INVESTIGATION OF ORGANIC NITROGEN ACTIVITY IN BIOLOGICAL PROCESSES OF WATER RESOURCE RECOVERY FACILITIES

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

The decrease in effluent inorganic nitrogen concentration in water resource recovery facilities (WRRFs) has been effectively achieved by including or finetuning the biological nutrient removal process. However, soluble organic nitrogen (sON) remains relatively unchanged, resulting in no reduction in the proportion of sON in effluent nitrogen. Therefore, for some WRRFs subjected to stringent nitrogen discharge limits, the removal of sON is crucial. Furthermore, the interest in sON is growing due to its impact on eutrophication, close relationship with the membrane fouling in wastewater treatment and the formation of nitrogenous disinfection byproducts in drinking water treatment. The overall aim of this research work was to determine the limits and capabilities pertaining to the removal and production of organic N in two different biological wastewater treatment processes, activated sludge process and moving bed biofilm reactors (MBBRs).

Three research tasks were performed. In the first task, the effect of sludge retention time (SRT) on the production of organic nitrogen fractions (particulate, colloidal and soluble) and the biodegradability of produced sON in an activated sludge process was investigated. SRT influenced each fraction (particulate, colloidal and soluble) of organic nitrogen along with the biodegradability of effluent sON. The second task investigated sON activity in batch reactors mimicking nitrifying MBBRs. Although net production of sON was observed, both production and ammonification coexisted in the reactors which regulated the sON concentration. Organic carbon bioavailability and/or ammonia concentration were found to influence the production and ammonification of sON. The task also identified the variation in the bacterial activity in the biofilm during nitrification when exposed to different C/N ratios in the influent. The third task examined sON activity under heterotrophic and nitrifying sludge to identify how one sludge type is better

than the other in removing sON under a simple aerobic reactor. Higher degradation of sON was identified in the presence of heterotrophic sludge than nitrifying sludge. Results from this dissertation research provide a better understanding of sON activity for two different biological wastewater treatment processes, which is critical for optimizing the removal of sON in WRRFs.

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It is a well-known fact that completing a doctorate can never be credited to just one person's effort. Being aware of all the people whose contributions have allowed me to come to this point, it is next to impossible to mention everyone's name here. Irrespective, I will be forever indebted to all the people I shared my journey with because I realize how far I have come with the help of the philosophies shared by these people, which was not possible otherwise.

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DEDICATION

To my:

Almighty *(Shirdi Sai Baba)*

Mother *(Mrs. Anita Joshi)*, Father *(Mr. J.B. Joshi)*

Brother *(Rohit Joshi)*

and

Husband *(Ankur Bhardwaj)*

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LIST OF ABBREVIATIONS

µg/L.....microgram per liter

µg .....microgram

µm .....micrometer

AAO .....anaerobic, anoxic, oxic

AbsON .....bioavailable sON

AHL .....acyl homoserine lactones

Anammox.....anaerobic ammonium oxidation

ANOVA .....analysis of variance

AO.....anaerobic, oxic

AOA .....ammonia oxidizing archaea

AOB .....ammonia oxidizing bacteria

AS .....activated sludge

ASP .....activated sludge process

ATP.....adenosine triphosphate

BAP.....biomass associated product

BNR .....biological nutrient removal

BOD .....biochemical oxygen demand

bsON/sON.....soluble organic nitrogen biodegradability

bsON .....biodegradable sON

C/N.....carbon to nitrogen ratio

C.....colloidal

°C .....degree Celsius

cCOD .....colloidal COD

CDF.....cumulative distribution functions

COD/N .....chemical oxygen demand/nitrogen ratio

COD .....chemical oxygen demand

cON .....colloidal organic nitrogen

CSTR.....continuous stirred tank reactor

d.....day

DCAA .....dissolved combined amino acid

DFAA.....dissolved free amino acid

DMA .....dimethylamine

DNF.....denitrification biofilters

DO.....dissolved oxygen

EDTA.....ethylenediaminetetraacetic acid

EPS.....extracellular polymeric substance

F/M.....food to microorganism ratio

FE.....final effluent

FF .....flocculation filtration

G.....gram

HCl.....hydrochloric acid

HPO-AS .....high purity oxygen activated sludge process

HR .....high range

h.....hour

HRT.....hydraulic retention time

HS .....heterotrophic sludge

HTC.....high-temperature combustion

L/d.....liter/day

L .....liter

LC-OCND.....liquid chromatography combined with organic carbon and nitrogen detection

LR .....low range

m2 .....square meter

m3 .....cubic meter

MBBR.....moving bed biofilm reactor

MBR.....membrane bioreactor

mg/L.....milligram per liter

MGD .....million gallons per day

min .....minute

MLSS .....mixed liquor suspended solids

MLVSS .....mixed liquor volatile suspended solids

MN .....Minnesota

MPa.....mega pascal

msON .....process-derived sON

MW .....molecular weight

N.....nitrogen

N2.....dinitrogen

NaHCO3 .....sodium bicarbonate

nbsON .....non-biodegradable sON

NH3.....ammonia

NH4Cl.....ammonium chloride

nm .....nano meter

NO2.....nitrite

NO3- .....nitrate

NOB .....nitrite oxidizing bacteria

NS .....nitrifying sludge

ON.....organic nitrogen

P .....particulate

pCOD .....particulate COD

PE.....primary effluent

pON.....particulate organic nitrogen

RAC .....risk-aversion coefficient

RLU.....relative light unit

S .....soluble

SAFL.....sludge alkaline fermentation liquid

SBR.....sequencing batch reactor

sCOD.....soluble COD

SD .....standard deviation

SE.....secondary effluent

SEC .....size-exclusion chromatography

sIN.....soluble inorganic nitrogen

SMP.....soluble microbial products

sON .....soluble organic nitrogen

SRT .....solids retention time

SWW.....synthetic wastewater

T .....total

TCOD.....total COD

TE.....tertiary effluent

TF.....trickling filter

TKN .....total Kjeldahl nitrogen

TN .....total nitrogen

TNT.....test and tube

TON .....total organic nitrogen

TSN.....total soluble nitrogen

TSS.....total suspended solids

UAP.....utilization associated product

UV.....ultraviolet

VSS .....volatile suspended solids

WRRF .....water resource recovery facility

WWTP .....wastewater treatment plant

ZnSO4.....zinc sulfate

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CHAPTER 1: GENERAL INTRODUCTION

1.1. Background

The decrease in effluent inorganic nitrogen concentration in water resource recovery facilities (WRRFs) has been effectively achieved by the advancement of wastewater treatment technologies, such as the retrofitting of a conventional bioprocess to biological nitrogen removal (BNR) process (Eom et al., 2017). However, soluble organic nitrogen (sON) remains unchanged, resulting in no reduction in the proportion of sON in effluent nitrogen (Pehlivanoglu-Mantas and Sedlak, 2006; Simsek et al., 2016). Therefore, the removal of sON is an objective needed to comply with the requirements of a tightened stringency of nitrogen discharge regulations (Simsek et al., 2012; Hendriks and Langeveld, 2017). Furthermore, the interest in sON is growing due to its impact on eutrophication (Qin et al., 2015), close relationship with the membrane fouling (Xu, Bellona et al., 2010) in wastewater treatment and the production of nitrogenous disinfection byproducts (N-DBPs) in drinking water treatment (Pehlivanoglu-Mantas and Sedlak, 2008; Hu et al., 2020; Kiattisaksiri et al., 2020). In surface water, sON concentration ranges from 0.07 to 0.62 mg N/L with a median value of 0.30 mg N/L (Xu, Ye et al., 2011; Krasner et al., 2012). Table 1.1 displays characteristics of sON in municipal wastewater and effluent from WRRFs.

Studies have shown that different biological processes can remove different levels of sON (Parkin and McCarty, 1981a; Simsek et al., 2012). About 60-70% removal of influent sON by activated sludge process (ASP) was reported (Parkin and McCarty, 1981a), whereas 37-50% removal was achieved by a two-stage trickling filter (TF) process (Simsek et al., 2012). Several studies on activated sludge (AS) have reported the influences of solids retention time (SRT) (Simsek et al., 2016), chemical oxygen demand to N ratio (COD/N or C/N) (Hu, Liao, Shi et al., 2018), and mixed liquor suspended solids (MLSS) concentration (Hu et al., 2020) on organic

nitrogen (ON) removal during biological treatment. For secondary wastewater effluent, sON concentration ranges from 0.30 to 3.33 mg N/L (Pehlivanoglu-Mantas and Sedlak, 2008; Chen et al., 2011; Simsek et al., 2013). Based on these previous studies, biological processes do not completely remove sON. Additional removal of sON is achievable through physicochemical process(es) (Parkin and McCarty, 1981a; Czerwionka et al., 2014).

Table 1.1. Characteristics of sON in municipal wastewater and WRRF effluent (Source: Zheng et al., 2021)

sON characteristics	Municipal wastewater	WRRF effluent	Reference
Total concentration	3-7 mg N/L	0.5-1.7 mg N/L	Pagilla et al., 2008; Sattayatewa et al., 2009; Czerwionka et al., 2012; Huo et al., 2013
sON/TSN	2-40 %	6-84%	Sattayatewa et al., 2010; Czerwionka et al., 2012; Liu et al., 2012; Hu et al., 2016; Eom et al., 2017
Composition	Proteins, amino acids and humic-like substances	Amino acids, EDTA and specific proteins newly produced	Pehlivanoglu-Mantas and Sedlak, 2008; Huo et al., 2013
MW distribution	LMW sON: 4.2-4.4 mg N/L	50-66% are LMW, ranging from 1.5 to 3.5 mg N/L	Eom et al., 2017; Lu et al., 2018
Hydrophobicity	N/A	80-85% are hydrophilic	Pehlivanoglu-Mantas and Sedlak, 2008; Huo et al., 2013
Bioavailability	N/A	Hydrophilic fractions are highly bioavailable (40-85%)	Liu et al., 2012; Huo et al., 2013
N-DBPs formation potential	N/A	Overall N-DBP formation potential: 16 mg/mg sON	Liu et al., 2019

Arnaldos and Pagilla (2010) reported that coagulation and microfiltration were efficient processes for sON removal. They investigated the effect of enhanced coagulation using different aluminum (Al(III)) dosages (0.8, 1.6, 2.4, 3.2 and 4.1 mg Al(III)/L) followed by microfiltration (using a 0.22 µm pore size filter) of the settled effluent on sON removal. Maximum sON removal (69%) was achieved by coagulation at 3.2 mg Al(III)/L and microfiltration (Arnaldos and Pagilla, 2010). Other processes, such as ion exchange, membrane processes (microfiltration, reverse osmosis), and chemical oxidation processes (e.g., with sodium permanganate, hydrogen peroxide, chlorination, ozonation, UV radiation and the process of Fenton) have been found to be less

effective (Parkin and McCarty, 1981a; Westerhoff and Mash, 2002; Pehlivanoglu-Mantas and Sedlak, 2006).

Effluent sON from biological treatment processes mainly includes two groups based on the source of its production: influent-derived nitrogenous compounds (influent-derived sON); and microorganism-derived nitrogenous compounds (process-derived sON). The influent-derived sON is the result of recalcitrant ON, which has not been biodegraded or removed during wastewater treatment. Process-derived sON is generated from microbial metabolism in the biological treatment processes, such as soluble microbial products (SMP) (Pehlivanoglu-Mantas and Sedlak, 2006; Simsek et al., 2016; Hu et al., 2020). Since process-derived sON is contributed by biomass growth and decay during the biological treatment processes, process-derived sON is unavoidable and more closely related to operational parameters than influent-derived sON (Sattayatewa et al., 2009). Approximately 67% of the effluent sON was residual from the influent wastewater, and 33% biological degradation products (Randtke et al., 1978; Novak, 2006). However, the extent of biological production of sON varies from one biological system to the next and the 67:33 estimate is not applicable to all WRRFs (Bratby et al., 2008). Therefore, reducing the formation of process-derived sON in the biological treatment processes is desirable to facilitate strict nitrogen discharge limits and to protect the receiving waters.

Parkin and McCarty (1981a) studied the effect of process control parameters such as MLSS concentration, organic loading, and aeration time on sON production during the treatment process. Higher MLSS concentrations led to faster sON degradation. In addition, at high MLSS concentrations, sON removal reached its maximum. Increase in organic loading of glucose, acetate, and a glucose-acetate mixture in the bioreactor increased sON production. The sON production was independent of aeration time. More sON was excreted during the logarithmic

growth stage compared to the stationary stage and sON produced during the logarithmic stage was removed during the stationary stage. Two-thirds of the refractory sON in the effluent of a conventional activated sludge plant was from the influent which passed through untreated while the rest was produced during the treatment process. About forty percent of effluent sON may be produced during the biological treatment (Parkin and McCarty 1981b). Sattayatewa et al. (2009) focused on determining whether the operating conditions (anoxic versus aerobic) influenced sON production in the bioreactor (Bardenpho process). sON production was reported in first anoxic zone and to a lesser extent in the first aerobic basin.

1.2. Research Problem and Justification

1.2.1. Effect of SRT on Organic Nitrogen Production and Biodegradability

The ON can be as high as 80 percent of the effluent total nitrogen (TN) for WRRFs producing low effluent TN (Sattayatewa et al. 2009; Chen et al., 2011; Simsek et al., 2016; Li et al., 2017). It consists of particulate (pON), colloidal (cON) and soluble (sON) fractions. Since the majority of pON is effectively removed through settling processes in WRRFs, cON and sON become the dominant forms of organic nitrogen in the effluent. Since effluent ON could be the majority nitrogen fraction in very low effluent TN (<3 mg N/L), identifying the contribution of cON versus sON fractions to effluent ON is very important. The size fractionation information can help the WRRF target the removal of specific fractions after the biological process. For example, employing granular activated carbon or chemical precipitation can help in sON removal, whereas anaerobic and anoxic compartments can help reduce effluent cON fractions (due to influent dilution and recirculated mixed liquor) (Arnaldos and Pagilla, 2010; Czerwionka et al., 2012). Although several studies have investigated the ON fractions in biological treatment processes (Pagilla et al., 2008; Mekinia et al., 2009; Sattayatewa et al., 2009; Pagilla et al., 2011; Czerwionka

et al., 2012), so far none has examined the effect of SRT on effluent ON fractions in an ASP using chemostat bioreactors. Therefore, there is a need to examine the effect of SRT on the production of ON and its fractions in an ASP.

sON is exceptionally complex and has been medially characterized. Urea, amino acids, peptides, amino sugars, purines, pyrimidines, and amides are few of the known sON compounds. Most of these compounds are biodegradable (can be mineralized to ammonia). sON in surface water stimulates algal growth and depletes dissolved oxygen (DO) if it undergoes ammonification and nitrification (Simsek et al., 2016). Previous studies have found that in the influent approximately 40 - 60% of sON is biodegradable whereas, in secondary effluent around 25 -33 % of sON is biodegradable (Murthy et al., 2006; Khan et al., 2009). Impact of SRT (0.7, 2, 3, 4, 5, 7, 8 and 13 d) on sON and its biodegradability was studied in treated wastewater using bench-scale chemostat bioreactors (Simsek et al., 2016). The authors reported that effluent sON biodegradability decreased with increasing SRT, highlighting the benefit of high SRTs in terms of producing effluent with less biodegradable sON (bsON), leading to relatively less oxygen consumption and nutrient support in receiving waters. However, the relative effectiveness of different biological treatment process technologies on degrading process-derived ON has not been studied. Therefore, there is a need to examine process derived sON biodegradability under different processes and conditions.

1.2.2. Production and Removal of sON by MBBR Process

Many studies have described the role of different operational conditions including SRT, temperature, bioreactor hydraulics and plant perturbations in ASP on degradation of sON (O'Shaughnessy et al., 2006; Simsek et al., 2016; Hu, Liao, Shi et al., 2018; Hu et al., 2019). Harper and Jenkins (2003) showed that although total effluent soluble nitrogen (sON + ammonia

+ nitrite + nitrate) was similar for anaerobic-aerated systems, and completely aerobic systems, effluent sON was significantly lower, at least 0.5 mg N/L lower, for anaerobic-aerated systems, compared with completely aerobic systems. Effluent ON concentration was reported to be influenced by both uncontrollable factors (temperature, start-up of unit processes) as well as controllable factors (SRT, chemical addition, changes in process operation) (O'Shaughnessy et al., 2006). Bratby et al. (2008) noted that sON concentration increases through aerobic biological treatment. Sattayatewa et al. (2009) observed that sON concentration increased under the anoxic zone of a BNR WWRF due to biomass metabolic and catabolic activities while a significant sON decrease was observed within the oxic zone. Sattayatewa et al. (2010) reported that majority of ON removal occurred in the biological treatment process of all four BNR plants studied. Factors including SRT, C/N ratio and temperature were reported to affect the effluent sON concentration (Hu, Liao, Shi et al., 2018; Hu, Liao, Geng et al., 2018; Hu et al., 2019). The majority of the research work related to sON degradation has been focused on conventional ASP.

In an MBBR, the biofilm activity plays critical roles in the biological conversion of nutrients. The C/N ratio is known as an important operational parameter that significantly affects biofilm activity (Zhou and Xu, 2020). Examining the influence of C/N ratio on sON degradation could lead to improvement of the nitrogen removal performance of MBBR. Simsek et al. (2013) investigated the fate of bsON and bioavailable sON (AbsON) in a full-scale WRRF consisting of both ASP and MBBR. Results showed that ASP removed 29% sON whereas MBBR removed only 4% sON. The study suggested that low C/N ratio might have affected the sON removal in the MBBR process along with other possibilities (production of soluble microbial products and hydrolysis of particulate organic matter entrapped in MBBR media) (Simsek et al., 2013). So far, no study has investigated the effect of C/N ratios on sON degradation in an MBBR process. Hence,

identifying the influence of C/N ratio on sON degradation as well as production can help in enhancing the efficiency of WRRF equipped with MBBR process in achieving low TN discharge limits.

1.2.3. Roles of Heterotrophic and Nitrifying Sludge in the Degradation of sON

The microbial consortia in wastewater treatment bioreactors are the combination of heterotrophic and autotrophic bacteria. As mentioned earlier, the bacterial population can be influenced by different parameters which can affect the biological processes in a bioreactor (Zielinska et al., 2012). Therefore, it will be interesting to examine how different microbial populations can influence sON degradation. To this date, no known study has evaluated sON degradation under different types of sludge (heterotrophic versus nitrifying) in an aerobic process. Understanding sON biodegradation as well as production under different types of sludge will help identify strategies for improving treatment efficiency at WRRFs required to achieve low TN discharge limits.

1.3. Research Aim, Tasks, Objectives, Hypotheses, and Approaches

The overall aim of this research work is to determine the limits and capabilities pertaining to the removal and production of organic N during two different biological wastewater treatment processes, i.e., ASP and MBBR process. Specifically, the three tasks below were conducted to achieve the overall aim of this dissertation. Each task has a dedicated objective and hypothesis as listed below. A brief experimental approach for each task is also provided.

1.3.1. Task I. Investigating ON Production in ASP: Size Fraction and Biodegradability

- *Objective:* Investigate the effect of SRT on the production of ON and its fractions in an activated sludge process.

- *Hypothesis:* Organic nitrogen production and its biodegradable portion decrease with the increase in SRT.
- *Approach:* To eliminate the role of influent-derived ON, activated sludge bioreactors were fed with ammonia only as a nitrogen source.

1.3.2. Task II. Investigation of the Production and Degradation of sON by Nitrifying Biofilm in an MBBR Process

- *Objectives*:
  a) Examine the effect of influent organic carbon on sON production; and
  b) Investigate the effect of C/N ratio (high, medium, and low) on sON degradation in a MBBR system.
- *Hypotheses*:
  a) sON production increases in the absence of influent organic carbon in the MBBR process.
  b) sON degradation is enhanced under low C/N ratios in the MBBR process.
- *Approaches:* Real media or biofilm carriers collected from an MBBR were used in bench-scale bioreactors for both objectives.
  a) To examine the effect of influent COD on sON production, MBBR media were fed without influent COD and ON to eliminate the role of influent derived COD and ON.
  b) To examine the influent sON degradation, bench-scale bioreactors received actual wastewater samples with different C/N ratios.

1.3.3. Task III. Comparison of sON Activities under Heterotrophic and Nitrifying Sludge

- *Objectives*:
    a) To evaluate the effect of food to microorganisms ratio (F/M) on the removal of sON by heterotrophic and nitrifying sludge.
    b) To quantify sON removal under different influent C/N ratios by heterotrophic and nitrifying sludge.
    c) To investigate the production of sON in the absence of influent ON and COD by heterotrophic and nitrifying sludge.
- *Hypotheses*:
    a) sON removal increases with increasing F/M ratio under both heterotrophic and nitrifying sludge.
    b) sON removal increases with increasing C/N ratio under both heterotrophic and nitrifying sludge.
    c) Heterotrophic sludge has higher sON removal capability than nitrifying sludge.
    d) Production of sON is higher under nitrifying sludge than heterotrophic sludge.
- *Approaches*:
    a) Two sets of bioreactor series were set up and fed with primary influent. Each series received three different concentrations of heterotrophic and nitrifying sludge to vary F/M ratio.
    b) Wastewater samples collected from three different stages of a WRRF representing high, medium, and low C/N ratios were fed to bioreactors, which were inoculated with heterotrophic and nitrifying sludge, respectively.
    c) To examine the production of sON, reactors were fed without ON and COD.

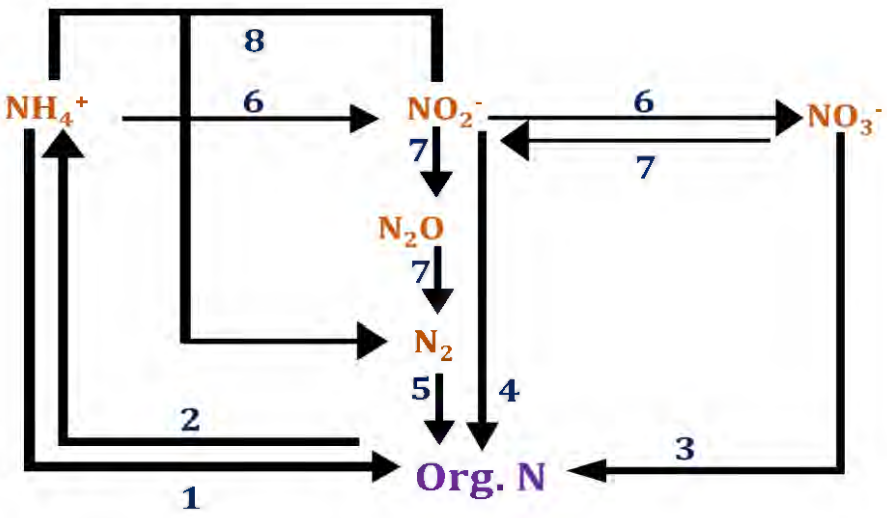
1.4. Dissertation Organization

This dissertation is divided into six chapters. This chapter (Chapter 1) includes background, research problem and justifications followed by research aim, objectives, hypotheses and approaches for three research tasks and dissertation organization (this subsection). A review of relevant literature about organic nitrogen in municipal wastewater, its fractions, biodegradability and, fate and removal under different biological processes is presented in Chapter 2. Chapter 3 describes sON production and biodegradation in an activated sludge process using chemostat reactors. The work described in Chapter 3 is based on a manuscript entitled "Investigating Organic Nitrogen Production in Activated Sludge Process: Size Fraction and Biodegradability." This manuscript has been published in *Science of the Total Environment* authored by Ruchi Joshi Bhardwaj, Eakalak Khan, Murthy Kasi and Tanush Wadhawan (Joshi et al., 2021a). The work described in Chapter 4 is based on a manuscript entitled "Production and Removal of Soluble Organic Nitrogen by Nitrifying Biofilm." This manuscript has been published in *Journal of Environmental Chemical Engineering* (Joshi et al., 2021b) authored by the same team as mentioned above. Chapter 5 compares sON activity under heterotrophic and nitrifying sludge and there is a plan to produce a manuscript entitled "Comparison of Soluble Organic Nitrogen Activities under Heterotrophic and Nitrifying Sludge" from this chapter for submission to a peer-reviewed journal. Lastly, Chapter 6 presents overall conclusions and recommendations for future work.

CHAPTER 2: LITERATURE REVIEW

2.1. Nitrogen in Municipal Wastewater

Multiple methods have been employed to segregate nitrogen in raw wastewater into many fractions such as organic, inorganic, biodegradable, and non-biodegradable. The inorganic forms of nitrogen (ammonia, nitrite and nitrate) in wastewater have been extensively examined. Ammonia (NH_3) can be toxic to aquatic life and even reduce the DO in the water. Similarly, nitrite (NO_2^-) can cause excess algae growth in the receiving water bodies. The excess algae consume oxygen and in turn adversely affect the aquatic life as well as water quality. Nitrate (NO_3^-) can cause methemoglobinemia in infants. Nitrogen in domestic wastewater comprises of around 60-70% NH_3-N and 30-40% ON. Ammonia is mostly contributed by urea, which degrades rapidly to NH_3in wastewater influent. Organic nitrogen in the municipal wastewaters is contributed by urine, feces, industrial process effluents, urban runoffs (animal excreta, plant matter, fertilizers, chemicals), microorganisms (bacteria, fungi, algae) and their exudates. Organic nitrogen can be in the forms of urea, uric acid, amino acids, cationic detergents, and proteins. Four major pathways have been identified for the formation of ON, namely NH_3 assimilation, nitrate assimilation, nitrite assimilation and nitrogen fixation (Figure 2.1).



1	Ammonia Assimilation	2	Ammonification
3	Nitrate Assimilation	4	Nitrite Assimilation
5	Nitrogen Fixation	6	Nitrification
7	Denitrification	8	Deammonification

Figure 2.1. Microbial pathways leading to the production of organic nitrogen (Adapted from Wadhawan, 2014).

2.2. Organic Nitrogen

Organic nitrogen is found to be the major nitrogen fraction in the effluent for WRRFs that are trying to achieve low effluent TN limits i.e.,, less than 5 mg N/L. The ON can be as high as 80% of the effluent TN for WRRFs producing low effluent TN (Sattayatewa et al. 2009; Chen et al., 2011; Simsek et al., 2016). The total ON (TON) consists of pON, cON and sON fractions (Figure 2.2). Since the majority of pON is effectively removed through settling processes in WRRFs, cON and sON become the dominant forms of ON in the effluent. About 45% of effluent ON was reported as cON (Sattayatewa et al., 2010), while sON ranged between 20 and 85% of the effluent ON (Pagilla et al., 2006, 2008; Pehlivanoglu-Mantas and Sedlak, 2004; Simsek et al.,

2016). Moreover, the concentrations of cON and sON combined in the final effluent can range between 0.5 and 3 mg N/L, posing a major challenge in achieving low levels of TN (Sattayatewa et al., 2009; Simsek et al., 2013; Czerwionka et al., 2014; Hu, Liao, Shi et al., 2018).

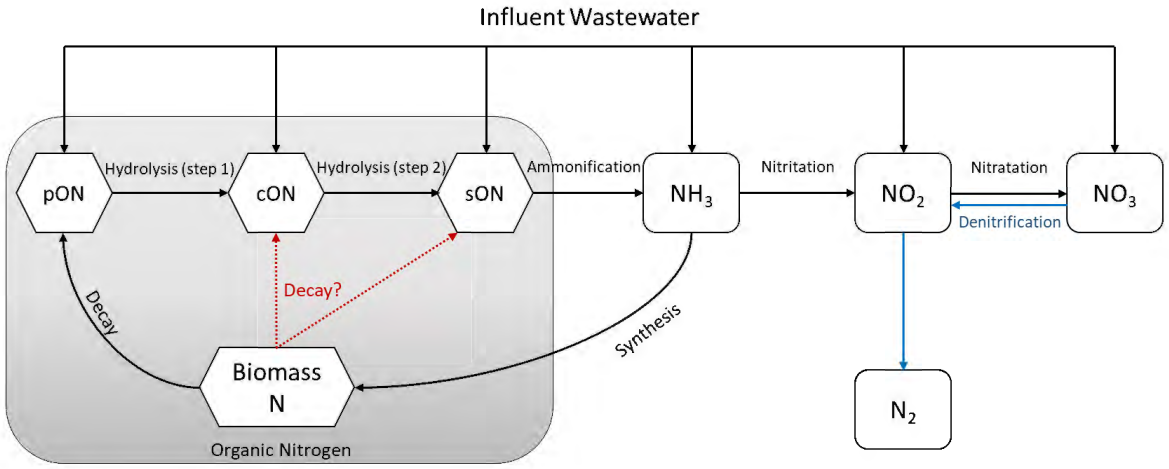


Figure 2.2. Possible N transformation pathways in bioreactors (Adapted from Makinia et al., 2009).

Influent fractions of cON and sON are removed mainly during biological treatment processes in WRRFs. Removal of ON in WRRFs is not well understood, because of lack of understanding of its production mechanism during the biological treatment processes (Parkin and McCarty, 1981 a, b, c; Pehlivanoglu-Mantas and Sedlak, 2006; Hu, Liao, Shi et al., 2018; Hu et al., 2020). Even though the average effluent ON concentration has been found to be in a range of 1 to 2 mg N/L in both BNR and non-BNR activated sludge plants, relative contribution from influent versus process-derived cON and sON is not completely clear (Pagilla et al., 2006; 2008, Sattayatewa, 2009, 2010). As mentioned earlier in the introduction chapter (subsection 1.1), it is estimated that approximately 67% effluent sON is influent derived and rest 33% is process derived (Randtke et al., 1978; Novak, 2006). However, this fraction may vary depending on the biological process and the operational conditions used (Bratby et al., 2008; Sattayatewa et al., 2009; Hu, Liao, Shi et al., 2018; Liao et al., 2019; Hu et al., 2019; Hu et al., 2020).

