

INVESTIGATING BIODEGRADABILITY OF DISSOLVED ORGANIC NITROGEN IN
OLIGOTROPHIC AND EUTROPHIC SYSTEMS

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State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Dissolved organic nitrogen (DON) in water and wastewater is a major public concern. In drinking water treatment plants (WTP), DON and biodegradable DON (BDON) may form carcinogenic by-products during disinfection and might also serve as a nutrient for microbiological growth in distribution systems. BDON in treated wastewater can promote algal growth in receiving water bodies. Understanding biodegradability of DON is important to develop strategies and processes capable of minimizing DON impact on the wastewater effluent receiving water bodies and drinking water. WTPs are nutrient-poor oligotrophic systems that receive source water with DON of about ≤ 2 mg N/L. Wastewater treatment plants (WWTPs) are nutrient-rich eutrophic systems which receive raw wastewater with DON of ≥ 8 mg N/L. At WWTPs, sidestream deammonification is a highly eutrophic system employed to treat highly concentrated streams of DON (≥ 100 mg N/L) and ammonia ($\geq 1,500$ mg N/L) generated from filtrate from anaerobically digested sludge dewatering. DON characteristics including biodegradability for different trophic levels could differ. The main goal of this dissertation is to investigate biodegradability of DON in these oligotrophic and eutrophic systems.

Three research tasks were performed. In the first task, a method to measure BDON in oligotrophic systems was developed and applied to determine the fate of BDON along four treatment stages of a WTP with ozonation prior to filtration. Optimum dose of inocula and incubation time were identified for the BDON measurement. The Moorhead WTP, Moorhead, MN on average removed 30% of DON and 68% of BDON. The second task involved investigating the role of four biological wastewater treatment processes in removing DON from eutrophic systems. Nitrification process biodegraded 70, 54, and 57% of DON in influent, primary effluent, and secondary effluent, respectively. Heterotrophic DON removal was less (1.7

to 38%) while denitrification and deammonification did not remove DON. For the third task, BDON biodegradability in highly eutrophic system was investigated using nitrifying sludge. About 45 to 90% of DON in sidestream effluent was biodegradable. Information from this dissertation provides a better understanding on DON and BDON fate through water and wastewater treatment processes representing different trophic levels.

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DEDICATION

I will like to dedicate my dissertation towards my late maternal grandmother Tripta Maini and
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LIST OF ABBREVIATIONS

AD.....	Anaerobic digestion
ATU.....	Allylthiourea
ANOVA.....	Analysis of variance
AOB.....	Ammonia oxidizing bacteria
AS.....	Activated sludge
BDON.....	Biodegradable dissolved organic nitrogen
BOD.....	Biochemical oxygen demand
COD.....	Chemical oxygen demand
CON.....	Colloidal organic nitrogen
DCAA.....	Dissolved combined amino acid
DDW.....	Distilled deionized water
DFAA.....	Dissolved free amino acid
DCAA.....	Dissolved combined amino acids
DI.....	Deionized
DIN.....	Dissolved inorganic nitrogen
DO.....	Dissolved oxygen
DOC.....	Dissolved organic carbon
DOM.....	Dissolved organic matter
DON.....	Dissolved organic nitrogen
DPA.....	Dissolved primary amine
EDTA.....	Ethylenediaminetetraacetic acid
ENR.....	Enhanced nutrient removal

EON.....	Effluent organic nitrogen
EPA.....	Environmental Protection Agency
EPS.....	Extracellular Polymeric Substances
GLM.....	General Linear Models
HS.....	Humic substances
MAD.....	Mesophilic anaerobic digestion
MGD.....	Million gallons per day
MLSS.....	Mixed liquor suspended solids
N.....	Nitrogen
NH ₃ -N.....	Dissolved ammonia nitrogen
NO ₂ -N.....	Dissolved nitrite nitrogen
NO ₃ -N.....	Dissolved nitrite nitrogen
NOB.....	Nitrite oxidizing bacteria
PON.....	Particulate organic nitrogen
RDON.....	Refractory dissolved organic nitrogen
SBOD.....	Soluble biochemical oxygen demand
SCOD.....	Soluble chemical oxygen demand
SRT.....	Solids retention time
TDN.....	Total dissolved nitrogen
TF.....	Trickling filter
THP.....	Thermal Hydrolysis Pretreatment
TKN.....	Total Kjeldahl nitrogen
TN.....	Total nitrogen

TSS.....Total suspended solids
WAS.....Waste activated sludge
WWTP.....Wastewater treatment plant

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CHAPTER 1. GENERAL INTRODUCTION

1.1. Background

Total nitrogen (TN) can be characterized into total particulate nitrogen (TPN) and total dissolved nitrogen (TDN) forms. TDN can be further divided into dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN). Ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) are the major constituents of DIN, while DON is extremely complex and medially characterized. DON cannot be directly quantified in water samples and is normally calculated by subtracting DIN from TDN. Commonly detected DON compounds in surface waters include urea, amino acids, peptides, amino sugars, purines, pyrimidines, and amides (Antia et al., 1991).

Biological transformation of DIN by microorganisms is better understood compared to DON biodegradation. The concentration, composition, and biodegradability of DON can vary among different types of water samples. The types of water samples can be divided into two trophic systems, nutrient-poor oligotrophic and nutrient-rich eutrophic systems. Ocean, rivers, streams, and lakes are oligotrophic in nature, DON concentration in these systems varies from 0.05 to 2 mg N/L, and the concentrations of organic carbon and other essential nutrients are also low (Antia et al., 1991). These oligotrophic water bodies serve as source water for drinking water treatment plants (WTPs). Wastewater, and agricultural and urban runoffs are eutrophic in nature and the DON concentration can vary from 2 to 100 mg N/L (Simsek et al., 2012; Cabezas et al., 2012). Biodegradability of DON in oligotrophic and eutrophic systems can differ due to composition and concentration of organics present. Also, a number of environment factors between these two systems such as residence time, type of microbial community, substrate complexity, temperature, pH, and dissolved oxygen (DO) that would lead to difference in biodegradability, can differ.

Release of DON and biodegradable DON (BDON) from eutrophic systems into oligotrophic systems is a major route of DON transport in the environment. Anthropogenic activities including wastewater treatment plants (WWTPs), and urban and agricultural lands discharge BDON into surface waters (Aitkenhead-Peterson et al., 2005; Pellerin et al., 2006; Seitzinger et al., 2002; Aitkenhead-Peterson et al., 2009; Wiegner and Seitzinger, 2004). Currently, more than 30 Mt per year of TN enters the coastal waters of which BDON can be a major portion. In oceans and other water bodies, about 20 to 50% of the TN pool is DON (Antia et al., 1991; Berman and Bronk, 2003).

DON in water and wastewater can cause major public health concerns. TDN including DON and BDON is an essential source of nutrient for microorganisms in the environment. DON is a dynamic constituent in water and is constantly being produced and utilized by microorganisms (Berman and Bronk, 2003). BDON can be mineralized by bacteria and DIN formed from BDON can support algal growth. High concentrations of DON including BDON in surface waters can lead to algal growth that can cause oxygen depletion affecting various aquatic life-forms, and commercial and recreational activities. To help in minimizing algal growth in receiving waters, the treatment plants should effectively remove BDON before discharging their effluent. A number of countries including the United States and United Kingdom have spent millions of dollars to rehabilitate algal impacted fresh waters (Hoagland et al., 2002; Pretty et al., 2003).

In WTPs, three major issues can arise due to presence of DON including BDON in the source water and treated water. First, during disinfection DON can react with chlorine or chloramine to form carcinogenic nitrogenous disinfectant by-products (N-DBPs) (Bond et al., 2011). Other drinking water treatment processes such as ozonation and ultra-violet disinfection

have also known to produce DON precursors such as dimethylamine for N-DBP formation. Second, if DON is untreated then it may serve as a nutrient for supporting microbial growth in the distribution systems. Third, DON can react with free chlorine and inorganic chloramine to form organic chloramines which are less bactericidal (Donnermair and Blatchley III, 2003). DON and BDON are major concerns for water and wastewater treatment systems. In order to remove DON from water, it is essential to understand its biotransformation in these oligotrophic and eutrophic systems.

1.2. Research Problem Statement

In water treatment, the fate of BDON has never been investigated. DON has only been determined for raw and finished waters (Lee et al., 2006; Lee et al., 2007; Lee and Westerhoff, 2006; Westerhoff and Mash, 2002; Xu et al., 2010; Xu et al., 2011). The difficulty in measuring BDON in this oligotrophic system is that there is no established method. For eutrophic systems such as wastewater, BDON can be quantified using a bioassay developed by Khan et al. (2009). The bioassay uses diluted mixed liquor suspended solids (MLSS) as a source of bacterial inocula and the sample is aerated daily and stored at 20°C for 20 to 28 days of incubation. However, the concentrations of DON and BDON are very low and the bioassay developed for wastewater will have to be modified for determining BDON in drinking water. The size of microbial inocula and time of incubation are two important parameters that require optimization. Determination of optimum microbial inocula is important because high concentrations of MLSS can add DON through exogenous decay promoted by insufficient substrate which can impact the BDON measurements. Time of incubation is important for a similar reason; as substrate gets exhausted, exogenous decay DON will be produced.

WWTPs are eutrophic systems that add TN including DIN, DON, and BDON to the oligotrophic systems. To protect receiving waters, nutrient discharge limits which require WWTPs to achieve 5 to 10 mg TN/L have been established. WWTPs equipped with advanced biological nutrient removal (BNR) processes including nitrification and denitrification processes achieve more than 95% removal of DIN and ≤ 10 mg/L of TDN in wastewater effluent (Urgun-Demirtras et al., 2008, Sattayatewa et al., 2009, Simsek et al., 2012). After the majority of DIN is removed from wastewater, most of the TDN comprises of DON. Lack of understanding of DON removal makes WWTPs that are required to meet TN discharge limits vulnerable.

WWTPs receive raw wastewater with chemical oxygen demand (COD) of about 300 mg/L and DON of about 8 to 12 mg N/L. A major portion of BDON in the influent is refractory to the current wastewater treatment technologies and therefore is discharged into receiving waters. Discharged BDON is mineralized by undergoing ammonification followed by assimilation and/or nitrification in the receiving waters. Recent studies indicate that the concentrations of DON in wastewater effluent can be up to 80% of the TDN (Pehlivanoglu-Mantas and Sedlak, 2004; Urgun-Demirtras et al., 2008; Sattayatewa et al., 2009; Simsek et al., 2012). Several studies have investigated fate and transformation of DON and BDON through WWTPs (Parkin and McCarty, 1981a, 1981b; Pehlivanoglu-Mantas and Sedlak, 2004; Sattayatewa et al., 2009; Simsek et al., 2012; Simsek et al., 2013) and effluent BDON (Murthy et al., 2006; Sattayatewa et al., 2009; O'Shaughnessy et al., 2006). BDON through 4- stage Bardenpho process (Sattayatewa et al., 2009), two-stage nitrification and denitrification process, step-feed biological nutrient removal process, and trickling filter (Khan et al., 2009; Simsek et al., 2012) was evaluated. These studies suggest that the type of treatment process can determine the fate of DON in the eutrophic system. However, there is no clear understanding of which

fundamental biological processes (such as organic carbon degradation, nitrification, and denitrification) remove more DON than the other. Currently, only heterotrophic processes are assumed to ammonify DON. Nitrification processes due to autotrophic nature of microorganisms involved are not believed to remove DON (Metcalf and Eddy, 2003). However, recent studies have observed that DON and BDON concentrations decrease after a nitrification stages at WWTPs suggesting the role of nitrifiers in biodegrading DON (Simsek et al., 2012 and Simsek et al., 2014). To meet the stringent TN discharge limits, it will be important to assess the role of biological wastewater treatment processes in removing DON.

Many WWTPs employ anaerobic digesters for sludge stabilization and practice energy recovery in a form of biogas. Anaerobically digested sludge dewatering produces highly eutrophic filtrate with high concentrations of DON (100 to 300 mg N/L) and ammonia (1,500 to 2,500 mg N/L). High levels of ammonia, especially free ammonia, is toxic to bacteria and is removed before recycling the filtrate through the treatment train of a WWTP. Sidestream processes such as deammonification are employed to treat high ammonia concentrations. DON from sidestream can have a major impact on DON removal performance of wastewater treatment processes and effluent concentration.

Understanding biodegradability of DON is important to develop strategies and processes capable of minimizing DON impact on the wastewater effluent receiving water bodies and drinking water. Information from this research will provide more understandings on DON and BDON fate through water and wastewater treatment processes representing different trophic levels.

1.3. Research Objectives

The main goal of this dissertation research is to investigate biodegradability of DON in eutrophic and oligotrophic systems. The specific objectives are:

1. To develop a BDON bioassay for drinking water and investigate fate of DON and BDON through a WTP.
2. To examine DON removal through an advanced WWTP and evaluate the roles of different biological processes in removing DON from wastewater; and
3. To investigate biodegradability of DON in a sidestream deammonification system receiving a high strength filtrate from the dewatering of anaerobically digested sludge which is thermally pretreated and evaluate its impact on inhibition of bacterial activity.

1.4. Hypotheses

Three hypotheses are formulated corresponding to the objectives.

1. Ozonation increases the concentration of BDON in water through refractory DON degradation while biologically active filters remove BDON due to activity of microorganisms.
2. Heterotrophic carbon and denitrification processes ammonify DON in wastewater more compared to autotrophic nitrification and deammonification processes.
3. Sidestream deammonification process being autotrophic in nature does not biodegrade DON.

1.5. Dissertation Organization

This dissertation is divided into 6 chapters. This chapter includes background, research problem statement, objectives, hypotheses, and dissertation organization. Chapter 2 provides a literature review on prevalence of DON and BDON in the environment. Chapter 3 presents

development of a method for determining BDON in drinking water and application of the method to determine the fate of BDON at a WTP with ozonation and biological active filtration processes. The work described in Chapter 3 is based on a manuscript entitled “Dissolved Organic Nitrogen and its Biodegradable Portion in a Water Treatment Plant with Ozone Oxidation.” This manuscript has been published in *Water Research* (Wadhawan et al., 2014). Chapter 4 is derived from a manuscript entitled “Biological Removal of Dissolved Organic Nitrogen during Different Wastewater Processes.” This manuscript will be submitted for publication in a peer reviewed journal. Chapter 5 is based on a manuscript entitled “Investigating Biodegradability of DON in the Effluent of a Sidestream Deammonification Process.” This manuscript will be submitted for publication in a peer reviewed journal. Conclusions and recommendations for future work are presented in Chapter 6.

CHAPTER 2. LITERATURE REVIEW

2.1. Microbial Pathways for DON and DIN Consumption and Production

DON and DIN play a very important role in various biochemical and physiological processes. DIN and DON both provide structural and functional stability to various cellular materials including proteins and nucleic acids. DON is an important part of the cellular system and can play a major role in regulating the nitrogen cycle. The dynamicity of the DON is to a great extent determined by microorganisms in the environment that are constantly producing and removing DON (Berman and Bronk 2003). DON is spontaneously produced, consumed, and transformed in various oligotrophic water bodies due to microbial and photolytic reactions. DON can consist of BDON and non-biodegradable or refractory portions (rDON). Complete mechanism of DON transformation in aquatic systems is still unknown. Biodegradation of DON by bacteria is an important part of a number of processes that are presented in Figure 2.1.

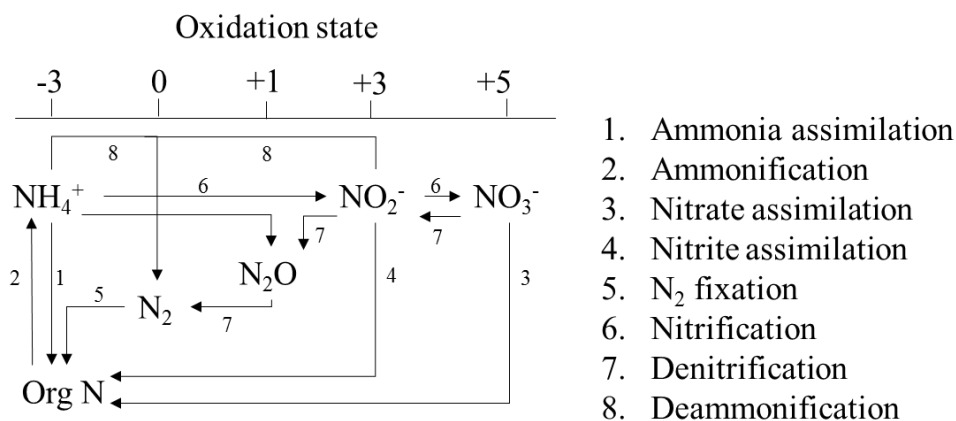


Figure 2.1. Eight major microbial pathways that result in organic nitrogen formation in the environment.

