appendix B: Supplementary information for chapter 4

Table 1: Flow split for PN-A process

<table>
<thead>
<tr>
<th>Temp-Time</th>
<th>50C-1hr</th>
<th>55-0.5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater_NH3</td>
<td>24.5</td>
<td>24.7</td>
</tr>
<tr>
<td>PN_NH3</td>
<td>0.4</td>
<td>7.4</td>
</tr>
<tr>
<td>PN_NO2</td>
<td>10.15</td>
<td>13.9</td>
</tr>
<tr>
<td>Wastewater_NO2-N</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Mix_Ammonia</td>
<td>5.943</td>
<td>9.3722</td>
</tr>
<tr>
<td>Mix_nitrite</td>
<td>7.8615</td>
<td>12.3382</td>
</tr>
<tr>
<td>Flow_Ammonia</td>
<td>230000</td>
<td>114000</td>
</tr>
<tr>
<td>PN flow</td>
<td>770000</td>
<td>886000</td>
</tr>
<tr>
<td>Moles of NH3</td>
<td>0.4245</td>
<td>0.669443</td>
</tr>
<tr>
<td>Moles of NO2</td>
<td>0.561536</td>
<td>0.8813</td>
</tr>
<tr>
<td>% Flow Influent</td>
<td>23</td>
<td>11.4</td>
</tr>
<tr>
<td>%Flow PN</td>
<td>77</td>
<td>88.6</td>
</tr>
<tr>
<td>Molar Ratio</td>
<td>0.755963</td>
<td>0.759608</td>
</tr>
</tbody>
</table>

Example calculation: R2 (50°C-2hr):

NH₃-N in Anammox reactor (mg/L) = ((Influent NH₃-N) \* Raw Wastewater Flow (\( \frac{L}{d} \)) + NH₃-N from PN (\( \frac{mg}{L} \)) * flow to PN (\( \frac{L}{d} \)))/Total flow

\[
= \frac{24.5 \times 230000 + 0.2 \times 770000}{1000000} = 5.9 \, mg/L
\]

NO₂-N in Anammox reactor (mg/L) = ((Influent NO₂-N) \* Raw Wastewater Flow (\( \frac{L}{d} \)) + NO₂-N from PN (\( \frac{mg}{L} \)) * flow to PN (\( \frac{L}{d} \)))/Total flow

\[
= \frac{10.15 \times 230000 + 13.9 \times 770000}{1000000} = 11.9 \, mg/L
\]
\[ \frac{0.2 \times 230000 + 10.15 \times 770000}{1000000} = 7.86 \text{ mg/L} \]

\[ \text{NH}_3-\text{N to Anammox (mole)} = \frac{\text{mg L} \text{ of NH}_3-\text{N to Anammox}}{\text{molecular weight of NH}_3-\text{N}} = \frac{5.9}{14} = 0.42 \text{ mole} \]

\[ \text{NO}_2-\text{N to Anammox (mole)} = \frac{\text{mg L} \text{ of NO}_2-\text{N to Anammox}}{\text{molecular weight of NO}_2-\text{N}} = \frac{7.86}{14} = 0.56 \text{ mole} \]

\[ \frac{\text{NH}_3-\text{N}}{\text{NO}_2-\text{N}} = \frac{0.42}{0.56} = 0.76 \]