Pagilla et al. (2008) examined secondary effluents from nitrifying plants in the USA, and BNR plants in Poland, to understand the fate of sON through different full-scale treatment trains. sON ranged between 56-95% of TON and cON was 5-44% of TON from 3 different WRRFs in the USA. In the 4 different WRRFs examined in Poland, sON ranged between 19-62 % of TON and cON ranged between 21-62% of TON (Pagilla et al., 2008). Czerwionka et al. (2014) evaluated secondary effluent concentrations in eight full-scale BNR activated sludge systems in Poland. They reported sON fraction of 12-45% of TON and cON was 35-44% of TON. Pagilla et al. (2011) reported negligible effluent cON while operating a bench-scale sequencing batch reactor (SBR) which was fed with SWW (27.9 mg N/L and 170.3 mg COD/L) at an SRT of 10 d. The SWW did not contribute any organic N to the bioreactors. While acknowledging that full-scale plants generate effluent cON fractions, they mentioned that effluent cON was absent in their bench-scale SBR bioreactor because it was fed SWW with no suspended solids. However, they suggested a further investigation on effluent cON generation (Pagilla et al., 2011).

Distinguishing between influent-derived and process-derived fractions is critical to optimize the removal of influent ON and to minimize the amount of process-derived ON. It is often desirable to minimize the generation of ON in a biological process, however, ON production is affected by the type of biological treatment employed and the operational conditions used (Sattayatewa et al., 2009; Liao et al., 2019; Hu et al., 2019; Hu et al., 2020). Considering the challenges associated with reducing the effluent ON, there is an imperative need to optimize the operational conditions to minimize process-derived ON generation. SRT, food to microorganism ratio (F/M), and C/N are some of the critical operational parameters that are commonly used in process optimization for WRRFs. During the adjustments to these parameters in full-scale WRRFs,

the focus is given at TN, and less attention is paid toward ON because effluent ON is not regulated and there is no direct method for measuring ON.

Studies have reported influences of SRT (Simsek et al., 2016; Parkin and McCarty, 1981b) and C/N (Hu, Liao, Geng et al., 2018) on ON during biological treatment. Simsek et al. (2016) fed primary effluent to a chemostat and did not find any correlation between SRT and effluent sON. However, a decreasing trend was observed between sON biodegradability and SRT (Simsek et al., 2016). Hu, Liao, Geng et al. (2018) fed secondary effluent to a denitrifying filter. They observed maximum effluent sON at C/N ratio of 3 and no impact on effluent sON was observed at higher C/N ratios (4, 5 and 6). The presence of ON in the influent and fluctuation in its concentration did not allow these studies to estimate the production of ON in the biological process. Sattayatewa et al. (2010) fed laboratory-based sequencing batch bioreactors with NH_3 as the only nitrogen source to eliminate influent ON. They reported that the majority of the effluent ON was soluble, probably due to the use of synthetic influent feed. However, they did not examine the impact of SRT on ON production and its fractions (pON, cON, and sON). Although considerable attention has been given to the generation of sON in biological treatment, surveys showed the presence of significant amounts of cON in full-scale WRRF effluents (Pagilla et al. 2008; Sattayatewa et al.; 2010; Pagilla et al., 2011; Czerwionka et al., 2012; 2014).

As mentioned earlier, pON is removed during settling processes, while cON and sON could remain in the final effluent (Sattayatewa et al., 2010). Since effluent ON could be the major nitrogen fraction in very low effluent TN (<3 mg N/L), identifying the contribution of cON versus sON fractions to effluent ON from influent or biological processes is very important. To understand the N fractions better, Czerwionka et al. (2014) evaluated secondary effluent concentrations in three full-scale BNR activated sludge systems in Poland. They reported an

average effluent sON concentration of 0.5 to 1.3 mg N/L (12- 45 % of TON), and average effluent cON concentration was 0.7 to 1.9 mg N/L (35- 44 % of TON). Therefore, the size-fractionation of process-derived ON fractions is necessary to determine the contribution of cON and sON to the WRRFs in order to meet stricter effluent TN limits. The obtained size fractionation information can help the WRRFs target the removal of specific fractions after the biological process. For example, employing chemical precipitation followed by effluent filtration can help in sON removal, whereas anaerobic and anoxic compartments can help reduce effluent cON fractions (Arnaldos and Pagilla, 2010; Czerwionka et al., 2012).

2.3. Biodegradability of Organic Nitrogen

Biodegradable sON is a biodegradable part of the sON that can be mineralized by bacteria when the optimum conditions are attained. sON is an important N source for bacteria which biomineralize it to NH_3, a preferred N source for algae and phytoplankton. Since excessive amounts of algal growth have a negative impact on aquatic ecosystems, bsON in the system needs to be minimized. There are many sources of bsON in the receiving water bodies with wastewater derived bsON as the main one (Simsek et al., 2016). sON concentration in treated effluent from WRRFs equipped with nitrification and denitrification processes typically ranges from 1 to 5 mg N/L. Although effluent sON is recalcitrant to the treatment processes, studies showed that about 50% of the effluent sON is biodegradable by bacteria (Murthy et al., 2006; Khan et al., 2009; Sattayatewa et al., 2009).

The level of bsON in the effluent is dependent on the type of biological treatment process. Effluents of non-nitrifying plants have more bsON than effluents of nitrifying plants (Chen et al., 2011). Studies have reported effluent sON biodegradability of 50% and 51% with AS and TF process respectively (Murthy et al., 2006; Simsek et al., 2012). Sattayatewa et al. (2009)

determined bsON in the final effluent of the Parkway wastewater treatment plant (WWTP) (Laurel, Maryland, US). They separated the samples into 2 sections; one set of samples stayed as original samples while NO_3- was removed (pretreated) from the other set of samples by ion exchange. Maximum bsON in sON was 41-43% for untreated samples and 45-57% for pretreated samples. These findings indicated that there was a slightly greater biodegradability detected in the samples without NO_3-. Possibly sON was converted to NH_3 and subsequently transformed to nitrate by nitrifiers. This shows that sON can be another N source for the bacteria to use in the absence of nitrate. After the incubation (40 d), nitrate concentration increased because of nitrification. Ammonia and NO_2-were not detected after the incubation. Rapid increase in NO_3- was observed in the first ten days of 40 d incubation. Inorganic carbon concentration also reduced during the incubation, possibly because it was used by nitrifiers (Sattayatewa et al., 2009).

In 2012, Simsek et al. investigated the fate of sON and bsON in a WWTP with a two-stage TF process. In this study, the procedure for bsON determination developed by Khan et al. (2009) was followed with slight modifications. A 20-day incubation time and a MLSS seed were used in the bsON procedure by Khan et al. (2009). However, a 28-day incubation time and a raw wastewater seed were used by Simsek et al. (2012). The rationale for choosing 28 d for incubation was to ensure that time was not a limiting factor for ammonification of sON in the sample. Raw wastewater was selected as the seed to be consistent with the treatment facility that uses it for regular BOD measurement. sON concentrations in the influent and effluent were 27% and 14% of total soluble nitrogen (TSN). The plant eliminated around 62% and 72% of the influent sON and bsON mainly by the TFs. The final effluent bsON values averaged 1.8 mg N/L. bsON was found to be between 51% and 69% of the sON in raw wastewater and after various treatment units. The study concluded that the TFs removed substantial amounts of sON and bsON; however, the

effluent concentrations were still high enough to be critical if a TN limit in the effluent is 5 mg N/L or below. The TF process by itself may not be able to meet the low TN standard. However, it can be combined with nutrient removal (denitrification) processes to achieve that (Simsek et al., 2012).

Simsek et al. (2016) investigated whether effluent sON, bsON, and sON biodegradability (bsON/sON) can be minimized by SRT and to evaluate the role of biokinetic parameters on the removal of sON and bsON at various SRTs through model simulations. Laboratory scale experiments were conducted to investigate the effects of SRT (0.3, 0.7, 2, 3, 4, 5, 7, 8, and 13 d) on the concentrations of sON and bsON in actual primary treated wastewater. Results indicated no trend between effluent sON and SRTs. Effluent bsON was comparable for SRTs of 0.3-4 d and had a decreasing trend with SRT after that. Effluent sON biodegradability ranging from 23% to 59% tended to reduce with SRT. Reactors during longer SRTs, however, were contributing to non-biodegradable sON (nbsON), and this fraction of sON increased with SRT longer than 4 d (Simsek et al., 2016).

With few exceptions (Parkin and McCarty 1981a; Parkin and McCarty 1981b), all past studies investigating biodegradability of sON have focused on using a high F/M method for bsON determination (Khan et al., 2009; Sattayatewa et al., 2009; 2010; Simsek et al., 2012; 2016). The widely used high F/M method for measuring bsON in wastewater measurement was developed by Khan et al. (2009) by adapting traditional biological oxygen demand (BOD) and biodegradable dissolved organic carbon (Khan et al., 1998) procedures. The method uses a small amount (240 mg TSS/L) of bacterial inocula of MLSS from aeration tanks for determining bsON in wastewater. sON in wastewater sample is measured before the inocula is added and after a long incubation period of 20 d. The low F/M based bioassay uses a high concentration of MLSS (250 to 1,500 mg

TSS/L) and a short incubation period of 5 to 10 hrs. Similar to the high F/M method, the low F/M method uses aerobic heterotrophic sludge as an inoculum and monitors sON reduction during the incubation.

The high F/M method is useful for indicating the effluent quality and its impact on receiving waters as the bioassay relies on low inoculum and long-term incubation conditions that are usually encountered in natural waters such as rivers and streams. The method is not the most suitable tool for evaluating the ability of biological processes to remove sON because of high concentrations of microorganisms involved (leading to relatively low F/M) and low hydraulic retention time during typical wastewater treatment conditions. The low F/M test is, therefore, better representation for the process condition. Thus, understanding sON biodegradation under different conditions will help identify strategies for improving treatment efficiency at WRRFs required to achieve low TN discharge limits.

2.4. Removal of Organic Nitrogen in Municipal Wastewater Treatment

More than 95% reduction of inorganic nitrogen in WRRFs has been successfully achieved by the upgrading of wastewater treatment technologies, such as the retrofitting of a conventional bioprocess to biological nutrient removal process (Simsek et al., 2012; Eom et al., 2017). However, removal of ON remains unimproved, increasing the proportion of sON in effluent nitrogen (Pehlivanoglu-Mantas and Sedlak, 2006; Simsek et al., 2016). Therefore, investigating sON activity under different variables (e.g., process type, SRT for AS, C/N ratio, and temperature) can help in understanding the performance of biological processes in the removal of sON.

The amount of sON in effluent ranges from 0.76 to 6.46 mg N/L (Simsek et al., 2013; Hu et al., 2016; Zhang et al., 2016). sON is commonly determined by subtracting soluble inorganic nitrogen (sIN, the sum of ammonium, nitrate, and nitrite) from the TSN. Low sON concentration

quantification in waters with high sIN/TSN ratio using existing methods tends to be inaccurate, and sON measurements often have high standard deviations (Lee and Westerhoff, 2005; Vandenbruwane et al., 2007). To increase the accuracy and precision of sON measurements, some pretreatment methods were used to remove sIN species in waters, such as dialysis (Lee and Westerhoff 2005) and nanofiltration (NF) (Xu, Li et al., 2010). The limited available measurement methods discouraged researchers from investigating the nature and behavior of sON in wastewater treatment plants. As an alternative to measuring sON as a bulk parameter in wastewater, researchers have quantified specific ON-containing compounds such as dissolved free and combined amino acids (DFAA and DCAA), dimethylamine (DMA), and ethylenediaminetetraacetic acid (EDTA) (Pehlivanoglu-Mantas and Sedlak 2008). Molecular weight (MW) distributions of sON have also been measured to characterize the unidentifiable wastewater-derived sON. Studies showed that about 70% of wastewater-derived sON still cannot be characterized with currently available methods (Pehlivanoglu-Mantas and Sedlak, 2006; 2008; Simsek et al., 2012).

Activated sludge process is the most common biological technique used to treat municipal wastewater. Solid retention time, a critical operational control parameter for the AS process, is known to affect the removal and characteristics of sON (Hu, Liao, Shi et al., 2018). To date, however, only limited studies have investigated the effect of SRT on removal of sON. Simsek et al. (2016) conducted bench-scale chemostat experiments to investigate the effect of SRT (0.3, 0.7, 2, 3, 4, 5, 7, 8 and 13 d) on sON and its biodegradability levels in treated wastewater. The authors reported no trend between SRT and effluent sON concentration (minimum sON was 4.75 mg N/L at SRT 0.3 d and maximum sON was 8.08 mg N/L at SRT 4 d). This observation could be attributed to different characteristics of influent wastewater and fluctuation in influent TN concentration.

However, a more conclusive trend was reported between effluent sON biodegradability and SRT. Effluent sON biodegradability decreased with increasing SRT until SRT of 8 d and then increased slightly on increasing the SRT to 13 d. This finding highlighted higher generation of non-biodegradable sON at longer SRTs, showcasing the importance of SRT in controlling biodegradable fraction of sON in the effluent (Simsek et al., 2016). Hu, Liao, Shi et al. (2018) investigated the effect of SRT on sON degradation using a bench-scale sequencing bench bioreactor. Results indicated that effluent sON decreased (4.9, 4.4, 3.7 and 3.6 mg N/L) with increasing SRTs (5, 13, 26 and 40 d) in an ASP. Although, prolonged SRT removed more sON, it could not control AbsON which stimulates algal growth in the receiving water bodies (Hu, Liao, Shi et al., 2018).

Hu, Liao, Geng et al. (2018b) investigated the effect of C/N ratios (3, 4, 5, and 6) on the removal of sON in pilot-scale post denitrification biofilters (DNFs) treating secondary effluent. Effluent sON accounted for 31.2−39.8% of TN wherein maximum effluent sON concentration was observed at a C/N ratio of 3 (1.91 mg N/L). Although effluent sON concentration decreased at C/N ratio of 4 (1.70 mg N/L), there was no significant difference in effluent sON concentrations at C/N ratios of 4, 5 (1.70 mg N/L), and 6 (1.69 mg N/L). This study highlighted the benefit of operating DNFs at higher C/N ratios to reduce the effluent sON contribution (Hu, Liao, Geng et al., 2018).

Microbial activity plays a vital role in metabolisms and biotransformation of organic compounds (Barker and Stuckey, 1999). As a source of and a sink for sON, bacteria can excrete enzymes that regulate nitrogen transport and catabolism (Chubukov et al., 2014). In response to environmental stress such as low temperatures, bacteria can directly release sON due to autolysis (decay or endogenous respiration), which occurs when they produce enzymes that destroy their cell membranes (Sipler et al., 2015).

Hu et al. (2019) investigated the effect of temperature (8 ℃, 15 ℃, and 25 ℃) on the characterization of SMPs in an ASP with special emphasis on sON. Larger concentrations of sON were exerted at lower temperatures i.e., 8 ℃ (1.17 mg N/L) and 15 ℃ (1.10 mg N/L), than at room temperature i.e., 25 ℃ (0.93 mg N/L). The authors explained that the high sON concentrations at low temperatures could be the higher production of nitrogenous organic compounds by the bacteria and/or the decomposed or lysed cells when the AS bioreactor encountered stressful conditions. The study highlighted that it is necessary to control sON during biological processes at a low temperature to decrease the potential effect of effluent SMPs on receiving waters or wastewater reuse (Hu et al., 2019).

Liao et al. (2019) evaluated the relationship between microbial activity, microbial communities, and sON and AbsON formation under cold (8 ℃ and 15 ℃) and room temperatures (25 ℃) in an ASP. Results from this study showed that sON and AbsON concentrations significantly increased at low temperatures. sON formation was significantly correlated to microbial activity only; however, AbsON formation was influenced by both microbial activity and microbial community structure. It was concluded that distinct differences exist between the formation of sON and AbsON under low temperatures, which may provide a basis for considering them separately in the strategy for improving water quality under low temperatures (Liao et al., 2019).

A previous study investigated the effect of sludge alkaline fermentation liquid (SAFL) addition on effluent sON in a BNR process treating municipal wastewater (Hu et al., 2020). Three bench-scale SBRs were operated with different C sources: 1) with no external C source 2) with sodium acetate and, 3) with SAFL (Hu et al., 2020). SAFL is the filtered liquid from the sewage sludge alkaline fermentation mixture that contains quantities of volatile fatty acids, which are an

excellent C source for the BNR process (Sun et al., 2016; Cao et al., 2019; Huang et al., 2020). Due to the high levels of biodegradable carbon and the economic feasibility, SAFL has been considered as an alternative carbon source for the BNR processes (Li et al., 2011; Liu et al., 2018; Wang et al., 2019). Results indicated that using SAFL as a BNR carbon source besides enhancing the removal of sIN reduced effluent sON and AbsON (Hu et al., 2020). Although effluent sON was slightly higher with SAFL (2.04 mg N/L) than with sodium acetate (1.79 mg N/L), the AbsON of the two bioreactors were similar (1.06 vs. 1.04 mg N/L, respectively). Studies have shown that SAFL contains a large quantity and broad diversity of N-containing organic matter that is difficult to biodegrade (Lu et al., 2018; Ma et al., 2019). Due to their refractory nature, these nitrogenous organic compounds might be rarely or partially degraded in the bioreactor. Thus, their presence may result in an increase in effluent sON compared with the bioreactor containing sodium acetate (Hu et al., 2020).

2.5. sON Transformation by Autotrophs and Heterotrophs

In the conventional and advanced treatment of wastewater, labile sON is biologically degraded to NH_3, NO_2^- and NO_3^- via ammonification and nitrification (Revsbech et al., 2006) followed by physiochemical transformation via adsorption, membrane filtration, ion exchange, and disinfection (Yang et al., 2014; Aryal, 2016; Zhu et al., 2018). Biologically produced sON or process-derived sON generated through amination and acylation processes have nitro, amino and amide functional groups present in abundance (Soh et al., 2020). Effluent TN largely comprises of untreated recalcitrant sON which primarily includes lignin and humic-like molecules, and process-derived sON (Urgun-Demirtas, Sattayatewa et al., 2008; Zhang et al., 2019). Since sON can be utilized as a source of nutrients and/or energy source by microorganisms in wastewater, removal

of sON in WRRFs has been a complex process because of the biological transformation paths of sON.

In biological treatment, sON can be used as an electron donor and/ or a nutrient source by both autotrophic and heterotrophic microorganisms. This dual function of sON allows it to be removed or utilized by the microorganisms in the wastewater. Autotrophs typically ammonify sON to NH_3 followed by nitrification whereas heterotrophs use sON as an energy source after hydrolyzing it into smaller organic molecules. sON, as a source of nutrients, can be utilized directly or indirectly via hydrolysis of sON into low MW molecules such as amino acids and urea.

Nitrifiers actively transform sON and have been found to reduce sON concentrations (1.1 mg N/L to 0.5 mg N/L) in different BNR processes (Krasner et al., 2009). When NH_3 has depleted or is limited in the reactor, cyanate, and urea can be used as electron donors by the autotrophic microorganisms such as ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and nitrite-oxidizing bacteria (NOB) (Spang et al., 2012; Sonthiphand and Neufeld, 2014; Mundinger et al., 2019). AOB and AOA are known to have urease whereas NOB possesses both cyanase and urease. Cynate and/or urea are hydrolyzed by the autotrophs to release free NH_3 which is eventually utilized as an electron donor (Palatinszky et al., 2015). Although sON plays an important role in species interactions between nitrifying autotrophs, removal of sON in the presence of both autotrophs and heterotrophs is still unknown.

Heterotrophic bacteria biologically degrade higher MW sON to lower MW sON which releases energy that is used by different microorganisms in the reactor (Zhang, Liang et al., 2015; Zhang, Zhang et al, 2015). Both direct (low MW sON such as DFAA and urea) and indirect (via hydrolysis of sON to NH_3) uptake of sON has been reported. Middelburg and Nieuwenhuize (2000) investigated uptake rates between DFAA and urea and found that heterotrophs preferred

DFAA (0.006 - 0.15 mmol N/L/h) over urea (0.0001 - 0.007 mmol N/L/h) as the nitrogen source. Mechanisms including amino acid transport system and energy-dependent urea uptake system transported sON into cells allowing heterotrophs to directly utilize sON (Armbrust et al., 2004). Lawson et al. (2017) reported significant expression of genes involved in these systems in heterotrophic bacteria in wastewater. In anaerobic reactors, hydrolysis is followed by deamination and transamination which include enzymes such as proteases, aminotransferase, L-amino acids oxidase, and L-amino acids deaminase. sON compounds with larger MW are hydrolyzed (such as proteins to amino acids) which are then transformed into NH_3 (via deamination) and volatile fatty acids (via transamination) (Li et al., 2020). Lawson et al. (2017) found *Chlorobi-related heterotrophs (anaerobic bacteria)* extensively dispersed in both conventional and BNR processes such as anaerobic ammonia oxidation (anammox) granules. These bacteria contain a vast range of peptidases (such as serine peptidase, metallopeptidase) and hence actively degrade protein during biological processes.

Generally, biological processes degrade hydrophilic and low MW sON, but recalcitrant sON which is less reactive to biological treatment is better removed via tertiary physiochemical technologies such as coagulation, adsorption via activated carbon, ultra- or nanofiltration, reverse osmosis (Boyer et al., 2008; Gur-Reznik et al., 2008; Yang et al., 2014; Liu et al., 2019). Studies have shown that biological processes (65 - 90%) are more efficient at removing sON than physiochemical processes (10 - 80%) (Sattayatewa et al., 2010; Huo et al., 2013; Czerwionka and Makinia, 2014; Liu et al., 2019). However, it is important to note that in municipal WRRFs physiochemical processes treat the wastewater that has already gone through the biological processes. Unlike physiochemical processes, biological processes are exposed to a larger concentration of biodegradable sON, leaving a higher fraction of recalcitrant sON for the

physiochemical processes to treat. That explains the difference in the sON removal efficiency between the two process types.

2.6. Biologically Synthesized sON

The effluent sON discharged by WRRF partially (~50%) comprises of nitrogenous SMPs which are primarily contributed by the biological treatment processes. SMPs are synthesized and released by microbial cells during their growth, decay and starvation period (Barker and Stuckey, 1999; Hu et al., 2020). SMPs are composed of intricate structures wherein their MW can range from less than 1 kDa to more than 100 kDa (Aquino et al., 2006; Janga et al., 2007). Shen et al. (2012) investigated SMP composition in effluent samples from ten full-scale membrane bioreactors and found that the major components of SMP included polysaccharides (3-18 mg/L) followed by humic substances (2-10 mg/L) and proteins (<5 mg/L). Maqbool et al. (2017) suggested that extended periods of starvation could result in hydrolysis of SMPs by the microbes for their survival.

SMPs are divided into two categories depending on their derivation as 1) utilization-associated products (UAPs) or 2) biomass-associated products (BAPs). Soluble microbial product produced via substrate metabolism and microbial growth is a UAP whereas SMP produced from microbial decay is a BAP (Namkung and Rittmann, 1986; Jarusutthirak and Amy, 2006). UAPs consist of low MW compounds that are rich in carbonaceous compounds and the production of UAPs can be affected by SRT or the substrate type (Urbain et al., 1998; Mesquita et al., 2010). Unlike UAPs, BAPs are made up of high MW cellular macromolecules containing both carbon and nitrogen (e.g., protein, humic substances). Activated sludge processes operating at longer SRTs release a higher fraction of BAPs (Simsek et al., 2016; Xie et al., 2016) which are less biodegradable than UAPs (de Silva and Rittmann, 2000; Ni et al., 2011).

Acyl homoserine lactones (AHL) are intercellular quorum-sensing signaling molecules used by bacteria to regulate the expression of virulence genes. A small fraction of sON (0.1 - 1.6 ng N/L) comprises of AHL which are synthesized by the AHL synthase enzyme (Galloway et al., 2011). The AS inhabits a diverse microbial population wherein particular bacterial populaces such as *Holophagaceae* and *Verrucomicrobia* participate more actively in the production of AHL (Panchavinin et al., 2019). The biologically synthesized AHL enhances the microbial communication system which assists in the formation of biofilm and mixed culture colonies, community assembly, regulation of population density, etc. among bacteria of different species or even between organisms from different domains (Riedel et al., 2001; Tan et al., 2015). Tan et al. (2015) investigated community quorum sensing in activated sludge since it represents diverse microbial communities. AHL were found to regulate the microbial assembly and also stimulated a shift from flocs to granules (Tan et al., 2015).

Several factors such as the source of nitrogen, substrate availability, operating parameters (e.g., temperature) can influence the biological synthesis of sON components by the microbes in the wastewater. He et al. (2018) enriched glutamate synthase gene from a reactor that was fed with SWW which contributed inorganic nitrogen (NH_4Cl) as the source of nitrogen as compared to the reactors that were fed with organic sources of nitrogen such as urea and alanine. Guo et al. (2018) reported that purine and pyrimidine metabolism pathways were enhanced to assist with the production of bacteria when the anaerobic ammonium oxidation (anammox) membrane bioreactors were fed with limited substrates. Lu et al. (2016) found that lowering the temperature (23 ℃ to 10 ℃) significantly impeded the production of amino acids (e.g., glutamic acid) in a nitrifying reactor.

2.7. Analytical Methods of sON Measurement

The concentration of sON in wastewater is calculated by subtracting either sIN from TSN or NH_3-N from total Kjeldahl nitrogen (TKN) wherein the former calculation is more frequently employed (Hu et al., 2017). In the TKN method, organic nitrogen or sON is converted to ammonium which is separated via distillation and can be analyzed colorimetrically using titration or with an ion-selective electrode (ISE) or through spectrophotometry (Kjeldahl, 1883; Westerhoff and Mash, 2002). sIN compounds can be quantified by using the colorimetric method, phenate method, ISEs or ultraviolet (UV) spectrophotometry (APHA et al., 2013). TSN is determined by oxidizing sON to NO_3^- or NO via photolytic oxidation, persulfate digestion and high-temperature combustion (HTC) method or alkaline potassium persulfate digestion with UV spectrophotometry (Chang et al., 2011; Huo et al., 2013; Liu et al., 2019).

The HTC method is becoming more popular than the traditional TKN method because HTC is a simple method that determines TSN rapidly and reliably. It is a precise method that requires a small volume of sample. During HTC, nitrogen species are oxidized to NO_2 that is determined via chemiluminescence. Higgins (2014) suggested that the accuracy of the HTC method may depend on the (organic and inorganic) nitrogen standards. Pathak et al. (2015) compared TKN with the HTC method to investigate analytical errors related to sON measurement. Unlike the HTC method, the TKN method displayed reduced accuracy and precision. Results from the HTC method showed that it was a better method in terms of reproducibility, interferences of dilution reagents, cumulative human errors related to sample dilution and recovery rate (>99%). Although results from the TKN method were reproducible and had a high recovery rate (95%), it was found to overestimate the sON concentration (~10% by average) and also had a positive bias (Pathak et al., 2015).

Considering the collective analytical errors associated with different methods used to measure TSN, sIN, TKN and sON, a novel method was developed to directly quantify the sON concentration, called the liquid chromatography combined with highly sensitive organic carbon and nitrogen detection (LC-OCND). This method fractionates a sample using a size-exclusion chromatography (SEC) column followed by the detection of dissolved organic carbon, UV absorbance, and sON. Based on the MW and charge, sON compounds are chromatographically separated followed by UV oxidation of separated fractions to nitrate which is measured by a UV detector at 220 nm (Huber et al., 2011; Cai et al., 2020). Li et al. (2019) examined the HTC and LC-OCND methods to measure the concentrations of both bulk and fractionated algal-derived sON. LC-OCND showed slight interference from sIN but directly measured both bulk and fractionated sON and sIN. Unlike HTC, LC-OCND displayed reduced efficiency for high MW compounds (<75%) which was attributed to incomplete oxidation of aromatic compounds by UV oxidation technique (Li et al., 2019).

The accuracy of quantifying sON concentration in wastewater is affected by the higher concentrations of sIN in TSN. The error in sON measurement increases if the sIN/TSN ratio is more than 0.6 (Lee and Westerhoff, 2005; Vandenbruwane et al., 2007; Hu et al., 2017). Thus, pretreatment techniques such as nanofiltration and dialysis are employed for removing sIN. Nanofiltration pretreatment is carried out under a nitrogen pressure of 0.5 MPa. Past studies have reported 26 – 56% removal efficiency of NH_3-N by two different nanofiltration membranes (NF90, NF270) (Xu, Li et al., 2010; Yu et al., 2013). Lee and Westerhoff (2005) conducted dialysis of wastewater sample against deionized water with a cellulose ester dialysis membrane and found 70–80% removal efficiency of NH_3-N whereas Vandenbruwane et al. (2007) found 83–96% removal efficiency when dialyzed against a buffer solution (H_3PO_4-KH_2PO_4; pH 2.2). Chon et al.

(2013) reported that electrodialysis can achieve a higher (>95%) removal of sIN through anion- and cation-exchange membranes. Currently, in spite of the efficient removal of sIN via pretreatment, sON is quantified indirectly, and cumulative analytical errors exist in the available techniques. Therefore, there is a need to optimize methods such as LC-OCND and develop effective techniques that allow direct quantification of sON in wastewater samples.

CHAPTER 3: INVESTIGATING ORGANIC NITROGEN PRODUCTION IN ACTIVATED SLUDGE PROCESS: SIZE FRACTION AND BIODEGRADABILITY[1]

3.1. Introduction

The concentration of sON in wastewater is calculated by subtracting either sIN from TSN or NH_3-N from total Kjeldahl nitrogen (TKN) wherein the former calculation is more frequently employed (Hu et al., 2017). In the TKN method, organic nitrogen or sON is converted to ammonium which is separated via distillation and can be analyzed colorimetrically using titration or with an ion-selective electrode (ISE) or through spectrophotometry (Kjeldahl, 1883; Westerhoff and Mash, 2002). sIN compounds can be quantified by using the colorimetric method, phenate method, ISEs or ultraviolet (UV) spectrophotometry (APHA et al., 2005). TSN is determined by oxidizing sON to NO_3^- or NO via photolytic oxidation, persulfate digestion and high-temperature combustion (HTC) method or alkaline potassium persulfate digestion with UV spectrophotometry (Chang et al., 2011; Huo et al., 2013; Liu et al., 2019).

The HTC method is becoming more popular than the traditional TKN method because HTC is a simple method that determines TSN rapidly and reliably. It is a precise method that requires a small volume of sample. During HTC, nitrogen species are oxidized to NO_2 that is determined via chemiluminescence. Higgins (2014) suggested that the accuracy of the HTC method may depend on the (organic and inorganic) nitrogen standards. Pathak et al. (2015) compared TKN with the HTC method to investigate analytical errors related to sON measurement.