There are four majorly identified and studied microbiological pathways which lead to organic nitrogen formation (Figure 2.1). BDON is mineralized by bacteria into ionized ammonia (NH_4^+) and the process is referred to as ammonification. Ammonification is the major pathway

of DON biodegradation which is performed by most of the heterotrophs and few phytoplanktons such as *Pleurochrysis* (Ohkouchi and Takano, 2014). These microorganisms ammonify DON including amino acids and generated NH_4^+ is then assimilated for cell growth. Enzymes such as aminase that are capable of breaking the amine groups of DON molecules are thought to be involved in DON ammonification (Ohkouchi and Takano, 2014). The produced NH_4^+ can take different routes and is available to bacteria, algae, and other planktonic cells for uptake. The NH_4^+ produced can either be directly assimilated into the bacterial biomass and/or nitrified to nitrite (NO_2^-) and nitrate (NO_3^-) which is further denitrified into nitrogen (N_2) gas.

Deammonification might be another route involved in complete removal of mineralized DON.

The NH_4^+ assimilation involves incorporation of NH_4^+ into two amino acids, glutamine and glutamic acid using the enzymes glutamate synthetase (GS) and glutamate dehydrogenase (GDH) (Figure 2.2). GS enzyme adds an amine group to the glutamic acid to form glutamine. While GDH converts 2-oxoglutarate into glutamic acid, but the enzyme is active only at higher NH_4^+ concentrations, due to its low affinity towards NH_4^+ . The glutamine is then converted back into glutamic acid using glutamine 2-oxoglutarate aminotransferase (GOGAT). The GS-GOGAT cycle produces 2 glutamic acid molecules which are converted into desired amino acid by aminotransferase, glutamic acid is also used to make tetrapyrrole. Tetrapyrrole is a heterocyclic complex DON compound that has been identified to be an important part of humic substance that are commonly found in natural waters. The GS-GOGAT biological pathway is one of the most essential mechanisms in which DON is biologically produced in the environment. This pathway is used by many species of bacteria in the environment (Ohkouchi and Takano, 2014).

Photoautotrophic microorganisms are abundant in oligotrophic water environments. The primary form of DON produced by photoautotrophs is the amide group of glutamine or the

amino group of glutamic acid. The amide and amino groups are transferred to α -keto acids to form amino acids. Glutamic acid forms most of the amino acids while glutamine adds the amide group to histidine, tryptophan, asparagine, purines, pyrimidine, amino sugars and NAD⁺. About 80% of the nitrogenous content of the cell is amino acids existing as proteins and peptides (Antia et al., 1991; Berman and Bronk, 2003).

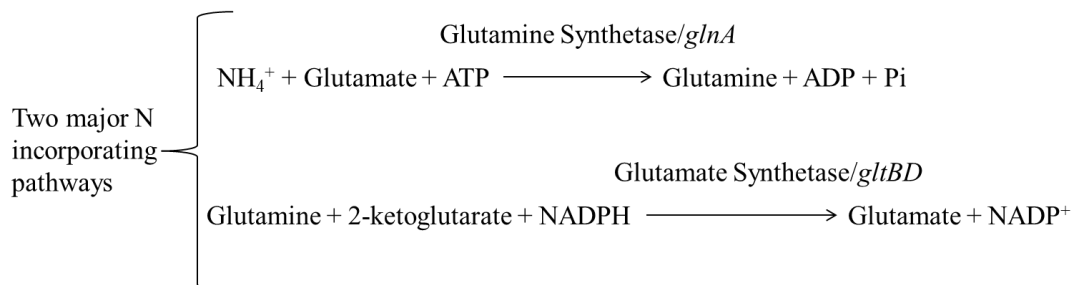


Figure 2.2. The ammonia assimilation pathway in microorganisms.

The most reduced form of nitrogen, NH_4^+ is the most preferred nitrogen source for autotrophs. Physical attributes such as temperature and chemical attributes including pH and salinity of the water determine if ammonia is present in its NH_4^+ or unionized form (NH_3). The pH of seawater is about 8.1 while for most of the wastewater receiving rivers and lakes its closer to 7. The logarithm dissociation constant for ammonia in water is about 9.34 (at 25°C), so most of the ammonia is ionized rather than unionized. There is a great debate in the scientific community about which form of ammonia is more assimilated by photoautotrophs. However, the unionized form, also referred as free ammonia is known to be an inhibitor of growth rate and in some cases toxic to many organisms (Torá et al., 2010).

Various bacteria and fungi in the ocean are also capable of performing NO_3^- assimilatory reduction to gain energy and produce DON molecules for cellular material. Assimilation of NO_3^- into nitrogenous organic matter is presented in Figure 2.3. Cells actively transport NO_3^- inside the cell where it reacts with nitrate reductase to form NO_2^- . The NO_2^- generated cannot be

oxidized back to NO_3^- and is further reduced to NH_4^+ using ferredoxin-dependent nitrite reductase, the generated NH_4^+ is then incorporated into amino acids following the pathway described above. A few autotrophs including algae and cyanobacteria in the environment can assimilate some forms of DON to produce other more desired nitrogenous organic materials. Urea, and some free amino acids such as alanine and asparagine that are found in water are commonly assimilated by microorganisms (Niu et al., 2013; Ohkouchi and Takano, 2014).

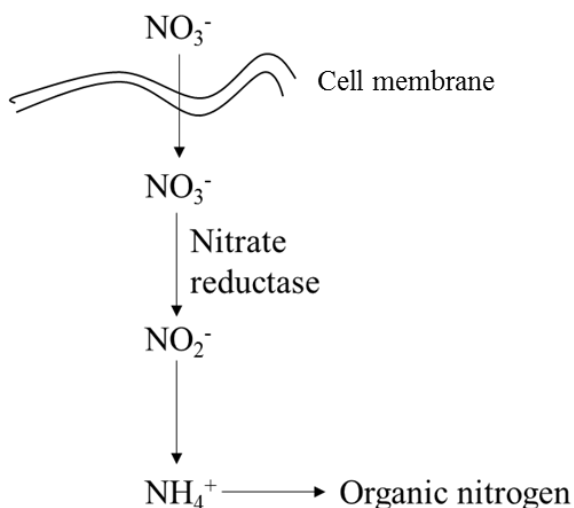


Figure 2.3. Assimilation of NO_3^- into nitrogenous organic matter.

Apart from photoautotrophs, obligate chemosynthetic bacteria also play a major role in the environment, they use nitrogen species for producing energy. It is theorized that nitrogen is limited in the oceans, and most of the nitrogen present is converted to photosynthates. Dinitrogen (N_2) is the most abundant form of dissolved nitrogen available for assimilation in the ocean. Most photoautotrophs in the aquatic environment along with some bacteria on the land are able to assimilate N_2 . Assimilation of N_2 into NH_4^+ using the enzyme nitrogenase, the biological nitrogen fixation in ocean is about 1.2×10^5 ton N per year (Zehr et al., 2007). In the tropical and subtropical oceans N_2 and NO_3^- are the important nitrogen substrates for the formation of organic nitrogen. It has been estimated that along the north-south intersect of the Atlantic Ocean as much

as 38 to 59% of the particulate organic nitrogen is produced through N₂ assimilation (Benavides et al., 2013; Ohkouchi and Takano, 2014).

2.2. DON in Oligotrophic Systems

DON is complex and has been partially characterized; some of the known DON compounds include urea, dissolved free amino acids (DFAA), dissolved combined amino acids (DCAA, such as oligopeptides and proteins, amino sugars, nucleic acids, and complex macromolecules such as humics) (Antia et al., 1991; Bronk, 2002). DON is commonly found in various aquatic environments including lakes, streams, rivers, estuaries, and oceans. DON is a dynamic constituent of the nitrogen cycle and its percentage can vary from 8 to 83% of TN. For the coastal areas, TN loadings of which 36-68 % can be DON to coastal waters have increased over the last century, resulting per year increase in DON concentrations (Boyer et al., 2006). DON sources to coastal waters include sewage and industrial wastewater effluent, agricultural and urban runoff, release from microorganisms, riverine delivery, groundwater discharge, atmospheric deposition, and biotic water column processes (Capone, 1988). DON is potentially a major nitrogen source for bacterial, algal, and phytoplankton communities in aquatic oligotrophic environment (Jansson, 1979; Crowder and Painter, 1991).

Sarmiento and Gruber (2006) estimated that about 9×10^9 ton N is fixed as nitrogenous organic matter every year in the oceans while about 1.4×10^8 ton N is fixed on land. About 3.6×10^7 ton N is deposited into the oceans via rivers from the land every year. Currently, flux of combined DIN and DON to coastal waters is about $30\text{--}36 \times 10^6$ ton per year of which $9.3\text{--}13.32 \times 10^6$ ton are due to the leaching of materials from the local catchment, wastewater effluents, and diffuse run-off from agricultural land and urban centers. A major portion of these deposited nitrogenous organic matter is biodegradable. Isotopic studies have identified that in ocean

disturbances or perturbations in the sedimentary layer can release BDON and rDON into the water. On the time scale of more than thousands of years the isotopic variation of DON is regulated by the sedimentary layer of the ocean.

WWTPs are a major contributor of DON into the receiving waters. Nam and Amy (2008) discovered that the DON levels downstream of a WWTP were 2-2.5 times higher than upstream DON levels and they concluded that wastewater effluent was the main contributor for this increase. About 10-20% of the wastewater effluent DON is DFAA and DCAA while a very small portion < 10% of DON is humic substances and ethylenediaminetetraacetic acid (EDTA) (Pehlivanoglu-Mantas and Sedlak, 2008). These substances identified are mostly biodegradable and can be directly or after degradation by bacteria bioavailable for growth of natural algae and plankton (Pehlivanoglu-Mantas and Sedlak, 2004, Sattayatewa et al., 2009, Simsek et al., 2013). To control algal growth in receiving waters, WWTPs should effectively remove BDON in wastewater. Algal growth negatively impacts aquatic ecosystems by causing oxygen depletion and increasing the concentration of nitrogenous organic matter (Ye et al., 2011).

Europe exports 8% of global DON of which 40% is anthropogenic Hoagland et al., 2002). This high TN loading into the water bodies adds to the economic cost. The United Kingdom spends about 225 million per year to rehabilitate fresh waters affected by eutrophication while the US has spent about \$1 billion over the last 20 years (Hoagland et al., 2002; Pretty et al., 2003). Due to increased cost and damages to the environments, regulations on wastewater effluent N limits are becoming very stringent for some receiving water bodies. This fact has forced some WWTPs to reduce effluent DON concentration prior to discharge. Many WWTPs are forced to reduce effluent TN concentration to 3-4 mg N/L or lower to protect receiving waters.

The compositions of DON in surface run-off are important in determining the extent of the bacterial degradation (McCallister et al., 2006). Urban and suburban runoffs have a higher proportion of BDON ($59\pm 11\%$) than agricultural ($30\pm 14\%$) or forest ($23\pm 19\%$) (Seitzinger et al., 2002; Wiegner and Seitzinger, 2004). In freshwater (lake) samples, DON gets mineralized into NH_4^+ and can transform into urea. Berman et al. (1999) conducted experiments with various DON compounds, especially amino acids and amino sugars, and monitored formation of NH_4^+ and urea during the incubation period of about 14 days. Glucosamine, guanine, hypoxanthine, lysine, or ornithine increased the ammonia concentration while hypoxanthine, guanine, or arginine samples increased urea concentrations. Because of its complex structure, DON is not readily available to some species in aquatic ecosystems. DON degradation converts high molecular weight compounds to low molecular weight compounds, and finally this degradation makes DON bioavailable to some species including algae, bacteria, micrograzers, bacterioplankton, cyanobacterium, and phytoplankton (Pehlivanoglu and Sedlak, 2004; Sattayatewa et al., 2009; Bronk et al., 2010; Loh et al., 2011). Degraded low molecular compounds could be NH_4^+ , amino acids, humic substances, and urea and these substances could be bioavailable to the species mentioned above (Bushaw-Newton and Moran, 1999; Wiegner et al., 2006; Bronk et al., 2010).

In surface water, DON can be up to 60 to 99% of the TDN whereas the percentage of DON to TDN decreases with depth (Wheeler et al., 1997). DON plays a major role in not only the nitrogen cycle but also dissolved organic matter cycle in the environment. For successful evaluation of dissolved organic matter (DOM) biodegradability, elemental composition analysis in terms of dissolved organic carbon (DOC) and DON is important. DON and DOC analyses of the Bay of Biscay and the northwestern Mediterranean revealed that at surface higher

concentrations of DOC and DON are present (Aminot and K  rouel, 2004). The concentrations decreased with depth, at about 2000 m about 2.6–2.8 mmol/L of DON was present and the ratios of DOC:DON was about 14 to 17. About 25–35% of DOC and 30–35% of DON were determined to be biolabile. Bacteria were identified to be directly responsible for mineralization of a high proportion of DON in the deep water. The same study revealed that the concentrations of DOC and DON had been increasing from 1990 to 2004 (Aminot and K  rouel, 2004). DON in the sedimentary layer of the estuaries can be adsorbed and released, and has an impact of the water quality. DON rich, especially amino acid rich hydrophobic organic matter, are known to sorb onto particles (Aufdenkampe et al., 2001). Freely suspended DON is more bioavailable (12–72%) than DON adsorbed onto the surface.

The dynamic nature of the surface water shoreline often causes dramatic shifts in the supply of DON and other nutrients to the plankton and bacterial community. Under growth limiting nitrogen conditions, DON is an essential nutrient and can lead to primary production. In deep oceans, DON is very refractory and can stay for up to thousand years before getting mineralized (Bauer et al., 1992). Wong et al. (2002) identified DON residence time in NE Pacific of about 180–1560 days which indicates the degree of refractory DON present in the ocean. A number of biological processes can lead to release of DON into the environment, extracellular production by growth and decay of microorganisms, viral cell lysis, solubilization of particles, and bacterial transformation. The main sinks of DON in estuaries include bacterial respiration, sorption to suspended particles, photochemical oxidation and phytoplankton uptake (Bronk, 2002).

The biodegradation of organic nitrogen by microorganisms is dependent on the molecular weight of inorganic and organic suspended particles in the water sample. High molecular weight

(HMW) particles have higher surface area and organic nitrogen on the surface of the particles might be more biodegradable (Fagerberg et al., 2009). Fagerberg et al. (2009) showed that the harmful dinoflagellate *Alexandrium minutum* and bacteria uptake larger molecules of river HMW DON, especially utilization of organic nitrogen from large DON molecules.

DON transformation in the dry season is less compared to the wet season. The wet season upsets the sediment bed and increases the concentration of suspended particles which have bacteria attached to them. The rates of complete DON transformation (ammonification) and nitrification have been observed during re-suspension of the sediments. At higher suspended sediment concentrations of 0, 5 and 10 g/L, the ammonification rates were 0.286, 0.332, 0.538 per day, respectively while the nitrification rate were 0.0018, 0.0038, 0.005 per day/mole/L (Xia et al., 2013).

Druon et al. (2010) showed that during summer there is a higher release of DON in the upper ocean layer that increases the regenerated primary production by 30-300%. The increase in primary production includes increase in DOC production in the upper layer which also leads to solubilization of organic matter in deeper water column. These observations suggest that DON is a major organic pool directly related to primary production and regulates the carbon and nitrogen cycles.

The composition of TDN varies with season, higher DON to TDN pool during the summer and autumn relative to winter and spring has been observed (Ribas-Ribas et al., 2011; Van Engeland et al., 2010). Concentrations of DON in two anthropogenically influenced estuarine systems, Yealm and Plym in southwest England were about 80 μM with 9.6 μM being biodegradable. Microbial degradation of DON and TDN is very prominent in these estuaries, both ammonification and nitrification have been observed. A study performed in the English

Channel showed seasonal variations of $< 5 \mu\text{M}$ in winter and up to $10 \mu\text{M}$ in summer (Butler et al., 1979). In coastal waters of the French Atlantic coast, DON concentration changes from less than $6 \mu\text{M}$ in winter to about $25 \mu\text{M}$ in spring and summer (Aminot and K  rouel 2004).

The concentration of TDN including DON not only changes with the season but also varies throughout the day. Higher DON concentrations peaking during the day while minimum detection during the night have been observed (Cellos et al., 1996). Along with DON, NO_3^- also shows a similar pattern which was due to prominence of nitrification during the day. There is evidence that nitrate is converted to DON biologically in water bodies. There is a significant inverse relationship between changes in DON and changes in nitrate. Ribulose biphosphate carboxylase (Rubisco) which is a surrogate of autotrophic activity and is used to identify the portion of nitrifying bacteria in the water sample. (Cellos et al., 1996). Cellos et al. (1996) observed lack of relationship between Rubisco and DON suggesting that the removal of DON in the environment is due to heterotrophic and not associated with autotrophic activity.