**Table 2: Energy Calculation**

<table>
<thead>
<tr>
<th>1) Aeration Energy</th>
<th>Complete Nitrification (23°C)</th>
<th>55°C-0.5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (L/d)</td>
<td>1000000</td>
<td>886000</td>
</tr>
<tr>
<td>Observed Yield (gVSS/gCOD)</td>
<td>0.36</td>
<td>0.11</td>
</tr>
<tr>
<td>Avg. Effluent Ammonia (mg/L)</td>
<td>0.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Influent total COD (mg/L)</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>Effluent COD (mg/L)</td>
<td>30</td>
<td>64</td>
</tr>
<tr>
<td>Avg. Influent TKN (mg/L)</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Biomass synthesis (mgVSS/L)</td>
<td>72</td>
<td>18.26</td>
</tr>
<tr>
<td>Maximum nitrite accumulation ratio (NAR)</td>
<td>0</td>
<td>0.79</td>
</tr>
<tr>
<td>N needed for synthesis (mg/L)</td>
<td>7.2</td>
<td>1.82</td>
</tr>
<tr>
<td>Ammonia Oxidized (mg/L)</td>
<td>29.3</td>
<td>27.8</td>
</tr>
<tr>
<td>Oxygen Saved (mgO₂/L)</td>
<td>-</td>
<td>25.1</td>
</tr>
<tr>
<td>Oxygen saved (kgO₂/d)</td>
<td>-</td>
<td>22.2</td>
</tr>
<tr>
<td>Density of air (kg/m³)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Oxygen content (%)</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Oxygen transfer rate</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Air flow saved (m³/d)</td>
<td>-</td>
<td>804.7</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Oxygen required for complete nitrification (mgO₂/L)</td>
<td>134</td>
<td>127</td>
</tr>
<tr>
<td>Oxygen saved for complete nitrification (kgO₂/d)</td>
<td>134</td>
<td>113</td>
</tr>
<tr>
<td>Air flow required for complete nitrification (m³/d)</td>
<td>4851</td>
<td>4075</td>
</tr>
<tr>
<td>Airflow required after PN (m³/d)</td>
<td>-</td>
<td>3270</td>
</tr>
<tr>
<td>Mechanical efficiency of diffuser (%)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Tank depth (m)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>P discharge (atm)</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Aeration Energy (kWh/d)</td>
<td>73</td>
<td>53</td>
</tr>
</tbody>
</table>

**2) Heat Energy**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSS (mg/L)</td>
<td>-</td>
<td>1440</td>
</tr>
<tr>
<td>HRT (d)</td>
<td>-</td>
<td>0.667</td>
</tr>
<tr>
<td>Effective volume of WW in the reactor (m³/d)</td>
<td>-</td>
<td>667</td>
</tr>
<tr>
<td>Biomass heated (MLSS) in the reactor, kg/d</td>
<td>-</td>
<td>960</td>
</tr>
<tr>
<td>RAS concentration (Assumed, 10kg/m³)</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Density of water (kg/m³)</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Heated RAS flow (m³/d)</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>Heat rate Qheat, MJ/d</td>
<td>-</td>
<td>269</td>
</tr>
<tr>
<td>Heat input for heating once in a day (kWh/d)</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Heat input for once in a 10 day (kWh/d)</td>
<td>0</td>
<td>7.5</td>
</tr>
<tr>
<td>Heat input for once in a SRT turnover, 7 days (kWh/d)</td>
<td>0</td>
<td>10.6</td>
</tr>
</tbody>
</table>

**3) Total Energy (Aeration+Heat)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>For daily heating (kWh/d)</td>
<td>73</td>
<td>148</td>
</tr>
<tr>
<td>For once in a 10-day heating (kWh/d)</td>
<td>73</td>
<td>60</td>
</tr>
<tr>
<td>For heating once in a 1-SRT turnover (kWh/d)</td>
<td>73</td>
<td>64</td>
</tr>
</tbody>
</table>

Example: **Aeration energy: For 55°C-0.5hr heating**

\[
\text{Biomass synthesis} = \text{Yield} \times (\text{Influent total COD} - \text{effluent soluble COD})
\]

\[
= 0.11 \times (230 - 64) = 18.3 \text{ mg/L}
\]
Nitrogen needed for biomass synthesis = 10% of synthesis = 0.1 * 18.3 = 1.83 mg/L

Ammonia Oxidized = Influent TKN - Effluent Ammonia-N - synthesis

= 37 - 1.83 - 7.4 = 27.8 mg/L

Oxygen saved (mg/L) = NAR * Ammonia oxidized * 4.75 * 0.25

= 27.8 * 0.79 * 4.57 * 0.25 = 25.1 mg/L

Oxygen saved (KgO2/d) = 25.1 mg/L * 886 m3/d = 22.21 kgO2/d

\[
Air\ flow, Q_{air} = \frac{Oxygen\ saved}{Oxygen\ transfer\ rate \times \%\ 02\ in\ air \times\ density\ of\ air} = \frac{22.21}{0.1 \times 0.23 \times 1.2} = 804.5 m^3/d
\]

Oxygen required for complete nitrification = Ammonia oxidized * 4.57 * 886 m3/d = 112 kgO2/d

Airflow for complete nitrification = 112 / (Oxygen transfer rate * 0.23 * 1.2) = 4075 m3/d

Airflow after Nitrite accumulation = Airflow for complete nitrification - Airflow for PN = 4075 - 804.5 = 3270 m3/d

\[
P_{discharge} = (1 + 0.0968 \times \text{depth}) \times 0.21 \text{ atm} = 0.29 \text{ atm}
\]