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[1] The material in this chapter was co-authored by Ruchi Joshi Bhardwaj and Dr. Eakalak Khan. Ruchi Joshi Bhardwaj had primary responsibility for collecting samples from the WRRF, methodology and formal analysis. Ruchi Joshi Bhardwaj was the primary developer of the conclusions that are advanced here. Ruchi Joshi Bhardwaj also drafted and revised all versions of this chapter. Dr. Eakalak Khan served as a project administrator, proofreader, and supervisor for validating the investigation conducted by Ruchi Joshi Bhardwaj.

Unlike the HTC method, the TKN method displayed reduced accuracy and precision. Results from the HTC method showed that it was a better method in terms of reproducibility, interferences of dilution reagents, cumulative human errors related to sample dilution and recovery rate (>99%). Although results from the TKN method were reproducible and had a high recovery rate (95%), it was found to overestimate the sON concentration (~10% by average) and also had a positive bias (Pathak et al., 2015).

Considering the collective analytical errors associated with different methods used to measure TSN, sIN, TKN and sON, a novel method was developed to directly quantify the sON concentration, called the liquid chromatography combined with highly sensitive organic carbon and nitrogen detection (LC-OCND). This method fractionates a sample using a size-exclusion chromatography (SEC) column followed by the detection of dissolved organic carbon, UV absorbance, and sON. Based on the MW and charge, sON compounds are chromatographically separated followed by UV oxidation of separated fractions to nitrate which is measured by a UV detector at 220 nm (Huber et al., 2011; Cai et al., 2020). Li et al. (2019) examined the HTC and LC-OCND methods to measure the concentrations of both bulk and fractionated algal-derived sON. LC-OCND showed slight interference from sIN but directly measured both bulk and fractionated sON and sIN. Unlike HTC, LC-OCND displayed reduced efficiency for high MW compounds (<75%) which was attributed to incomplete oxidation of aromatic compounds by UV oxidation technique (Li et al., 2019).

The accuracy of quantifying sON concentration in wastewater is affected by the higher concentrations of sIN in TSN. The error in sON measurement increases if the sIN/TSN ratio is more than 0.6 (Lee and Westerhoff, 2005; Vandenbruwane et al., 2007; Hu et al., 2017). Thus, pretreatment techniques such as nanofiltration and dialysis are employed for removing sIN.

Nanofiltration pretreatment is carried out under a nitrogen pressure of 0.5 MPa. Past studies have reported 26 – 56% removal efficiency of NH_3-N by two different nanofiltration membranes (NF90, NF270) (Xu, Li et al., 2010; Yu et al., 2013). Lee and Westerhoff (2005) conducted dialysis of wastewater sample against deionized water with a cellulose ester dialysis membrane and found 70–80% removal efficiency of NH_3-N whereas Vandenbruwane et al. (2007) found 83–96% removal efficiency when dialyzed against a buffer solution (H_3PO_4-KH_2PO_4; pH 2.2). Chon et al. (2013) reported that electrodialysis can achieve a higher (>95%) removal of sIN through anion- and cation-exchange membranes. Currently, in spite of the efficient removal of sIN via pretreatment, sON is quantified indirectly, and cumulative analytical errors exist in the available techniques. Therefore, there is a need to optimize methods such as LC-OCND and develop effective techniques that allow direct quantification of sON in wastewater samples.

3.2. Materials and Methods

3.2.1. Synthetic Wastewater Composition

Synthetic wastewater (SWW) recipe was modified from Nagaoka et. al. (1996) to mimic a medium-strength domestic wastewater composition. The composition included glucose (450 mg COD/L), ammonium chloride (50 mg N/L), orthophosphate using monopotassium phosphate and disodium phosphate (5 mg P/L), calcium chloride (11 mg/L), magnesium sulfate heptahydrate (17 mg/L), ferric chloride hexahydrate (4 mg/L) and sodium bicarbonate (350 mg/L). Trace metals including zinc chloride (0.2 mg/L), nickel chloride (1.5 mg/L), manganese chloride (2 mg/L) and cobalt chloride (3 mg/L) were added. Vitamins were contributed by RPMI 1640 solution (Sigma-Aldrich, St. Louis, Missouri).

3.2.2. Inoculum Source

Mixed liquor suspended solids were collected from a WRRF treating municipal wastewater located in Moorhead, MN, United States. and used as an inoculum for the experimental work. The treatment facility removes organic carbon through a high purity oxygen activated sludge process which is operated at an SRT of 3 d. The treatment facility receives an average flow of 15,000 m^3/day.

3.2.3. Experimental Setup

Four laboratory-scale chemostat reactors were set up with an initial total suspended solids (TSS) concentration of 1,800 mg TSS/L and operated in parallel. The reactors were set up by mixing the MLSS collected from the Moorhead WRRF with lab-grade de-ionized water. Figure 3.1 shows the bench-scale experimental setup employed for this task. In parallel, the four reactors were fed with SWW using peristaltic pumps operated at different flow rates to achieve 2, 5, 10 and 20 d of hydraulic residence times (HRT). Since the reactors were operated as chemostat with no wasting or recycling of sludge, HRT would be equivalent to SRT at steady-state conditions. Mixing and aeration were achieved by magnetically stirring the sludge mixture and by supplying air though a coarse bubble diffuser, respectively. Sludge inside the reactors was magnetically stirred to avoid sludge settling or the creation of dead zones within the reactors.

Dissolved oxygen (DO) concentration was maintained between 2 and 4 mg/L. pH in the reactors was maintained between 7.2 and 7.8 using $NaHCO_3$ and HCl. Effluent from each reactor was analyzed daily for TSS, volatile suspended solids (VSS), COD and nitrogen species (NH_3, NO_2^-, NO_3^- and TN). Steady-state conditions were assumed to be achieved when less than 10 percent variation was observed in the concentrations of effluent quality parameters (TSS, VSS,

COD and NH_3) consecutively for five days. At steady state, effluent samples were fractionated and analyzed for particulate, colloidal and soluble fractions of COD and nitrogen species.

Figure 3.1. Bench-scale experimental setup for chemostat operation on day 1 (top) and day 20 (bottom). SWW – synthetic wastewater water; Eff – effluent; SRT – solids retention time.

3.2.4. Fractionation Technique

Effluent samples were separated into particulate, colloidal and soluble fractions using different size fractionation techniques to analyze for COD and nitrogen species. Initially, the unfiltered effluent sample was analyzed for a total fraction (T). Samples were then filtered through a 1.2 µm pore-size glass microfiber filter (GF/C, Whatman Inc., Kent, UK) to obtain colloidal plus soluble fraction (C + S) in the filtrate (the particulate fraction was retained on the filter).

Conventional filtration technique using a 0.45 µm pore size membrane filter to obtain soluble fraction can contain colloidal fraction that ranges between 0.1 and 1.0 µm diameter. Hence,

the flocculation-filtration technique (Mamais et al., 1993) was employed to remove both particulate and colloidal fraction from the effluent. Zinc sulfate ($ZnSO_4$) and sodium hydroxide (NaOH) were added to the effluent sample to flocculate colloids and particulates followed by filtration with a 0.45 μm pore size cellulose acetate membrane filter (PALL Co., Port Washington, NY, USA) to obtain true soluble (S) fraction. Particulate (P) and colloidal (C) fractions were calculated using the following equations:

$$\text{Particulate fraction (P)} = T - (C + S) \quad (3.1)$$

$$\text{Colloidal fraction (C)} = T - (S + P) \quad (3.2)$$

where, T= total fraction, C = colloidal fraction, S = soluble fraction and P = particulate fraction.

The effluent sample obtained from each reactor represented the total fraction (T) which was not filtered and was measured as it is. The filtrate of the effluent sample through a 1.2 μm pore size cellulose acetate membrane filter represented a combination of colloidal and soluble fraction (C+S). The particulate fraction (P) was calculated using equation 3.1. The soluble fraction (S) was obtained via the flocculation-filtration method, as described above. The colloidal fraction (C) was obtained by using equation 3.2. At each fractionation step, samples were analyzed for COD and nitrogen species.

Since sON concentration is determined indirectly, the reliability of the employed methods should be identified (Pathak et al., 2015). The accuracy of sON determination was verified by preparing standard solutions with known quantities of ammonia (1 mg NH_3-N/L), nitrate (1 mg NO_3^--N/L) and urea (1 mg N/L) mixed in deionized water. Table 3.1 displays the error analysis results. The measured sON concentration (1.04 ± 0.03 mg N/L) was close to the urea concentration (1 mg N/L) added to the standard solution.

Table 3.1. Error analysis for sON measurement

Sample	NH_3 (mg N/L)	NO_3^- (mg N/L)	TSN (mg N/L)	sON (mg N/L)
1	1.10	1.03	3.14	1.01
2	1.09	1.01	3.17	1.07
3	1.10	1.03	3.21	1.08
4	1.13	1.02	3.13	0.98
5	1.10	1.05	3.21	1.06
Avg. ± Std. Dev.	1.10 ± 0.01	1.03 ± 0.01	3.17 ± 0.03	1.04 ± 0.03
Coeff. of Var.	1%	1%	1%	4%

3.2.5. Analytical Techniques

All the monitored parameters were determined in triplicate, and average values ± standard deviation values are reported. Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed gravimetrically according to Standard Methods (APHA et al., 2005). COD was determined using HACH TNT kits (HACH Company, Colorado, USA). COD was measured using the USEPA bioreactor digestion method (HACH method 8000) with low range (3-150 mg COD/L) and high range (20-1500 mg COD/L) testing kits.

Inorganic nitrogen species were measured using the HACH TNT plus kits. Ammonia concentration was measured using the salicylate method (TNT plus method 10205) for ultra-low range (0.015-2.0 mg NH_3-N/L), low range (1.0-12.0 mg NH_3-N/L) and high range (2-47 mg NH_3-N/L). Nitrite concentration was measured using the diazotization method for both low range (0.015-0.6 mg NO_2^--N/L) and high range (0.6-6.0 mg NO_2^--N/L). TNT plus method 10207 was used for measuring low range nitrite whereas TNT plus method 10237 was used for measuring high range nitrite. Nitrate concentration was measured using the dimethylphenol method (TNT plus method 10206) for both low range (0.23-13.5 mg/L NO_3^--N) and high range (5-35 mg NO_3^--N/L). Total N concentration was measured using the persulfate digestion method (TNT plus

method 10208) for low range (1-16 mg TN/L), high range (5-40 mg TN/L), and ultra-high range (20-100 mg TN/L).

To determine concentration, a HACH DR 5000 spectrophotometer was used. The spectrophotometer was calibrated using blank samples and standard solutions as referred in the manual (HACH Company). Benchtop meters were used to continuously monitor the pH (model 250A+, Thermo Scientific Orion) and DO (model 850 Thermo Scientific Orion) in the reactors. The pH meter was calibrated daily using the three-point pH calibration method with three different buffer solutions. The DO meter was calibrated daily using the water-saturated air method.

3.2.6. Biodegradability Assay

The bsON was measured following the method developed by Khan et al. (2009). The bsON bioassay was performed in triplicates for all samples. A blank control was included by using deionized distilled water as a sample. The difference in sON reduction of the sample and the control during the incubation was bsON as shown in equation 3.3:

$$\text{bsON (mg N/L)} = (sON_i - sON_f) - (sONb_i - sONb_f) \quad (3.3)$$

where sON_i and sON_f are sON before and after the incubation for effluent samples; and $sONb_i$ and $sONb_f$ are sON before and after incubation for control. nbsON was calculated using the following equation:

$$\text{nbsON (mg N/L)} = \text{sON} - \text{bsON} \quad (3.4)$$

3.3. Results and Discussion

3.3.1. Performance of Reactors at Different SRTs

This subsection discusses the basic reactor performances observed during the experiments whereas the following two subsections highlight the main findings pertaining to ON fractions and sON biodegradability. Initially, each reactor for SRTs 2, 5, 10 and 20 d was seeded with 1,800 mg

TSS/L of mixed liquor, which gave dark color to each reactor. Upon achieving steady- state, the mixed liquor appearance inside each reactor changed from dark brown to light brown (Figure 3.1). Differences in the reactor appearances indicated faster washout at shorter SRTs compared to longer SRTs. All the reactors were fed with 50 mg/L NH_4Cl (no organic nitrogen in the feed). Nitrification increased with increasing SRT as expected (Figure 3.2). Partial nitrification observed at lower SRTs could be due to the presence of nitrifiers in the MLSS, which was used as a seed in the reactors. MLSS was collected from the aeration basin of Moorhead WRRF, and the past operational data of the plant indicated the presence of nitrifiers in the mixed liquor, which was evident by the significant removal of NH_3 in the aeration basin (Simsek et al., 2016).

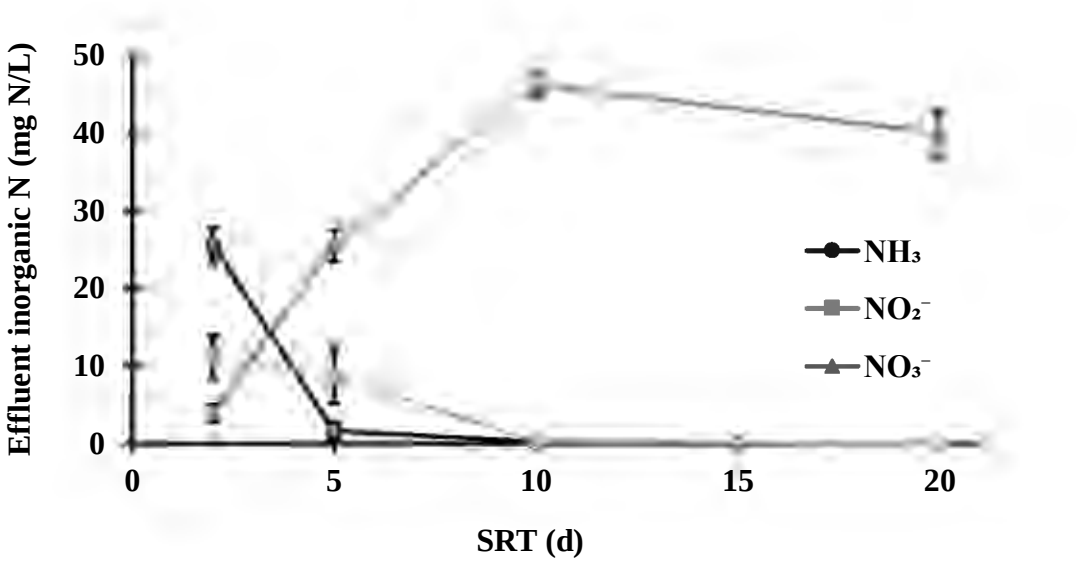

Figure 3.2. Nitrification performance under SRTs 2, 5,10 and 20 d.

All reactors were fed with 450 mg sCOD/L in the form of glucose with no cCOD and pCOD in the SWW. During steady state, the majority of influent sCOD was transformed into pCOD (76 to 86% pCOD/TCOD), suggesting that influent COD was readily consumed by the biomass in all the reactors (Figure 3.3). Effluent pCOD decreased significantly from SRT of 2 d to 5 d, after which it did not change much (201.2, 140.5, 141.1, 143.4 mg pCOD/L). At SRT of 2 d, maximum washout was expected (1.0, 0.4, 0.2 and 0.1 L/d was the influent flowrate) because

of which the rate of hydrolysis in the floc matrix was restricted (Jimenez et al., 2007), thus leading to a higher concentration of pCOD in the effluent. The higher concentration of pCOD in the effluent due to washout at SRT of 2 d is also supported by effluent VSS concentration (195, 110, 98 and 104 mg VSS/L) which was the highest for SRT of 2 d and did not change much at SRT of 5, 10 and 20 d confirming that the maximum amount of biomass discharged at SRT of 2 d. The colloidal fraction of COD was the smallest out of three fractions investigated in the effluents of each reactor (2 to 9% cCOD/TCOD). The sCOD fraction drop from SRT of 2 d to 10 d (44.6, 29.2, 16.3 mg sCOD/L), after which it went up at SRT of 20 d (25.9 mg sCOD/L). The increase in effluent sCOD at SRT of 20 d is attributed to the endogenous respiration occurring in the reactor due to starvation.

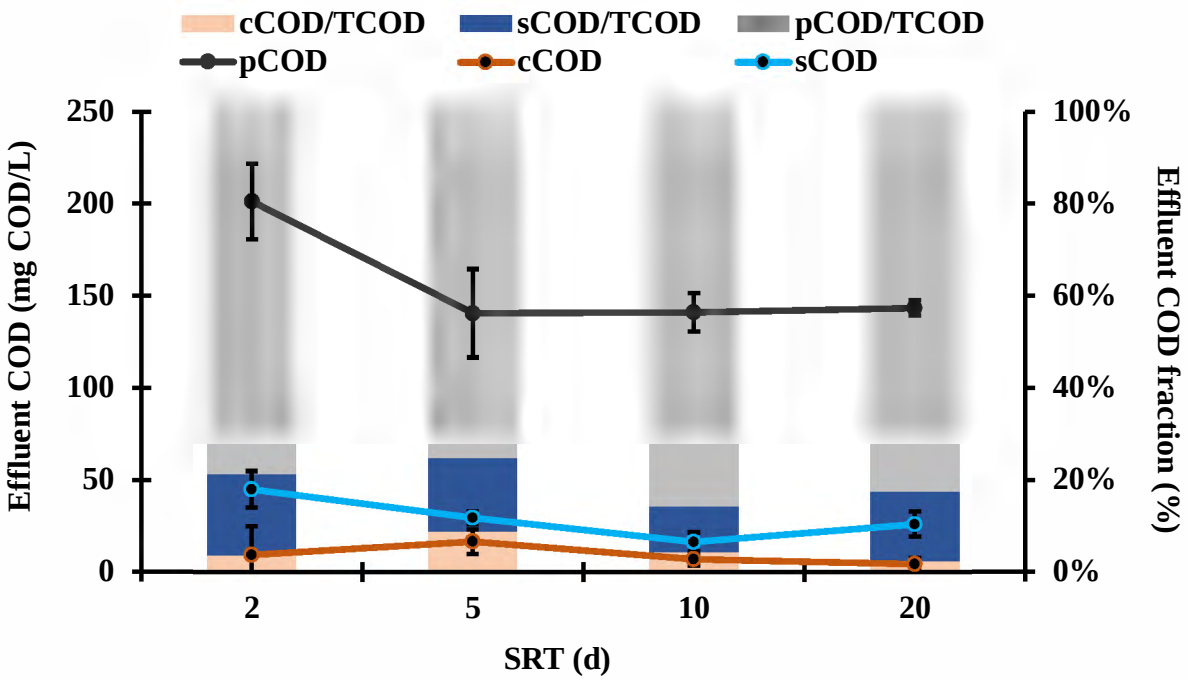

Figure 3.3. Fractions of effluent total COD (TCOD) under SRTs 2, 5, 10 and 20 d.

Overall, influent COD was not completely degraded in any reactor leaving approximately 37 to 57% COD in the effluent (Figure 3.4). It is important to mention that laboratory-scale chemostat reactors were operated and could only partially degrade the influent COD (450 mg/L)

due to their limited capability. From a different perspective, this finding of incomplete COD degradation was consistent with suggestions by Hu et al. (2020). They investigated process-derived sON formation for the purpose of modeling at both bench and full scales. They operated AS systems at SRT of 20 d under different concentrations of MLSS (1,000, 2,000 and 3,000 mg/L) and NH_3-N (10, 20 and 30 mg N/L). They suggested that carbonaceous compounds were excreted during the microbial metabolism because of which COD (influent SWW contained 300 mg COD /L) was not completely degraded (Hu et al., 2020).

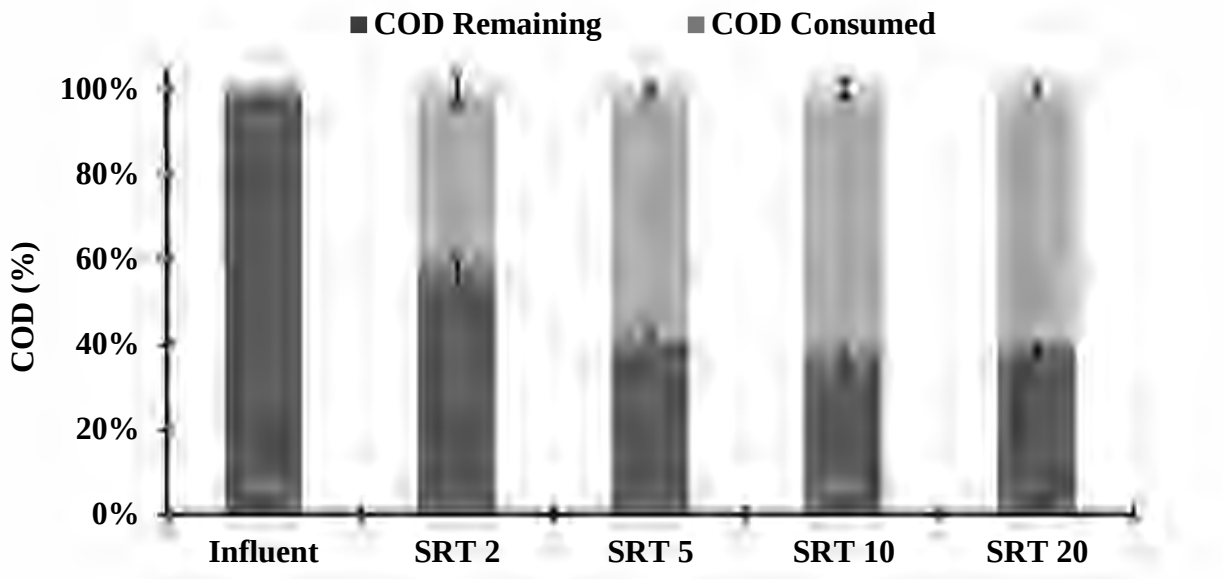

Figure 3.4. Influent COD fraction consumed versus remaining in each reactor under SRT 2, 5, 10 and 20 d.

Results from this task showed that SRT influences the influent COD degradation efficiency in an activated sludge process. SRT can be used to control both settleable (pCOD) and non-settleable (cCOD and sCOD) fractions. Operating reactors at higher SRTs (>5 d) can help in reducing the non-settleable fractions, which usually contribute towards final effluents in a WRRF. Therefore, both colloidal and soluble COD fractions should be the target for the advanced wastewater treatment technologies applied after the activated sludge process in a treatment facility.

3.3.2. Effect of SRT on Effluent ON Fractions

Overall, the TON/TN ratio declined with increasing SRT (36%, 30%, 21% and 24% at SRTs 2, 5, 10 and 20 d, respectively) as displayed in Figure 3.5. This result suggests that operating an ASP at lower SRTs (2 and 5 d) generates a larger fraction of TON/TN, which would be degraded further if operated at longer SRTs (10 to 20 d). Effluent ON fractions under different SRTs are presented in Figure 3.6. Organic nitrogen was produced in all the reactors when no ON was fed in the influent which is consistent with past studies (Parkin and McCarty, 1981b; Pehlivanoglu-Mantas and Sedlak, 2006; Pagilla et al., 2011, Hu et al., 2020). For effluent TON, the highest contribution was from pON (45-60% of TON) followed by cON (18-31% of TON) and sON (19-25% of TON). For effluent TON, the highest contribution was from pON (45-60% of TON) followed by cON (18-31% of TON) and sON (19-25% of TON).

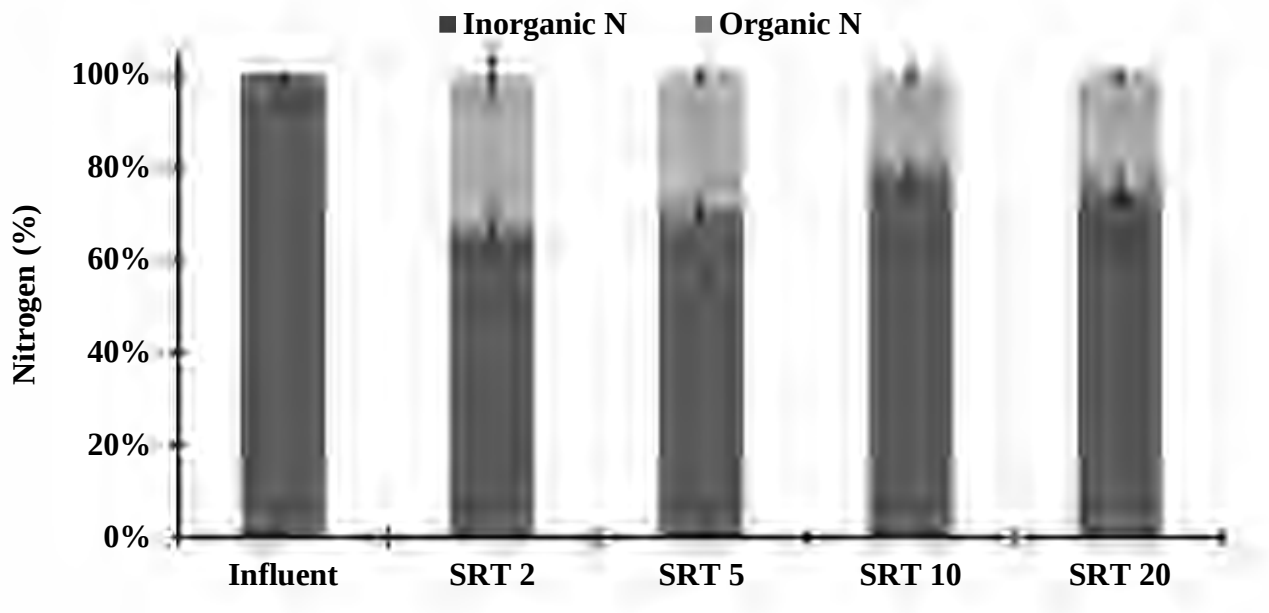

Figure 3.5. Nitrogen mass balance for influent and effluent from each reactor.

Pagilla et al. (2008) examined nitrifying plants in the U.S., and BNR plants in Poland, to understand the fate of sON through different treatment trains. Secondary effluent samples were fractionated into cON and sON fractions for the plants in the U.S. and pON, cON and sON for the

Polish plants by filtering the samples through different pore-sized (0.1, 0.22, 0.3, 0.45 and 1.2 µm) membrane filters. sON ranged between 56-95% and cON was 5-44% of TON for the effluent from 3 different WRRFs in the U.S. All three fractions, sON (19-62 % of TON), cON (21-62% of TON) and pON (17-27% of TON), were observed for the effluent from 4 different WRRFs in Poland. Effluent sON (19-25% of TON) and cON (18-31% of TON) fractions obtained from this task are relatively closer to the fractions observed for Polish plants compared to plants in the U.S. The effluent pON (17-27% of TON) from Poland was much smaller than that reported in this task (45-60% pON of TON). It is important to note that the reported fractions from the Poland WRRFs were obtained from the effluent of secondary clarifiers, which further remove particulates and in turn the pON.

Effluent sON fraction range obtained from the U.S. WRRFs was higher than that from the Polish facilities as well as this task. Pagilla et al. (2008) suggested that higher fractions of sON were contributed by different types of secondary treatment processes employed by the WRRFs in the U.S. The effluent sON samples in the U.S. WRRFs were determined strictly by 0.45 µm pore-size membrane filtration unlike filtration-flocculation method used in this task, which is expected to further remove colloidal fractions. Overall, effluent cON fraction range contributed by the U.S. WRRFs was large; however, the highest (44% cON of TON) fraction was contributed by one facility whereas the other two facilities contributed smaller fractions (5 and 14% cON of TON). The cON fractions in these facilities were reduced due to use of coagulant in the secondary clarifiers (Pagilla et al., 2008). The cON fractions obtained from this task fall within the range reported by the U.S. WRRFs.

The effluent cON concentration was almost similar in each reactor (3.6, 3.8, 3.7 and 3.8 mg cON/L). On comparing effluent cON with sON concentrations, except at SRT of 2 d (3.6 mg

cON/L < 4.6 mg sON/L) the concentrations of cON were always larger than sON in all other reactors (SRT 5 d: 3.8 > 3.2 mg/L, 10 d: 3.7 > 2.3 mg/L, and 20 d: 3.8 > 3.1 mg/L). This finding suggests that cON is the larger fraction generated in an activated sludge process when operating between SRT of 5 and 20 d. However, when operating at smaller SRT of 2 d, sON is the larger fraction of effluent TON. It could be possible that the rate of hydrolysis (pON to cON) or cell lysis increases with increasing SRTs causing a reduction in pON fraction and increasing the cON fraction (Pagilla et al., 2006; Mękinia et al., 2009). However, the rate of hydrolysis of pON to cON must be different from the hydrolysis of cON to sON because unlike cON fraction which increased with increasing SRT, sON fraction declined with increasing SRTs (Fig. 4). Pagilla et al. (2011) reported negligible effluent cON while operating a bench-scale SBR which was fed with SWW (27.9 mg N/L and 170.3 mg COD/L) for an SRT of 10 d. The SWW did not contribute any organic N to the reactors. While acknowledging that full-scale plants generate effluent cON fractions, they mentioned that effluent cON was absent in their bench-scale SBR reactor because it was fed with SWW with no suspended solids. However, they suggested a further investigation on effluent cON generation (Pagilla et al., 2011).

Figure 3.6 displays a decreasing trend in sON concentration from SRT of 2 d to 10 d (4.6, 3.2, 2.3 mg sON/L) after which an increase was observed at SRT of 20 d (3.1 mg sON/L). These results are different from the results reported by both Hu et al. (2018) and Simsek et al. (2016), that investigated the effect of SRT on sON degradation using bench-scale reactors. Hu et al. (2018) found that effluent sON decreased (4.9, 4.4, 3.7 and 3.6 mg sON/L) with increasing SRTs (5, 13, 26 and 40 d), whereas Simsek et al. (2016) reported no trend between SRT (0.3, 0.7, 2, 3, 4, 5, 7, 8 and 13 d) and effluent sON concentration (minimum sON was 4.75 mg sON/L at SRT 0.3 d and maximum sON was 8.08 mg sON/L at SRT 4 d). The absence of a trend between sON

concentration and SRT was expected by Simsek et al. (2016) due to fluctuation in the influent TN. Hence, effluent bsON was normalized by sON, reported as biodegradability (bsON/sON), and plotted against SRT highlighting a conclusive trend (Simsek et al., 2016).