Presence of metals and ions in the water affects the transformation of the organic nitrogen. The redox-sensitive metals can regulate the concentration of fixed nitrogen species including DON in marine sediments. Iron oxides have shown to accelerate degradation of organic nitrogen in water bodies. Iron can play a role in regulating the oceanic production and removal of reactive nitrogen species while it preserves some of the organic matter preventing it from degradation through the formation of strong Fe–organic matter complexes (Barber et al., 2014).

Although found in low concentrations, DON compounds can undergo biological and photochemical transformations to form either labile or refractory light absorbing chromophoric dissolved organic matter (CDOM). Exposure to sunlight has an important impact on the fate of

DON in the environment. Photochemical degradation of DON is an important step to produce nutritious and biolabile component in estuaries and near shore marine waters (Wetzel et al., 1995). The dilute pools of dissolved amino acids and amino sugars could provide suitable conditions for the natural formation of CDOM. Amino sugars form fluorescent and labile organic matter while tryptophan transforms into non-fluorescent refractory humic-like fluorophore. The CDOM formed from the collection of labile N-containing compounds is heterogenic and is more likely to produce molecules with a greater potential to absorb light than carbonaceous compounds (Klapper et al., 2002). In natural aquatic systems, DON is susceptible to photooxidation, suggesting that at least some components may be light-absorbing molecules (Bushaw et al., 1996; Vähätalo and Zepp, 2005; Xie et al., 2012). Microbial formation of CDOM from N-rich compounds is greater than photochemical formation except for tryptophan which rapidly formed significant CDOM. Chemically identical amino sugars, mannosamine, galactosamine, and glucosamine might form varying CDOM with different optical characteristics (Biers et al., 2007).

2.3. DON in Eutrophic Environments

The conventional activated sludge process is capable of removing substantial amounts of BDON from raw wastewater leaving mainly rDON in the effluent (Parkin and McCarty, 1981a; Murthy et al., 2006). However, the mechanism of BDON removal from wastewater is not completely known. It is not clear which operational parameter in activated sludge process is responsible for DON removal. Growth and decay of organisms, sludge retention time, hydraulic retention time, and MLSS concentration can influence the concentration and composition of DON in the effluent of wastewater (Parkin and McCarty, 1981a; Simsek et al., 2013).

To meet stringent nutrient discharge limits, utilities are increasingly designing biological nitrogen removal (BNR) plants for nitrogen and phosphorus removal. Most of the BNR plants use processes focused on removing inorganic nitrogen from wastewater. TN concentration in treated effluent from WWTPs equipped with nitrification and denitrification processes typically ranges from 5 to 10 mg N/L. However, inorganic nitrogen removal results in an increased fraction of DON in the final effluent of BNR plants. DON concentrations in treated wastewater effluent can vary from 0.7 and 2.1 mg N/L (Parkin and McCarty, 1981a; Murthy et al., 2006; Urgun-Demirtas et al., 2008; Pehlivanoglu-Mantas and Sedlak, 2008; Simsek et al., 2012). Although effluent DON is recalcitrant to the treatment processes, studies showed that about 50% of the effluent DON is biodegradable by bacteria (Murthy et al., 2006; Khan et al., 2009; Sattayatewa et al., 2009). Previous studies have focused on understanding DON removal or production by different processes that make up a BNR process (Czerwionka et al., 2012; Simsek et al., 2013).

For evaluating biodegradability of DON in wastewater, two bioassays have been developed (Parkin and McCarty 1981a; Khan et al., 2009). The bioassays are based on two different food to microorganism (F:M) ratios. Different information can be extracted depending on the type of F:M test used for understanding BDON exertion (DON ammonification). A high F:M ratio is the most commonly and widely used bioassay developed by Murthy et al. (2006) and Khan et al. (2009). While a low F/M bioassay for BDON analysis is more crucial for understanding the kinetics of DON removal through activated sludge process (Parkin and McCarty 1981a). The low F/M is a good test for optimizing activated sludge process for DON removal. While a high F/M test is a good representative test for quantification of residual DON in a wastewater treatment plant which might be biodegradable in receiving waters.

An example of the high F:M ratio is the BOD based approach used by Murthy et al. (2006). An un-optimized BOD based approach was applied to determine BDON in the effluent of a denitrifying plant. The effluent samples were seeded with settled raw wastewater and reduction in DON was monitored over a period of 20 days. The samples were filtered through a 1.0 μm pore-size glass fiber filter, BDON was estimated by measuring DON before and after the incubation. It has been observed that a large decrease in DO (4 to 6 mg/L) during the 20 days of incubation did not correspond to small DON reduction. Presence of more biodegradable carbonaceous sources for the bacterial inoculum could result in the decrease of the DO and not the DON.

Khan et al. (2009) modified and improved the BOD based bioassay for BDON measurement. The bioassay uses diluted MLSS as a source of bacterial inocula and the sample is manually aerated daily and stored at 20°C for 20 to 28 days of incubation. The modified procedure was investigated on the concentration of bacterial inoculum (2 mL of diluted MLSS at the concentrations of 30, 60, 120, and 240 mg /L in 300 mL sample) incubation period (2 to 180 days), and filtration requirement after the incubation. The optimized method used the 240 mg/L of MLSS as an inoculum source with an incubation time of 20 days. Khan et al. (2009) identified that no filtration was required after the incubation. The BDON bioassay is very useful and has been used to provide a good understanding on the fate and transformation of DON and BDON through WWTPs. The modified BDON procedure by Khan et al. (2009) is summarized in Figure 2.4 and equations 2.0 as follows:

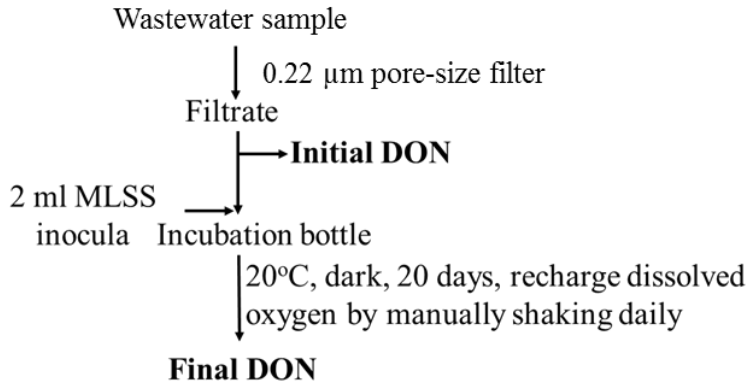


Figure 2.4. BDON procedure developed by Khan et al. (2009).

$$\text{BDON (mg N/L)} = (\text{DON}_i - \text{DON}_f) - (\text{DON}_{bi} - \text{DON}_{bf}) \quad (2.0)$$

Where, DON_i and DON_f are initial and final DON concentrations, respectively in the wastewater sample, while DON_{bi} and DON_{bf} are initial and final DON in a control sample, respectively.

Parkin and McCarty 1981a and 1981b evaluated the factors that affect DON production and removal during activated sludge treatment and the sources of DON in WWTP effluents. Parkin and McCarty 1981a operated a semi-continuously fed bench scale activated sludge reactor to examine the impact of different operational conditions on DON removal during activated sludge treatment process. Parkin and McCarty (1981b) focused on performing a low F:M test, and studied DON removal in wastewater samples from a WWTP. Both Parkin McCarty 1981a and 1981b defined DON in raw influent and treated wastewater using Equations 2.1 to 2.3.

$$\text{DON}_{\text{influent}} = \text{BDON} + \text{RDON} \quad (2.1)$$

$$\text{DON}_{\text{effluent}} = \text{RDON} + \text{DON}_{\text{produced}} + f^*(\text{BDON}) \quad (2.2)$$

$$\text{DON}_{\text{produced}} = \text{DON}_{\text{growth}} + \text{DON}_{\text{decay}} + \text{DON}_{\text{equilibrium}} \quad (2.3)$$

Where $\text{DON}_{\text{influent}}$ and $\text{DON}_{\text{effluent}}$ are DON concentrations in the raw influent and treated effluent, respectively. $\text{DON}_{\text{produced}}$ is DON that is generated during the activated sludge process

due to release of DON during growth ($\text{DON}_{\text{growth}}$) and decay ($\text{DON}_{\text{decay}}$), and f is the fraction of BDON from the influent not removed during the process. $\text{DON}_{\text{equilibrium}}$ is the concentration of DON adsorbed on the sludge.

Parkin and McCarty (1981a) suggested that better control of operating parameters might help minimize the amount of DON in effluent from of a wastewater treatment plant. They studied the effect of process control parameters such as MLSS concentration, organic loading, and aeration time on DON production during the treatment process. Higher MLSS concentrations showed faster DON reduction. In addition, at high MLSS concentrations DON removal reached its maximum. They also identified that increase in organic loading of glucose, acetate, and a glucose-acetate mixture in the reactor increased DON production. Also, it was identified that more DON was excreted during the logarithmic growth stage compared to the stationary stage and DON produced during the logarithmic stage was removed during the stationary stage (Parkin and McCarty, 1981a). Two-thirds of the refractory DON 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 DON may be produced during the biological treatment (Parkin and McCarty 1981a).

Parkin and McCarty (1981b) performed all DON removal experiments using concentrated MLSS obtained from the Palo Alto WWTP, California. The low F:M tests were performed by spiking high concentrations of MLSS (TSS of $> 1,000$ mg/L) in wastewater samples and incubating at room temperature for 8 to 10 hrs. In the low F:M tests, DON concentration initially decreased and then increased suggesting possible production of DON during the activated sludge process. At 1,390 mg/l MLSS, DON concentration reached a low value and then increased to about 1.5 times. At lower MLSS concentrations, a significant

increase of DON during the first 6 hours of aeration was observed while the soluble COD decreased. During the test, DON removal of 61-70% was observed.

Sattayatewa et al. (2009) reported that BDON in the final effluent of the Parkway WWTP (Laurel, Maryland, US) was about 41-57% of the DON. The Parkway WWTP uses a four-stage Bardenpho process. In absence of NO_3^- during the incubation, BDON exerted was slightly higher compared to samples with NO_3^- . They explained that NO_3^- competes with DON as a nitrogen source to microorganisms in the BDON test. A rapid NO_3^- increase was observed in the first 10 days of incubation. They explained that DON was ammonified and the NH_4^+ produced was then nitrified to NO_3^- . An increase in organic nitrogen was observed in the primary anoxic zone which could be due to either microbial activities or heterotrophic denitrification. Dilutions from return activated sludge and internal recycling added DON in the treatment process. DON, produced in the primary anoxic zone, did not substantially change in the next three sections, which were primary aerobic, secondary anoxic, and secondary aerobic.

2.4. DON in Soil

DON along with DOC plays a crucial role in driving biogeochemical processes in soils; however, minimal information is available on microbial mineralization of DON in soils. DON can be more than 57% of the TDN pool in soils (Christou et al., 2005). In soils, understanding the transformation of DON might be the key to determining its role in ecosystem functioning. It helps identify routes DON might take from the land to the oceans (Worrall et al., 2012). It has been identified that DON, including peptides and amino acids, is more abundant in low-productivity relative to high-productivity grassland ecosystems. During summer more concentration of DON with a molecular weight >100 kDa can be attributed to conversion of DIN to DON through assimilation. Addition of DIN to the soil has shown to significantly increase the

labile components and decreased recalcitrant components of DON especially in the top layer of soil (Farrell et al., 2011; Fang et al., 2014).

DON and DOC are nutrient sources in soils which are essential for plant growth. Chlorophyll (which contains DON) patterns of the leaves can indicate the nutrient availability and predict variations in DOC and DON in soil (Albrechtova et al., 2008). The composition of DOC and DON in soil is regulated by pH and the microbial activity. In some cases, DON compounds are utilized by the microbial community for the carbon source rather than a source of nitrogen (Christou et al., 2006). Organics can leach from the soil depending on several factors such as molecular size and aromaticity of the organic molecules, acid strength, charge density and the presence of polyvalent cations.

In Europe and North America, increasing the pH using lime resulted in higher DOC and DON from the organic topsoil (Andersson et al., 2000). Andersson et al. (2000) performed studies in Northern Sweden, where they changed the pH using lime and studied the effect on the microbial activity. The plots with pH 5.4 and 4.3 were incubated at 48°C and 158°C for more than 30 days. The microbial activities, and release of DON and DOC were estimated based on the CO₂ evolved which increased at higher pH and temperature. It was identified that pH was more important than temperature for the leaching of DOC and DON for the first 30 days while temperature was more important after that. A higher pH increased the content of hydrophobic acid leachate and increased the dissociation of acid functional groups resulting in more soluble DON and DOC.

In natural temperate fens, a type of wetlands, due to lower temperature and waterlogged conditions the decay rates of organic matter is slow which leads to accumulation of refractory organic matter including nitrogen in the long-term. Due to more refractory organic presence, the

amount of eutrophication downstream of the aquatic ecosystem is kept under control. For example, in NE Germany fens accumulates about 4.4 - 11.9 kg N/ha/y which accounts for 28 to 75% of TN export to the Baltic Sea. Removal of the highly decomposed peat layer and rewetting of the peat from the fens has been recommended for controlling the release of nitrogenous organic matter; however, this can hamper the N removal potential (Cabezas et al., 2012).

Temperature and vegetation cover on soil affect DIN and DON concentrations and transformations. Mineralization of DON in soil increases with temperature irrespective of the vegetation cover. However, about 4 times higher leachate of DON is observed with vegetation than without (Delgado-baquerizo et al., 2006; Chapman et al., 2001). In some cases, DON turnover rate is within minutes, suggesting microbial activity being a major player in DON transformation in soils (Wilkinson et al., 2014).

2.5. DON from Atmosphere

Organic N ubiquitously exists in the atmosphere in the form of gas and particle and can be a major contributor to the open waters. Amines have been one of the major DON compounds identified in the atmosphere. The atmospheric deposition of nitrogen can be a major concern as it can increase the concentration of DON in surface waters. Atmospheric DON deposition can impact the nitrogen cycle through acidification of soils and aquatic systems, and nutrient enrichment (Cornell et al., 2003; Cape et al., 2011). Along with natural, anthropogenic release of recalcitrant organic nitrogen through combustion has been found to have a significant effect on different streams across North America. The export of DON and nitrogenous organic matter to the ocean might affect the microbial community structure and the nitrogen cycle in aquatic environments (Ding et al., 2014).

Pelster et al. (2009) identified that annually fluxes in throughfall, a process by which wet leaves shed excess water onto the ground, and bulk deposition were 216, 80, 114 and 410 mg N/m² for NH₄⁺, NO₃⁻, DON and TDN, respectively on the Boreal Plain, Canada. The fluxes of NH₄⁺ and NO₃⁻ in throughfall were approximately 50% of the bulk deposition fluxes, while the TDN throughfall flux was about 70% of the bulk deposition flux. The highest DON concentration was observed in the snowpack and it was suggested that some of the NH₄⁺ was transformed to DON within the snowpack.

CHAPTER 3. DISSOLVED ORGANIC NITROGEN AND ITS BIODEGRADABLE PORTION IN A WATER TREATMENT PLANT WITH OZONE OXIDATION

3.1. Introduction

DON is a major portion of the aquatic nitrogen pool and the concentration of DON in estuaries and coastal waters averages 13% and 18%, respectively (Berman and Bronk, 2003). In ocean water and freshwater, DON can be >50% of the TDN pool (Antia et al., 1991). The amount of DON in rivers in U.S. and Sweden ranges from 14 to 1,260 $\mu\text{g L}^{-1}$ as N (Wiegner et al., 2006; Stepanauskas et al., 1999; Smith et al., 1991). DON is a dynamic participant in water and is constantly being produced and utilized by microorganisms (Berman and Bronk, 2003). Biolabile DON is consumed by bacteria and/or phytoplankton.

Wastewater discharge, and urban, agricultural, and forestry runoffs increase the concentrations of DON and biolabile DON in water (Aitkenhead-Peterson et al., 2005; Pellerin et al., 2006; Seitzinger et al., 2002; Aitkenhead-Peterson et al., 2009; Wiegner and Seitzinger, 2004). The concentrations of biolabile DON in urban, agricultural and forest runoffs were as high as 168, 1,092, and 532 $\mu\text{g L}^{-1}$ as N, respectively (Seitzinger et al., 2002). Seitzinger et al. (2002) used a mixture of bacteria and phytoplankton to study DON utilization and observed both mineralization and uptake. Flood events increase concentrations of biolabile DON in river water from 280 to 770 $\mu\text{g L}^{-1}$ as N (Stepanauskas et al., 2000). Wastewater treatment plants discharge up to 1,800 $\mu\text{g L}^{-1}$ as N of BDON into the receiving waters (Simsek et al., 2012).