Aeration Energy required =

\[
Q_{air} \times \frac{1 \text{ mol}}{0.0224 \text{ m}^3} \times RT \left[ \left( \frac{P_d}{P_{\text{pinlet}}} \right)^{0.283} - 1 \right] \times 2.78 \times 10^{-7} \text{ KWH/J}
\]

= 53 kWh/d

Aeration energy for complete nitrification:

\[
Q_{air} \times \frac{1 \text{ mol}}{0.0224 \text{ m}^3} \times RT \left[ \left( \frac{P_d}{P_{\text{pinlet}}} \right)^{0.283} - 1 \right] \times 2.78 \times 10^{-7} \text{ KWH/J}
\]

= 73 kWh/d

Heat Energy: For 55°C-0.5hr heating

HRT in the system = 0.667 days
Volume of wastewater in the reactor = $1000 \times 0.667 = 667$ m$^3$/d

Biomass in the reactor (MLSS) = $667 \times 1440 \times \text{mg/L}/1000 = 960$ kg/d

Considering the RAS concentration 10,000 mgTSS/L =10 kg/m$^3$

The biomass flow=$940/10=96$m$^3$/d

Heat energy = $96$ m$^3$/d* $1000$ kg/m$^3 \times 4.2$ Kj/Kg°C× (55-23) *0.5/24 = 75 kWh/d

**Heat Optimization calculation:**

The aeration energy would be same, but the heating is provided in every 10 days

Heat Energy = $75/10=7.5$ kWh/d

Energy requirement condition:

- Aeration energy required without PN=73 kWh/d
- Total energy (aeration plus heating once a day) required=74+53=128 kWh/d
- Total energy (aeration plus heating once in 10 days) = 53+7.5= 61 kWh/d

Sensitivity analysis:

- Total energy (aeration plus heating once in a SRT turnover, 7 days) = 53+11=64 kWh/d
- Total energy (aeration plus heating once in 4 days) =53+19=72 kWh/d
- Total energy if WW temperature is 12°C
  1.  Aeration plus heating once in 10 days=53+10= 63 kWh/d
  2.  Aeration plus heating once in 7 days=53+13.3=66.2 kWh/d

**Table-3**

<table>
<thead>
<tr>
<th>effective volume (m$^3$)</th>
<th>Scenario 1: Sidewalls and top slab insulated</th>
<th>50°C-1hr</th>
<th>55°C-0.5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>667 667</td>
<td>Sidewalls</td>
<td>Sidewalls</td>
</tr>
<tr>
<td>Length, Width, height(m)</td>
<td>17,10,4</td>
<td>Top slab</td>
<td>Top slab</td>
</tr>
<tr>
<td>Heat loss efficiency (%)</td>
<td>80 80</td>
<td>17,10,4</td>
<td>17,10,4</td>
</tr>
<tr>
<td>Wall thickness (m)</td>
<td>0.3 0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Coefficient of heat conduction (W/m.K)</td>
<td>0.1 0.1</td>
<td>0.1 0.1</td>
<td>0.1 0.1</td>
</tr>
<tr>
<td>Thickness of sidewall and top slab</td>
<td>0.3 0.3</td>
<td>0.3 0.3</td>
<td>0.3 0.3</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>386</td>
<td>154</td>
<td>386</td>
</tr>
<tr>
<td>----------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Power (W)</td>
<td>222.3</td>
<td>88.7</td>
<td>259.4</td>
</tr>
<tr>
<td>Heat loss energy (kWh)</td>
<td>0.22</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Total Energy for PN (kWh)</td>
<td>63</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Total heat energy requirement</td>
<td>63.3</td>
<td>60.2</td>
<td></td>
</tr>
<tr>
<td>Percent heat loss (%)</td>
<td>0.50%</td>
<td>0.40%</td>
<td></td>
</tr>
</tbody>
</table>

Scenario 2: Sidewalls and top slab was not insulated

<table>
<thead>
<tr>
<th>Effective volume (m³)</th>
<th>667</th>
<th>667</th>
<th>667</th>
<th>667</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, Width, height (m)</td>
<td>17,10,4</td>
<td>17,10,4</td>
<td>17,10,4</td>
<td>17,10,4</td>
</tr>
<tr>
<td>Heat loss efficiency (%)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Wall thickness (m)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Coefficient of heat conduction (W/m.K)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Thickness of sidewall and top slab</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>386</td>
<td>154</td>
<td>386</td>
<td>154</td>
</tr>
<tr>
<td>Power (W)</td>
<td>4864</td>
<td>1552</td>
<td>5674</td>
<td>1811</td>
</tr>
<tr>
<td>Heat loss energy (kWh)</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total Energy for PN (kWh)</td>
<td>69.5</td>
<td>63.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total heat energy requirement</td>
<td>9.35%</td>
<td>5.87%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scenario 3: Sidewalls not insulated and the top is open