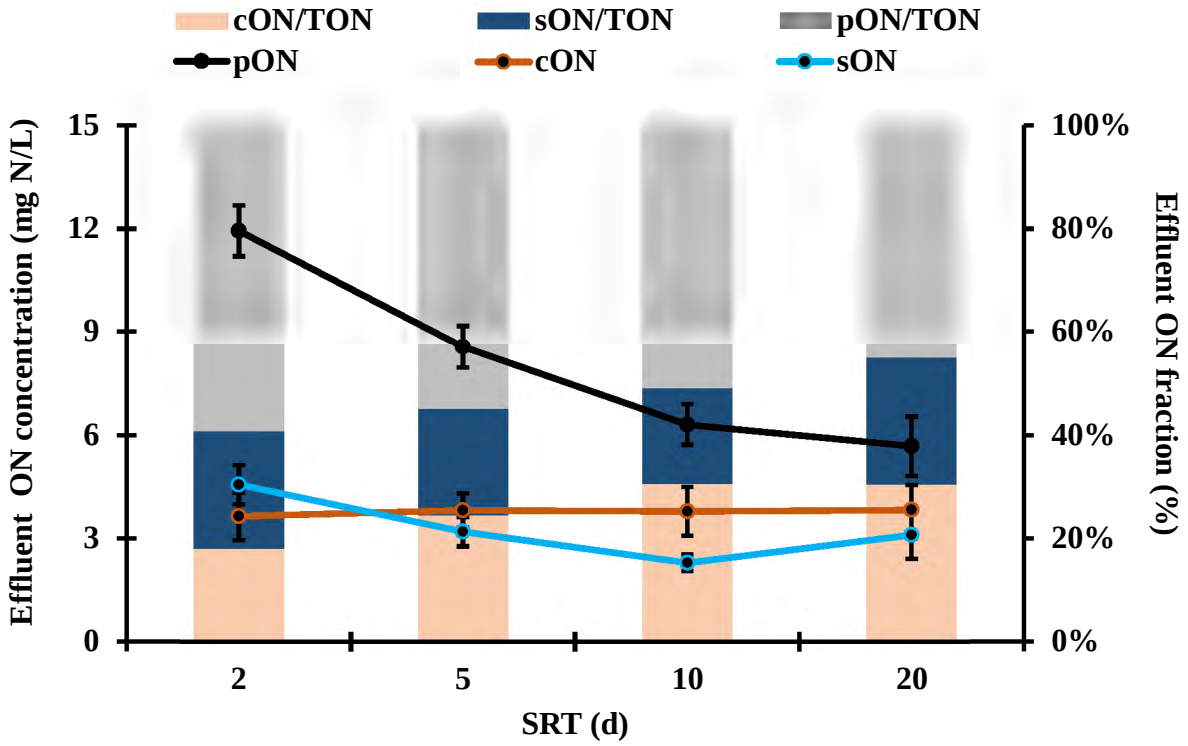

Figure 3.6. Concentration (left axis) and fractions (right axis) of effluent total organic N (TON) under SRTs 2, 5, 10 and 20 d.

The magnitude of sON concentrations observed in this task are comparable with results concluded by Hu, Liao, Shi et al. (2018) study. Also, the decreasing trend of effluent sON with increasing SRT observed in this task is similar with a trend reported by Hu, Liao, Shi et al. (2018) study. Unlike this task wherein chemostat reactors were operated, sequencing batch reactors were employed by Hu, Liao, Shi et al. (2018). The working volume (2 L) and the initial mixed liquor suspended solids concentrations (1,800 mg/L) of the reactors were similar in both studies. In addition, no sludge was recirculated or reinoculated in both studies. On the other hand, sON concentrations reported in this task as well as Hu, Liao, Shi et al. (2018) were smaller than those reported by Simsek et al. (2016). Although no trend was observed between SRT and effluent sON,

a more conclusive trend was reported between effluent sON biodegradability (effluent biodegradable sON/effluent sON) and SRT by Simsek et al. (2016). Effluent sON biodegradability decreased with increasing SRT until SRT of 8 d and then increased slightly on increasing the SRT to 13 d. This finding highlighted higher generation of non-biodegradable sON at longer SRTs, showcasing the importance of SRT in controlling biodegradable fraction of sON in the effluent. The difference in the effluent sON concentrations reported by Simsek et al. (2016) and this task is attributed to different characteristics of influent wastewater and fluctuation in influent TN concentration.

Unlike pCOD concentration, which did not decrease much after SRT of 5 d (201.2, 140.5, 141.1 and 143.4 mg pCOD/L), effluent pON dropped gradually with increasing SRT (11.9, 8.6, 6.3 and 5.7 mg pON/L). Increasing the SRT could lead to higher hydrolysis of pON into cON and sON, hence the drop in pON concentration as hypothesized by Pagilla et al. (2006). However, similar to pCOD/TCOD, pON/TON was also the largest fraction in the effluents of each reactor. Unlike WRRFs wherein the activated sludge processes are followed by secondary clarifiers, the bench-scale reactors operated in this task were chemostats (SRT= HRT) i.e., concentration of N components will be the same inside the reactor and in the effluent, because the collected effluent was not separated from the solids. Secondary clarifiers remove a major fraction of particulates via activated sludge settleability (Pagilla et al., 2008). As a result, the effluent pON concentrations achieved in this task via chemostat reactors will always be higher than effluent pON from WRRFs.

This task shows that operating at different SRTs can affect the generation of different fractions of effluent ON in an activated sludge process. However, it should be noted that contribution of effluent cON fraction can be larger than sON in the secondary effluent TON. This is crucial because WRRFs can then identify appropriate technologies for further lowering effluent

TN. For instance, polymer addition to water containing colloidal material enhances flocculation, thus removing colloidal particles from the solution via sedimentation. Therefore, considering the generations of sON and cON fractions during AS process, treatment technologies employed at WRRFs to meet stricter effluent TN limits should target these fractions.

3.3.3. Effect of SRT on Effluent sON Biodegradability

The goal behind examining the biodegradability of effluent sON was to determine the fraction of sON generated in the activated sludge system that is biodegradable and likely be reduced with more prolonged treatment time in a chemostat reactor wherein HRT is equal to SRT. The biodegradability assay quantified the bsON and nbsON fractions of the effluent sON from each reactor (Figure 3.7). More than 50% of effluent sON was bsON at the SRTs of 2 d (58%), 5 d (62%) and 10 d (53%), whereas effluent sON at SRT of 20 d was the least biodegradable (31%). Although no specific trend was observed between SRT and sON biodegradability, larger fractions of sON at lower SRTs (2 d and 5 d) were biodegradable, and the biodegradability decreased with increasing SRTs (10 d and 20 d). Operating the reactor at SRT of 10 d, generated the least amount of sON (2.29 mg sON/L), which was partially biodegradable (53%). At SRT of 20 d, a larger concentration of sON (3.10 mg sON/L) was generated when compared with SRT of 10 d and was highly non-biodegradable (69%). Operating reactors at longer SRTs enhanced nitrifiers and provided a longer time for the biomass to degrade sON fraction in the reactor. However, it is important to note that in this task the chemostat reactors were operated without wasting or recycling any sludge which means that during longer SRTs such as SRT of 20 d, endogenous decay is expected. A F/M ratio of 0.2 (mg COD/mg VSS d) corresponded with SRT of 20 d, indicating that the effluent sludge was old and perhaps decayed due to starvation, thus releasing non-biodegradable endogenous residues.

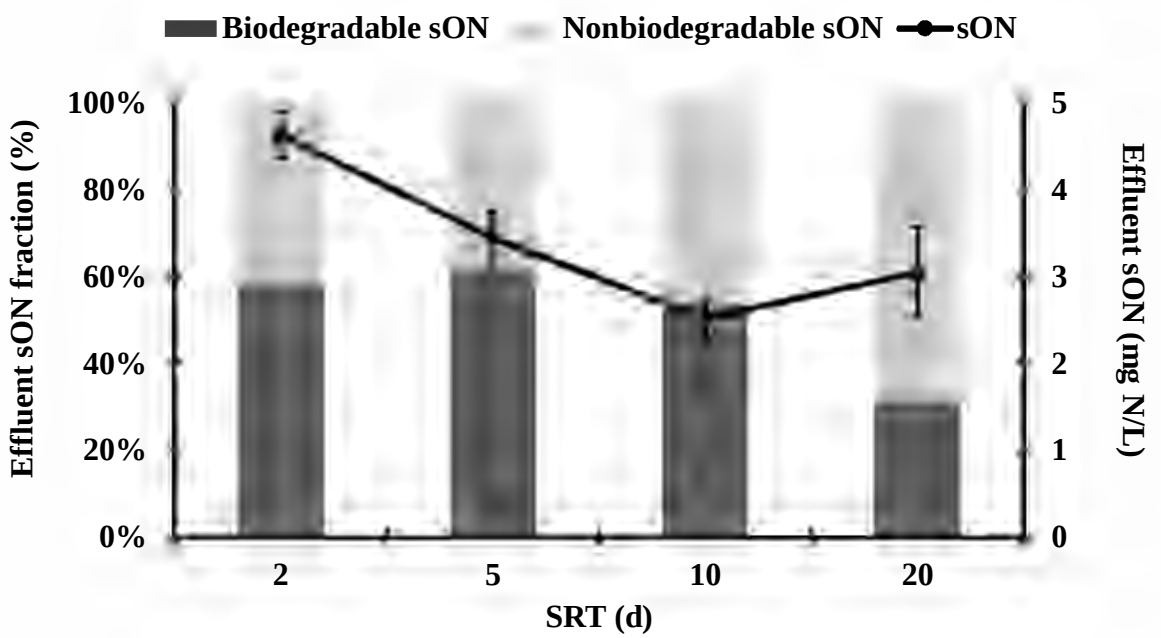

Figure 3.7. Biodegradable (bsON) and non-biodegradable (nbsON) fractions of effluent sON (left axis) and sON concentration (right axis) under SRTs 2, 5, 10 and 20 d.

At SRT of 20 d, only 31% of the produced sON was biodegradable. With minimum biodegradable fraction produced, this observation sounds promising from an effluent quality perspective (to be discharged in water bodies). However, several studies have found that endogenous residue undergoes slow biodegradation (Jones et al., 2007; Laera et al., 2005; Lubello et al., 2009; Ramdani et al., 2010). This suggests that the effluent sON from SRT of 20 d could degrade even further. On examining the active biomass fraction and the endogenous decay rate under aerobic conditions, Ramadani et al. (2012) found that the active biomass fraction (based on VSS/TSS) reduced from 68% to 59% from SRT of 5.2 d to 10.4 d. In contrast, the endogenous decay rate increased from 32% to 41%. Therefore, SRT affects both active biomass fraction as well as endogenous decay rate. Results from this task are agreeable with the previous study (Ramadani et al., 2012) suggesting that endogenous decay rate may increase with increasing SRTs. This agreement is based on the effluent sON concentration (2.2 and 3.1 mg sON/L) and active biomass fraction (0.90 and 0.60 VSS/TSS) at SRT of 10 d and 20 d. On increasing the SRT from

10 d to 20 d, active biomass fraction declined significantly (90% to 60%) which can be attributed to increased endogenous decay, thus increasing the effluent sON concentration.

Simsek et al. (2016) reported the increase in effluent nbsON at longer SRTs being contributed by SMPs and extracellular polymeric substances (EPSs). SMPs include organic material such as proteins, which are released by biomass. SMPs can be categorized as i) UAPs, associated with biomass growth and ii) BAPs, associated with biomass decay (Ni et al., 2011). EPSs include proteins and are subject to hydrolysis and contribute to BAPs (Aquino and Stuckey, 2004). Several studies conducted in the past provided several conclusions about the contributions made by EPSs and SMPs. For example, more EPSs were produced by biomass under stressful conditions (Aquino and Stuckey, 2004), higher amounts of SMPs were produced at higher SRTs, and UAPs decreased while BAPs increased with increasing SRT (Ni et al., 2010). These results suggest that at SRT of 20 d, large fractions of nbsON could be contributed by EPSs and SMPs, specifically BAPs. Ni et al. (2010) reported higher accumulation of BAPs than UAPs when a continuous flow activated sludge reactor was operated and simulated at an SRT of 20 d under famine condition. This finding by Ni et al. (2010) further confirms the presence of BAPs at SRT of 20 d due to low availability of substrate. Effluent BAPs can be targeted post biological treatment via techniques such as membrane filtration, adsorption using granular activated carbon, coagulation and filtration (Kiser et al., 2010; Ni et al., 2011).

SRT affects the non-biodegradability of produced sON due to changes in the endogenous residue characteristics (e.g., production and degradation of EPSs and SMPs). SMP fractions (UAPs and BAPs) and their biodegradability are known to change with SRT (Jarusutthirak and Amy, 2006; Ni et al., 2010). Therefore, based on the results obtained from this task as well as past studies, it is inferred that SRT and (non-) biodegradability of sON are related irrespective of the source of

sON (influent-derived or process-derived). Increasing the SRT enhances the non-biodegradability of sON. This finding is in agreement with a previous study (Simsek et al., 2016) which investigated the biodegradability of effluent sON under different SRTs (0.3, 0.7, 2, 3, 4, 5, 7, 8 and 13 d) in a chemostat reactor that was fed with primary treated wastewater with approximately 13 mg sON/L. Larger fractions of nbsON in the effluent were observed at longer SRTs (Simsek et al., 2016). Therefore, operating an aerobic reactor at an SRT of 20 d or longer will generate sON that will be recalcitrant in receiving waters for a longer period of time. The WRRFs attempting to achieve lower TN in the final effluent should be aware about the production and recalcitrant nature of sON, especially when operating under longer SRTs.

3.4. Summary

The results provided by this task show that pON, cON and sON were generated in an AS process when the influent contained no ON. SRT affects each fraction of organic nitrogen along with the biodegradability of effluent sON. Besides effluent sON, which has already garnered attention in the past two decades, effluent cON also deserves attention from the research community because it contributes a significant fraction to effluent total organic nitrogen in the biological process. This research task demonstrated sON and cON production for a simple aerobic system. Depending on the biological treatment processes configuration, generation of organic nitrogen and its fractions will vary. Therefore, WRRFs should account for the ON production, particularly when they aim to reduce more TN by increasing the SRT.

CHAPTER 4: PRODUCTION AND REMOVAL OF SOLUBLE ORGANIC NITROGEN BY NITRIFYING BIOFILM[1]

4.1. Introduction

Recent guidelines for discharging TN are approaching ≤ 5 mg TN/L for several parts of the United States. These guidelines aim to curb the hypoxic conditions and eutrophication issues in vulnerable receiving water bodies. With advancements in science and technology, WRRFs are capable of removing > 95% of inorganic nitrogen resulting in sON being a major nitrogen fraction (> 50%) of the effluent TN. Several studies have described that about 60-70% of the total influent sON is removed by ASP (Parkin and McCarty,1981a; Sattayatewa et al., 2010; Pagilla et al., 2011) while Simsek et al. (2012) found that 37-50% of the influent sON is biodegraded by a TF system. The majority of the research work related to sON degradation has been focused on conventional ASP (Bratby et al., 2008; Simsek et al., 2016; Hu, Liao, Shi et al., 2018) while few studies have touched on the fixed film processes, mainly on TF and DNF (Simsek et al. 2012, 2013; Hu, Liao, Geng et al., 2018).

At WRRFs, MBBRs are employed usually as a separate stage nitrification process (to nitrify wastewater with a lower C/N ratio). Considering the consequences of elevated fraction of sON in the effluent (complication with permit compliance and impairment of receiving water quality), it will be reasonable to identify the available strategies in an MBBR process to control the concentration of sON while avoiding the need for (additional) advanced removal technologies.

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[1] The material in this chapter was co-authored by Ruchi Joshi Bhardwaj and Dr. Eakalak Khan. Ruchi Joshi Bhardwaj had primary responsibility for collecting samples from the WRRF, methodology and formal analysis. Ruchi Joshi Bhardwaj was the primary developer of the conclusions that are advanced here. Ruchi Joshi Bhardwaj also drafted and revised all versions of this chapter. Dr. Eakalak Khan served as a project administrator, proofreader, and supervisor for validating the investigation conducted by Ruchi Joshi Bhardwaj.

Simsek et al. (2013) investigated the fate of bsON and AbsON in a full-scale WRRF consisting of both ASP and MBBR. The biodegradable fraction of sON or bsON can be biochemically oxidized by bacteria to produce NH_3-N (Khan et al., 2009) whereas the bioavailable fraction of sON or AbsON can be uptaken by algae or other aquatic plant species for growth (Pehlivanoglu-Mantas and Sedlak, 2004, 2006). Nitrogen cycling can be influenced by the form of sON in the effluent. For instance, DFAA can be directly uptaken by (bioavailable to) the algae; however, other forms of sON might have to be first hydrolyzed and/or mineralized (biodegraded) by bacteria making them bioavailable to the algae or other phytoplanktons in the receiving waters (Simsek et al., 2013; Zheng et al., 2021). Results from the study by Simsek et al. (2013) concluded that ASP removed 29% sON whereas MBBR removed only 4% sON. The authors suggested that a low C/N ratio, solubilization of particulate organics from the biofilm, and/or release of soluble microbial products (SMPs) might have affected the sON removal in the MBBR process along with other possibilities (Simsek et al., 2013).

Hu, Liao, Geng et al. (2018) investigated the effect of different C/N ratios (3, 4, 5 and 6) on the removal of sON and AbsON in DNFs. They fed secondary effluent to the filters and noticed the maximum effluent sON at C/N ratio of 3 (1.91 mg sON/L) and no impact on effluent sON for higher C/N ratios i.e., 4 (1.70 mg sON/L), 5 (1.70 mg sON/L) and 6 (1.69 mg sON/L). However, effluent AbsON decreased with increasing C/N ratio suggesting that sON produced by DNFs at higher C/N ratios will be less bioavailable, a scenario favorable for the receiving waters (Hu, Liao, Geng et al., 2018). The studies of Simsek et al. (2013) and Hu, Liao, Geng et al. (2018) indicated that relatively less sON removal should be expected under a lower C/N ratio. However, no study has explicitly investigated the removal of sON in an MBBR process under different C/N ratios.

Effluent sON from biological treatment processes is primarily from influent- and process-derived sources. The influent-derived sON is the result of recalcitrant ON, which has not been biodegraded or removed during wastewater treatment. Process-derived sON is released by metabolic activities associated with the biological processes (e.g., SMPs and extracellular polymeric substances) (Pehlivanoglu-Mantas and Sedlak, 2006; Simsek et al., 2016; Hu et al., 2020). Since process-derived sON is contributed by the growth and decay of microorganisms during the biological treatment processes, process-derived sON is unavoidable and more closely related to operational parameters than influent-derived sON (Sattayatewa et al., 2009). Approximately 33% of the effluent sON are process-derived while the rest of it is from the influent (Randtke et al., 1978; Novak, 2006). However, the extent of the biological production of sON varies from one biological system to the other (Bratby et al., 2008). Therefore, reducing the formation of process-derived sON in the biological treatment processes will be beneficial in achieving the low TN discharge limits and eventually safeguard the water bodies receiving the treated wastewater. Parkin and McCarty (1981b) investigated the influence of organic loading (glucose, acetate, glucose-acetate mixture) on sON production and found that an increase in organic loading increased sON production in the ASP (Parkin and McCarty, 1981b). Although there have been several studies investigating the effect of organic loading (measured as chemical oxygen demand (COD)) on nitrification in an MBBR process (Kermani et al., 2008; Bassin et al., 2012; Torkaman et al., 2015; Bassin et al., 2016), no study has investigated the effect of organic loading on the production of sON by biofilm particularly those in an MBBR.

Simsek et al. (2013) reported sON removal of 29% by ASP and 4% by MBBR (for nitrification) in a full-scale WRRF. Their study suggested that in an MBBR, lower ammonification of sON occurred due to less ability of ammonifying bacteria to compete for oxygen compared to

nitrifiers (Simsek et al., 2013). Ammonification is a major pathway for sON degradation and is considered to be achieved primarily by heterotrophs and phytoplanktons (Ohkouchi and Takano, 2014). Ammonia produced from ammonification is transformed via nitrification and/or assimilated into biomass. Since nitrifiers are primarily known autotrophs, they are not believed to be directly associated with sON degradation (Metcalf and Eddy, 2003; Simsek et al., 2013). Hence, sON degradation is largely considered to be a heterotrophic bacterial process.

Few studies reported that while heterotrophic processes remove higher fraction of sON, reduction in sON concentration was also observed after nitrification stages at full-scale WRRFs highlighting the involvement of nitrifiers in sON biodegradation (Simsek et al., 2012; 2013). Wadhawan et al. (2015) reported 57% sON removal through the nitrification process in secondary effluent and lesser removal through the heterotrophic process (38%). The nitrification process biodegraded higher concentration of sON compared to the heterotrophic process. The study also claimed that during the nitrification, AOB rather than NOB were responsible for sON degradation and it is the first study that reported the involvement of nitrification in sON degradation. Therefore, based on the results from these previous studies (Simsek et al., 2012; 2013; Wadhawan et al., 2015), this task aimed at exploring the production of sON and the effect of C/N ratios on sON degradation during nitrification in a MBBR.

The objective of this task was to identify the influence of organic loading and different C/N ratios on sON activity (production and removal) in bench-scale reactors that mimic the nitrification process of MBBRs. Specifically, this task examined the effect of readily bioavailable COD on the production of sON by feeding synthetic wastewater with no organic nitrogen. This task also investigated the effect of different C/N ratios on sON degradation for which the reactors received real wastewater samples representing different C/N ratios. Results from this task could extend the

knowledge on the fixed film process with respect to sON activity to regulate and optimize reactor operation in order to achieve low TN discharge limits.

4.2. Materials and Methods

4.2.1. MBBR Media and Wastewater Sample Sources and Collections

The biofilm carriers used in this task were collected from the nitrifying MBBR basin of the Moorhead wastewater treatment plant (MWWTP), Moorhead, MN (Figure 4.1). The biofilm carriers were collected from the basin in bulk using a 5 L bucket and transported within 15 min to a laboratory where experimental work was conducted. The carriers were separated from the liquid phase using a stainless-steel strainer. The separated carriers were weighed, and equal amounts (50% of the reactor volume) were added immediately to four 1 L beakers (batch reactors).

To represent varying C/N ratios, effluent grab samples were collected from the equalization basin (C/N = 4.2:1), primary clarifier (C/N = 1.5:1), activated sludge effluent (C/N = 0.8:1) and MBBR effluent (C/N = 0.2:1) as shown in Figure 4.2. The C/N ratios were obtained by dividing sCOD with TSN (C/N: 4.2 = 168/39.6, C/N: 1.5 = 74/48.4, C/N: 0.8 = 33/40.1; and C/N: 0.2 =8/38.8). The collected wastewater samples were used in the experiments immediately after they were brought to the laboratory. Portions of collected wastewater samples were used for the analyses of TSS, VSS, inorganic nitrogen species and sON. Experimental work (operation of batch reactors as described in subsection 4.2.3) for each objective (production versus removal) was triplicated. The biofilm carriers as well as wastewater samples were collected three times for each objective corresponding to the triplication.

Figure 4.1. Biofilm carriers (A) collected from MBBR basin (B), magnified image of a single biofilm carrier with accumulated biofilm inside (C).

The MWWTP has a peak pumping capacity of 38,000 m^3/day and an average flow of 15,000 m^3/day. The facility must be in compliance with the discharge limits for BOD and NH_3 and is not regulated for the TN limits. The facility employs high purity oxygen ASP (HPO-ASP) for removing BOD. A 3,024 m^3 MBBR is used to nitrify NH_3 in the treated wastewater from HPO-AS. The hydraulic retention time and sludge retention time of the MBBR are 3.2 hand 32 d respectively. The reactor is filled approximately 32% with biofilm carriers (21 mm in diameter) that move throughout the reactor with the mixing action caused by the aeration system. More detailed information regarding the MBBR basin and the media is tabulated in Table A1 (under Appendix). The media provides a surface for nitrifying bacteria to attach and grow while a screen on the discharge keeps the media in the reactor.

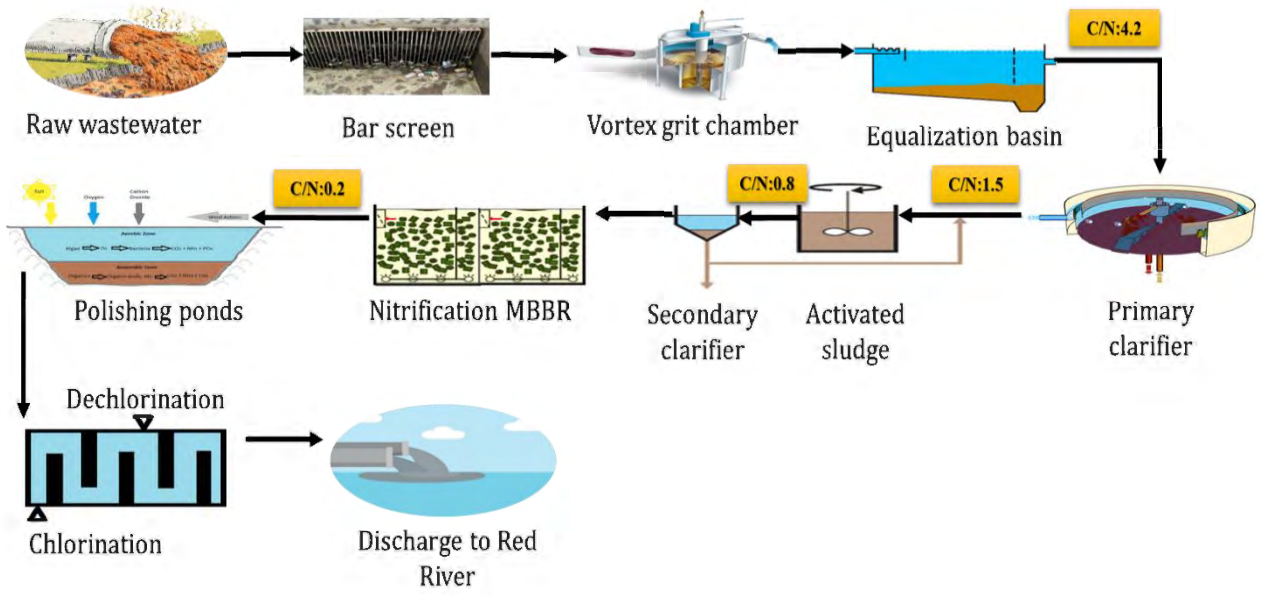

Figure 4.2. Graphical representation of the MWWTP. Sampling locations with respective C/N ratios are indicated by yellow boxes.

4.2.2. Synthetic Wastewater Recipe

The synthetic wastewater (SWW) recipe was modified from Nagaoka et. al. (1996) to mimic medium-strength domestic wastewater composition. SWW was employed to identify the effect of organic loading (readily bioavailable COD) on sON production in an MBBR process. Therefore, two different solutions of SWW (A and B) were prepared wherein the basic composition remained same as described in Chapter 3 under subsection 3.2.1. SWW A received no-COD, whereas SWW B received 400 mg COD/L using glucose. SWW contributed inorganic nitrogen (40 mg N/L) via NH_4Cl, and no ON was added in the SWW solution.

4.2.3. Experimental Setup

This task was divided into two parts with two separate focuses: effect of organic loading on sON production and effect of C/N ratio on sON degradation.

4.2.3.1. Effect of Organic Loading on sON Production

For the first part, bench-scale experiments were conducted to identify the production of sON when the reactors were fed with SWW containing no organic nitrogen (Figure 4.3). Glucose

is a readily bioavailable source of COD which was added in the SWW that was fed to one of the two reactors. Two 1 L reactors were filled 50% (reactor volume) with biofilm carriers (~118 g/reactor) collected from the nitrifying MBBR basin of the MWWTP. Each reactor was fed with SWW-A (0 mg COD/L) and SWW-B (400 mg COD/L) to make up the final volume to 1 L. The resulting organic loading rates were 0 and 2 g sCOD/m^2 of carrier surface whereas NH_3 loading rate in each reactor was 0.2 g NH_3-N/m^2 of carrier surface. Both reactors were aerated using air stone-diffusers to maintain a DO concentration between 2-4 mg O_2/L at room temperature (~20°C). pH inside the reactors was maintained at 7.2 - 7.8 using HCl and $NaHCO_3$. Samples were collected from each reactor every 30 minutes until NH_3 concentration reached below the detection limit (0.015 mg NH_3/L).

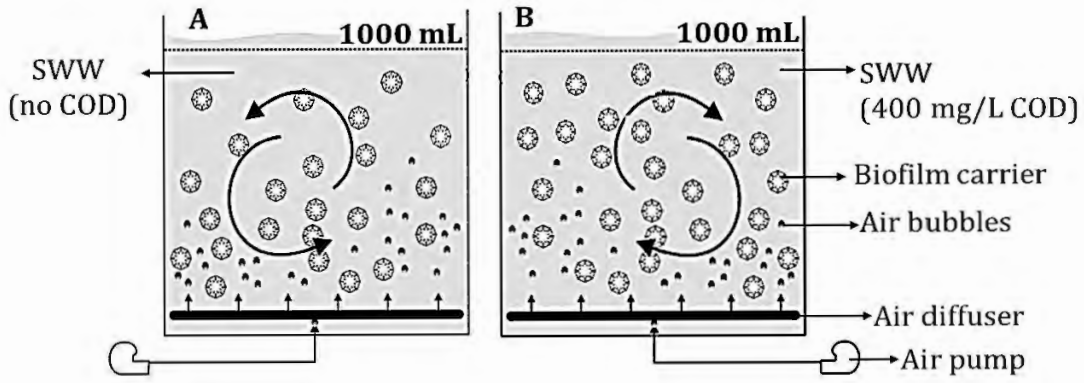

Figure 4.3. Experimental setup to investigate the effect of organic loading (readily bioavailable COD) on sON production. Reactors were fed SWW containing 0 mg COD/L (A) and 400 mg COD/L (B).