Loadings of DON and biolabile DON from wastewater effluent discharge and runoffs in receiving waters could cause major water quality concerns. DON causes algae blooms (Glibert et al., 2004; Fang et al., 2010) in waters, which decreases DO and in turn is harmful to aquatic animals. A number of studies have investigated biolability of DON in rivers and streams

(Wiegner and Seitzinger, 2004; Stepanauskas et al., 2000; Kroer et al., 1994; Wiegner et al., 2006; Seitzinger and Sanders, 1997; Stepanauskas et al., 1999; Kaushal and Lewis Jr., 2005; Stepanauskas et al., 2002). In the Delaware and Hudson rivers, 40% ($176 \mu\text{g L}^{-1}$ as N) and 72% ($47 \mu\text{g L}^{-1}$ as N) of the DON was biolabile (Seitzinger and Sanders, 1997). A study observed that in water samples from six rivers in New Jersey, New York, Georgia, and Maryland, biolabile DON was eight times higher than biolabile DOC (Wiegner et al., 2006). These studies indicate high concentrations of biolabile DON in river waters, which could affect drinking water quality.

DON is extremely complex and has been medially characterized. Detailed composition of DON and BDON and its impact on drinking water is presented above in Chapter 2 Section. The major water quality concerns due to DON should be addressed and understanding them requires monitoring the fate of DON and BDON during water treatment. Previous studies have investigated the presence of DON only in raw and finished water (Lee et al., 2006; Lee et al., 2007; Lee and Westerhoff, 2006; Westerhoff and Mash, 2002; Xu et al., 2010; Xu et al., 2011).

There is no established bioassay for determining BDON in drinking water. Incubation period, and type and size of inoculum are two important parameters for a successful bioassay. Inoculum selection for performing BDON in water samples is essential, as different microbial communities will have organisms with different transport and enzyme systems for utilizing a specific set of DON substrates. A few studies have used bioassays based on fresh or marine water bacteria for evaluating biolability of DON (Wiegner and Seitzinger, 2004; Wiegner et al., 2006; Seitzinger and Sanders, 1997; Stepanauskas et al., 1999). However, the bacterial inocula used were not preconditioned to various DON molecules which could have been present in different water samples examined. For example, Wiegner et al. (2006) collected bacterial inocula from a single freshwater source to evaluate biolability of DON from nine different rivers. Some

studies have also used mixed liquor suspended solids (MLSS) as the bacterial inocula for determining BDON in wastewater (Simsek et al., 2012; Khan et al., 2009; Sattayatewa et al., 2009; Sattayatewa et al., 2010). Use of MLSS provides an advantage of having a mixed culture bacterial inoculum with a capability of utilizing broad spectrum of DON substrates.

The above described existing bioassay methods for wastewater, which has high DON (1,800 to 8,000 $\mu\text{g L}^{-1}$ as N), require high inoculum concentrations and longer incubation periods. Therefore, the bioassay for wastewater lack precision and accuracy for drinking water samples which normally have low DON concentrations ($\leq 1,000 \mu\text{g L}^{-1}$ as N). The bioassays used for determining biolabile DON in fresh and marine water samples use inocula from river water, which might not be able to degrade a wide variety of DON.

The objective of the work described in this chapter was to develop a bioassay for BDON determination in drinking water and determine the fate of BDON in a water treatment plant (WTP). To meet this objective, a BDON bioassay for drinking water was adapted from the bioassay developed by Khan et al. (2009) for BDON determination in wastewater. The optimum inoculum size and incubation period were identified. As DON and BDON in drinking water is lower than in wastewater, more sensitive method for measuring the nitrogen species for DON determination were developed in this study. The adapted BDON bioassay was then applied to examine the evolution of BDON through an ozonation-biologically active filtration WTP.

3.2. Materials and Methods

3.2.1. Glassware and Standards

All glassware and chemicals in Chapter 3 were obtained from VWR International LLC, IL, USA except otherwise noted. Glassware were washed with soap, rinsed with tap water, kept in a 10% v/v hydrochloric acid (HCl) bath overnight and rinsed with copious amounts of de-

ionized (DI) water. The washed glassware (not sensitive to heat) were dried overnight at 105°C, covered with aluminum foil, and baked for 1 hr at 550°C. Ammonium sulfate, sodium nitrite, potassium nitrate, and glutamic acid were used as standards for ammonia (NH₃-N), nitrite (NO₂-N), nitrate (NO₃-N), and TDN, respectively. Glycine and glutamic acid were used to identify the sensitivity of the BDON method.

3.2.2. Description of the Moorhead WTP, and Sample Collection and Preparation

The City of Moorhead WTP has a capacity of 60,566 m³/d. The plant uses surface water from the Red River of the North as its major source of raw water. Occasionally, the raw water is blended with groundwater from the Buffalo or Moorhead aquifer to reduce the organic content.

Figure 3.1. shows a process diagram of the Moorhead WTP and sampling locations.

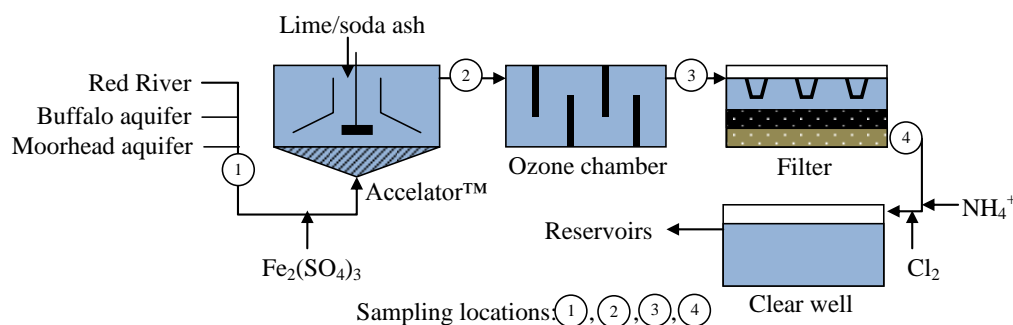


Figure 3.1. Process diagram of the City of Moorhead WTP.

Table 3.1. presents the sampling dates and corresponding raw water sources. Basic characteristics of the raw water during the two year sampling period are presented in Table 3.2. A one or four liter sample was collected from each location on each sampling date.

Table 3.1. Raw water sources for the Moorhead WTP during the sampling period.

Raw water sources	Sampling dates
Only River	10/23/2010^a , 12/7/2010, 12/21/2010, 1/17/2011, 10/27/2011, 1/7/2012, 1/9/2012, and 1/16/2012.
River:Buffalo	11/1/2010^a (6.1:3.9) ^b , 4/3/2011 (3.6:6.4), 4/29/2011 (3.9:6.1), and 8/10/2011 (7.0:3.0).
River:Moorhead	10/11/2010^a (8.4:1.6) ^c , 11/1/2010 (6.1:3.9), 11/8/2010 (8.6:1.4), 4/3/2011 (3.6:6.4), and 6/9/2011 (8.3:1.7).

^aSampling dates for the optimization experiments.

^bBlending ratio of River and Buffalo Aquifer.

^cBlending ratio of River and Moorhead Aquifer.

Table 3.2. Basic characteristics of raw water obtained from the Moorhead WTP.
Data are from daily sampling in 2010-2012.

Parameters	Average \pm standard deviation	Range
UV absorbance at 254 nm (cm^{-1})	0.24 ± 0.05	0.18 - 0.31
Alkalinity (mgL^{-1} as CaCO_3)	197.1 ± 25.4	100 – 296
Hardness (mgL^{-1} as CaCO_3)	392.41 ± 58.74	172 – 520
pH	8.11 ± 0.34	7.2 - 9.1
Turbidity (NTU)	40.61 ± 35.74	3.4 - 240

The sample was filtered through a 0.2 μm pore-size cellulose acetate membrane filter (PALL Co., Port Washington, NY, USA) for all water analyses performed in the study except for SUVA. For SUVA analysis, a 0.45 μm pore-size cellulose acetate membrane filter (PALL Co., Port Washington, NY, USA) was used. Due to high solid concentrations in the raw water, samples were filtered through a 1.2 μm pore size glass microfiber filter (Whatman Inc., Kent, UK) prior to the filtration through the 0.45 or 0.2 μm pore-size filter. The filtered samples were used for determining dissolved inorganic nitrogen species (DIN; $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$), TDN, DON, BDON, DOC, BDOC, UV absorbance at 254 nm (UV_{254}), DOC specific UV_{254} ($\text{SUVA}_{254\text{-DOC}}$), and DON specific UV_{254} ($\text{SUVA}_{254\text{-DON}}$). Spectrophotometer (DR 5000™ UV-Vis Spectrophotometer, Hach®, CO, USA) was used for required analytical methods.

3.2.3. Analytical Methods

3.2.3.1. DIN and TDN Analysis and DON Determination

The 4500- NH_3 phenate method (APHA, 2005) was modified to measure $\text{NH}_3\text{-N}$ in the samples. To a 5 ml sample, 200 μL of a phenol solution prepared by adding 1.11 mL of 89% liquefied phenol in 8.89 mL of 95% (v/v) ethyl alcohol was added followed by 200 μL of a 5% (w/v) sodium nitroprusside solution. Finally, 500 μL of an alkaline hypochlorite solution prepared by adding 10 mL of 20% (w/v) alkaline sodium citrate to 2.5 mL of 5% (v/v) sodium hypochlorite was added to the sample and absorbance was measured spectrophotometrically at 640 nm after 1 hr of incubation.

Nitrite in the samples was measured spectrophotometrically using a colorimetric method described by Bronk et al. (2000). Briefly, 250 μ L of a colorimetric reagent was pipetted onto a 5 mL sample which was then incubated in the dark for 10 min before being measured for absorbance at 540 nm. The colorimetric reagent was prepared by dissolving sulfanilamide (5 g) and N-(1-naphthyl)ethylenediamine dihydrochloride (0.5 g) in 500 mL of a 1.46 M phosphoric acid solution.

Both $\text{NO}_3\text{-N}$ and TDN in samples were analyzed by converting to $\text{NO}_2\text{-N}$ which was then measured using the colorimetric method (described above). The spongy cadmium reduction method (Jones, 1984) was modified and used to reduce $\text{NO}_3\text{-N}$ to $\text{NO}_2\text{-N}$. The acid-washed zinc sticks were dropped into a 20% (w/v) solution of cadmium sulfate for a minimum of 12 hrs. The cadmium deposited on the zinc sticks was scrapped and used for $\text{NO}_3\text{-N}$ analysis. To a 15 mL centrifuge tube containing pre-weighted >0.5 g of scrapped cadmium and 1 mL of ammonium chloride solution (4.7%), 5 mL of sample was added. The tube was shaken at 200 rpm for 90 min at room temperature after which the samples were analyzed for $\text{NO}_2\text{-N}$ (reduced from $\text{NO}_3\text{-N}$). The cadmium was regenerated by washing with HCl (6N) and rinsing it with DI until pH > 5 .

TDN was analyzed by the persulfate oxidation method described by Bronk et al. (2000), in which 2 mL oxidizing reagent was added to a 15 mL sample and autoclaved for 30 min at 121°C and 15 lbin^{-2} pressure. The oxidizing reagent was prepared by dissolving 45 g potassium persulfate and 27 g boric acid into 900 mL of 0.35 M sodium hydroxide. The oxidation converts TDN into $\text{NO}_3\text{-N}$ which is then reduced to $\text{NO}_2\text{-N}$ using the cadmium reduction method and the $\text{NO}_3\text{-N}$ reduced $\text{NO}_2\text{-N}$ is determined using the colorimetric method.

DON was determined from the difference between measured TDN and sum of measured DIN species using equation 3.1.

$$\text{DON } (\mu\text{g L}^{-1} \text{ as N}) = \text{TDN} - (\text{NH}_3\text{-N} + \text{NO}_2\text{-N} + \text{NO}_3\text{-N}) \quad (3.1.)$$

3.2.3.2. BDON Bioassay

3.2.3.2.1. BDON Procedure

Two hundred milliliters of the filtered sample were mixed with 2 mL of the inoculum in a 250 mL amber bottle. The solution in the bottle was shaken thoroughly to aerate and incubated in the dark at 20°C for a period of time. The BDON procedure relies on the change of DON in the sample before and after incubation. The BDON bioassay was performed in triplicates for all samples. BDON was calculated using Equation 3.2.

BDON was determined from

$$\text{BDON } (\mu\text{g L}^{-1} \text{ as N}) = (\text{DON}_i - \text{DON}_f) \quad (3.2.)$$

Where,

DON_i and DON_f are DON before and after the incubation.

3.2.3.2.2. Optimization Studies: Inoculum Size, Incubation Period, and Standard DON

Compounds

BDON analysis is susceptible to the amount of inoculum used (Khan et al., 2009). Less inoculum can result in BDON underestimation while high inoculum can add error due to endogenous decay. In this study, MLSS (3,000 mgL⁻¹ total suspended solids) was collected from the City of Moorhead WWTP and three dilutions 1, 5, and 10% (v/v) of the MLSS in DI were prepared. Along with inoculum size, the incubation time was also varied at 2, 7, 14, 21, and 28 days. The effects of inoculum size and incubation time were tested on raw water samples. The incubation time was tested for the selected inoculum size for samples from all four locations of the plant. When testing the incubation time, samples were sacrificed at each time interval. BDON in two standard DON compounds, glycine (50, 100, and 250 $\mu\text{g L}^{-1}$ as N) and glutamic

acid (50, 100, and 250 $\mu\text{g L}^{-1}$ as N) were tested. For application of the method to study fate of BDON in a drinking water treatment plant, the BDON analysis was performed on the samples using optimized inoculum size and incubation time.

3.2.3.3. DOC and BDOC determinations

DOC was analyzed using an ultraviolet/persulfate oxidation total organic carbon (TOC) analyzer (Phoenix 8000, Tekmar Dohrmann, OH, USA) with a detection limit of 2 $\mu\text{g L}^{-1}$ as C. The TOC analyzer was calibrated according to the instrument manual. The DOC measurements were based on calibration with potassium hydrogen phthalate. All the standards and samples were preserved by adjusting to ≤ 2 pH using phosphoric acid. Analysis was performed according to Standard Methods (APHA, 2005). Raw or un-normalized UV_{254} was determined spectrophotometrically. $\text{SUVA}_{\text{UV}_{254}\text{-DOC}}$ or $\text{SUVA}_{\text{UV}_{254}\text{-DON}}$ are indicators of relative unsaturated carbon and nitrogen amounts which were determined by dividing UV_{254} by DOC or DON, respectively. BDOC was evaluated in accordance with a protocol by Khan et al. (2005).

3.2.4. Bacterial Growth

The BacTiter GloTM microbial cell viability assay was used to confirm the growth of inoculum during the BDON bioassay. The assay uses adenosine triphosphate (ATP) as an indicator of bacterial biomass. The assay was performed based on a protocol provided by the manufacturer of BacTiter GloTM with a minor modification on sample incubation period. One hundred microliters of the samples were placed in a 1.5 mL plastic centrifuge tube, an equal volume of the BacTiter GloTM reagent was added, and the sample tube was placed in a luminometer and the luminescence intensity (RLU) was recorded after 3 min of incubation at room temperature using a TN20/20 luminometer (Turner Designs, Sunnyvale, CA, USA). The

cell viability method is previously standardized and commonly used for estimating bacterial growth (Sule et al., 2009; Wadhawan et al., 2010).

3.2.5. Statistical Analysis

Two-way analysis of variance (ANOVA) procedure was conducted using Microsoft Excel to determine statistical differences followed by a two sample *t*-test, assuming equal variances with Bonferroni correction. This statistical analysis was performed to differentiate results from different conditions including inoculum size, incubation period, and treatment stages (sampling locations). Results that were statistically analyzed included those for the following parameters: DON, BDON, TDN, SUVA_{UV254_DOC}, SUVA_{UV254_DON}, and ratios of DON to TDN, DON to DOC, BDON to DON, and BDON to BDOC. Statistical analysis on triplicate of 68 samples was performed.

3.3. Results and Discussion

3.3.1. Optimization Studies: Inoculum Size, Incubation Period, and Standard DON Compounds

3.3.1.1. Inoculum Size and Incubation Period

Average BDON in three raw water samples inoculated with the three inoculum sizes and for five incubation periods ranged from 26 to 236 $\mu\text{g L}^{-1}$ as N (Fig. 3.2).