<table>
<thead>
<tr>
<th>Effective volume (m³)</th>
<th>667</th>
<th>667</th>
<th>667</th>
<th>667</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, Width, height (m)</td>
<td>17,10,4</td>
<td>17,10,4</td>
<td>17,10,4</td>
<td>17,10,4</td>
</tr>
<tr>
<td>Heat loss efficiency (%)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Wall thickness (m)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Coefficient of heat conduction (W/m.K)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Thickness of sidewall and top slab</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Area (m²)</td>
<td>386</td>
<td>154</td>
<td>386</td>
<td>154</td>
</tr>
<tr>
<td>Power (W)</td>
<td>4864</td>
<td>4990</td>
<td>5674</td>
<td>6426</td>
</tr>
<tr>
<td>Heat loss energy (kWh)</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total Energy for PN (kWh)</td>
<td>63</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total heat energy requirement</td>
<td>72.9</td>
<td>66.1</td>
<td></td>
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<tr>
<td>Percent heat loss (%)</td>
<td>13.5%</td>
<td>9.2%</td>
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<td></td>
</tr>
</tbody>
</table>
Appendix C: Supplementary information of Chapter 5

\[ y = 5.0676x + 7.0257 \]
\[ R^2 = 0.9642 \]

\[ y = 0.503x + 0.584 \]
\[ R^2 = 0.1861 \]

\[ y = 4.9196x + 7.9342 \]
\[ R^2 = 0.8844 \]

\[ y = -5.66x + 8.32 \]
\[ R^2 = 0.9289 \]
\[ y = 4.1221x + 8.7812 \]
\[ R^2 = 0.8438 \]

\[ y = -1.5071x + 5.4998 \]
\[ R^2 = 0.8572 \]

\[ y = 4.9518x + 11.512 \]
\[ R^2 = 0.9415 \]

\[ y = -5.0162x + 12.738 \]
\[ R^2 = 0.9978 \]

\[ y = -0.1585x + 1.0572 \]
\[ R^2 = 0.2568 \]

\[ y = 3.0271x + 7.2433 \]
\[ R^2 = 0.9811 \]
\[ y = -4.44x + 7.33 \quad R^2 = 0.8154 \]

![Graph of NH4 concentration over time with regression equation and R^2 value.](image)

\[ y = 4.6162x + 6.3622 \quad R^2 = 0.9906 \]

![Graph of NO3 concentration over time with regression equation and R^2 value.](image)

\[ y = 0.03x + 0.93 \quad R^2 = 0.0084 \]

![Graph of NO2 concentration over time with regression equation and R^2 value.](image)

\[ y = 3.8703x + 10.403 \quad R^2 = 0.8798 \]

![Graph of NO2 concentration over time with regression equation and R^2 value.](image)
\[ y = \text{-}4.4081x + 11.669 \quad \left( R^2 = 0.9522 \right) \]

\[ y = 1.8081x + 3.2311 \quad \left( R^2 = 0.9549 \right) \]

\[ y = 1.5446x + 7.1109 \quad \left( R^2 = 0.7363 \right) \]

\[ y = 3.3527x + 10.342 \quad \left( R^2 = 0.8829 \right) \]
$y = -1.5256x + 9.8103$
$R^2 = 0.9134$

$y = 0.6358x + 2.9207$
$R^2 = 0.7393$

$y = 1.6028x + 6.3948$
$R^2 = 0.8399$

$y = 2.1407x + 9.4413$
$R^2 = 0.8753$
Figure S1: Specific rates
Figure S2: Confirmation of complete nitrification in control reactor in entire operation
Figure S3: MLSS and MLVSS in heated reactors
Figure S4: Prediction of COD in PN reactors
Figure S5: Prediction of phosphorus in heated reactors
Curriculum Vitae

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2022-2023

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2021

The Ross and Jean Clark Scholarship
2022

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1. Niema, A., Effect of Thermal Shock and Sustained Heat Treatment on Mainstream Partial Nitrification and Microbial Community in Sequencing Batch Reactors

   http://dx.doi.org/10.1080/09593330.2022.2082324