4.2.3.2. Effect of C/N Ratio on sON Degradation

For the second part, batch experiments were conducted to investigate the effect of different C/N ratios on sON degradation (Figure 4.4). Fifty percent of the 1 L beakers were filled with biofilm carriers (~115 g/reactor) collected from the MBBR basin. Each reactor was filled to make up a final volume of 1 L with wastewater sample collected from four different locations, i.e., after equalization basin (C/N=4.2), after primary clarifier (C/N=1.5), after activated sludge (C/N=0.8),

and after MBBR basin (C/N=0.2) (Figure 1). The reactors were operated at room temperature (~20°C) while maintaining the pH between 7.2-7.8 and DO between 2-4 mg O_2/L. All the reactors were operated continuously until the concentration of NH_3 was below the detection limit (0.015 mg NH_3/L). Samples were collected from each reactor every 30 min and were analyzed for sON. To examine if different C/N ratios affected the microbial activity in the biofilm attached to the biofilm carriers, an adenosine triphosphate (ATP) assay was used. The ATP assay is an indirect measurement for active cells, including non-culturable cells, based on their metabolic activity. It determined relatively an amount of active biomass in the biofilm attached to the biofilm carrier that was collected immediately before and after the operation of reactors.

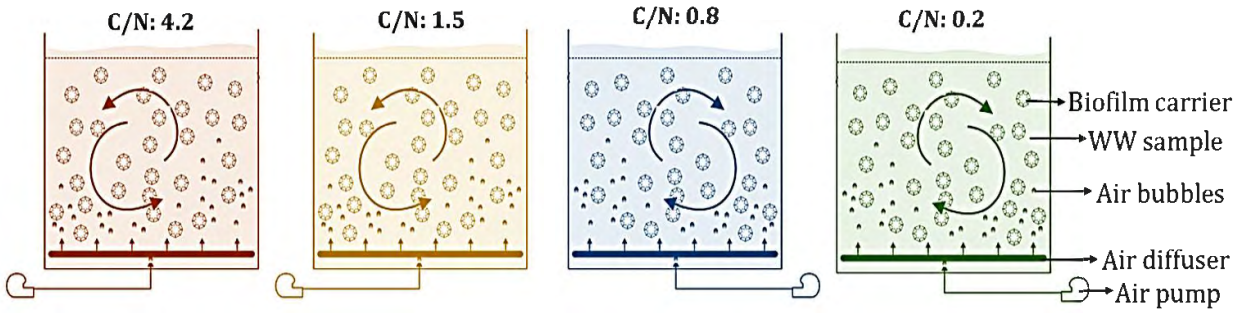

Figure 4.4. Experimental setup for investigating the effect of different C/N ratios on sON in bench-scale MBBRs.

4.2.4. Analytical Techniques

The analytical techniques employed in this task are presented in Chapter 3 under subsection 3.2.5.

4.2.5. Measuring Soluble Fraction and sON

Filtering a wastewater sample through a 0.45 µm pore size membrane filter to obtain soluble fraction can allow colloidal fraction ranging between 0.1 and 0.45 µm diameter to pass through in the filtrate. Hence, the flocculation-filtration technique (Mamais et al., 1993) was employed to remove both particulate and colloidal fractions from the samples. Zinc sulfate

($ZnSO_4$) and sodium hydroxide (NaOH) were added to the sample to flocculate colloids and particulates followed by filtration with a 0.45 μm pore size cellulose acetate membrane filter (PALL Co., Port Washington, NY, USA) to obtain true soluble fraction. sON concentration was determined as the difference between TSN and total inorganic nitrogen (ammonia + nitrite + nitrate).

4.2.6. Bacterial Growth Assessment

The QuenchGone21^{TM} wastewater test kit (Luminultra, New Brunswick, Canada) was used to assess the bacterial activity over a period of time (start and end of reactor operation) in the biofilm attached to the biofilm carriers in each reactor. The assay uses ATP as an indicator of biomass activity. The protocol provided in the QuenchGone21^{TM} wastewater test kit was followed. The ATP assay measures light produced from a luminescent reaction between ATP (from the wastewater sample) and a mixture of luciferin, luciferase (an enzyme which naturally occurs in the tails of fireflies to produce light), and magnesium. Since the oxidation of one molecule of ATP produces one photon, concentration of ATP in a sample is proportional to the emitted light. The light output i.e., relative light units (RLU) was measured with a luminometer. The obtained RLU values were converted to ATP concentrations (μg ATP/g biofilm).

4.3. Results and Discussion

4.3.1. Effect of Organic Loading on sON Production

4.3.1.1. MBBR Performance and sCOD Profile

Figure 4.5 shows the sCOD profile in reactors A and B when the reactors were operated for a duration of 4.5 h. Although no COD was added in reactor A, sCOD concentration of nearly 70 mg sCOD/L was detected in the reactor at t = 0 min. The presence of sCOD was attributed to biofilm carriers added to the reactors from the full-scale MBBR basin. Since media-contributed

COD is a residual content from two biological treatment processes (ASP and MBBR), it is assumed to be biorefractory in nature. The media-contributed COD could be released by following two routes. The recalcitrant sCOD leached from the biofilm into the bulk phase and/or readily hydrolysed particulate matter from the biofilm detached into the bulk phase (Sawyer and Hermanowicz, 1998; Hunt et al., 2004). Both the leaching and detachment were likely promoted by agitation via aeration in the reactors. The presence of recalcitrant COD identified in both reactors was unavoidable, therefore, from further on, reactor A will be referred to as "control" whereas reactor B will be referred to as "glucose" highlighting the presence of readily bioavailable COD source that was added through SWW. Hence, the operation of reactor A began with internal COD whereas reactor B operation began with both internal as well as an external source of COD.

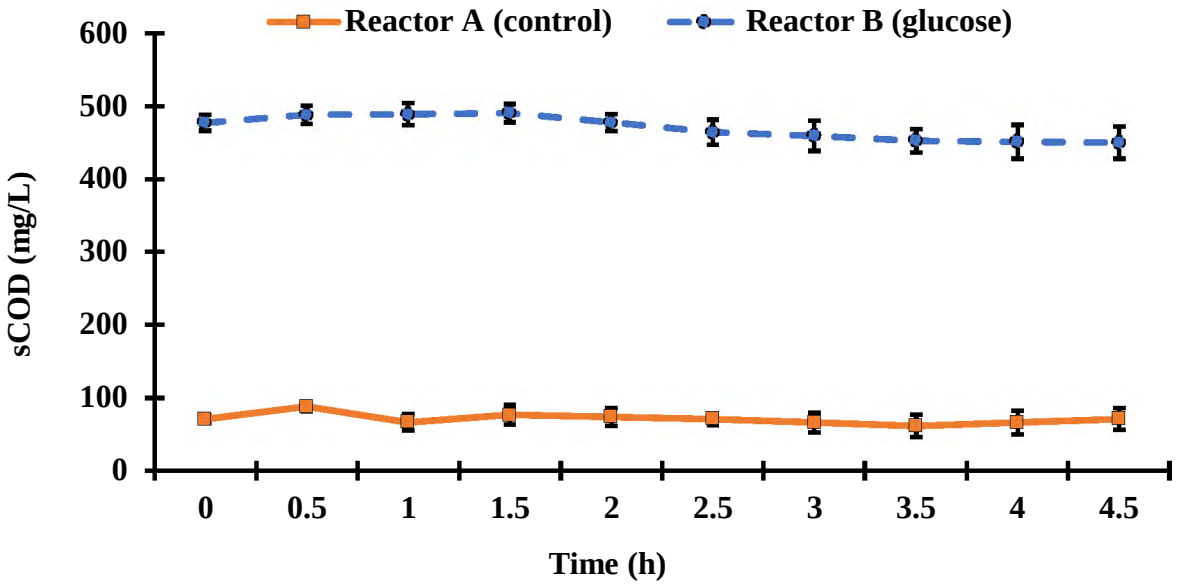

Figure 4.5. COD removal during the nitrification process in reactors A (internal COD) and B (internal + external COD).

Graphically, no significant sCOD reduction was observed in the bulk phase of each reactor (Figure 4.5). However, over a period of 4.5 h of operation, a net concentration of 26 mg sCOD/L was removed from the bulk phase of reactor B whereas no reduction was observed for reactor A.

Two possibilities have been postulated for the decrease of sCOD concentration observed in the bulk phase of reactor B. Since the addition of glucose was the only difference between the two reactors, the first possibility is that the growth of heterotrophs in the biofilm of reactor B could have enhanced the sCOD removal. The second possibility is that sCOD might have diffused from the liquid phase into the biofilm to equilibrate the concentration gradient. The biofilm composition was not examined, hence, these are only speculations based on bulk phase sCOD analysis.

4.3.1.2. Nitrification and sON Profile

Figure 4.6 displays the nitrification profile of each reactor wherein by 4.5 h of operation, MBBR media added to each reactor successfully nitrified NH_3 (>99%) to below detection limits. In reactor B (glucose), nitritation (NH_3 to NO_2^-) and nitratation (NO_2^- to NO_3^-) were achieved relatively earlier as compared to reactor A (control). At 2.5 h of operation, higher removal of influent NH_3 was observed under reactor B (94.6%) than reactor A (54.2%). Bassin et al. (2012) reported that less time was required to achieve complete removal of NH_3 in the presence of organic carbon during the initial operation of a laboratory-scale MBBR. The authors tied the heterotrophic condition (presence of casein peptone, meat extract and urea) to the enrichment of nitrifiers in the biofilm. The presence of higher amounts of organic carbon resulted in greater EPS production by heterotrophs, which promoted the attachment of nitrifying bacteria to biofilm and reduced loss of nitrifiers through detachment, thereby resulting in an increased nitrification rate (Bassin et al., 2012). Considering the availability of readily bioavailable organic carbon and NH_3 in the reactor, the explanation by Bassin et al. (2012) agrees with the difference in the nitrification rates observed in this task.

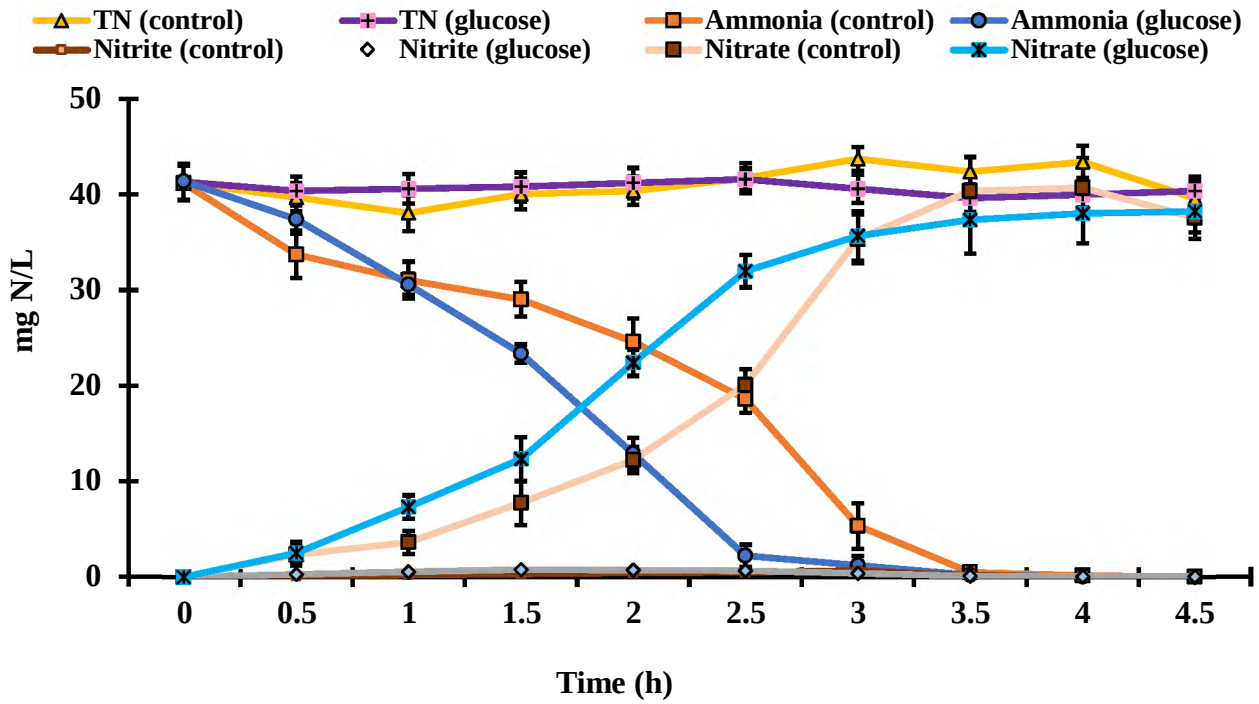

Figure 4.6. Nitrification profile of bench-scale MBBRs operated for a duration of 4.5 h. Operation of reactor A (control) started with internal COD whereas reactor B (glucose) started with a combination of internal + external COD.

Figure 4.7 shows the sON profiles observed in each reactor. Net production of process-derived sON was identified at every time point in each reactor when no organic nitrogen was fed. Similar to the presence of internal COD in each reactor at t = 0 h, 0.36 mg sON/L in reactor A and 0.57 mg sON/L in reactor B were observed. The presence of residual sON in both reactors at t = 0 h is for the same reason given above for residual COD. After 4.5 h of operation, reactor A (control) produced 1.63 mg sON/L of whereas reactor B (glucose) produced 1.58 mg sON/L.

In reactor A, sON concentration increased during the first 30 minutes of reactor operation after which it gradually decreased with some minor fluctuations. sON production in the first half-hour of operation is attributed to the prevalence of biomass starvation or decay because organisms in the biofilm possibly got stressed due to change of environmental conditions (real wastewater or feast versus SWW or famine) and insufficient bioavailable organic carbon in the reactor (Hao et al., 2010; Curtin et al., 2011). Under stressful periods, excretion of sON to establish a concentration

of equilibrium across the cellular membrane increases sON concentration in the reactor (Parkin and McCarty, 1981b). Moreover, starvation has the potential to induce bacterial death via programmed cell death or self-destruction of cells under stressful conditions (Hao et al., 2010) which may as well contribute to sON in the reactor.

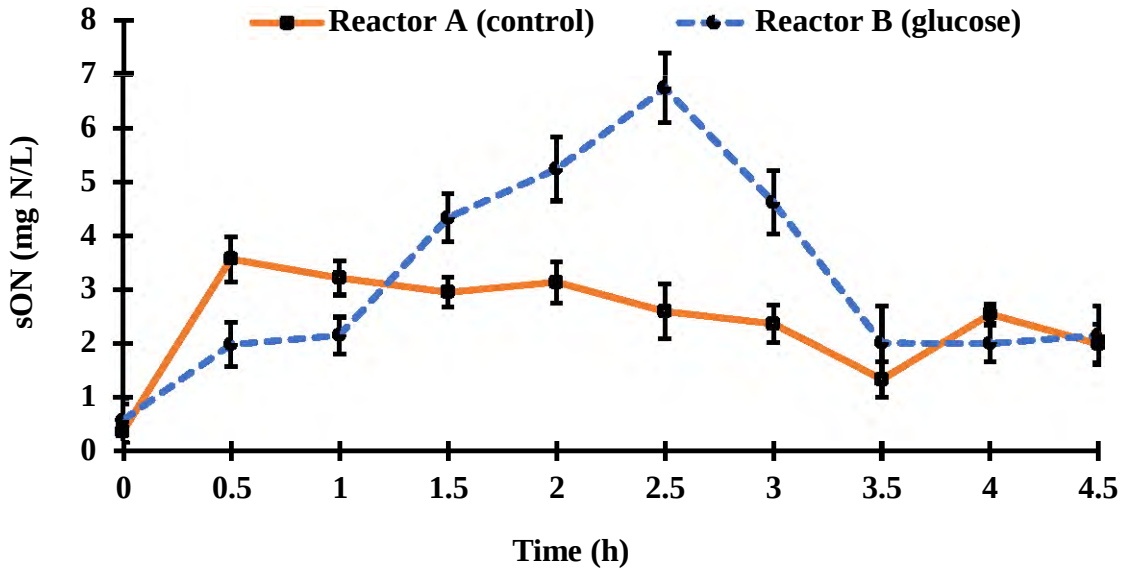

Figure 4.7. sON profile in a nitrifying MBBR system in the presence of an internal source of COD (reactor A) and internal + external source of COD (reactor B).

Unlike autotrophs, lack of organic carbon is expected to significantly affect the heterotrophs whereas change in environment would affect both heterotrophs and autotrophs. Since NH_3 is added in the SWW, autotrophs are assumed to face less stress i.e., no starvation, as compared to heterotrophs. Considering that biomass decay releases soluble microbial products (specifically biomass associated products that originate from decay), fraction of which contribute cellular macromolecules containing organic carbon and nitrogen (Namkung and Rittmann, 1986; de Silva and Rittmann, 2000; Ni et al., 2011). This organic carbon which might be partially bioavailable, could be oxidized by the heterotrophs as a substrate to support growth or cell maintenance. However, since no increase in COD concentration was observed in reactor A (Figure 5) and the organic carbon (released from decay) is expected to be available in small amounts, the

heterotrophic growth is possibly minimal. Besides, the growth of nitrifiers in the presence of NH_3 could have as well contributed to sON since nitrification was happening in the reactor (Figure 4). Therefore, larger fraction of sON release is attributed to heterotrophic activities particularly the decay than autotrophic growth in the reactor in the first half hour of operation.

The sON concentration after t = 0.5 h of operation reduced steadily in the bulk phase which could be attributed to the dominance of ammonification of sON in the reactor over sON release. Although ammonification dominates after 0.5 h, the heterotrophic decay in the reactor was likely to continue to take place, although submissively beyond 0.5 h of reactor operation. In addition, nitrification was also in progress between 0.5 to 3.5 h of reactor operation. After 3.5 h of operating the reactor, externally added NH_3 had significantly depleted (Figure 4) and the sON concentration increased slightly followed by a small decrease during the last hour of reactor operation. Since NH_3 was essentially gone and sON was present in low concentrations, an increase in sON concentration (t = 3.5 h) is attributed to lack of required nutrients or starvation leading to endogenous respiration for the autotrophic bacterial cells to obtain energy for maintenance (Hao et al., 2010). Since nitrification was reduced after 3.5 h of reactor operation, endogenous respiration of both autotrophs and heterotrophs (pre-existing decay) seems to dominate in the reactor. Thereafter, the excreted sON was possibly ammonified by the surviving bacteria in the next half hour (t = 4.0 to 4.5 h) to support growth or cell maintenance. Overall, between 0 (0.36 mg sON/L) and 4.5 h (2.0 mg sON/L) of operating reactor A, a net production of 1.64 mg sON/L was observed. The overall analysis of sON activity highlights the cycling of organic nitrogen under a nutrient-limited environment. This dynamic behavior of sON was also observed by Khan et al. (2009) wherein the sON activity of a sample collected from a full-scale WRRF was monitored over a period of 180 d. Heterotrophic growth and decay, and autotrophic growth (nitrification) and

ammonification co-exist in the nitrifying MBBR wherein the dominating process determines the fate of sON concentration.

In reactor B, sON concentration increased consistently until 2.5 h of operation followed by a sharp decline in the next hour and then stayed relatively constant until the end of the reactor operation. During the first 2.5 h of operation, the externally added NH_3 in reactor B was nitrified (Figure 4.6). The drastic increase in sON concentration in 2.5 h of operation highlights that the production overtook the ammonification of sON in the reactor. Unlike reactor A, wherein an increase in sON concentration was primarily attributed to the decay of heterotrophs in the first half-hour, the increase in sON concentration in reactor B is attributed to the growth of heterotrophs. Contrary to reactor A, reactor B was fed with readily available organic carbon which possibly enhanced the growth of heterotrophs, releasing sON as a result of substrate oxidation (Parkin and McCarty, 1981c). In addition, the availability of NH_3 supported nitrification, further releasing sON from autotrophic growth in the reactor. The higher net production of sON in reactor B compared to reactor A is mainly due to the heterotrophic growth in reactor B agreeing with the fact that heterotrophs are much faster growers than autotrophs.

In reactor B, sON concentration after 2.5 h of operation declined considerably suggesting that ammonification of sON trumped sON production. The depletion of readily available NH_3 in the reactor (after 2.5 h) triggered ammonification of the available sON (to generate $NH_3$for growth). Since the externally added $NH_3$was the primary source of nitrogen for the bacteria, after depletion, NH_3 was generated from the ammonification of the available sON in the reactor. The NH_3 generated from ammonification of available sON is assumed to be rapidly nitrified, hence, no increase in the NH_3 concentration was observed in the reactor after 2.5 h of operation (Figure 4.6). After its decline, the sON concentration did not vary much in the last hour of operating reactor B.

Overall, between 0 (0.57 mg sON/L) and 4.5 h (2.15 mg sON/L) of operating reactor B, a net production of 1.58 mg sON/L was observed which was quite close to that observed in reactor A (1.64 mg sON/L).

The net concentration of the sON in each reactor suggests that irrespective of the presence of organic substrate in the reactor, the MBBR will be contributing sON to the reactor during the nitrification process. However, unlike the batch setup in this objective, in full-scale nitrifying MBBRs, both organic carbon (small fraction of bioavailable carbon) and NH_3 (large fraction of TN) are continuously fed. Therefore, there is never really a dearth of those nutrients in the reactor which suggests that the production of sON may dominate over ammonification of sON which is less needed when NH_3 is always available. The organic carbon in the MBBR influent, besides being low in concentration, is mostly recalcitrant in nature. It indicates that heterotrophic growth will not be significantly enhanced under such an environment, thus resulting in reduced ammonification of sON. Moreover, sON activity is expected to vary with changes in operating and/or substrate conditions. For example, the sON production may increase if COD is not removed well upstream due to organic loading increase and/or inhibition of the biological organic carbon process. Although the final concentration of sON in this task was close at a specific duration, it may as well fluctuate if the reactors were further operated which is attributed to the difference in the microbial activity occurring within each reactor that eventually dictates the organic nitrogen cycling. Findings from this objective display a strong dependence between substrate concentrations (organic carbon and NH_3) and bacterial activity and these two related factors firmly influence the production or ammonification of sON in a nitrifying MBBR.

4.3.2. Effect of C/N Ratios on sON Degradation

4.3.2.1. Nitrification and sON Profile

Figure 4.8 displays the nitrogen profiles of the four reactors when fed with real wastewater samples representing C/N ratios of 4.2, 1.5, 0.8 and 0.2. Unlike the previous section, which focused on the production of sON using SWW, this objective focused on sON ammonification by using real wastewater samples. After 4 h of operation, NH_3 in each reactor was successfully nitrified to below the detection limit. Consistent nitritation and nitratation were observed in reactors with C/N ratios 4.2, 1.5 and 0.8, however in the reactor with a C/N ratio of 0.2, after 2 h of operation, NH_3 concentration increased from 0.07 to 0.25 mg NH_3/L possibly due to ammonification of sON and NO_3^- concentration decreased from 40.33 to 34.39 mg NO_3/L suggesting reduced nitrification. Considering the extremely low initial concentration of NH_3 in the reactor (1.66 mg NH_3/L), the nitrification rate (0.66 mg NO_3^-/L/h) was expected to be lower than those observed in the other reactors. The C/N ratio of 0.2 is based on the final effluent sample in which the majority of NH_3 has already been removed. Hence, the low concentration of NH_3 likely limited the nitrification rate at the C/N ratio of 0.2.

The nitrification rate at the highest C/N ratio of 4.2 was lower (5.62 mg NO_3^-/L/h) than those observed at the C/N ratios of 0.8 (7.18 mg NO_3^-/L/h) and 1.5 (7.98 mg NO_3^-/L/h). This finding of lower nitrification rate at the highest C/N ratio (Figure 4.8c) aligns with the findings from past studies wherein similar results were reported while using different methods including biofilters, activated sludge system, MBBRs with different types of media (Carrera et al., 2004; Ling and Chen, 2005; Torkaman et al., 2015; Bassin et al., 2016). The C/N ratio of 4.2, besides offering the highest concentrations of organic carbon and NH_3, also represents a large pool of bioavailable nutrients, which are expected to enhance the heterotrophic growth substantially. This

pool of bioavailable nutrients decreases with decreasing C/N ratios and is expected to affect the bacterial population and activity in each reactor. Nitrification process is significantly influenced by the concentration of organic carbon in the reactor (Hanaki et al. 1990; Cheng, 1994). Since the outer layers of biofilm are primarily inhabited by heterotrophs, increasing the organic carbon load in the bioreactor further decreases the rate of nitrification (Satoh et al., 2000). Whenever influent organic carbon load was increased in an oxic bioreactor, nitrifiers were mostly found in the inner layers of a biofilm wherein only limited oxygen is available for the bacteria to thrive (Jing et al., 2009). In this task, the increasing C/N ratio represents increasing concentration of organic carbon and its bioavailability and hence, enhanced heterotrophic growth in the reactors. The domination of heterotrophs took away the available space and dissolved oxygen for the nitrifiers, thus reducing the nitrification rate in each reactor. Therefore, at the highest C/N ratio (4.2), reduced nitrification rate was observed. Although nitrification was successfully achieved in each reactor, the rates were highly influenced by the concentrations and availability of organic carbon.

The nitrification rate results observed in objective 2 conflict with those observed in objective 1 (Figure 4.6), i.e., a relatively higher nitrification rate was observed in the presence of externally added organic carbon (8.48 mg NO_3^-/L/h) than that in the reactor that was not fed with any external carbon source (8.33 mg NO_3^-/L/h). Since the reactors in objective 1 were fed with SWW instead of real wastewater, the difference between the characteristics of the two carbon sources may lead to this contrast. The increased nitrification rate in the presence of higher organic loading was also reported by a study of Bassin et al. (2012) that used SWW containing readily available carbon (Casein peptone, meat extract, and urea).

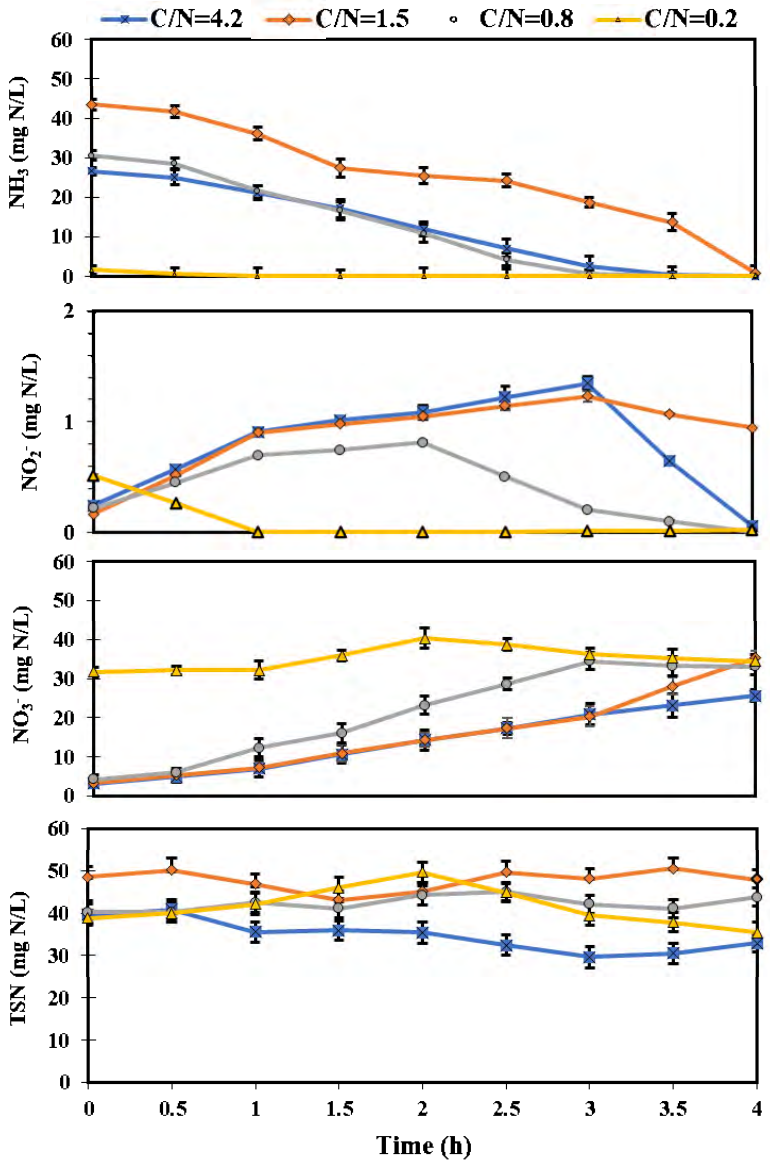

Figure 4.8. Nitrogen profile of bench-scale MBBRs when operated under different C/N ratios.

The C/N ratios tested (4.2, 1.5, 0.8, and 0.2) basically vary because of the sCOD concentrations rather than the TSN concentrations. The decreasing trend reflects the change in sCOD concentration after going through different treatment processes at the MWWTP. The concentration of sCOD changes drastically compared to the TSN, which is relatively stable but consists of different nitrogen fractions. Not only the level of COD decreased throughout the treatment train, but also its biodegradability decreased (or its biorecalcitrance increased) after the

activated sludge and MBBR (C/N = 0.8 and 0.2). Therefore, at C/N = 0.2, which represents the MBBR effluent, sCOD besides being lowest in concentration, is the most biorecalcitrant. Regarding the changes in N fractions, organic nitrogen and NH_3 dominate the influent (C/N = 4.2) while NH_3 concentration dominates after the activated sludge (C/N = 0.8) and nitrate is the major fraction of the MBBR effluent (C/N = 0.2).