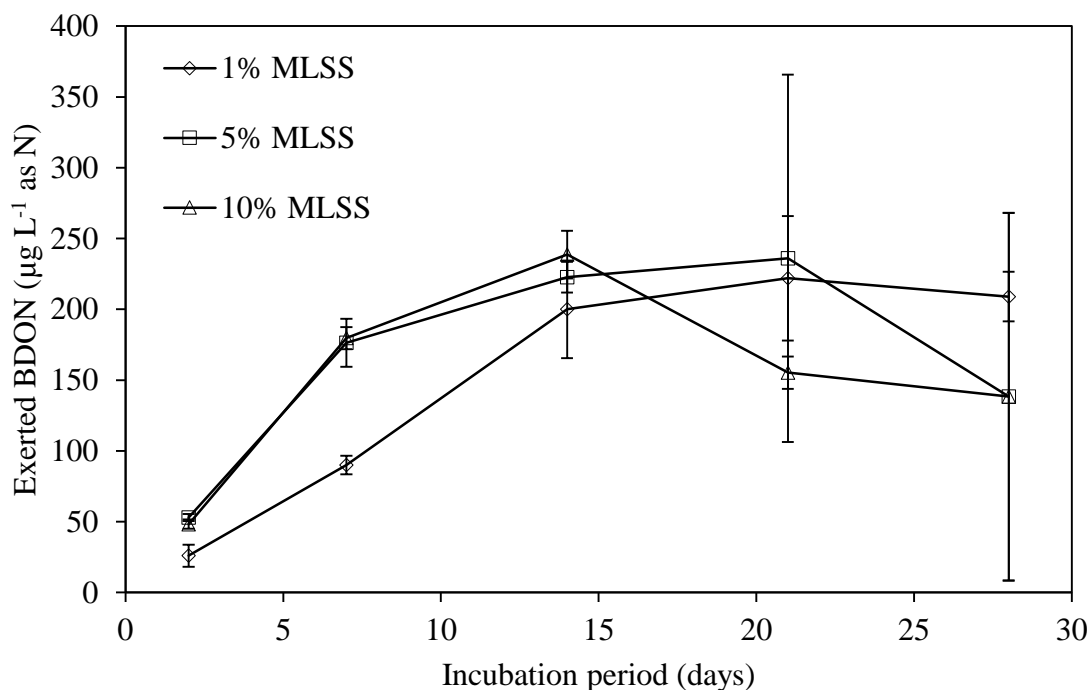


Figure 3.2. Amount of BDON in raw water samples inoculated with 1, 5, or 10% MLSS for different incubation periods.

These raw water samples were collected on three different dates (Table 3.1.) and each of the samples was analyzed in triplicate. The error bars represent standard deviations based on the total of 9 replicates. Limited BDON exertion and DON reduction were observed during the first 2 days of incubation. At day 7, the amount of BDON exerted in the sample with 1% MLSS was significantly less than the samples incubated with 5% and 10% MLSS ($p = 0.0002$ and 0.0017 , respectively). The BDON exerted at day 14 for all the samples were significantly more compared to samples at day 7, for 1, 5, and 10% MLSS inocula ($p = 0.024$, 0.024 , and 0.014 , respectively). The amount of BDON exerted at day 14 was not significantly different among all inocula ($p > 0.05$). Standard deviations of the results for samples inoculated with 10% MLSS on days 21 and 28 were higher compared to the cases for the 1% and 5% MLSS inocula. At days 21 and 28, organic carbon and nitrogen were not sufficient for the 10% MLSS inoculum. The increased standard deviation is inferred to exogenous release of nitrogen containing soluble microbial

products from the inocula. MLSS concentrations of 1% and 5% were more reliable as they impart statistically less error to the samples compared to 10% MLSS. To prevent possible underestimations of BDON, the 5% MLSS inoculum was chosen for further analysis of all the samples.

MLSS was chosen as an inoculum source since it contains a diverse bacterial population capable of degrading a variety of complex natural and synthetic organics. MLSS contains bacterial species commonly found in natural water and soils (Sanapareddy et al., 2009). MLSS has been proven to be a reliable inoculum for determining BDOC in drinking water (Khan et al., 2005; Khan et al., 2006; Ratpukdi et al., 2009). Another benefit of the MLSS inoculum is minimal or no acclimation time.

A number of studies investigating the biolability of DON in fresh waters have identified the optimum incubation time with bacteria and/or phytoplankton for complete DON utilization to be 10 to 14 days (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999; Stepanauskas et al., 2000; Wiegner and Seitzinger, 2001; Seitzinger et al., 2002; Stepanauskas et al., 2002). The amount of DON decreased rapidly in the water samples for the first 7 days of incubation and then slowly for the next 7 days after which no change was observed. For raw water samples, 56.7% and 68.4% decreases in DON by days 7 and 14 were observed, respectively. The rate of DON reduction to day 7 and day 14 was 20.5 and 7.5 $\mu\text{g L}^{-1}$ as N d^{-1} , respectively. Seitzinger and Sanders (1997) observed a similar trend in water samples from the Delaware River. They reported the amount of DON rapidly decreased by 63% through day 4 and more slowly through day 15 to give an overall 72% DON decrease (initial DON = 180.6 $\mu\text{g L}^{-1}$ as N). As the total amounts of BDON exerted on days 21 and 28 were not significantly different from those on day 14 regardless of the inoculum size ($p > 0.05$), an incubation period of 14 days was identified to

be sufficient for complete BDON exertion. However, the incubation time used in this study might not be true universally for other drinking water plants. The incubation time can vary depending on the ionic strength of water samples, biodegradability of DON, and the type of bacterial seed used for inoculation. Hence, it encouraged to determine the incubation time for each type of water sample should be determined before BDON experiments are performed.

Average BDON ranging from 4.6 to 226.3 $\mu\text{g L}^{-1}$ as N for different incubation periods for samples from all four locations of the Moorhead WTP was determined using the 5% MLSS inoculum (Fig. 3.3.). The amount of BDON exerted and DON utilized by bacteria did not significantly change between 14 and 20 days of incubation in any of the samples ($p > 0.05$). Average BDON concentrations on day 28 were much lower but not statistically different from those on days 14 and 20 due to high standard deviations associated with the concentrations on day 28. The decreases in BDON on day 28 could be because of substrate depletion resulting in cellular release of organics including DON (Khan et al., 2009). The incubation period of 14 days was sufficient for BDON exertion for all the samples. BDON determined based on 14 days of incubation were 226 ± 13 , 130 ± 7.4 , 172 ± 16 , and 47 ± 7.4 $\mu\text{g L}^{-1}$ as N in raw water, before ozonation, after ozonation, and after filtration water samples, respectively.

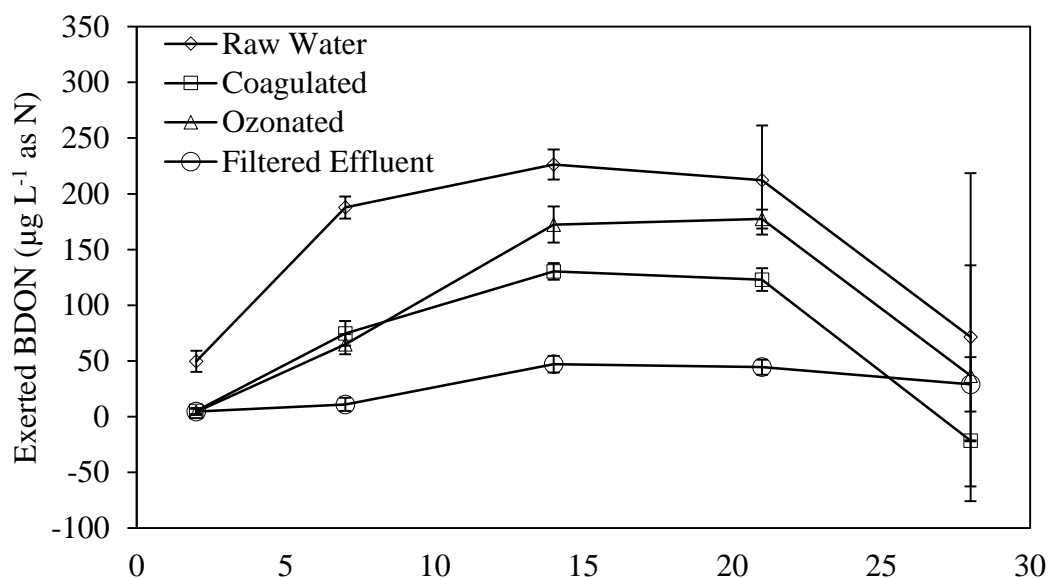


Figure 3.3. BDON in water samples inoculated with 5% MLSS for different incubation periods.

For the bacterial growth, there was a sudden peak within the first day of incubation in raw water samples and after that the growth became steady at around 8 days of incubation (Fig. 3.4.). Consumption of readily biodegradable organics (C and/or N) during the first day could have caused the bacterial growth to suddenly peak. For the rest of the samples, the growth slowly increased and reached steady state at 8 days (minimal or no growth after that) except for DI water sample. No growth was observed in the DI water sample.

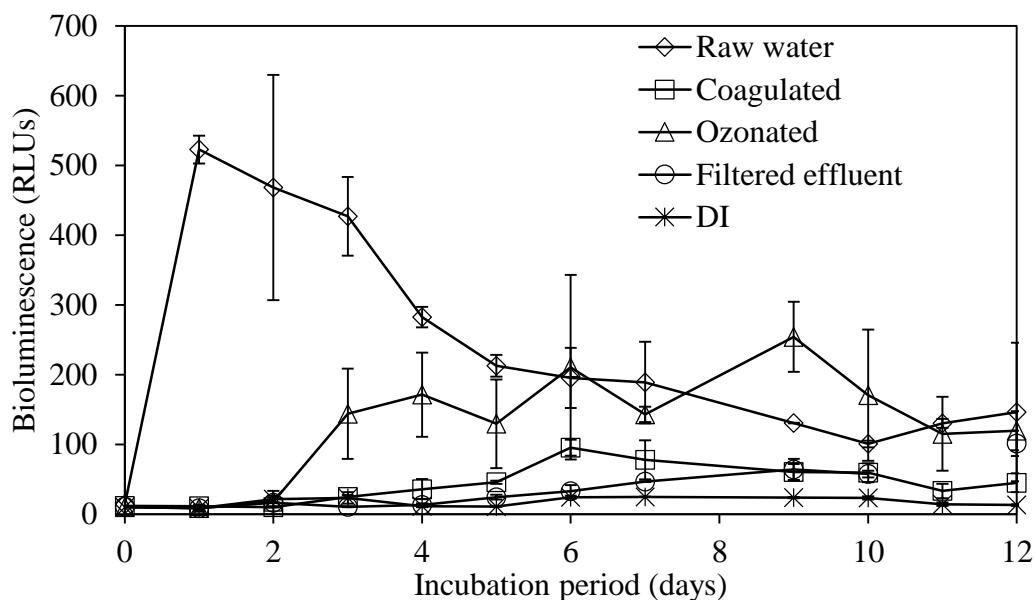


Figure 3.4. Bacterial growth as RLUs in water samples inoculated with 5% MLSS for different incubation periods.

The bacterial growth results concurred with the exerted concentration of BDOC, a surrogate parameter for bacterial growth, in each sample (Fig. 3.5.). Ozonation significantly increased the amount of BDON ($p = 0.0043$) while the filter significantly removed BDON from the ozonated water samples ($p = 0.0005$).

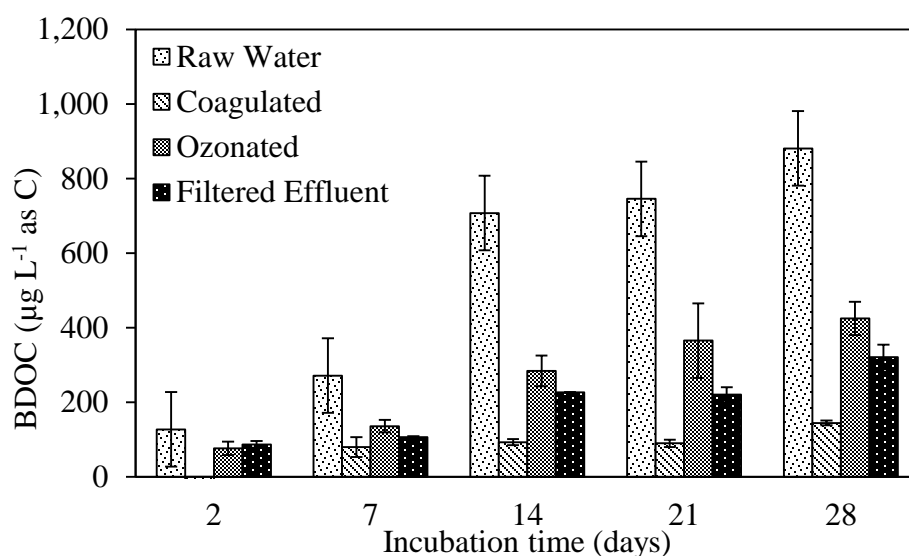


Figure 3.5. BDOC in water samples inoculated with 5% MLSS.

After ozonation, samples showed higher growth of bacteria which could be due to the transformation of complex nonbiodegradable organics into simpler biodegradable organics by ozone. Limited bacterial growth was observed in the filtered effluent after a long lag period of about 4 days. As the bacterial culture in this study was from a WWTP, the bacteria were used to consumption of higher concentrations of more readily biodegradable organic matter. In the filtered effluent, which was likely dominated by recalcitrant organics, the inoculum might need more acclimation time leading to this delay in growth. A control experiment was performed by inoculating the 5% MLSS in DI water. No bacterial growth was observed in DI water over an incubation time of 12 day.

3.3.1.2. Standard DON Compounds

When incubated with 5% MLSS for 14 days, almost complete BDON exertion of 50 and 100 $\mu\text{g L}^{-1}$ as N of glycine was observed while 76% of 250 $\mu\text{g L}^{-1}$ as N of glycine was mineralized into $\text{NH}_3\text{-N}$ (Fig. 3.6.).

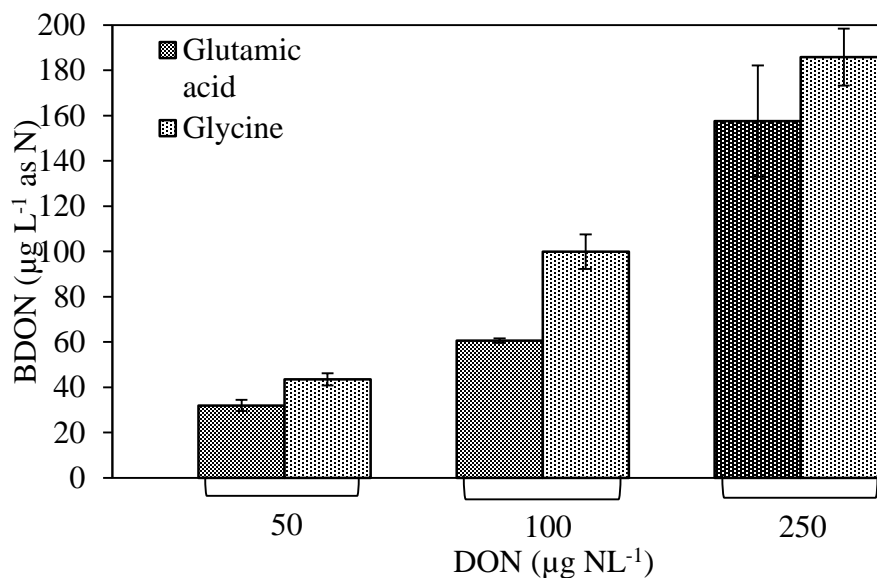


Figure 3.6. BDON in standard DON compounds, 50, 100 and 250 $\mu\text{g L}^{-1}$ as N of glycine and glutamic acid, inoculated with 5% MLSS.

About 60% of glutamic acid was mineralized into $\text{NH}_3\text{-N}$, irrespective of their initial concentrations. DON in glycine and glutamic acid had different sensitivities to biodegradation. Glycine was more sensitive to complete DON biodegradation. The sensitivity difference between the two compounds can be attributed to the ratio of carbon to nitrogen in each of the compounds (Dias and Alexander, 1971). Glycine is one of the simplest amino acids with a short structure and minimal side chain of only one hydrogen. On the contrary, glutamic acid has a long structure with more side chains making it less amenable to biodegradation (Dias and Alexander, 1971).

3.3.2. DON, BDON, DOC, BDOC, and TDN Profiles

BDON and DON for the four treatment stages of the Moorhead WTP are presented as box plots (Fig. 3.7. a and b). In raw water samples, DON ranged from 217 to 434 $\mu\text{g L}^{-1}$ as N with an average of 329 $\mu\text{g L}^{-1}$ as N. A survey of 28 WTPs found that raw water DON ranged from < 50 to 430 $\mu\text{g L}^{-1}$ as N with an average DON of 190 $\mu\text{g L}^{-1}$ as N (Lee et al. 2006). The amount of DON at different stages of the Moorhead WTP did not significantly change ($p > 0.05$) compared to the raw water. However, filtration did lower the average DON (229 $\mu\text{g L}^{-1}$ as N) by 30% compared to DON in raw water. Lee et al. (2006) reported average DON removal of 21% by 28 WTPs surveyed. Another study showed that an ozonation-sand filtration WTP removed only 22% of 340 $\mu\text{g L}^{-1}$ as N of DON in its raw water (Xu et al., 2011). Biologically active filters play an essential role in removing organic matter, $\text{NH}_3\text{-N}$ and microorganisms (Metcalf and Eddy, 2003).

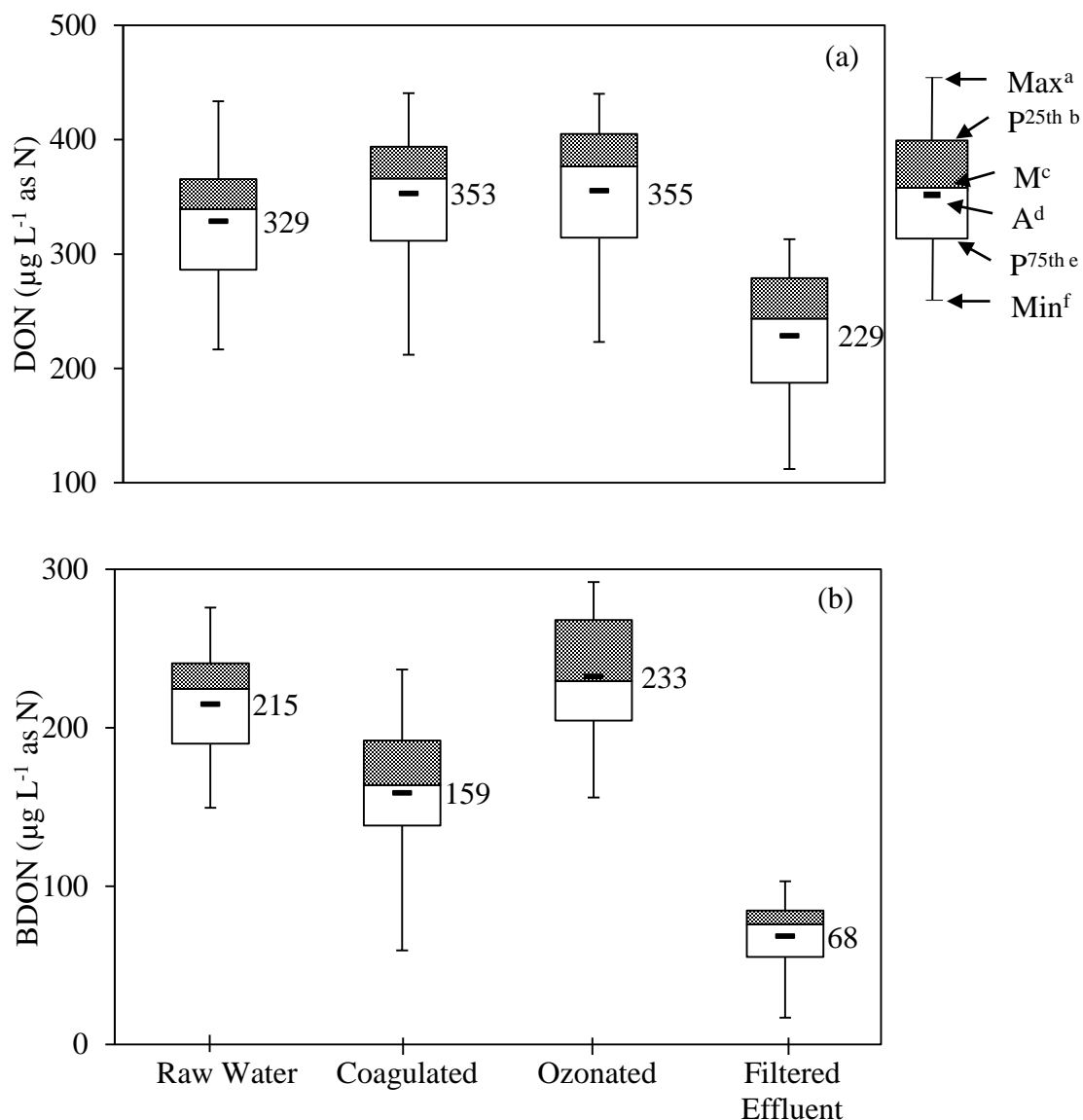


Figure 3.7. A box plot representing (a) DON_i and (b) BDON removal at the Moorhead WTP. Max^a is the maximum value, P25^{th b} is the 25th percentile, M^c is the median, A^d is the average, P75^{th e} is the 75th percentile, and Min^f is the minimum value.

BDON in the raw water ranged from 150 to 276 µg L⁻¹ as N with an average of 215 µg L⁻¹ as N. BDON in water treated by coagulation and ozonation was not significantly different from raw water BDON ($p = 0.38$ and 0.81). However, the average BDON in the coagulated water decreased by 26% compared to that in the raw water and while the average BDON in the ozonated water increased by 147% from that in the coagulated water. DON is part of

heterogeneous and aromatic natural organic matter (Świetlik and Sikorsa, 2005). Ozonation breaks down aromatic organics into aliphatic organics (Morrison and Boyd, 1983, Westerhoff et al., 1999). The increase in BDON by ozonation is anticipated possibly because of conversion of the aromatic DON into aliphatic BDON which is more biodegradable. Ozone attacks the double bonds, aromatic ring and amino group to give simpler aliphatic compounds and/or compounds with reduced aromaticity. For example, tetracycline an antibiotic is a predominant pharmaceutical aromatic DON in wastewater which consists of four aromatic rings. On ozonation tetracycline produces end-products with fewer aromatic rings (Khan et al., 2010). Also, generation of very reactive free hydroxyl radicals during ozonation is responsible for conversion of aromatic DON to aliphatic compounds. Song et al. (2007) showed *p*-nitrotoluene a biorefractory hazardous nitroaromatic DON is ozonated to acetic acid which is a known readily biodegradable compound. Average BDON in the filtered effluent was $68 \mu\text{g L}^{-1}$ as N which was significantly less compared to BDON in the raw and ozonated water ($p = 0.0267$ and 0.0243). Filtration removed 71% of BDON from the ozonated water. The filters at the Moorhead WTP were biologically active and performed nitrification. Fig. 3.8.a and b shows a box plot presenting $\text{NH}_3\text{-N}$ and $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ concentrations in ozonated and filtered effluent samples. After filtration, the amount of $\text{NH}_3\text{-N}$ decreased by an average of $185 \mu\text{g L}^{-1}$ as N while the concentration of $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ increased by $144 \mu\text{g L}^{-1}$ as N.

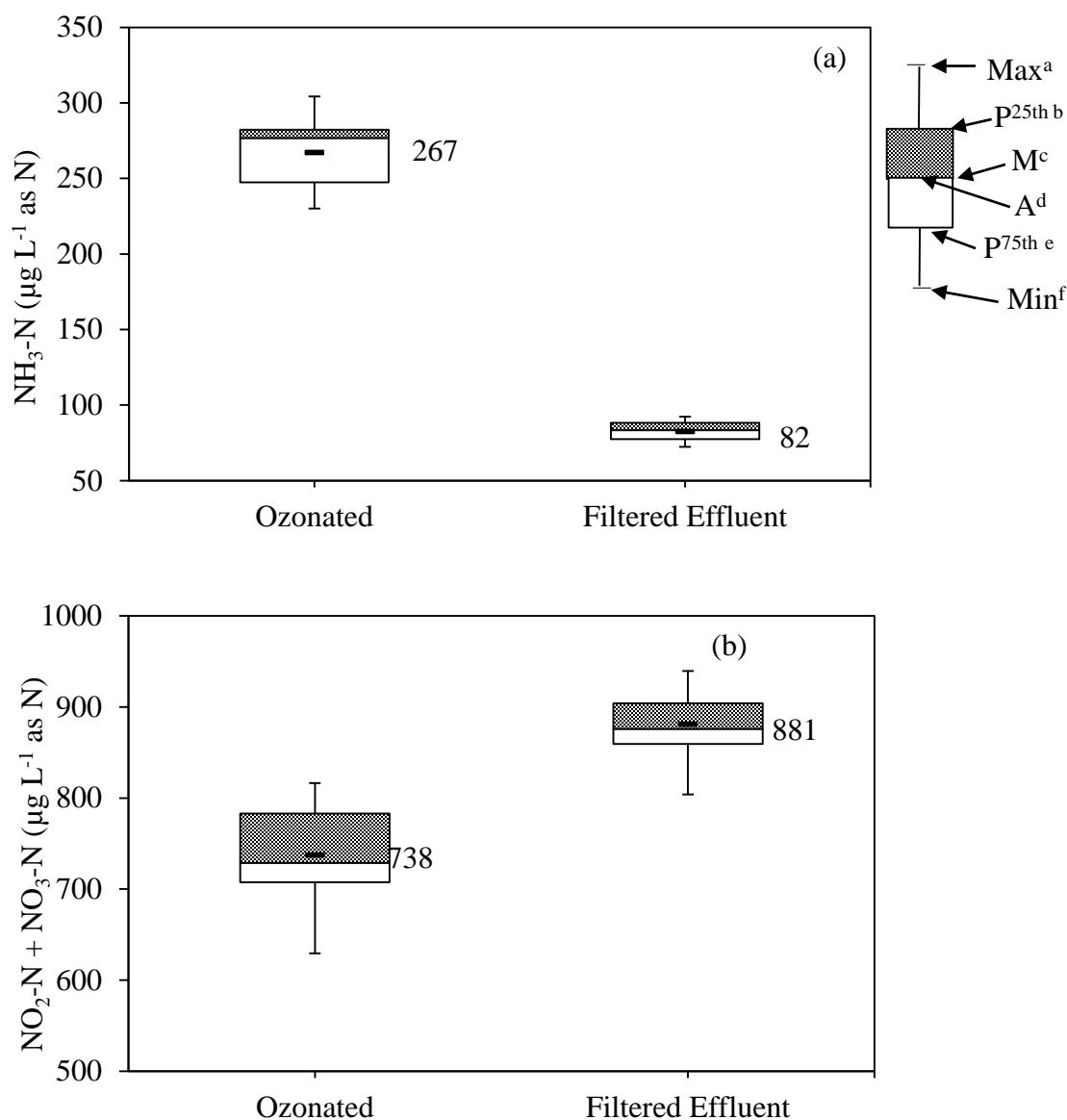


Figure 3.8. A box plot for the amounts of (a) $\text{NH}_3\text{-N}$ and (b) $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ in ozonated water and filtered effluent.

Fig. 3.9. in Supplementary Data shows a box plot for TDN. In the raw water, TDN ranged from 1,201 to 1,426 $\mu\text{g L}^{-1}$ as N with an average of 1,335 $\mu\text{g L}^{-1}$ as N. The plant lowered the TDN on average by 10.8%.

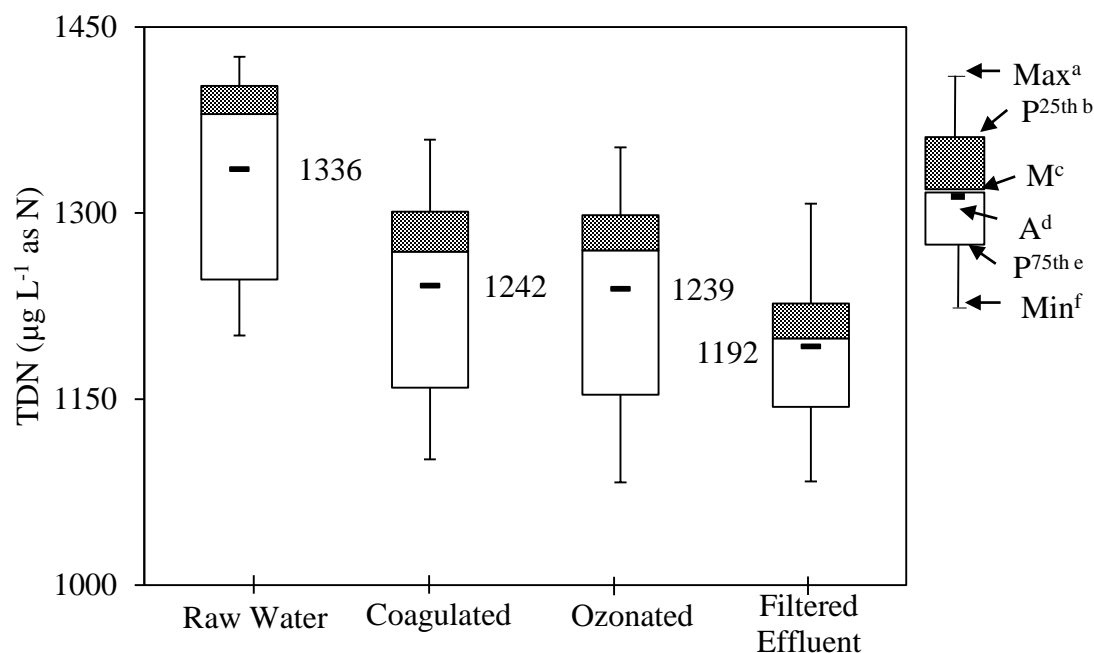


Figure 3.9. A box plot representing TDN in water samples collected from the Moorhead WTP. Max^a is the maximum value, P25^{th b} is the 25th percentile, M^c is the median, A^d is the average, P75^{th e} is the 75th percentile, and Min^f is the minimum value.

Table 3.3. shows ratios of DON/TDN, DON/DOC, BDON/DON, and BDON/BDON for all the water samples. Low DON/TDN suggests that water had more DIN rather than DON.

Please note some studies suggest an error associated with DON determination when the DIN to TDN ratio is high (Lee and Westerhoff, 2005, Graeber et al., 2012). Lee and Westerhoff (2005) and Graeber et al. (2012) report a ratio of DIN/TDN >0.8 and >0.6, respectively to effect DON determination. In this study for more than 75% of the samples the ratio of DIN/DON was at least <0.75 while the rest were about 0.8. The error in DON determination might vary depending on the type of sample. The information about the influence of DIN/DON ratio on DON measurement is recent and complex which requires a lot more work before a substantial conclusion can be made.

The concentration of DON ranged from 19 to 29% of the TDN in the water samples. The ratios of DON/TDN and DON/DOC increased in the coagulated water by only 0.03 and 0.04

compared to the raw water, respectively. Ozonation barely increased the ratios of DON/TDN and DON/DOC by 0.01 and 0.02, respectively. This suggests that coagulation and ozonation remove more DIN and DOC compared to DON. Lee and Westerhoff (2006) reported that aluminum salt based coagulation removes more DOC than DON. They experimented with a dual coagulation approach by using a cationic polymer and aluminum sulfate, and observed DON removal improvement by 15%.

In the raw water, 67% of DON was biodegradable. DON biodegradability (BDON/DON) decreased after coagulation to 43% while ozonation increased it back to 67%. Filtration reduced BDON/DON to 38%. The ratio of BDON/BDOC was 0.11 in the raw water. Ozonation increased BDON/BDOC to 0.15 while filtration decreased it to 0.06. Ozonation breaks down complex organics to simpler molecules which are readily biodegradable (Albidress et al., 1995). The results suggest that ozonation might be producing more N containing biodegradable organics rather than C containing organic.

Table 3.3. Ratios of DON to TDN, DON to DOC, BDON to DON, and BDON to BDOC.

Ratio	Raw Water			Coagulated			Ozonated			Filtered Effluent		
	A ^a	R ^b	P ^(25th,75th) c	A	R	P ^(25th,75th)	A	R	P ^(25th,75th)	A	R	P ^(25th,75th)
DON/TDN	0.25	0.18-0.31	0.22,0.27	0.28	0.19-0.35	0.25,0.31	0.29	0.20-0.34	0.25,0.31	0.19	0.10-0.36	0.16,0.22
BDON/DON	0.67	0.45-0.96	0.58,0.76	0.43	0.21-0.65	0.37,0.50	0.67	0.55-0.87	0.59,0.77	0.29	0.07-0.58	0.21,0.34
DON/DOC	0.04	0.02-0.05	0.03,0.04	0.08	0.05-0.14	0.07,0.09	0.10	0.07-0.17	0.08,0.10	0.07	0.04-0.10	0.06,0.08
BDON/BDOC	0.11	0.04-0.20	0.08,0.14	0.09	0.03-0.21	0.08,0.10	0.15	0.11-0.27	0.12,0.16	0.06	0.01-0.10	0.05,0.07

^aA is the average

^bR is the range

^cP^(25th,75th) is percentile, 25th and 75

In the raw water, DOC ranged from 7,703 to 10,172 $\mu\text{g L}^{-1}$ as C with an average of 8,818 $\mu\text{g L}^{-1}$ as C (Fig. 3.10.a). BDOC was 25.7% of DOC in the raw water (Fig. 3.10. b).