Unlike the sCOD/TSN ratio wherein the variation is primarily attributed to change in sCOD concentrations, the corresponding sCOD/sON ratio reflects variations contributed by both sCOD and sON concentrations. The sCOD/sON ratios (15.7, 47.3, 6.6, and 1.6) besides being higher than the sCOD/TSN ratios showcases the influence of sON fraction on the ratio. An overall decreasing trend is observed in the sCOD/sON ratios, however, a significant jump in the ratio (15.7 to 47.3) indicates ammonification of biodegradable sON prevailing in the primary clarifier. The decreasing ratios (15.7, 6.6, and 1.6) reflect the production and slower degradation of sON concentrations which is assumed to be less biodegradable after the major ammonification process observed at sCOD/sON ratio of 47.3. Ammonification, unlike other biological processes, is more spontaneous and can exist under both oxic and anoxic conditions (Selecky, 2005). For instance, ammonification takes place faster than nitrification in terms of kinetics because nitrifying bacteria have relatively slow growth rates and a small acceptable pH-range (Rittstieg et al., 2001; Stefanakis et al., 2014). The majority of organic nitrogen before the ASP is urea which is readily oxidized to ammonium to derive metabolically useful energy by the bacteria (Pehlivanoglu-Mantas and Sedlak, 2006; Urgun-Demirtas, Pagilla et al., 2008). Therefore, substantial ammonification in the primary clarifier is not uncommon.

This task will focus primarily on four biological processes i.e., heterotrophic growth, heterotrophic decay, nitrification and ammonification, in terms of their involvements in regulating

the sON activity. The sON profile observed under different C/N ratios is displayed in Figure 4.9. Altogether, in reactors with the C/N ratios of 4.2 and 0.2, net removal of 3.9 and 4.1 mg sON/L was observed, respectively, whereas, in the reactors with C/N ratios of 1.5 and 0.8, net production of 5.6 and 8.7 mg sON/L was observed, respectively.

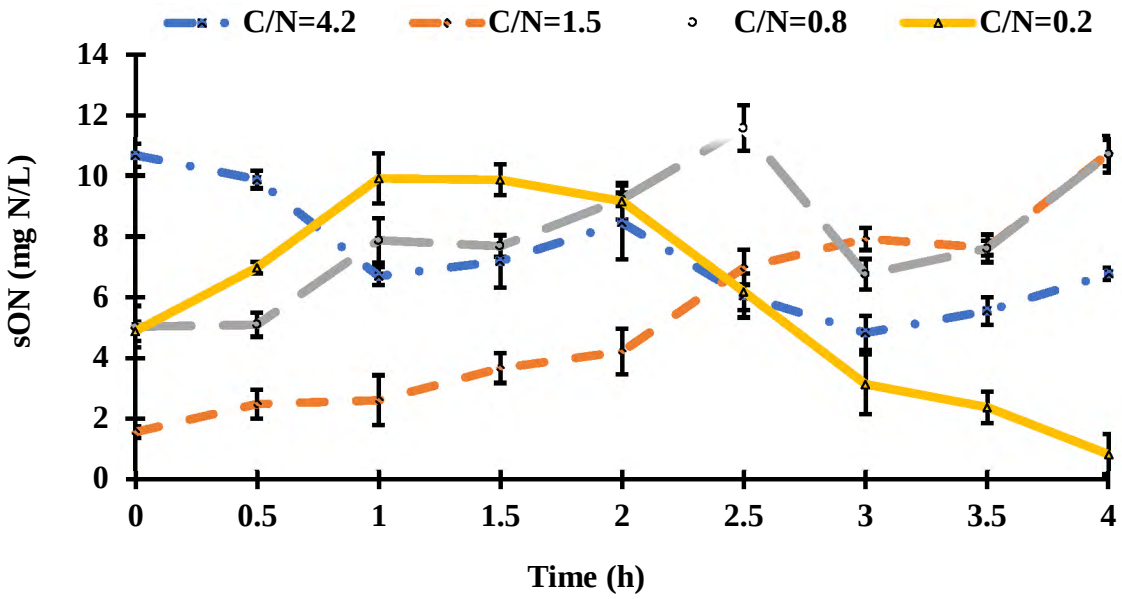

Figure 4.9. Soluble organic nitrogen profile of bench-scale MBBRs when operated under different C/N ratios.

Overall, the production of sON in batch reactors with C/N ratios of 1.5 and 0.8 is associated with carbon oxidation and nitrification occurring in the reactors. Although the initial availability of organic carbon, ON, and NH_3 fluctuates within the two reactors, active metabolic activity (carbon oxidation and nitrification) due to the presence of readily available nutrients results in the net production of sON. Besides, ammonification also co-existed in the reactor but was less dominant. Unlike the reactor with a C/N ratio of 1.5 wherein mainly production dominated the reactor throughout the operation period, in the reactor with a C/N ratio of 0.8, ammonification did overtake the sON production briefly (2.5 to 3 h), causing a decrease in sON concentration. For the C/N ratio of 0.8, between 2.5 and 3.0 h, ammonification surpassed the production of sON due to

the significant depletion of NH_3 in the reactor. Thereafter, sON concentration increased in the last hour of operation, however, there was no increase in NH_3 concentration (from ammonification). Therefore, nitrification could not have enhanced the sON concentration in the last hour. Lack of both, NH_3 and organic carbon likely stimulated endogenous respiration of both autotrophs and heterotrophs, thus releasing sON in the reactor (Romillac, 2019). Hence, the net production of sON was observed in the reactor with a C/N ratio of 0.8.

The net removal of sON concentration observed in the reactors with C/N ratios of 4.2 and 0.2 is attributed to the dominance of ammonification over the production of sON overall in each reactor. However, ammonification observed in each reactor was attributed to different reasons. The wastewater sample contributing a C/N ratio of 4.2 provided a large concentration of organic carbon which must have enhanced the heterotrophic growth in the reactor consequently resulting in accelerated ammonification of readily available sON (contributed by the sample), predominantly by the heterotrophs. Therefore, with the accelerated growth of heterotrophs, ammonification of available sON must have dominated over nitrification resulting in higher removal of sON than production. On the contrary, a decline in sON concentration in the reactor with the C/N ratio of 0.2 was attributed to ammonification (of produced sON and sON contributed by the sample) due to the depletion of NH_3 in the reactor. Since bioavailable organic carbon concentration was extremely low, therefore no significant heterotrophic growth was expected, further supporting the dominance of ammonification over the production of sON from heterotrophic growth. The decrease in ATP concentration of the biofilm after 4 h of reactor operation further supports the reduced growth of heterotrophs at C/N ratio of 0.2. More information on the ATP results under each C/N ratio can be found under subsection 4.3.2.2. Although ammonification dominated the reactor for 3 h (t = 1 to 4 h), sON production also co-

existed in the reactor which was attributed to endogenous respiration of the biomass due to the lack of readily available nutrients in the reactor unlike those contributed by the reactor with the C/N ratio of 4.2.

The ammonification of sON in the presence of NH_3 in the reactors with the C/N ratios of 4.2 and 0.2 suggests that ammonification is not solely influenced by the absence or depletion of NH_3, as observed under the first objective. The presence of bioavailable organic carbon also governs the ammonification process. Unlike in the first objective wherein sON was biologically produced, in this objective, in the reactor with the C/N ratio of 4.2, sON was influent-derived. Studies have shown that influent sON is highly biodegradable (>80%) whereas biologically produced sON is less biodegradable (up to 60%) (Parkin and McCarty, 1981b; Pehlivanoglu-Mantas and Sedlak, 2004; Urgun-Demirtas, Sattayatewa et al., 2008; Sattayatewa et al., 2009). Although in this task, sON was ammonified in both objectives irrespective of how it was derived, the reasons behind ammonification existing in each reactor were different between the two objectives. Therefore, the variations in sON concentration in each reactor highlight the combined roles of C/N ratio, absolute concentrations of organic carbon and nitrogen, and nutrient biodegradability in influencing the sON activity. The observations from this objective suggest that a nitrifying MBBR can degrade more sON when fed with influent containing low C/N ratio. Biomass in a nitrifying biofilm includes both heterotrophs and nitrifiers. Heterotrophs require organic carbon and organic nitrogen for growth whereas nitrifiers need inorganic nitrogen (NH_3) and inorganic carbon (alkalinity). In a low C/N ratio, most of the organic carbon is recalcitrant, hence, heterotrophs target sON for ammonification (to obtain nitrogen and carbon via hydrolysis) whereas nitrifiers rapidly nitrify the available NH_3 eventually causing ammonification of

available/produced sON (to obtain NH_3). Therefore, ammonification in the reactor was responsible for higher degradation of sON under the lowest C/N ratio of 0.2.

4.3.2.2. ATP Analysis

To evaluate the effect of batch-fed C/N ratio on the biofilm of a nitrifying MBBR, the ATP assay was used to assess the bacterial activity occurring in the biofilm extracted from the carriers during the start and the end of the MBBR operation (Figure 4.10). ATP concentration measured immediately after starting the operation (t = 0 h) in each reactor was almost similar because the biofilm carriers were collected from the same basin and at the same time for each run. After 4 h of operation, the ATP concentration increased in the reactors with C/N ratios of 4.2 and 1.5 whereas decreased concentrations were observed in the reactors with C/N ratios of 0.8 and 0.2. As mentioned earlier, the decreasing C/N ratios represent the decreasing concentration and biodegrdability of organic carbon along with fluctuations in the inorganic and organic nitrogen fractions depending on the treatment stage in the WRRF from where the wastewater sample was collected. The wastewater samples representing different C/N ratios were exposed to attached-nitrifying biofilm that were collected from a nitrification basin which operates at a low C/N ratio.

Unlike the lower C/N ratios (0.8 and 0.2), higher C/N ratios (4.2 and 1.5), as expected, seemed to enhance more heterotrophic activity in the biofilm due to the higher bioavailability of organic carbon as a substrate, thus enhancing the microbial activity in these two reactors. The increased ATP concentration in the reactor with a C/N ratio of 4.2 also supports the enhanced ammonification of initial sON as observed in the first hour of reactor operation (Figure 4.9). The ammonification in the reactor was attributed to amplified heterotrophic growth in the reactor due to the presence of readily bioavailable organic carbon. On comparing the ATP concentrations after 4 h of operation in each reactor, a decreasing trend was observed suggesting that the influent C/N

ratio influenced the microbial activity of the nitrifying MBBRs. Nogueira et al. (2002) had reported that during the nitrification process, the influent C/N ratio can influence both utilization of oxygen and carbon, and the distribution of heterotrophic and nitrifying population within the biofilm layers. The decrease in ATP concentrations with decreasing C/N ratio observed in this task aligns with the results from other studies as well (Miqueleto et al., 2010; Bassin et al., 2012; Zhou and Xu, 2020). Therefore, the influent C/N ratio along with independent characteristics (concentration and bioavailability) of carbon and nitrogen, can influence the microbial activity in the biofilms of a nitrifying MBBR which possibly regulates the sON activity in the bulk phase as well.

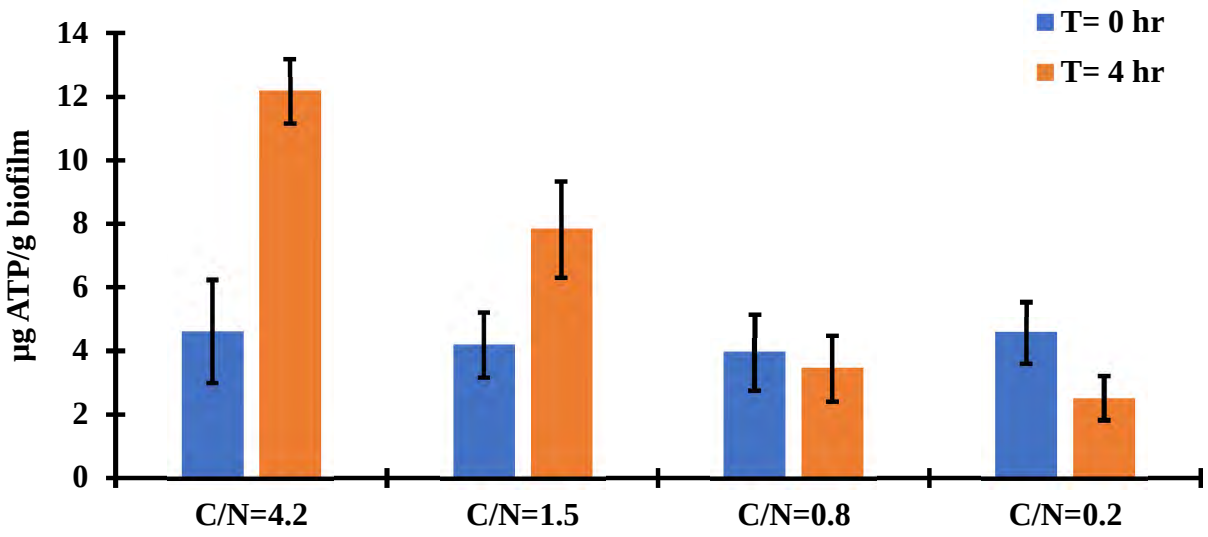

Figure 4.10. Change in ATP concentrations measured in the biofilm from reactors operated under different C/N ratios.

4.4. Summary

This task investigated sON activity in batch nitrifying reactors mimicking MBBRs. The production of sON was examined by feeding SWW (no organic nitrogen), whereas sON degradation was analyzed by feeding actual wastewater samples with different C/N ratios. The task also identified the variation in the activity of the biofilm during nitrification when exposed to different C/N ratios in the influent. This is the first research demonstrating that influent organic

carbon concentration influences the production of sON in a nitrifying biofilm reactor. The batch nitrifying biofilm reactors fed with readily biodegradable organic carbon generated a higher concentration of process-derived sON, which may contribute to effluent TN concentration in the absence of an additional treatment process. For the effect of C/N ratio on sON degradation, operating a nitrifying biofilm reactor at a C/N ratio of 0.2 degraded more sON than the other C/N ratios tested. At the higher C/N ratios of 0.8 and 1.5, the production of sON was higher than the degradation, whereas, at the C/N ratio of 4.2, sON degradation was limited. In addition, higher microbial activities of the nitrifying biofilm were observed when fed with influent containing higher C/N ratios. Overall, this task suggests that MBBR, which is known for its ability to provide high nitrification efficiency, can be beneficial in minimizing effluent sON when operated under a lower C/N ratio.

CHAPTER 5: COMPARISON OF SOLUBLE ORGANIC NITROGEN ACTIVITIES UNDER HETEROTROPHIC AND NITRIFYING SLUDGE

5.1. Introduction

Biological nutrient removal processes have been successfully implemented by water WRRF to achieve low TN concentrations (≤ 10 mg N/L) in the wastewater effluents. Soluble organic nitrogen is a major portion of TN especially for WRRFs that are highly efficient in TIN removal. A considerable portion of sON can be utilized by algae and bacteria, resulting in eutrophication in receiving waters (Eom et al., 2017). Moreover, for wastewater reuse, effluent sON contributes to a precursor for disinfection by-products and membrane fouling (Pehlivanoglu-Mantas and Sedlak, 2006; Krasner et al., 2009; Kiattisaksiri et al., 2020). Less sON in effluent is therefore desirable to minimize the negative impact on reused wastewater and receiving water quality.

In WRRFs, effluent sON consists of influent-derived and microorganism-derived nitrogenous compounds (Pehlivanoglu-Mantas and Sedlak, 2006; Sattayatewa et al., 2009). Distinguishing between these two types of sON fractions is critical to optimize the removal of influent sON and to minimize the amount of microorganism-derived sON. It is estimated that approximately 67% of effluent sON is influent-derived and the rest is microorganism-derived (Randtke et al., 1978; Novak, 2006). However, this fraction may vary depending on the biological processes and the operational parameters employed at individual WRRFs (Bratby et al., 2008; Sattayatewa et al., 2009; Hu, Liao, Shi et al., 2018; Liao et al., 2019; Hu et al., 2019; Hu et al., 2020). Although several studies have examined influent-derived sON degradation (Simsek et al., 2013; Wadhawan et al. 2015, Simsek et al., 2016; Hu, Liao, Geng et al., 2018), no study examined microorganism-derived sON activity under different biological processes.

Studies have reported that some forms of sON such as SMPs can be minimized by process parameters (Ni et al., 2010; Xu, Sheng et al., 2011; Simsek et al., 2016). F/M ratio and C/N ratio are among common operational parameters employed in process optimization for biological carbon and nitrogen removal processes, respectively. During the adjustments to these parameters in WRRFs, the focus is given to TN and less attention is paid toward ON because effluent ON is not directly regulated. While few studies have investigated the effect of C/N ratio on sON degradation (Wadhawan et al., 2015; Hu, Liao, Geng et al., 2018), no study has explored the effect of F/M ratio on sON degradation under different biological processes.

Simsek et al. (2013) showed that the carbon-removing process (ASP) removed 29% sON whereas the NH_3-removing (nitrification) process (MBBR) removed only 4% sON. They suggested that a low C/N ratio might have affected the sON removal in the MBBR process along with other possibilities including solubilization of particulate organics from the biofilm, and/or release of SMPs (Simsek et al., 2013). Hu, Liao, Geng et al. (2018) investigated the effect of different C/N ratios (3, 4, 5 and 6) on the removal of sON, but on post-denitrification filters. Both Simsek et al. (2013) and Hu, Liao, Geng et al. (2018) indicated that relatively less sON removal should be expected under a lower C/N ratio.

Wadhawan et al. (2015) evaluated sON degradation under different types of processes (heterotrophic, nitrification, denitrification, and deammonification). The nitrification process biodegraded more sON than the heterotrophic process whereas very limited sON biodegradation was observed under both denitrification and deammonification processes. sON degradation experiments were performed under different C/N ratios (22, 6.2, and 1.5) based on wastewater samples from different stages of a WRRF to compare sON degradation efficiency between the heterotrophic and nitrification process. The rate of sON degradation decreased with decreasing

C/N ratios under both heterotrophic and nitrification process. sON was more rapidly degraded under nitrification process (0.99, 0.46, 0.40 mg sON/L/h) than under heterotrophic process (0.41, 0.29, 0.10 mg sON/L/h).

Although Wadhawan et al. (2015) examined sON degradation under different C/N ratios, the inoculum used for nitrification process was collected from a nitrification basin that was fed with return activated sludge (containing both nitrifiers and denitrifiers). Consequently, the degradation of sON observed was a product of nitrifying and denitrifying sludge and not just nitrifying sludge. It is possible that removal of sON by nitrifying sludge (no denitrifiers) may differ from the results observed under Wadhawan et al. (2015). sON activities could fluctuate because different microorganism populations compete for oxygen and different types of substrates. A previous study has shown that removal of sON is affected by the different concentrations of external carbon source in denitrifying process (Hu, Liao, Geng et al., 2018). In addition, bioreactor configurations employed at BNR WRRFs also influence the sON removal as reported by a study of Czerwionka et al. (2012) that sON concentration reduced to a minimum in a denitrifying basin and further increased in a nitrifying basin. Therefore, it is worth exploring sON degradation by strictly nitrifying sludge because a significant portion of non-BNR WRRFs is subject to only nitrification.

Since sON is a major organic nitrogen fraction contributing to effluent TN, it is important to acknowledge that the presence of sON in the effluent is a result of a combination of degradation and production of sON that exists and varies under different biological processes. Although the majority of attention has been garnered by sON degradation (Bratby et al., 2008; Pagilla et al., 2008; Sattayatewa et al., 2009; Sattayatewa et al., 2010; Chen et al., 2011; Simsek et al., 2013; Czerwionka and Mękinia, 2014; Wadhawan et al., 2015; Simsek et al., 2016; Hu, Liao, Shi et al.,

2018; Hu, Liao, Geng et al., 2018; Kiattisaksiri et al., 2020) than sON production (Parkin and McCarty, 1981; Pagilla et al., 2011; Hu et al., 2019; Liao et al., 2019; Hu et al., 2020), degradation and production of sON are like two sides of a coin. Besides, studies have focused on either of the two activities (production or degradation) and not them together, which fails to render a complete picture of the sON activity. For instance, reduced sON removal between different biological processes has been associated with growth and/or decay of biomass (Simsek et al., 2013; Wadhawan et al., 2015); however, production (contributed by growth and/or decay) of sON between different biological processes has not been investigated. Therefore, this task endeavors to investigate both degradation and production occurring between two different biological processes (carbon and NH_3 removal) to identify the difference in the sON activity between the two processes.

This task was divided into three parts wherein bench-scale batch reactors were operated to investigate sON activity under carbon-removing (heterotrophic) and NH_3-removing (nitrifying) processes. Since no study has compared the degradation of sON between heterotrophic and nitrifying sludge under different F/M and C/N ratio, the first two objectives of this task focused on examining the sON degradation between these two biological processes. The first objective investigated the effect of F/M ratios on sON degradation by heterotrophic and nitrifying sludge when fed with influent wastewater. The second objective evaluated the effect of C/N ratios on sON degradation by heterotrophic versus nitrifying sludge. The third objective examined the production of sON under heterotrophic and nitrifying sludge when fed with SWW that contributed no organic nitrogen and organic carbon. This objective was designed to examine how the production of sON may vary between the two sludge types with respect to the removal of sON investigated in the previous two objectives. Overall, this task aims to understand the sON activity under two different

types of sludge to possibly identify operational strategies for improving treatment efficiency at WRRFs required to achieve low TN discharge limits.

5.2. Materials and Methods

5.2.1. Sludge Source

Two different types of sludge samples were used in this task including heterotrophic and nitrifying sludge. Heterotrophic sludge (HS) was collected from a HPO-ASP whereas nitrifying sludge (NS) was extracted from the biofilm carriers collected from the nitrifying MBBR of MWWTP. The HPO-ASP is operated at an SRT of 3 d whereas MBBR is operated at an SRT of 32 d. Since the HPO-ASP is employed mainly for BOD removal, mixed liquor collected from this process is rich in carbon removing organisms, thus representing HS in this task. The MBBR process is employed for nitrification, hence, biofilm on the suspended carriers is assumed to be rich in NH_3 removing organisms, thus representing NS. Figure 5.1 displays visual differences (color and texture) between the two sludge types when diluted in deionized water.

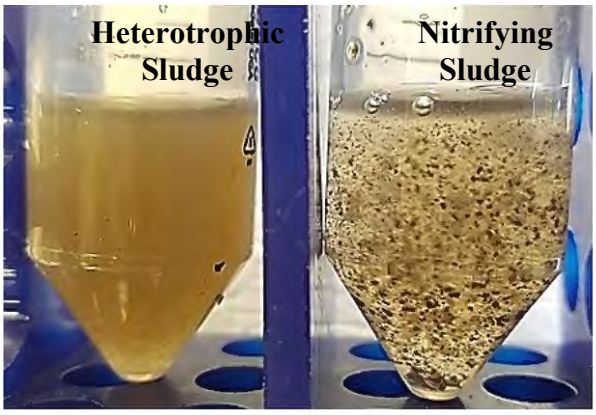

Figure 5.1. Heterotrophic sludge was collected from carbon removing basin whereas nitrifying sludge was collected from ammonia removing basin at the MWWTP.

For comparison, equal concentrations of biomass (determined by VSS) from each type of sludge were required for inoculating the reactors. For HS, measuring VSS was convenient because it was collected from a suspended solution. However, for NS, it was challenging to determine

biomass concentration from an attached biofilm as it may differ from one biofilm carrier to another, thus, affecting the consistency of inoculum concentration while replicating the reactor operation. Therefore, for NS, attached biofilm from the carriers was manually scrapped off (Figure 5.2) using a thin stainless-steel spatula to determine initial VSS contributed by the nitrifying biofilms. Both, HS from the ASP and NS from the MBBR process were diluted using deionized water to bring them to an initial concentration of approximately 3,000 mg VSS/L.

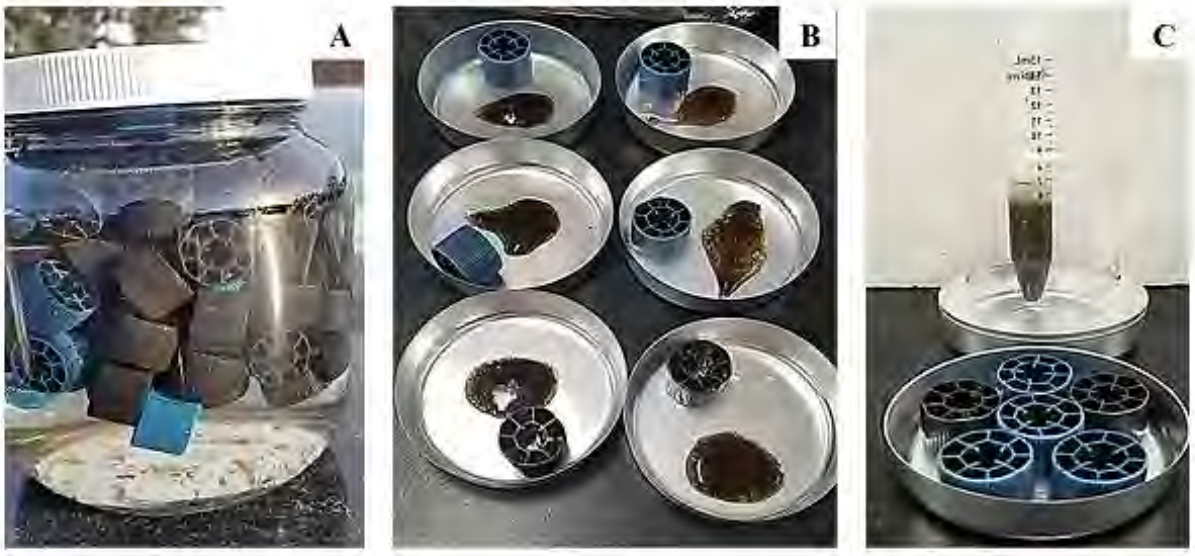

Figure 5.2. Biofilm carriers collected from the MBBR basin (A), biofilm from the carriers were scrapped off manually (B) and, approximately 6 mL biofilm was extracted from 6 biofilm carriers (C).

5.2.2. Synthetic Wastewater Recipe

Synthetic wastewater (SWW) was used as a feed for examining the production of sON in the batch reactors when seeded with different types of sludges i.e., HS versus NS. The SWW did not contribute any sources of organic carbon or ON to the reactors. The SWW recipe was modified from Nagaoka et. al. (1996) to mimic the medium-strength domestic wastewater composition wherein the basic composition remained same as described in Chapter 3 under subsection 3.2.1.

5.2.3. Experimental Setup

This task was divided into 3 objectives wherein the first two objectives focused on sON degradation under different F/M and C/N ratios. The third objective was designed to assess the production of sON in the presence of HS and NS. Batch reactors for the first two objectives were fed with real wastewater samples whereas SWW was fed for the third objective. Experiments under each objective were conducted in triplicates and the resulting values for the assessed parameters were recorded and displayed graphically as average ± standard deviation.

5.2.3.1. Effect of F/M Ratio on sON Degradation under HS and NS

The effect of different F/M ratios (0.1, 0.3, 0.7) on sON biodegradation was evaluated by operating six batch reactors (Figure 5.3). To calculate F/M ratio (mg BOD_5/mg VSS), amount of substrate (mg of BOD_5) contributed by the effluent wastewater sample collected from the equalization basin was divided by the biomass (mg of VSS) contributed by HS and NS (0.1 = 136/1,200; 0.3 = 181/600; 0.7 = 204/300). The BOD_5 values were calculated from the measured COD concentration using a common COD/BOD_5 ratio of 2.1 for municipal wastewater as reported in literature (Henze et al., 2008; Metcalf and Eddy, 2013). To gain the different ratios, the amount of the substrate was increased in each reactor by increasing the volume of substrate added to the reactors and making it up to a total reactive volume of 1 L in each reactor by adding the sludge (HS or NS).

The reactors were operated aerobically wherein air was provided via air stone-diffusers to maintain a DO concentration between 2 and 4 mg O_2/L at room temperature (20°C). The pH was maintained between 6.9 and 7.8 using HCl and $NaHCO_3$. Samples were collected from each reactor every 30 min for a period of 5 h. Samples from each reactor were analyzed for nitrogen species (NH_3, NO_2^-, NO_3^-, TSN and sON).

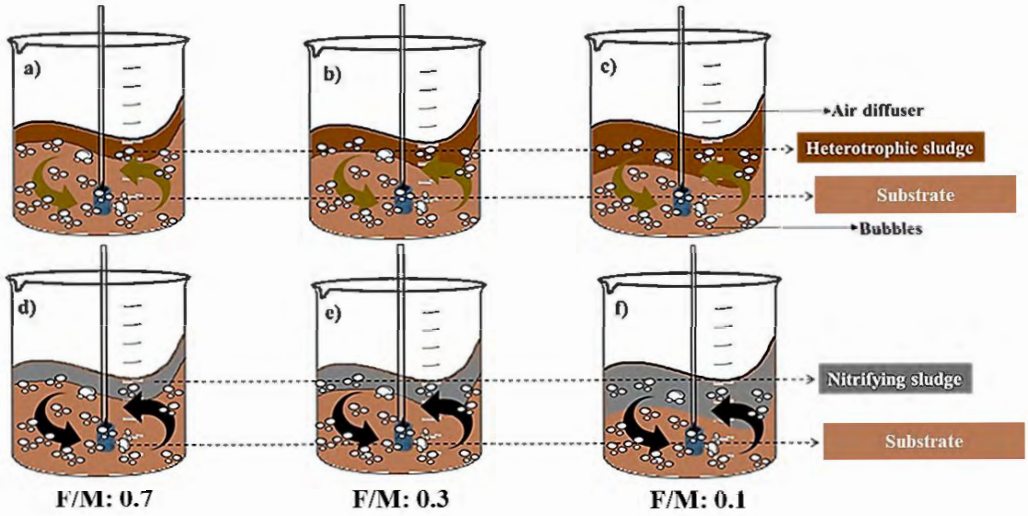

Figure 5.3. Experimental setup for investigating the effect of F/M ratios on sON degradation. Effluent collected from equalization basin (substrate) was inoculated with HS and NS to obtain F/M ratios of 0.7 (a, d), 0.3 (b, c) and 0.1 (c, f).

5.2.3.2. Effect of C/N Ratio on sON Degradation under HS and NS

The effect of different C/N ratios (4, 2, 0.4) on sON degradation was evaluated in batch reactors by feeding real wastewater samples to represent different C/N ratios (Figure 5.4). The C/N ratios were obtained by dividing the concentrations of sCOD with TSN determined from each wastewater sample (C/N: 4 = 168/40, C/N: 2 = 80/38, C/N: 0.4 = 15/35). Effluent wastewater samples were collected from three different locations of the WRRF, i.e., equalization basin (C/N = 4), primary clarifier (C/N = 2) and ASP clarifier (C/N = 0.4). A total of six reactors i.e., one reactor per C/N ratio were used. Each reactor was inoculated with 200 mL of either HS or NS while maintaining a working volume of 1 L. The reactor operation and, collection and analysis of samples was same as described under subsection 5.2.3.1.