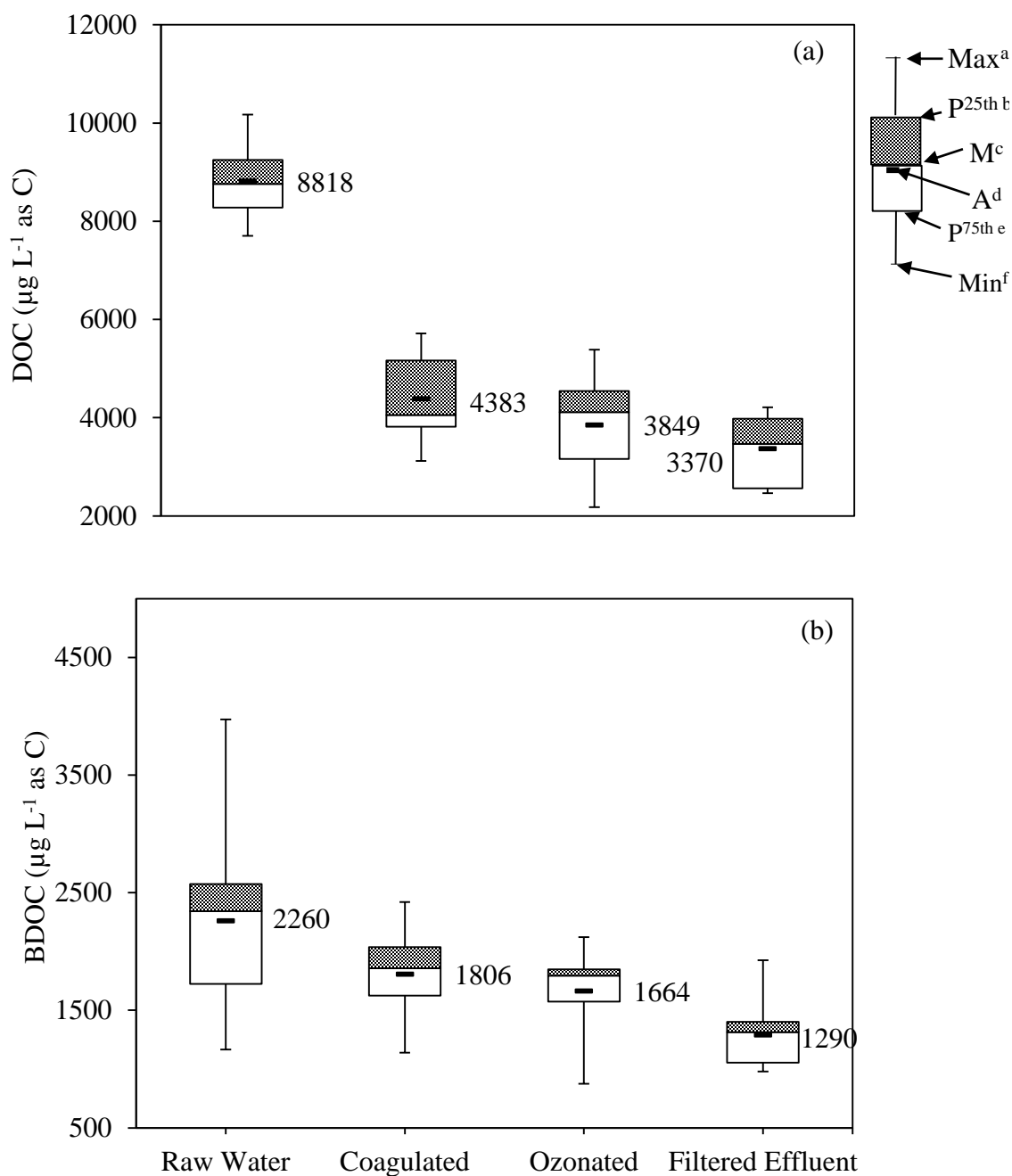


Figure 3.10. A box plot for the amounts of (a) DOC and (b) BDOC in water samples.

The coagulated water and filtered water had 50% and 62% less DOC than the raw water, respectively. The concentration of BDOC in the filtered effluent ranged from 979 to 1,926 $\mu\text{g L}^{-1}$

as C with an average of $1,290 \mu\text{g L}^{-1}$ as C. This high filtered effluent BDOC was expected since DOC concentrations in the raw water (7,703 to $10,171 \mu\text{g L}^{-1}$ as C) and filtered effluent (2,464 to $4,213 \mu\text{g L}^{-1}$ as C) were high.

Fig. 3.11. in Supplemental Data shows SUVA_{DOC} and SUVA_{DON} values for the raw water which averaged at 3 and $77 \text{ Lmg}^{-1}\text{m}^{-1}$. Both SUVA_{DOC} and SUVA_{DON} representing relative aromaticity of the organic matter carbon and nitrogen, respectively, were substantially less in the coagulated water samples. The reduction of SUVA_{DOC} and SUVA_{DON} suggested that the UV absorbing humic compounds including aromatics and to a lesser degree unsaturated aliphatics were removed from the raw water. The SUVA_{DOC} and SUVA_{DON} of the coagulated water was lowered by 68% and 33% in comparison to the raw water, respectively.

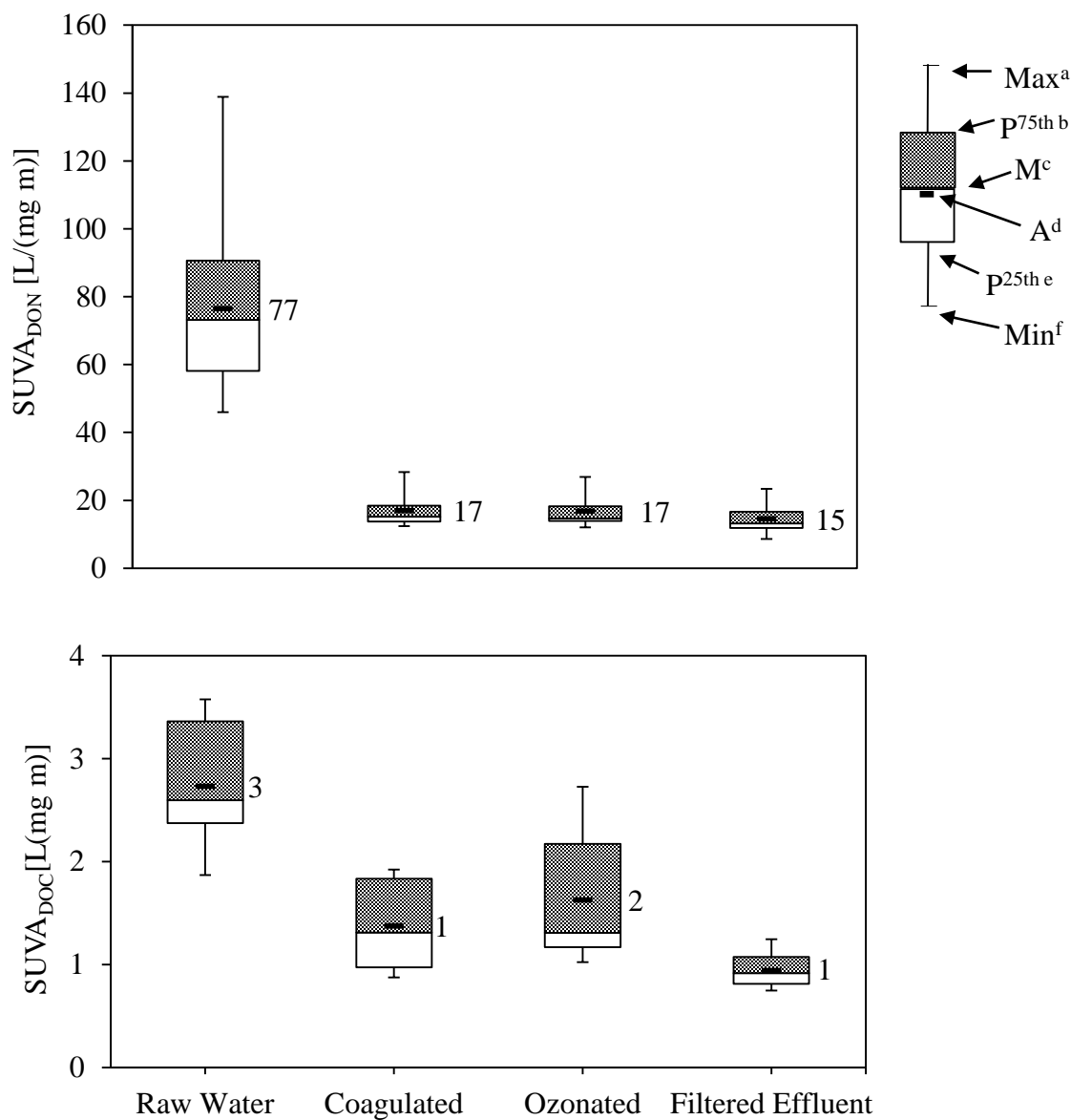


Figure 3.11. A box plot for (a) SUVA_{DON} and (b) SUVA_{DOC} for water samples.

3.3.3. Nitrogen Mass Balance

Throughout the BDON incubation, the TDN in the samples should be balanced. Any loss in TDN during the incubation could be from denitrification and/or microbial assimilation. The difference of TDN before and after the incubation ((TDN_(i-f)) for samples from all four stages of the Moorhead WTP in Table 3.4. show that during the 14-day incubation the TDN was quite balanced. The range of TDN_(i-f) in the raw water samples was -4 to 4 $\mu\text{g L}^{-1}$ as N with an average

of $-1 \mu\text{g L}^{-1}$ as N. The negative $\text{TDN}_{(i-f)}$ indicate increase in the TDN concentration which might be inferred as production of microbial exogenous products during the BDON experiments. The average $\text{TDN}_{(i-f)}$ of coagulated, ozonated, and filtered effluent water samples was -22, 13, and -16 $\mu\text{g L}^{-1}$ as N. Hence, TDN losses during the incubation of samples from all the four treatment stages were minimal. All the samples were aerated every 12 hrs to prevent denitrification and minimum bacterial growth, as indicated by small RLUs, was observed during the incubation (as discussed in Section 3.1) which suggests very little or no microbial assimilation.

The data for the differences between the concentrations of ammonia ($\text{NH}_3\text{-N}_{(i-f)}$) and nitrate plus nitrite ($\text{NO}_3\text{-N} + \text{NO}_2\text{-N}_{(i-f)}$) before and after the BDON bioassay shows that DON biodegraded (ammonified) during the incubation was later partially nitrified as decreases in $\text{NH}_3\text{-N}$ (positive $\text{NH}_3\text{-N}_{(i-f)}$ values) and increases in $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ (negative $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}_{(i-f)}$ values) were observed. In the raw, coagulated, ozonated, and filtered effluent water samples, the average decreases in $\text{NH}_3\text{-N}$ were 90, 70, 121, and 66 $\mu\text{g L}^{-1}$ as N, respectively. $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ increases were on average at least 136.3 $\mu\text{g L}^{-1}$ as N in the filtered effluent while nitrification was the highest in the raw water in which the concentration of $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ increased on average by 306 $\mu\text{g L}^{-1}$ as N.

Table 3.4. TDN_(i-f), NH₃-N_(i-f), and NO₂-N + NO₃-N_(i-f) values (µg L⁻¹ as N).

Sample	TDN _(i-f)				NH ₃ -N _(i-f)				NO ₂ -N + NO ₃ -N _(i-f)			
	A ^a	R ^b	M ^c	P ^(25th,75th) d	A	R	M	P ^(25th,75th)	A	R	M	P ^(25th,75th)
Raw Water	<u>1.0</u>	<u>4</u> -3.6	<u>1.6</u>	<u>2.8</u> , 0	90.2	54.2-122.7	87.8	78.3, 103.9	<u>306.1</u>	<u>368.5</u> - <u>220.1</u>	<u>316.1</u>	<u>337</u> , <u>277.8</u>
Cogulated	<u>22.1</u>	<u>84</u> -4.3	<u>6.6</u>	<u>47.8</u> , <u>0.3</u>	69.5	59-80	72	64, 73.16	<u>250.5</u>	<u>312.5</u> - <u>177.5</u>	<u>250.1</u>	<u>276</u> , <u>238</u>
Ozonated	13.4	<u>14.3</u> -42.2	18.5	<u>4.5</u> , 24.6	121.4	62-165	123.6	106.8, 140.8	<u>219.4</u>	<u>245.6</u> - <u>177.6</u>	<u>222.3</u>	<u>234.8</u> , <u>204.9</u>
Effluent	<u>16.3</u>	<u>97</u> -30.3	<u>6.3</u>	<u>26.3</u> , 7	65.5	45.3-77.6	67.3	57.5, 75.5	<u>136.3</u>	<u>238.3</u> - <u>64.3</u>	<u>140.6</u>	<u>170</u> , <u>105.5</u>

^aA is the average

^bR is the range

^cM is the median

^dP^(25th,75th) is percentile, 25th and 75th

^eNumber is a negative number.

3.4. Summary

A bioassay to determine BDON in drinking water was successfully optimized to examine the evolution of BDON through a WTP employing ozonation and biologically active filtration. An inoculum size of 5% MLSS and 14 days of incubation were sufficient to completely exert BDON in the water samples. The amount of BDON determined in water samples varied from 17-275 $\mu\text{g L}^{-1}$ as N. During the bioassay incubation, DON was biodegraded (ammonified) and partially nitrified. Coagulation removed BDON while ozonation increased BDON. BDON decreased through biologically active filters (immediately after ozonation). The studied WTP on average removed 68% of BDON. The optimized bioassay can serve as a simple tool to determine the fate of BDON through WTPs.

CHAPTER 4. BIODEGRADATION OF DISSOLVED ORGANIC NITROGEN UNDER DIFFERENT BIOLOGICAL WASTEWATER TREATMENT PROCESS CONDITIONS

4.1. Introduction

BNR processes have been successfully implemented by WWTPs to achieve low TN concentrations (≤ 10 mg N/L) in the wastewater effluents. TN is composed of total inorganic nitrogen (TIN) and total organic nitrogen (TON). TIN is normally equivalent to DIN, while TON is divided into particulate organic nitrogen (PON) and DON. BNR WWTPs achieving low TN effluent quality effectively remove $\geq 95\%$ of DIN from raw wastewater. PON can undergo hydrolysis turning into DON or is removed through solid-liquid separation process (clarification) and ends up in the sludge which is stabilized and landfilled. DON that is not removed on the other hand ends up in the effluent.

Fate of DON in wastewater treatment processes has not been comprehensively investigated compared to DIN. A major portion of DON in wastewater effluent is hydrophilic and has low molecular weights (Huo et al., 2013). About 50% of effluent DON is biodegradable and can promote algal growth in receiving waters (Halis et al., 2013; Pehlivanoglu and Sedlak, 2008). Even for WWTPs with low TN effluents (4 to 5 mg N/L), algae and bacteria can still utilize 18 to 61% of their effluent DON (Urgun-Demirtas et al., 2008). Discharge of wastewater effluent DON into drinking water sources is a major disconcerting issue for WTPs. As discussed in Chapter 3, DON in source water might have major repercussions for drinking water. DON can lead to the formation of N-DBPs during disinfection and might promote growth of microorganisms in the distribution system (Lee et al., 2007; Krasner et al., 2009; Chen et al., 2011, Bond et al., 2011).

The DON to TDN (DON/TDN) ratio in WWTP effluent is an indicator of the nitrogen fractions dominating the effluent. The effluent DON/TDN ratio depends on the type of treatment process used by the WWTP. For example, WWTPs using only a single stage non-nitrifying activated sludge process or lagoons (Tyler et al., 2001) have low effluent DON/TDN while those with advanced treatment processes tend to produce effluents with high DON/TDN. In BNR plants, DON is 52 to 85% of the final effluent TDN (Sattayatewa et al., 2009; Pehlivanoglu-Mantas and Sedlak, 2006; Urgan-Demirtras et al., 2008). In the United States, as the effluent TN requirements is becoming more stringent requiring a number of WWTPs to meet 3 mg N/L which will make DON a significant contributor (to the effluent TN), understanding DON and its biodegradability is very important for improving the treatment efficiency of DON for both water and wastewater.