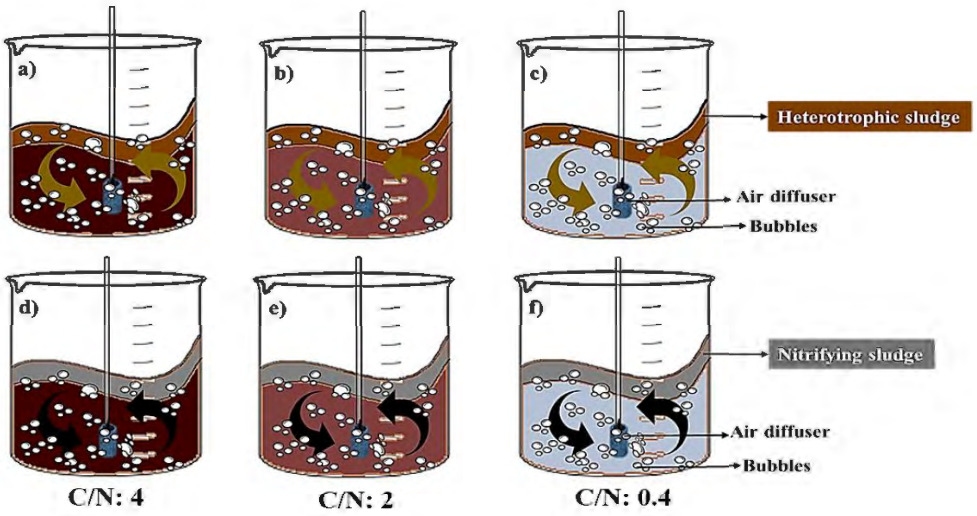

Figure 5.4. Experimental setup for examining the effect of different C/N ratios on sON degradation. Wastewater samples with C/N 4 (a and d), C/N 2 (b and e), and C/N 0.4 (c and f) were inoculated with HS and NS.

5.2.3.3. Production of sON under HS and NS

Two 2 L reactors (1 L working volume) were filled with SWW and inoculated with each type of sludge to bring the final sludge concentrations to 1,000 mg VSS/L in each reactor. Reactor A was inoculated with HS and reactor B was inoculated with NS (Figure 5.5). The reactor operation and, collection and analysis of samples was same as described under subsection 5.2.3.1.

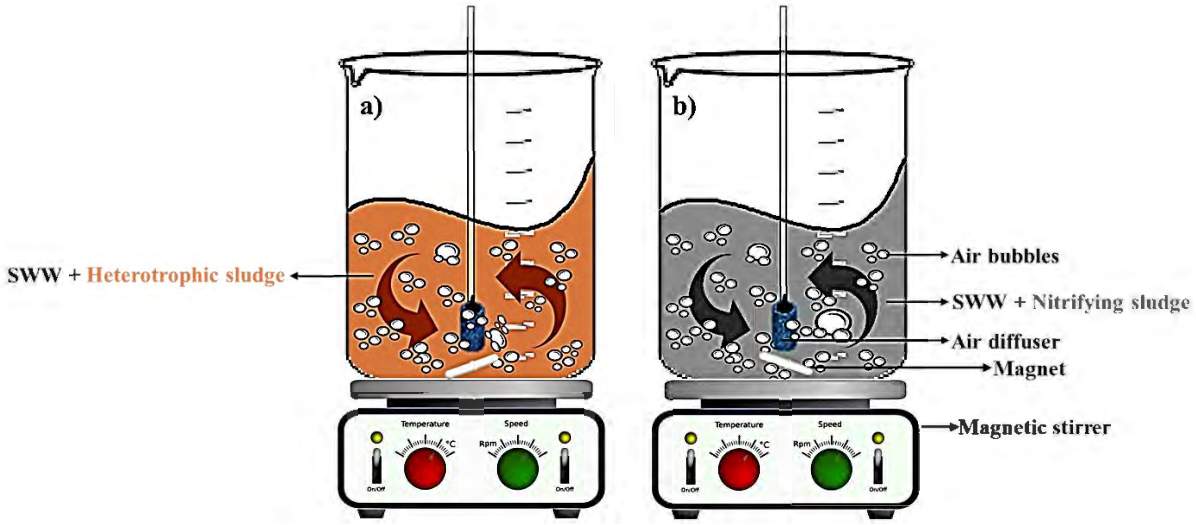

Figure 5.5. Experimental setup to investigate the sON activity in SWW inoculated with a) HS and b) NS.

5.2.4. Analytical Techniques

The analytical techniques employed for this task are described in Chapter 3 under subsection 3.2.5.

5.2.5. Measuring Soluble Fraction and sON

Soluble fractions of COD and ON were measured using the flocculation-filtration technique (Mamais et al., 1993) as described in Chapter 4 under subsection 4.2.5 along with the equation for calculating sON concentration.

5.2.6. Statistical Analysis

Results from this task were analyzed using two different statistical techniques; analysis of variance (ANOVA) and stochastic dominance (SD). ANOVA results were estimated using the software program R whereas SD results were estimated using Simetar software. Unlike ANOVA, which determines the amount of variability in groups of data and identifies if the variability is greater between groups than within groups, SD focusses on ranking which group of data is the best choice. The SD approach fits distribution functions to groups of data from an experiment and performs statistical test to determine which group of data is preferred. The spread of a distribution function or deviation from the mean is a measure of variability or risk. The advantage of using SD over ANOVA is that ANOVA only includes mean and variance while SD includes mean, variance, skewness, kurtosis, and a preference parameter (called the risk aversion parameter or aversion for less desirable outcomes).

SD is a statistical approach of determining the superiority of one distribution over another. Generalized SD with respect to a function (SDRF) determines which distribution with desirable outcome is preferred (e.g., less sON from HS or NS). Cumulative distribution functions (CDF) are estimated for each subset of data (e.g., HS and NS). The advantage of the CDF is that it can be

defined for any kind of random variable (discrete, continuous, and mixed) and makes it easy to compare different distributions or group of data. This task deals with biological processes wherein the sON concentration fluctuates randomly under different processes (HS and NS). Therefore, CDF for HS and NS will assist in ranking the degradation of sON under a specific variable being tested (e.g., F/M, C/N ratios).

Stochastic dominance was employed in this task for ranking between HS and NS based on the removal of sON in terms of F/M ratios (0.3, 0.7, 0.1) and C/N ratios (4, 2, 0.4). In addition, superiority of HS versus NS was tested based on the least production of sON. The distributions in figure 5.6 are a good representation of the data from this experiment, where HS and NS represent biological processes involved in the removal or production of sON. The graph shows that F(HS) is preferred or dominates G(NS) from zero to A followed by G(NS) dominating from A to B and again F(HS) dominates for sON values > B.

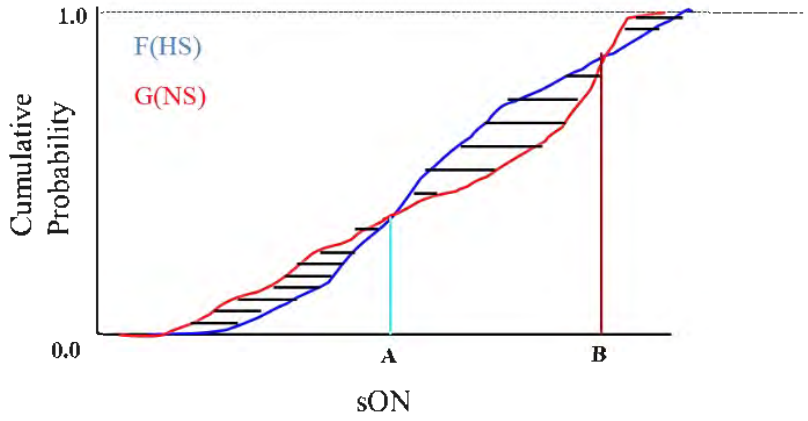

Figure 5.6. Schematic illustrating the applicability of stochastic dominance to the obtained experimental data.

For example, WRRFs are regulated differently for discharging effluent TN and/or NH_3 concentrations, which will dictate different preferences for each biological process employed by the WRRF targeting the removal of TN and/or NH_3. ANOVA will be limited in capturing the differences in preference. When comparing the means of three or more groups (e.g., NH_3

concentration under different F/M or C/N ratios), ANOVA can show if at least one pair of means is significantly different, but it cannot identify which pair. Also, ANOVA requires that the dependent variable be normally distributed in each of the groups and that the variability within groups is similar across groups.

Stochastic dominance overcomes the limitations of ANOVA and evaluates the robustness of the results. SD uses a flexible distribution that fits the exact distribution associated with the data. SD can be used to both compare and rank which distribution is optimal. Robinson and Barry (1987) indicated that SD is a methodology based on expected utility maximization theory or optimality. In this study, the lower-risk aversion coefficient (RAC) is set at zero (0), indicating the decision process only considers the average or mean performance. The upper-RAC is set at 0.45 based on the literature (Cochran et al., 1985), representing that the decision process incorporates average performance, variability and preferences/aversion for risk or variability.

5.3. Results and Discussion

5.3.1. Effect of F/M Ratio on sON Degradation by HS and NS

Figure 5.7 shows the nitrogen species and sON profiles observed under F/M ratios of 0.1, 0.3 and 0.7 in the presence of HS whereas figure 5.8 shows them in the presence of NS. The nitrification performance of the batch reactors in terms of NH_3, NO_2^-, NO_3^- and TSN concentrations is shown in figure 5.7a, b and c for HS; and figure 5.8a, b and c for NS. Broadly, the nitrification performance under both sludge types was influenced by different F/M ratios. As expected, NS nitrified higher fraction of NH_3 than HS, however, opposite trends were observed in terms of removal of NH_3 with respect to increasing F/M ratio. Under HS, 11% to 29% NH_3 was removed wherein both concentration and rate of removal increased with increasing F/M ratio. At F/M ratio of 0.1, 0.3 and 0.7, overall, HS removed 2.80, 4.09 and 7.04 mg NH_3/L at the rates of

0.56, 0.82 and 1.41 mg NH_3/L/h, respectively. Under NS, 46% to 100% NH_3 was removed wherein both concentration and rate of removal decreased with increasing F/M ratio. At F/M ratio of 0.1, 0.3 and 0.7, overall, NS removed 23, 18 and 11 mg NH_3/L at the rates of 4.60, 3.60 and 2.21 mg NH_3/L/h, respectively.

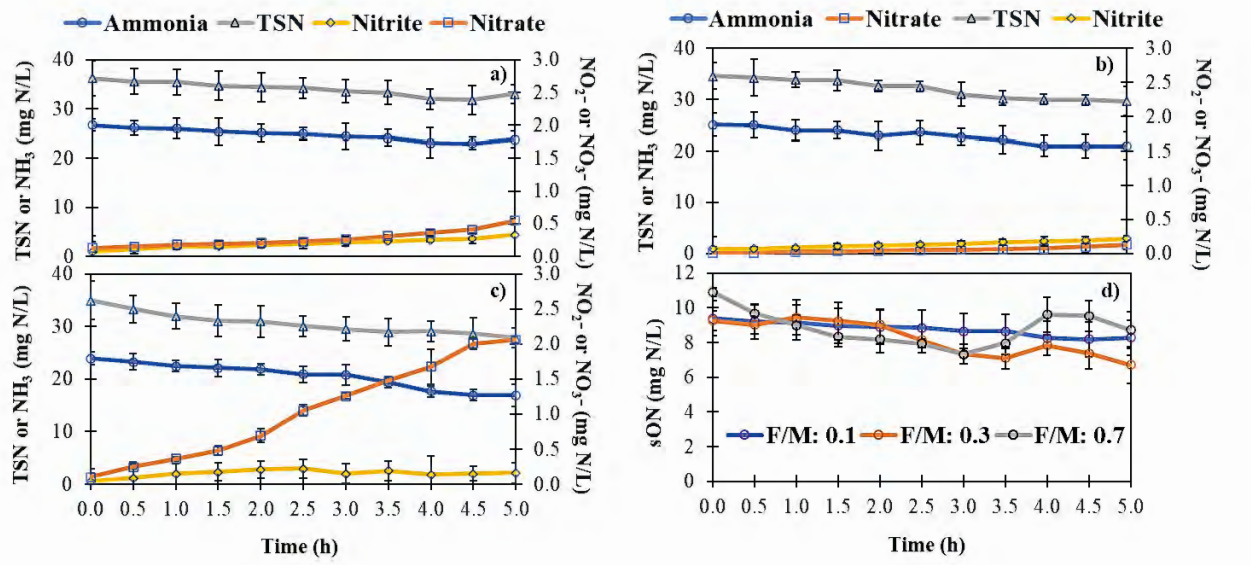

Figure 5.7. Nitrogen species profile under F/M ratios of (a) 0.1, (b) 0.3 (c) 0.7 when inoculated with HS and (d) sON activity under different F/M ratio.

sON activity is a combination of production and degradation of sON wherein one activity dominates the other under different conditions and periods of a biological process (Parkin and McCarty, 1981c; Pagilla et al., 2011; Liao et al., 2019; Hu et al., 2020). For this part of the task, degradation of sON was more prevalent than production under both the sludge types when fed with real wastewater. Under both HS and NS, net removal of sON increased on increasing the F/M ratio from 0.1 to 0.3 and then decreased at F/M of 0.7. However, HS removed relatively higher fraction of sON (12-28%) than NS (6-25%). At F/M ratios of 0.1, 0.3 and 0.7, overall, HS removed 1.10, 2.55 and 2.19 mg sON/L at the rates of 0.22, 0.51 and 0.44 mg sON/L/h, respectively while NS removed 0.56, 2.37 and 1.85 mg sON/L at the rates of 0.11, 0.47 and 0.37 mg sON/L/h,

respectively. The net sON removal was the highest at F/M of 0.3 for both sludge types but it was relatively higher and faster under HS than under NS.

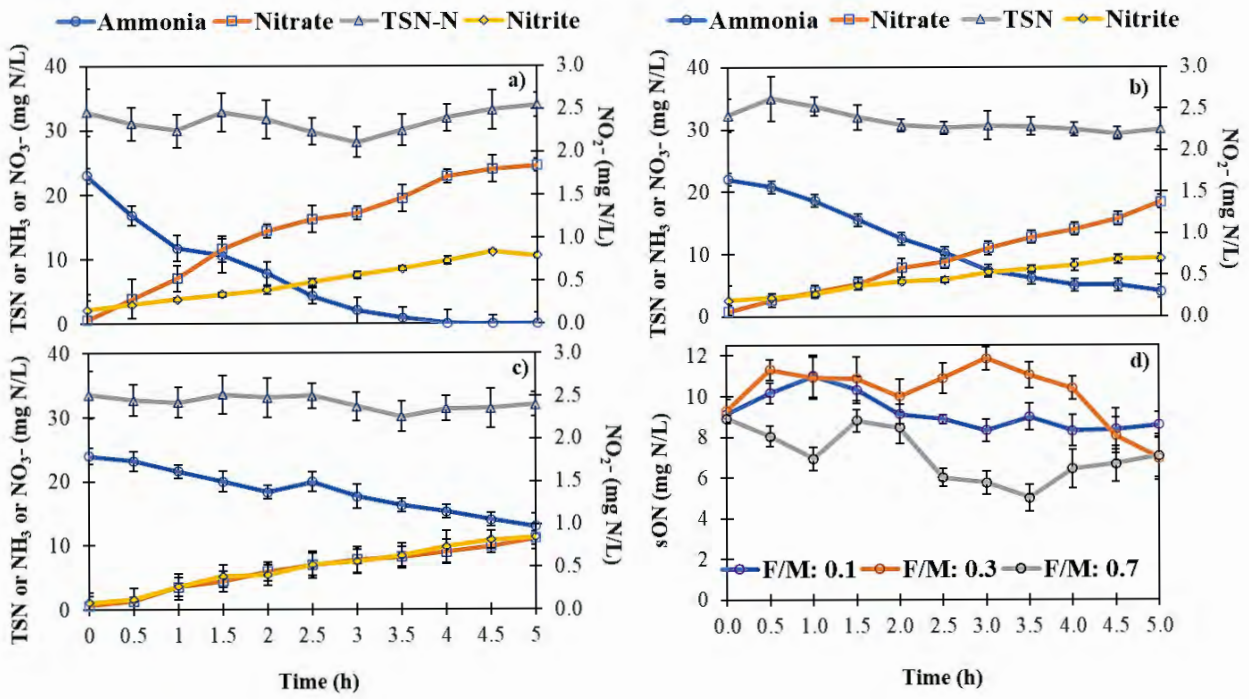

Figure 5.8. Batch reactor performance of nitrogen species under different F/M ratios of (a) 0.1, (b) 0.3 (c) 0.7 when inoculated with NS and (d) sON activity under different F/M ratio.

The sON results under different F/M ratios (Figure 5.7d and 5.8d) indicated higher removal of sON under F/M of 0.3 unlike F/M 0.1 and 0.7 which showed reduced removal of sON, possibly because of higher decay of biomass due to starvation and higher growth of biomass due to greater availability of food, respectively (Sattayatewa et al., 2009; Czerwionka et al., 2012). The increase in the removal of sON from F/M of 0.1 to 0.3 and then the removal reduction from F/M of 0.3 to 0.7 suggest that the production (release) of sON under F/M of 0.3 is relatively low due to less rates of biomass decay or growth. The removal of sON under different F/M ratios agrees with the finding from a previous study (Simsek et al., 2016) which examined sON degradation under different SRTs, which is analogous to the F/M ratio (SRT increases, F/M decreases). They reported that sON removal increased with increasing SRT reaching a maximum level after which it gradually

decreased. The decrease in sON removal at higher SRTs was attributed to an increase in the non-biodegradable fraction of sON (Simsek et al., 2016).

Based on the results from this part of the task, operating a simple aerobic system at F/M of 0.3 will reduce a higher fraction of biodegradable sON as compared to the other F/M ratios tested. The reduced removal of sON under F/M of 0.1 (famine condition) could result in the release of BAPs which are SMPs produced from biomass decay. Studies have reported higher production of BAPs at a lower F/M ratio and found them less biodegradable (de Silva and Rittmann, 2000; Ni et al., 2010; Ni et al., 2011). Therefore, the reduction in sON removal observed at F/M of 0.1 could be due to the presence of non-biodegradable sON contributed by endogenous respiration (Simsek et al., 2016). Similarly, the reduction in sON removal observed at F/M of 0.7 (feast condition) could be attributed to enhanced production of UAPs, another category of SMPs that are released in the presence of readily available substrate for metabolism and growth of biomass (Namkung and Rittmann, 1986; Aquino and Stuckey, 2004; Ni et al., 2011). Therefore, the observations from this objective suggest that F/M ratio of 0.3 is the optimum ratio among the three investigated ratios for achieving higher removal of sON under both, HS and NS. More studies will be needed to explore the variations in sON removal performance under the F/M ratios not investigated in the current objective e.g., F/M ratios of 0.2, 0.4, 0.5 and 0.6 to identify the exact optimal value.

The SD analysis showed that among the two sludge types tested for sON degradation; HS was the more preferred sludge for all the three F/M ratios investigated (Table A2 under Appendix). This preference was supported by both lower (0) and upper (0.45) RACs under each F/M ratio. The lower RAC indicates that there is no preference for lesser or higher concentration of sON when ranking the two processes while the upper RAC indicates that there is a strong preference for lower sON concentration. This analysis confirms the findings from the experimental work

which observed higher sON degradation under HS; hence, HS is the preferred sludge irrespective of the F/M ratio being analyzed. ANOVA does not differentiate lower or upper variability from the mean (e.g., removal or addition of sON) and consequently ANOVA is not an effective methodology for ranking.

Next, the SD of sON degradation was compared within the F/M ratios (Table A3 under Appendix) to identify which ratio resulted in the highest degradation of sON under HS and NS respectively. The results showed that F/M ratio of 0.3 was the preferred value among the three ratios for both the sludge types. The experimental results also align with the SD analyses because the highest degradation of sON was observed at F/M ratio of 0.3 for both HS and NS. Although F/M ratio of 0.3 was the most preferred value, the preference of F/M ratios of 0.1 and 0.7 for HS was not clear because of the rankings for the lower and upper RACs were different. On the contrary, for NS, the second preference was given to F/M ratio of 0.7. The experimental results also indicated the second-highest sON degradation under F/M ratio of 0.7 for both HS and NS. ANOVA result as (Figure A1 under Appendix) showed that the average sON degradation efficiencies observed for HS at F/M ratios of 0.1 and 0.7 were not significantly different (p-value 0.98) unlike those observed for NS (p-value 0.001). Therefore, it clarifies that because degradation of sON under HS between F/M ratios of 0.1 and 0.7 was not statistically significant, the preferences between the two RACs differed based on the SD analysis (Table A3 under Appendix). Overall, statistically, higher sON degradation was observed in the presence of HS specifically under F/M ratio of 0.3.

5.3.2. Effect of C/N Ratio on sON Degradation by HS and NS

The C/N ratios tested (4, 2 and 0.4) were based on varied sCOD concentrations and relatively constant TSN concentrations (but contributed by different concentrations of nitrogen

forms). The decreasing trend reflects the change in sCOD concentration after going through different treatment processes at the MWWTP. Not only the level of COD decreased throughout the treatment train, but also its biodegradability decreased (or its biorecalcitrance increased) after the activated sludge (C/N 0.4). Therefore, at C/N 0.4, sCOD besides being lowest in concentration, is the most biorecalcitrant. Regarding the changes in N fractions, organic nitrogen and NH_3 dominated the effluent from the equalization basin (C/N 4) and eventually decreased with increasing C/N ratios.

Figure 5.9 shows the nitrogen species and sON profiles observed under C/N ratios of 4, 2 and 0.4 in the presence of HS whereas figure 5.10 shows them in the presence of NS. The nitrification performance of the batch reactors in terms of NH_3, NO_2^-, NO_3^- and TSN concentrations is shown in figure 5.9a, b and c for HS; and figure 5.10a, b and c for NS. The nitrification performance under both sludge types was affected by different C/N ratios. As expected, NS nitrified higher fraction (>98%) of NH_3 than HS; however, no trends were observed in terms of removal of NH_3 with respect to increasing C/N ratios. Under HS, 13% to 27% NH_3 was nitrified. At C/N ratio of 4, 2 and 0.4, overall, HS nitrified 7.83, 4.18 and 7.30 mg NH_3/L at the rates of 1.57, 0.84 and 1.46 mg NH_3/L/h, respectively, whereas, NS nitrified 35.00, 32.41 and 27.98 mg NH_3/L at the rates of 7.01, 6.47 and 5.60 mg NH_3/L/h, respectively.

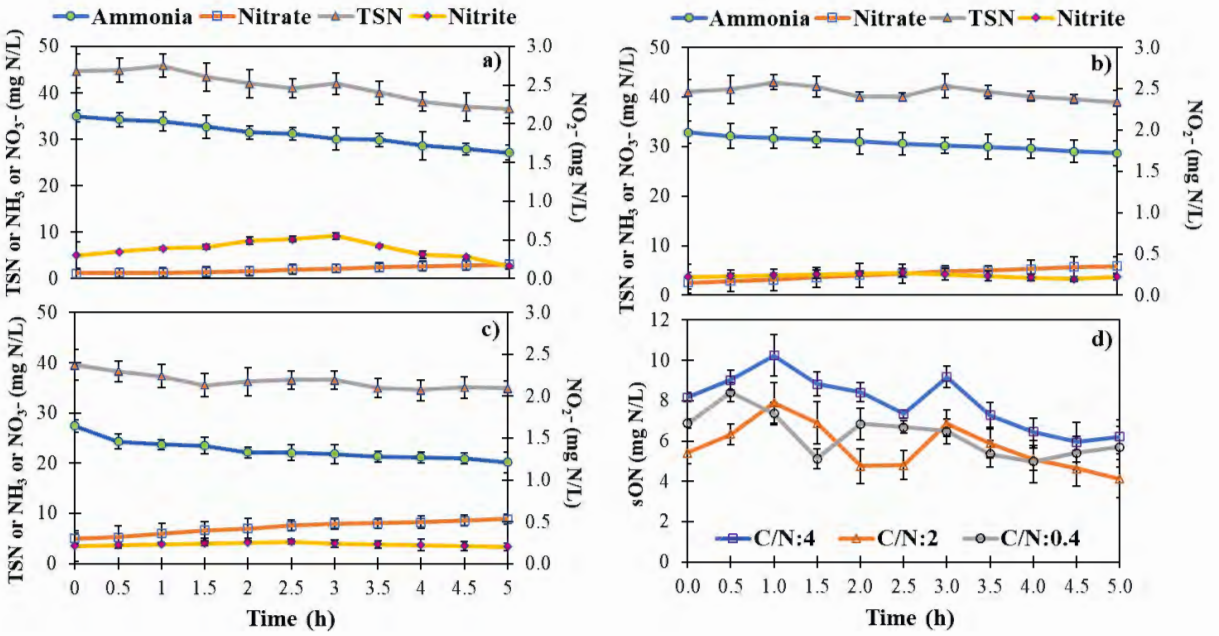

Figure 5.9. Batch reactor performance on nitrogen species under different C/N ratios of (a) 4, (b) 2 and (c) 0.4 when inoculated with HS; and (d) sON activity under different C/N ratio.

As mentioned earlier, sON activity is a blend of production and degradation of sON and in this subsection, similar to the preceding subsection, degradation of sON was more dominant than production under both the sludge types when fed with real wastewater. Under both HS and NS, net removal of sON concentration decreased on decreasing the C/N ratio from 4 to 0.2. Although both HS (17-24%) and NS (14-23%) removed comparable fractions of sON, the concentration of sON removed by HS was always relatively higher than NS under each C/N ratio. At C/N ratios of 4, 2 and 0.4, overall, HS removed 1.96, 1.30 and 1.17 mg sON/L at the rates of 0.39, 0.26 and 0.23 mg sON/L/h, respectively while NS removed 1.84, 1.06 and 0.92 mg sON/L at the rates of 0.37, 0.21 and 0.18 mg sON/L/h, respectively. The net sON removal was the highest at C/N of 4 for both sludge types but it was relatively higher and faster under HS than under NS.

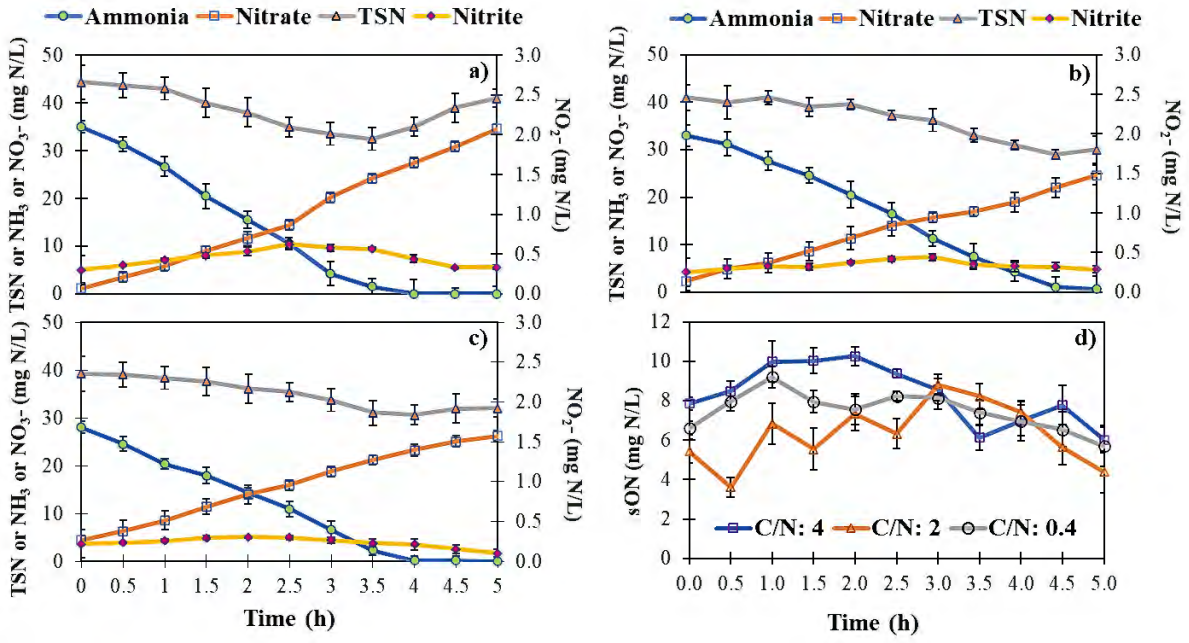

Figure 5.10. Batch reactor performance of nitrogen species under C/N ratios of (a) 4, (b) 2 and (c) 0.4 when inoculated with NS; and (d) sON activity under different C/N ratio.

The variations in sON concentration in each reactor highlight the combined roles of C/N ratio, and absolute concentrations and biodegradability of organic carbon and organic nitrogen in influencing the sON activity. The observations from this objective suggest that a simple aerobic system fed with suspended HS or NS can degrade more sON when fed with influent containing high C/N ratio. The wastewater sample with the C/N ratio of 4, unlike those with the C/N ratios of 2 and 0.4, provided a large concentration of organic carbon which must have enhanced the heterotrophic growth in the reactor consequently resulting in accelerated ammonification of readily biodegradable sON (contributed by the sample). Therefore, with the accelerated growth of heterotrophs, ammonification of available sON must have dominated (over sON production) resulting in higher removal of sON than production.

The decrease in the removal of sON with decreasing C/N ratios agreed with Wadhawan et al. (2015), however unlike results from this task, they found higher degradation of sON under

nitrification process than heterotrophic carbon removal process. The higher sON removal under nitrification process could also be contributed by the presence of denitrifying sludge in the inoculum (Wadhawan et al., 2015) as mentioned in the introduction section. Czerwionka et al. (2012) also reported higher degradation of sON under denitrifying sludge than the nitrifying sludge in different BNR WRRFs and batch reactors. sON removal results from these two studies (Czerwionka et al. 2012; Wadhawan et al., 2015) suggest that possibly denitrifiers in Wadhawan et al. (2015) were responsible for degrading sON in the presence of both nitrifiers and denitrifiers. It may also suggest that removal of sON by denitrifiers dominated the reactor over production of sON by nitrifiers when inoculated together by Wadhawan et al. (2015). Besides, findings from the two studies indirectly suggest that denitrifying heterotrophs remove a higher concentration of sON than carbon-removing heterotrophs which are two different processes that have not been investigated so far and should be examined in the future.

Results from Simsek et al. (2013) compared sON degradation between carbon removal (ASP) versus NH_3 removal (MBBR) processes at a non-BNR facility and found that MBBR removed less sON than ASP. The two processes were operated at different C/N ratios (influent in ASP was fed with a higher C/N ratio than MBBR). They suggested that higher removal of sON under ASP, which primarily involved HS, was attributed to the dominance of ammonification of sON. They also suggested that the reduced removal of sON under MBBR or NS could be due to low C/N ratio, production of SMPs, and or hydrolysis of particulate organic matter entrapped in the biofilm (Simsek et al., 2013). Results from this part of the task suggest that variation in the influent C/N ratio in a simple aerobic system will affect the removal of sON under both the sludge types. Out of the three ratios investigated for both types of sludge, the removal of sON was the highest at the C/N ratio of 4 and kept dropping with decreasing C/N ratio. This finding supports a

presumption made by Simsek et al. (2013) that possibly a low C/N ratio in the MBBR reduced the removal of sON due to more production of sON. Between the two sludge types for all three C/N ratios, relatively lower sON removal was observed under NS than HS. The presumption made by Simsek et al. (2013) about sON production affecting the removal of sON between carbon removal and nitrification processes will be experimentally elucidated in the third part of this task.

The SD analysis showed that among the two sludge types tested for sON degradation, HS was the more preferred sludge for all the three C/N ratios investigated (Table A4 under Appendix). This preference was supported by both lower (0) and upper (0.45) RACs under each C/N ratio. This analysis confirms the findings from the experimental work which observed higher sON degradation under HS. Irrespective of the C/N ratio being assessed, HS is the preferred sludge. Next, the SD of sON degradation was compared within the C/N ratios (Table A5 under Appendix) to identify the ratio that provided the highest degradation of sON under HS and NS respectively. The SD results showed that C/N ratio of 4 was the optimal value among the three ratios under both HS and NS, agreeing with the experimental observations. For both HS and NS, the second-best C/N ratio was 0.4. Unlike the difference in the preferences observed in the SD analysis for F/M ratio (Table A3 under Appendix), the preferences for the C/N ratios were consistent between the lower and upper RACs for both the sludge types (Table A4 under Appendix). The experimental findings contradict with the SD results; the second-highest degradation was observed under C/N ratio of 2 rather than 0.4. One possible explanation is that the SD considers the variation between time intervals while the descriptive statistical analysis of the experimental data only considers beginning and end values. ANOVA result showed that the difference in the degradation of sON between C/N ratios of 2 and 0.4 under both HS and NS was not statistically significant (p-values of 0.492 and 0.145 for HS and NS, respectively) (Figure A2 under Appendix).

5.3.3. Production of sON under HS and NS

Figures 5.11a and b show the nitrogen species profile observed under HS and NS when fed with SWW (no organic nitrogen and carbon). After 5 h of operating the batch reactors, unlike NS, which nitrified influent NH_3 to below detection limits (>99%) at the rate of 6.73 mg NH_3/L/h, HS nitrified only 12% of it at 0.92 mg NH_3/L/h. Figure 5.11c displays sCOD profiles under HS and NS. The sCOD concentrations observed at t = 0 h are contributed by each sludge type. Overall, sCOD profiles had opposite trends wherein HS degraded (53%) the available sCOD whereas sCOD concentration increased under NS (35%). During the 6 h of reactor operation, HS degraded 36.62 mg sCOD/L at the rate of 6.10 mg sCOD/L/h while NS released 16.67 mg sCOD/L at 2.78 mg sCOD/L/h. Figure 5.11d shows the sON profiles observed under each sludge type. Overall, the sON activities between the two sludge types seemed to have contradicting trends; when sON concentration increased under HS, a decreasing trend was observed under NS. At t = 0 h, 4.71 mg sON/L in the HS reactor and 7.59 mg sON/L in the NS reactor were observed which were contributed by the inocula. At the end of the operation, net removal of 3.09 mg sON/L was observed in the HS reactor whereas net production of 1.96 mg sON/L was identified in the NS reactor.

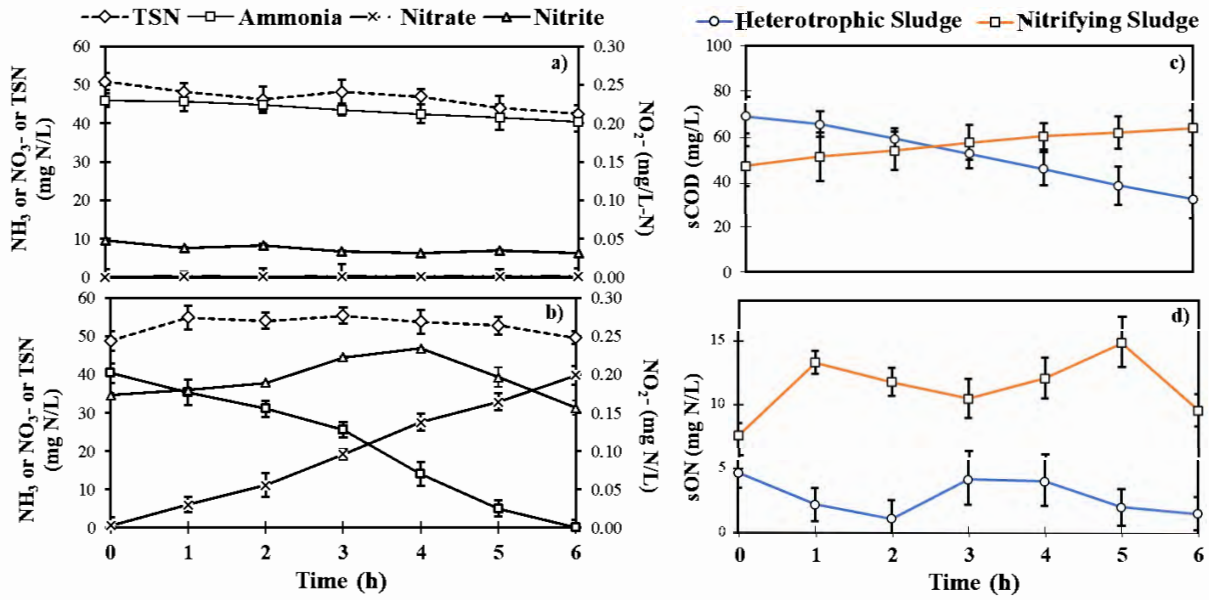

Figure 5.11. Nitrogen species profile under (a) HS and (b) NS. sCOD (c) and sON (d) profiles under HS and NS.

Under HS, in the first two hours of operation, sON concentration steadily decreased due to ammonification of available sON. The HS was collected from the ASP that is operated at a short SRT of 3 d. Since no significant nitrification was observed in the reactor, the involvement of nitrifiers in ammonification, if any, was insignificant. An increase in sON concentration in the third hour suggests the production of sON possibly contributed by the decay of heterotrophs due to the absence of a readily available organic carbon source in the reactor. Although residual sCOD was contributed by HS (70 mg sCOD/L), it was probably not readily biodegradable or not enough considering the biomass (1,000 mg VSS/L) added in the reactor, hence the prevalence of biomass decay in the reactor is expected. Overall, during the 6 h of reactor operation, only 53% of sCOD was degraded by HS very slowly (6.10 mg sCOD/L/h) considering that heterotrophs readily oxidize organic carbon for energy and growth (Figure 5.11c). Studies have reported that biomass decay releases SMPs (specifically BAP), a fraction of which contribute cellular macromolecules containing organic carbon and nitrogen (Namkung and Rittmann, 1986; de Silva and Rittmann,

2000; Ni et al., 2011). The organic carbon from the SMPs might be partially bioavailable and could be oxidized by the heterotrophs as a substrate to support growth or cell maintenance.

The concentration of sON did not change much between 3 and 4 h under HS but a gradual decrease in sON concentration was observed after the fourth hour until the end of the reactor operation suggesting hydrolysis of sON by extracellular enzymes in the reactor (Burgess and Pletschke, 2008). Heterotrophic bacteria require organic carbon as an electron donor or substrate and energy provider, however, residual sCOD was not readily biodegradable or possibly inadequate in quantity (Figure 5.11c). Therefore, to compensate for the scarcity of available organic carbon source in the reactor, heterotrophic bacteria possibly took the route of hydrolyzing other available organic sources to obtain carbon and energy. The reactor was not fed with organic carbon and organic nitrogen; therefore, the residual and process-derived sCOD and sON in the reactor were the only available organic sources. sON is composed of both labile and recalcitrant high molecular weight and low molecular weight molecules (Neff et al., 2003; Jones et al., 2004). Therefore, bacteria degraded the available sON in the reactor by the action of extracellular hydrolases to use it as a source of carbon.

In biofilms, nitrifying bacteria coexist with heterotrophic bacteria (Rittmann and McCarty, 2001; Navada et al., 2020). In this task, biofilm was scrapped off from the biofilm-carriers transforming attached cells into planktonic and/or flocculated cells. In the NS reactor, sON concentration increased in the first hour of the reactor operation possibly due to the change of environmental conditions (attached versus suspended and real wastewater versus SWW) (Hao et al., 2010; Curtin et al., 2011). Unlike NS, which was detached from the biofilm carriers, HS was already in the suspended form, therefore HS was exposed to relatively less degree of change (physiologically). Hence, sON concentration did not increase in the first hour of reactor operation

under HS as observed under NS (Figure 5.11d). Differences in the physiological status between biofilm and planktonic cells have been reported in terms of growth rates and bacterial metabolism **(**Ellwood et al., 1982; van Loosdrecht et al., 1990; Harrison et al., 2004; Bester et al., 2005; Grujić et al., 2017). Under stressful periods, excretion of sON to establish a concentration of equilibrium across the cellular membrane increases sON concentration in the reactor (Parkin and McCarty, 1981c). sON concentration gradually decreased in the second and third hours, possibly due to the dominance of ammonification of biodegradable sON. Ammonification, unlike other biological processes, is more spontaneous and can exist under both oxic and anoxic conditions (Selecky, 2005).

Release of sON was observed in the next two hours (t = 3-5 h) possibly due to the growth of nitrifiers since nitrification was actively in progress (Figure 5.11d). In addition, heterotrophic decay may as well have contributed to the sON production because unlike nitrifiers, the lack of readily available organic carbon source in the reactor was expected to significantly affect the heterotrophs. Figure 5.11c shows constant increase in sCOD concentration under NS which suggests that residual sCOD in the reactor was not or minimally degraded and also that heterotrophic decay resulted in increased sCOD concentration. Based on the sCOD results, the organic carbon contributed by the NS was more recalcitrant than that contributed by the HS. In the last hour of the reactor operation, more than 85% of NH_3 was nitrified indicating a dearth of electron donor or a required nutrient for the nitrifiers resulting in malnourishment. The depletion of NH_3 in the reactor (t = 5 h) triggered ammonification of the available sON (to generate NH_3 for growth). The NH_3generated from the ammonification of available sON is assumed to be rapidly nitrified, hence, no increase in the NH_3 concentration was observed in the reactor.

Based on the SD analysis, HS was superior to NS in term of less released sON (Table A6 under Appendix). The SD results agree with the experimental observation on higher production of sON by NS. Results from ANOVA showed a p-value of 0.0000036, which was below the significance level criterion (alpha = 0.05), thus implying that the average production of sON by HS was significantly different from that by NS (Figure A3 under Appendix).

5.4. Summary

This task examined the degradation and production of sON under two different sludge types or two different biological processes to identify how one sludge type is better than the other in removing sON under a simple aerobic reactor. It addressed sON activity under heterotrophic and nitrifying sludge wherein sON degradation was investigated at different ratios of F/M (0.1, 0.3, 0.7) and C/N (4, 2, 0.4), and sON production was investigated by feeding SWW with no sON. Overall, higher degradation of sON was observed in the presence of HS than NS. Out of the three F/M ratios investigated, the F/M ratio of 0.3 was the optimum ratio that provided the highest removal of sON. In terms of the C/N ratios, the degradation of sON was the highest under the C/N ratio of 4 and diminished with decreasing C/N ratios. Since HS removed a higher concentration of sON compared to NS through the domination of removal over sON production as observed in this task, WRRFs looking to achieve high sON removal should include or focus on heterotrophic process or carbon removal process.

CHAPTER 6: CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1. Conclusions

The municipal WRRFs, with upgrades in the conventional treatment processes or finetuning of the BNR techniques, are able to achieve significant removal (>95%) of dissolved inorganic nitrogen leading to sON becoming a major nitrogen form (>65%) of the total effluent dissolved nitrogen. sON removal from wastewater is very important because sON and its biodegradable fraction can promote algal growth in receiving waters causing oxygen depletion and/or eutrophication. To minimize the impact of total nitrogen species including sON on sensitive receiving waters, WRRFs are subject to more stringent effluent TN limits. Since organic nitrogen is contributed by the influent and produced during biological processes, controlling effluent organic nitrogen has been a challenge for WRRFs. Therefore, distinguishing between influent-derived and process-derived fractions is critical to optimize the removal of influent ON and to minimize the amount of process-derived ON. Comprehensive research, divided into three tasks, was conducted to identify the generation of ON fractions and investigate both production and degradation of sON by operating laboratory-scale batch reactors for short durations to mimic two different biological processes (ASP and MBBR) that are employed at WRRFs.

First, the effect of SRT on the production of ON fractions (particulate, colloidal and soluble) and the biodegradability of produced sON in a bench-scale continuous reactor mimicking an ASP was investigated. The effluent TON contained 45-59 % pON, 18-31% cON and 19-25% sON. cON fraction can be larger than the sON fraction in the secondary effluent. Therefore, besides focusing on sON fraction, WRRFs aiming to meet stricter effluent TN limits should also identify appropriate technologies to target cON fraction in the secondary effluent. More than 50% of effluent sON was biodegradable under SRTs of 2, 5, and 10 d but the biodegradability decreased

to 31% at 20 d SRT. Large fractions of non-biodegradable sON (69%) at SRT of 20 d are suggested to be contributed by EPS and SMP, specifically BAPs due to endogenous respiration. Therefore, operating ASP at long SRTs will produce large fractions of sON that may take longer to degrade in receiving waters.

In the second task, the activity of sON in batch reactors mimicking nitrifying MBBRs indicated that irrespective of the presence of influent ON (with 0 versus 400 mg COD/L) in the reactors, sON was contributed by the biofilm during nitrification. Although net production of sON was observed, both production and ammonification coexisted which regulated the sON concentration. When actual wastewater was fed to the reactors to investigate sON degradation under different carbon to nitrogen (C/N) ratios, results suggested that organic carbon bioavailability and/or NH_3 concentration influenced the production and ammonification of sON. This research is the first to explore the sON activity by MBBR and the findings could extend knowledge on the fixed film process with respect to sON activity to regulate and optimize reactor operation in meeting stringent TN discharge limits.

For the last task on examining which sludge type is better at removing sON under a simple aerobic reactor, higher degradation of sON was observed in the presence of heterotrophic sludge than nitrifying sludge. Out of the three F/M ratios investigated, the F/M ratio of 0.3 was the optimum ratio that provided the highest removal of sON. In terms of the C/N ratios, the degradation of sON was the highest under the C/N ratio of 4 and diminished with decreasing C/N ratios. Since heterotrophic sludge removed a higher concentration of sON compared to nitrifying sludge, WRRFs looking to achieve high sON removal should include or focus on heterotrophic process or carbon removal process.

6.2. Future Recommendations

In addition to issues addressed in this research, there are topics that require further investigation and are recommended for future studies as given below.

1. The mixed liquor inoculum used in this research was collected from an ASP operated at an SRT of 3 d. Studies using inoculum from processes operated at different SRTs will represent a different bacterial community. Longer SRTs benefit the proliferation of slow-growth microorganisms and consumption of a wide range of substrates. Therefore, future research work should identify how the influent- and process-derived fractions of effluent organic nitrogen (pON, cON and sON) vary when inoculated with mixed liquor collected from biological processes representing a gradient of SRTs.
2. The majority of the past studies focused on sON and its biodegradability (Murthy et al., 2006; Khan et al., 2009; Sattayatewa et al., 2009; Simsek et al., 2012). This research found that the contribution of process-derived cON fraction can be larger than sON in the effluent of an aerobic ASP. Therefore, studies should focus on colloidal fraction and its biodegradability under different biological processes because it might be a fraction contributing towards final TN and hence needs to be degraded before discharging in sensitive water bodies.
3. Based on previous studies (Pehlivanoglu and Sedlak, 2004, 2006; Simsek et al., 2013; Hu, Liao, Shi et al., 2018), portions of sON in wastewater effluent are available to bacteria and algae over retention time that is relevant to rivers and estuaries, which would increase the risk of eutrophication and harm the water quality. In addition, effluent sON bioavailability increased with increasing SRT when the reactors were fed with primary influent and inoculated with *Selenastrum capricornutum* as the algal inoculum. *S. capricornutum* has been used as a standard test organism for algal growth studies. Therefore, considering the bioavailability of

sON in natural waters, uptake of both influent and process-derived sON at different SRTs should be investigated with mixed algal culture (which exists in the receiving waters) instead of using a single culture. Since algal capabilities to uptake sON are not only related to the characteristics of sON but also to algal species, future work should employ artificial mixed algal cultures which represent dominant species of algae to further understand the role of effluent sON in eutrophication potential.

4. A considerable fraction of effluent sON consists of combined amino acids, SMPs, and other biomolecules. These macromolecular nitrogen-containing organic compounds are produced during biological wastewater treatment processes, as proteins are metabolized, and microbial products are released by bacteria. Humic substances derived from source water and introduced with wastes during biological wastewater treatment accounts for relatively more recalcitrant sON than sON derived from proteins and SMPs with respect to microbial transformation. Liu et al., (2011) found that a large fraction (~80%) of effluent sON from different BNR facilities consisted of hydrophilic (more reactive) forms. The hydrophobic sON (less reactive) exhibits characteristics of humic substances and is likely to persist for long periods in the aquatic environment. Future work should separate the process-derived sON into hydrophilic and hydrophobic fractions under different biological processes to optimize the treatment plant configuration with the processes that contribute relatively lower fractions of hydrophilic sON in the effluent.
5. Examining how much of the produced sON under different SRTs is contributed by SMP and EPS can further enhance the understanding of the relative importance of these process-derived sON sources. This recommendation is extended to anoxic and anaerobic processes associated with BNR facilities.

6. Production of process-derived sON, in this dissertation, was investigated under aerobic conditions wherein different parameters were found to influence the sON concentration. However, these findings only apply to conventional aerobic systems. Since BNR facilities involve different biological processes to target different compounds (e.g., anoxic conditions for denitrification; anaerobic condition for phosphorous removal), research work from this dissertation should be explored under anoxic and anaerobic conditions to further understand how the process-derived sON may vary under different processes to optimize the wastewater treatment plant configuration for the removal of sON.
7. The influent C/N ratio influenced sON degradation by nitrifying biofilm. However, the effect of C/N ratio on the degradation of AbsON by nitrifying biofilms is unknown considering that AbsON stimulates algal growth in the receiving water bodies and affects the nitrogen cycling. Previous studies have shown that AbsON concentration decreased with increasing C/N ratio in post-denitrification biofilters (Hu, Liao, Geng et al., 2018) and that the formation of AbsON was significantly influenced by microbial activity and microbial community structure (Liao et al., 2019). Therefore, the chemical composition and molecular weight distribution of sON should be investigated to better understand the effect of different C/N ratios on effluent AbsON from nitrifying biofilm processes. The chemical composition of sON can be analyzed via advanced analytical techniques such as Fourier-transform ion cyclotron resonance mass spectrometer (FTICR-MS) whereas sON size fractionation can be conducted using membrane filters with different molecular weight cutoffs. Based on the molecular composition, sON molecules can be identified as bioavailable and refractory. Studies have reported that sON molecules >1 kDa are potentially less bioavailable unlike smaller sON molecules which are

more bioavailable and difficult to remove via advanced treatment processes and hence end up in the receiving water bodies (Eom et al., 2017; Hu, Liao, Shi et al., 2018).

8. This dissertation investigated nitrifying biofilm attached to a specific carrier type (high-density polyethylene (HDPE)-ActiveCell450). Bassin et al. (2016) reported that the type of biofilm-carrier affected the quantity and distribution of attached biofilm, which influenced the activity of specific microbial groups in the biofilm. Therefore, the sON activities observed under different C/N ratios in this dissertation (Chapter 4) may differ when different biofilm-carrier types are used. Future work should examine the biofilms to identify and characterize the microbial community and enzymes involved in sON production and ammonification under different C/N ratios using different types of biofilm carriers. Molecular biology tools such as the next generation sequencing and real-time polymerase chain reaction can be used to identify the presence and expressions of genes involved in the production and removal of sON under different C/N ratios and biofilm carrier-types.
9. Investigation of sON activity under heterotrophic and nitrifying sludge showed relatively higher production of sON by nitrifiers than heterotrophs. Reactors in this research were operated at optimal pH (6.9-7.9) wherein sON production dominated over removal under nitrifying sludge. A recent study reported an AOB that was able to grow and oxidize NH_3 at pH 2.5, which is way below the optimal pH limits known for the growth of nitrifiers. Therefore, future studies should explore the effect of lower pH values on sON degradation during nitrification.
10. Temperature is a key and uncontrollable operational parameter in WRRFs. In ASP, low temperatures are problematic to the biological nitrogen removal processes. Past studies have demonstrated that changes in enzymatic activity and microbial community composition are

significant contributors to reduced removal of soluble inorganic nitrogen under cold conditions. Also, previous studies have shown that sON concentration increases with a decrease in temperature in an ASP (Hu et al., 2019; Liao et al., 2019). Since all the experiments in this dissertation were conducted under room temperature, future studies should investigate the changes in rates of production and degradation of sON under temperatures higher and lower than room temperature to cater to the WRRFs operating in warmer and colder locations.

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APPENDIX: SUPPLEMENTAL TABLES AND FIGURES

Table A1. Basic characteristics of biofilm carriers (MBBR media) and MBBR basin at the Moorhead WWTP.

Type	Floating biofilm carrier (HDPE) – ActiveCell450
Manufacturer	Headworks/Hydroxyl
Specific surface area	388 m^2/m^3
Amount of media used in the reactors	938 m^3
Nominal height	16 mm
Nominal diameter	21 mm
Basin area	42 m * 24 m = 1,008 m^2
Basin volume	42 m * 24 m * 3 m = 3024 m^3
Basin pH	6.6 -7.2

Table A2. Comparisons of stochastic dominance of sON degradation between HS and NS under F/M ratios of 0.1, 0.3, and 0.7.

Efficient Set Based on SDRF at		**Efficient Set Based on SDRF at**	
Lower RAC	**0**	**Upper RAC**	**0.45**
Name	Level of Preference	Name	Level of Preference
1 HS F/M 0.1	Most Preferred	1 HS F/M 0.1	Most Preferred
2 NS F/M 0.1	2nd Most Preferred	2 NS F/M 0.1	2nd Most Preferred
1 HS F/M 0.3	Most Preferred	1 HS F/M 0.3	Most Preferred
2 NS F/M 0.3	2nd Most Preferred	2 NS F/M 0.3	2nd Most Preferred
1 HS F/M 0.7	Most Preferred	1 HS F/M 0.7	Most Preferred
2 NS F/M 0.7	2nd Most Preferred	2 NS F/M0.7	2nd Most Preferred

***Efficient set:** It is the set with the dominant characteristics (e.g., higher removal of sON with lowest variability). In this task two sets were tested i.e.,, HS and NS.
***Risk aversion coefficient (RAC):** defined under subsection 5.2.6.
***Stochastic dominance with respect to function (SDRF):** defined under subsection 5.2.6.

Table A3. Comparing stochastic dominance of sON degradation between HS and NS within the F/M ratios of 0.1, 0.3, 0.7.

Efficient Set Based on SDRF at		**Efficient Set Based on SDRF at**	
Lower RAC	**0**	**Upper RAC**	**0.45**
Name	Level of Preference	Name	Level of Preference
1 HS F/M 0.3	Most Preferred	1 HS F/M 0.3	Most Preferred
2 HS F/M 0.1	2nd Most Preferred	2 HS F/M 0.7	2nd Most Preferred
3 HS F/M 0.7	3rd Most Preferred	3 HS F/M 0.1	3rd Most Preferred
1 NS F/M 0.3	Most Preferred	1 NS F/M 0.3	Most Preferred
2 NS F/M 0.7	2nd Most Preferred	2 NS F/M 0.7	2nd Most Preferred
3 NS F/M 0.1	3rd Most Preferred	3 NS F/M 0.1	3rd Most Preferred

Table A4. Comparisons of stochastic dominance of sON degradation between HS and NS under C/N ratios of 4, 2, and 0.4.

Efficient Set Based on SDRF at		**Efficient Set Based on SDRF at**	
Lower RAC	**0**	**Upper RAC**	**0.45**
Name	Level of Preference	Name	Level of Preference
1 HS C/N 4	Most Preferred	1 HS C/N 4	Most Preferred
2 NS C/N 4	2nd Most Preferred	2 NS C/N 4	2nd Most Preferred
1 HS C/N 2	Most Preferred	1 HS C/N 2	Most Preferred
2 NS C/N 2	2nd Most Preferred	2 NS C/N 2	2nd Most Preferred
1 HS C/N 0.4	Most Preferred	1 HS C/N 0.4	Most Preferred
2 NS C/N 0.4	2nd Most Preferred	2 NS C/N 0.4	2nd Most Preferred

Table A5. Comparisons of stochastic dominance of sON degradation between HS and NS within the C/N ratios of 4, 2, 0.4.

Efficient Set Based on SDRF at		**Efficient Set Based on SDRF at**	
Lower RAC	**0**	**Upper RAC**	**0.45**
Name	Level of Preference	Name	Level of Preference
1 HS C/N 4	Most Preferred	1 HS C/N 4	Most Preferred
2 HS C/N 0.4	2nd Most Preferred	2 HS C/N 0.4	2nd Most Preferred
3 HS C/N 2	3rd Most Preferred	3 HS C/N 2	3rd Most Preferred
1 NS C/N 4	Most Preferred	1 NS C/N 4	Most Preferred
2 NS C/N 0.4	2nd Most Preferred	2 NS C/N 0.4	2nd Most Preferred
3 NS C/N 2	3rd Most Preferred	3 NS C/N 2	3rd Most Preferred

Table A6. Comparison of stochastic dominance of sON production between HS and NS.

Efficient Set Based on SDRF at		**Efficient Set Based on SDRF at**	
Lower RAC	**0**	**Upper RAC**	**0.45**
Name	Level of Preference	Name	Level of Preference
1 HS	Most Preferred	1 HS	Most Preferred
2 NS	2nd Most Preferred	2 NS	2nd Most Preferred

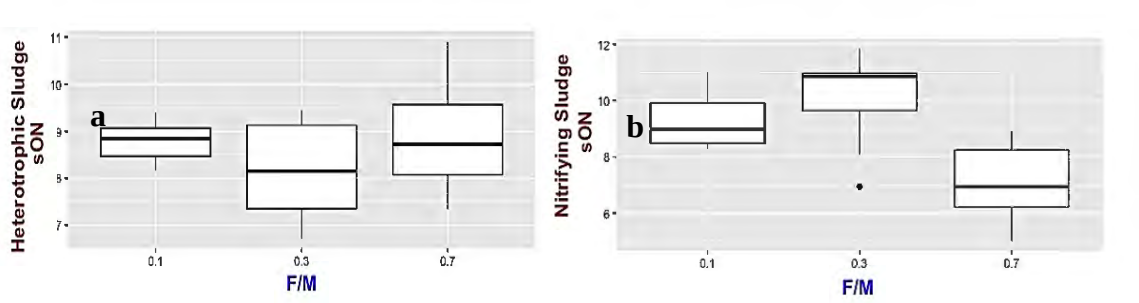

Figure A1. Box plots with error bars constructed using 95% confidence interval of the mean for different F/M ratios under a) HS and b) NS.

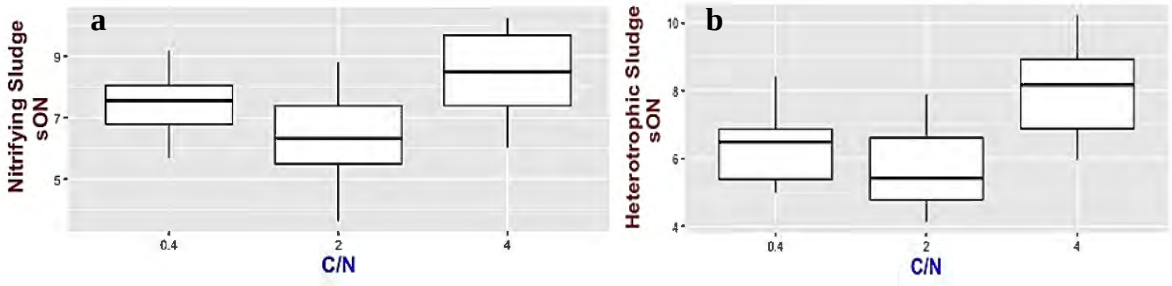

Figure A2. Box plots with error bars constructed using 95% confidence interval of the mean for different C/N ratios under a) HS and b) NS.

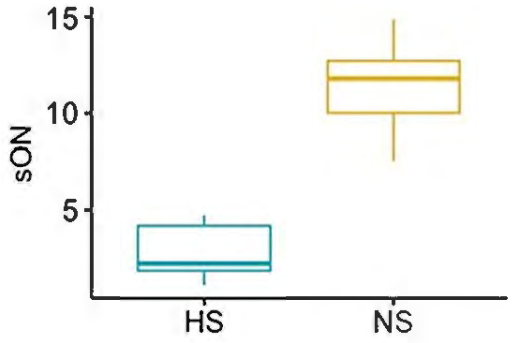

Figure A3. Box plots with error bars constructed using 95% confidence interval of the mean for HS and NS.