DON compounds are medially characterized and identified. The correct mechanisms and effective processes in removing DON from wastewater are not completely known. The concentration of DON in raw wastewater ranges from 7.67 to 8.64 mg N/L (Parkin and McCarty, 1981a, 1981b; Pehlivanoglu-Mantas and Sedlak, 2006; Sattayatewa et al., 2009; Simsek et al., 2012; Simsek et al., 2013). Parkin and McCarty (1981a) showed that influent DON is highly biodegradable (> 80%). However, during the treatment process DON might be produced by bacteria during growth and decay. Bacterially derived DON can be either biodegradable or refractory (Parkin and McCarty, 1981a; Sattayatewa et al., 2009). Complete removal of DON has never been reported.

The level of BDON in the effluent is dependent on the type of biological treatment process. Effluents of non-nitrifying plants have more BDON than effluents of nitrifying plants (Chen et al., 2011). Chen et al. (2011) surveyed 23 wastewater treatment plants in the U.S. for

effluent DON. The effluent DON concentrations ranged from 0.69 to 1.56 mg N/L. Parkin and McCarty (1981a) reported that up to 40% of effluent DON may be produced during the biological treatment plant operation and suggested that additional physical and/or chemical treatment following biological treatment would be needed to remove DON completely from the wastewater effluent.

With few exceptions (Parkin and McCarty 1981a; Parkin and McCarty 1981b), all past studies investigating biodegradability of DON have focused on using a high F/M method for BDON determination (Simsek et al., 2012; Khan et al., 2009; Sattayatewa et al., 2009b; Sattayatewa et al., 2010). The widely used high F/M method for measuring BDON in wastewater measurement was developed by Khan et al. (2009) by adapting traditional BOD and biodegradable dissolved organic carbon (Khan et al., 1998) procedures. The method uses a small amount of bacterial inocula of MLSS from aeration tanks for determining BDON in wastewater. DON in wastewater sample is measured before the inocula is added and after a long incubation period of 20-28 days. The low F/M based bioassay uses a high concentration of MLSS (250 to 1,500 mg total suspended solids (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 DON 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 DON 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 a better representation for the process condition.

Many studies have described the role of different operational conditions including SRT, temperature, reactor hydraulics and plant perturbations in activated sludge processes on DON and BDON removal (Parkin and McCarty 1981a; Parkin and McCarty 1981b; Simsek et al., 2013; Sattayatewa et al., 2013). Sharp et al. (2009) found that SRT and temperature may impact DON concentrations in the plant effluent. A few studies have pointed out the differences in DON degradation due to the type of sludge present in an aerobic versus anoxic process (Bratby et al., 2008; Sattayatewa et al., 2013; O'Shaughnessy et al., 2006). Sattayatewa et al. (2013) observed DON concentration increased under the anoxic zone of a BNR WWTP due to biomass metabolic and catabolic activities while a major DON decrease was observed within the oxic zone. Bratby et al. (2008) noted that DON concentration increases through aerobic biological treatment. To this date, no known study has evaluated biodegradation of DON under different biological process conditions other than heterotrophic process such as nitrification, denitrification, and deammonification. Understanding DON biodegradation under these processes will help identify strategies for improving treatment efficiency at WWTPs required to achieve low TN discharge limits.

The objective of this study was to perform a low F/M bioassay to investigate biodegradation ability of DON by different biological wastewater treatment process conditions including heterotrophic carbon removal, nitrification, denitrification, and deammonification. A modified BDON bioassay was adapted from the existing bioassay developed by Parkin and McCarty (1981a). The optimum inoculum size and incubation period were identified. The

adapted BDON bioassay was then applied to examine the evolution of BDON through an advanced WWTP.

4.2. Materials and Methods

4.2.1. Sample Source and Characteristics, and Plant Description

About 20 L of influent, primary effluent (PE) and secondary effluent (SE) samples (grab samples) were collected from the Blue Plains Advanced Wastewater Treatment Plant (AWTP), District of Columbia, USA in February and March 2014. Sampling locations and dates for different biological processes evaluated are provided in Table 4.1.

Table 4.1. Wastewater sampling locations and dates.

Biological process tested	Sampling dates
Heterotrophic	2/22 ^b , 3/1 ^{a,c} , 3/10 ^{a,b,c} , 3/14 ^{a,b,c} , 3/15 ^{a,b,c} , 3/16 ^{a,b,c} , 3/17 ^{a,b,c}
Nitrification	2/24 ^b , 3/1 ^{a,c} , 3/10 ^{a,b,c} , 3/14 ^{a,b,c} , 3/15 ^{a,b,c} , 3/16 ^{a,b,c} , 3/17 ^{a,b,c}
Denitrification	3/10 ^{a,b,c} , 3/12 ^c , 3/14 ^{a,b,c} , 3/15 ^{a,b,c}
Deammonification	3/8 ^c , 3/9 ^{a,b} , 3/10 ^{a,b,c} , 3/14 ^{a,b,c} , 3/15 ^{a,b,c}

^aInfluent

^bPrimary Effluent

^cSecondary Effluent

The Blue Plains AWTP is the largest advanced wastewater treatment utility in the world. It is designed to treat an average daily flow of 1,400,000 m³/d and a peak flow of 2,800,000 m³/d to the advanced treatment system, and a peak plant flow of 4,090,000 m³/d, and discharges to the Potomac River, a major tributary to the Chesapeake Bay. It is a two-stage activated sludge plant with a high rate stage removing carbon followed by a combined nitrification-denitrification stage. Wastewater is subjected to chemically enhanced primary treatment before the high rate stage. The high rate is operated at 3-day SRT while the nitrification-denitrification employs 15-day SRT. A simplified process diagram of the plant is shown in Figure 4.1.

The plant has to meet stringent seasonal NH₃-N, TN, and total phosphorus (TP) effluent discharge limits and satisfies the nutrient load reduction goals of the Chesapeake Bay

Agreement. The maximum monthly discharge limit for nitrogen species is higher in the winter ($\text{NH}_3\text{-N}$ of 6.5 mg/L and TN of 8.58 mg/L) compared to the summer ($\text{NH}_3\text{-N}$ of 1.0 mg/L and TN of 5.5 mg/L) while the TP limit is 0.18 mg/L year around.

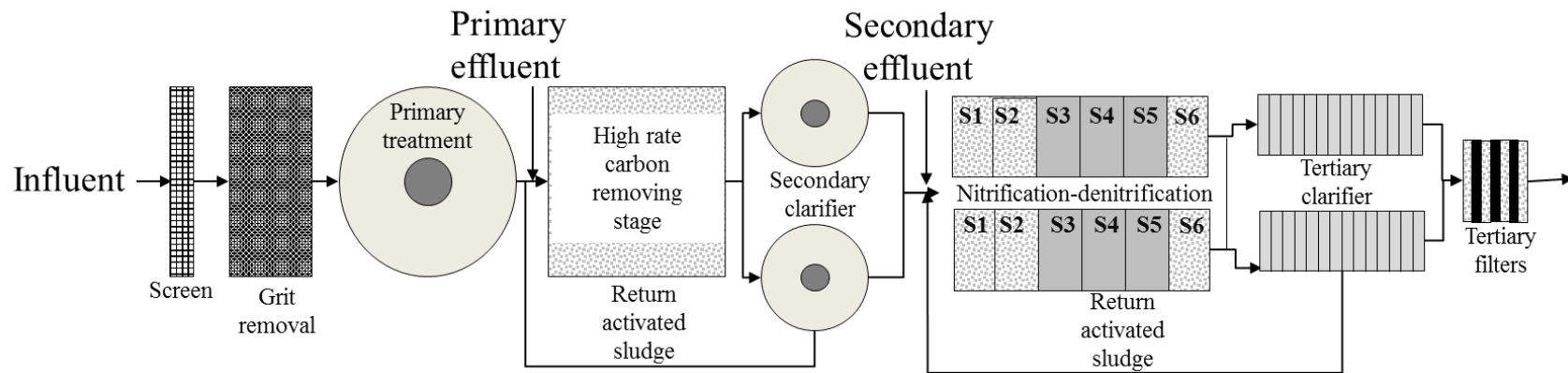


Figure 4.1. Process diagram of the Blue Plains AWTP.

4.2.2. Experimental Design

The effect of different concentrations (80, 160, and 410 mg TSS/L) of heterotrophic sludge from the high rate carbon removal process and nitrifying sludge from the nitrification-denitrification process on DON biodegradation was evaluated. After optimum concentrations of MLSS were identified, effect of different COD to nitrogen ratios on DON biodegradation was evaluated.

Wastewater samples were collected from three different locations of the plants (influent, PE, and SE) to represent high, medium, and low COD:N ratios. Along with heterotrophic carbon removal and nitrification processes, denitrification and deammonification processes were also evaluated for DON biodegradation in samples with different COD to N ratios. Role of denitrification in biodegrading DON was evaluated using sludge from the denitrification reactors of the Blue Plains. Deammonification was performed using sludge from Zillertal WWTP, Strass, Austria that performs deammonification for DIN removal.

Further, DON biodegradation in samples filtered through 1 μm and followed by 0.45 μm pore-size cellulose acetate membrane filters (PALL Co., Port Washington, NY, USA) were evaluated with the optimal type and concentration of sludge identified based on above mentioned experiments. Also, the fate of DON and BDON through the Blue Plains plant was examined. The BDON measurements were performed according to a batch BDON (DON biodegradation) test described below.

4.2.3. Batch BDON Test Protocol and Setup

A batch BDON test was modified from the bioassay used by Parkin and McCarty (1981a). The concentrations of inocula (MLSS) in the modified test were lower than that used by Parkin and McCarty (1981a) at 1,300 mg TSS/L. The lower inoculum concentrations were used to minimize impact of DON release by MLSS on the batch BDON test. The tests were performed

in 2 liter settleometers. The settleometers were attached with pH probe and meter (HACH Company, Colorado, USA), DO probe and meter (HACH Company, Colorado, USA), an air pump (Cole-Parmer Inc., Illinois, USA), and a nitrogen gas tank (Praxair Technology, Inc., Connecticut, USA). For all the tests, pH was maintained between 6.9 and 7.2 using sodium hydroxide and hydrochloric acid. Heterotrophic and nitrification processes were tested under aerobic condition of $\text{DO} > 2 \text{ mg O}_2/\text{L}$ (maintained by the air pump). For denitrification and deammonification tests, anoxic conditions were attained by purging the sample and sludge with N_2 gas. Different MLSS concentrations of heterotrophic and nitrification sludge MLSS (80, 160, or 410 mg TSS/L) was mixed with influent, SE, or PE to achieve a final volume to 1,800 ml. For denitrification tests, 410 mg TSS/L of denitrification sludge was used and for deammonification, 84 mg TSS/L of sludge was used. Before mixing with the samples, the MLSS concentration of the heterotrophic, nitrification, and denitrification sludge was around 3,000 TSS mg/L, while the deammonification sludge had a TSS concentration of about 30,000 TSS mg/L. During all the tests, a 10 ml sample for DON analysis was taken immediately after mixing the sample and MLSS and more samples were collected every hour up to 8 to 10 hrs.

4.2.4. Role of Nitrification Bacteria in DON Degradation

Nitrification consists of nitritation (conversion of NH_4^+ to NO_2^-) and nitratation (NO_2^- to NO_3^-). Tests were designed to understand the impact of DON degradation by nitritation and nitratation. Allythiourea (ATU) is well known to selectively inhibit AOB by inhibiting activity of ammonia monooxygenase (AMO), the enzyme involved in nitritation. ATU is known to inhibit by chelating the copper of the AMO active site. To identify the role of nitritation, DON degradation batch tests were conducted as described in the previous section using nitrification sludge and influent. Test samples were spiked with 1.8 ml of an ATU ($\text{C}_4\text{H}_8\text{N}_2\text{S}$) solution

prepared by dissolving 2 g of ATU in about 500 mL DI water and diluting it to 1 L. The solution was freshly prepared before the test. The tests with no ATU addition served as control experiments. To identify the role of nitrification, DON degradation tests were performed by adding the ATU spiked and control tests samples with $\text{NO}_2\text{-N}$ to reach a concentration of 10 mg N/L. Ten milliliters of sample was collected for DON determination at time zero and after 8 hrs of incubation.

4.2.5. Analytical Methods

All samples were analyzed in triplicates. The glassware were washed with soap, rinsed with tap water, kept in a 5% v/v hydrochloric acid bath overnight and rinsed with DI water before use. DIN species were measured using the HACH TNT (HACH Company, Colorado, USA) kits. $\text{NH}_3\text{-N}$ was measured using the Ammonia HR TNT 832 (HACH Company, Colorado, USA) kit which allows $\text{NH}_3\text{-N}$ to react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol, a blue colored solution which can be quantified spectrophotometrically at 690 nm.

$\text{NO}_2\text{-N}$ was measured using the Nitrite LR TNT 839 and Nitrite HR TNT 840 kits. Nitrite in the sample reacts with a primary aromatic amine in an acidic solution to form a diazonium salt. The salt reacts with an aromatic compound to form a colored complex that is directly proportional to the amount of $\text{NO}_2\text{-N}$ present. The colored complex between the salt and $\text{NO}_2\text{-N}$ in the sample is quantified spectrophotometrically at 515 nm. $\text{NO}_3\text{-N}$ was measured using the Nitrate LR TNT 835 and Nitrate LR TNT 836 kits. The $\text{NO}_3\text{-N}$ measurement kits contain sulfuric and phosphoric acids which catalyze the reaction of $\text{NO}_3\text{-N}$ in the sample with 2,6-dimethylphenol to form 4-nitro-2,6-dimethylphenol. The latter formed phenolic compound is quantified spectrophotometrically at 345 nm. A HACH DR 2800 spectrophotometer was used for

all absorbance measurements. DON and BDON were calculated based on equations 3.1. and 3.2., the only difference was the units for DON and BDON were mg/L rather than $\mu\text{g/L}$. COD, and TSS measurements were performed according to Standard Methods (APHA et al., 2005).

4.2.6. Statistical Analysis

Two-way analysis of variance (ANOVA) followed by *t*-test Two-Sample assuming equal variances with Bonferroni correction was conducted using Microsoft Office Profession Plus 2013 Excel to differentiate results from different conditions including inoculum size, incubation period, process types, and treatment stages (sampling locations). Results that were statistically analyzed included those for the following parameters: DON, BDON, TDN, and ratios of DON to TDN, and BDON to DON.

4.3. Results and Discussion

4.3.1. Optimal Inoculum Concentration and Incubation Period

Different inocula were tested to identify a workable time period in hours to perform the BDON test and also to determine the effect of increasing MLSS concentration on the rates of BDON exertion or DON biodegradation. The concentration of the sludge was increased by increasing the volume of sludge added to the batch test while maintaining the total volume (1,800 ml). Only PE was used as a substrate for evaluating the effect of MLSS concentration on DON biodegradation rates. Parkin and McCarty (1981a) reported that the factors affecting DON biodegradation in a batch study was the aeration time and MLSS concentration. They observed that for DON biodegradation there is an aeration time at which DON concentration reaches its minimum value and beyond which DON concentration increases. The increase is suspected to be due to DON production from microbial growth and decay surpass DON biodegradation in the system.

For heterotrophic sludge no trend for DON biodegradation was observed with increasing concentration of MLSS (Figure 4.2.). The DON biodegradation rates for 80, 160, and 410 mg TSS/L of sludge were about 0.378, 0.472, and 0.297 mg N/L/hr, respectively. No change in DON biodegradation was observed after about 8 hrs of incubation, which was perhaps the maximum aeration time beyond which no change or increase in DON was observed. BDON measured in the samples for the three concentrations of MLSS was about 24 to 38% of DON. An increasing trend of NH₃-N assimilation with MLSS was observed; the NH₃-N assimilated was 2.6, 3.4, and 5.6 mg N/L for 80, 160, and 410 mg TSS/L of sludge, respectively. The assimilation was calculated based on Equation 4.1. A small magnitude of nitrification was observed during the batch test as NO₂-N and NO₃-N increased during the test, 0.26 to 1.3 mg N/L. Although at an SRT of 2 days, no nitrification is possible as the low SRT does not allow for slow growing nitrifiers to grow in the system. However, at the Blue Plains AWTP, the A-stage (mainly organic carbon removal) is bioaugmented with nitrification-denitrification sludge for achieving nitrogen removal using the higher COD present in the PE.

$$\text{DIN}_I - \text{DIN}_F = \text{DIN assimilated} \quad (4.1.)$$

Where DIN_I is initial DIN and DIN_F is final DIN.

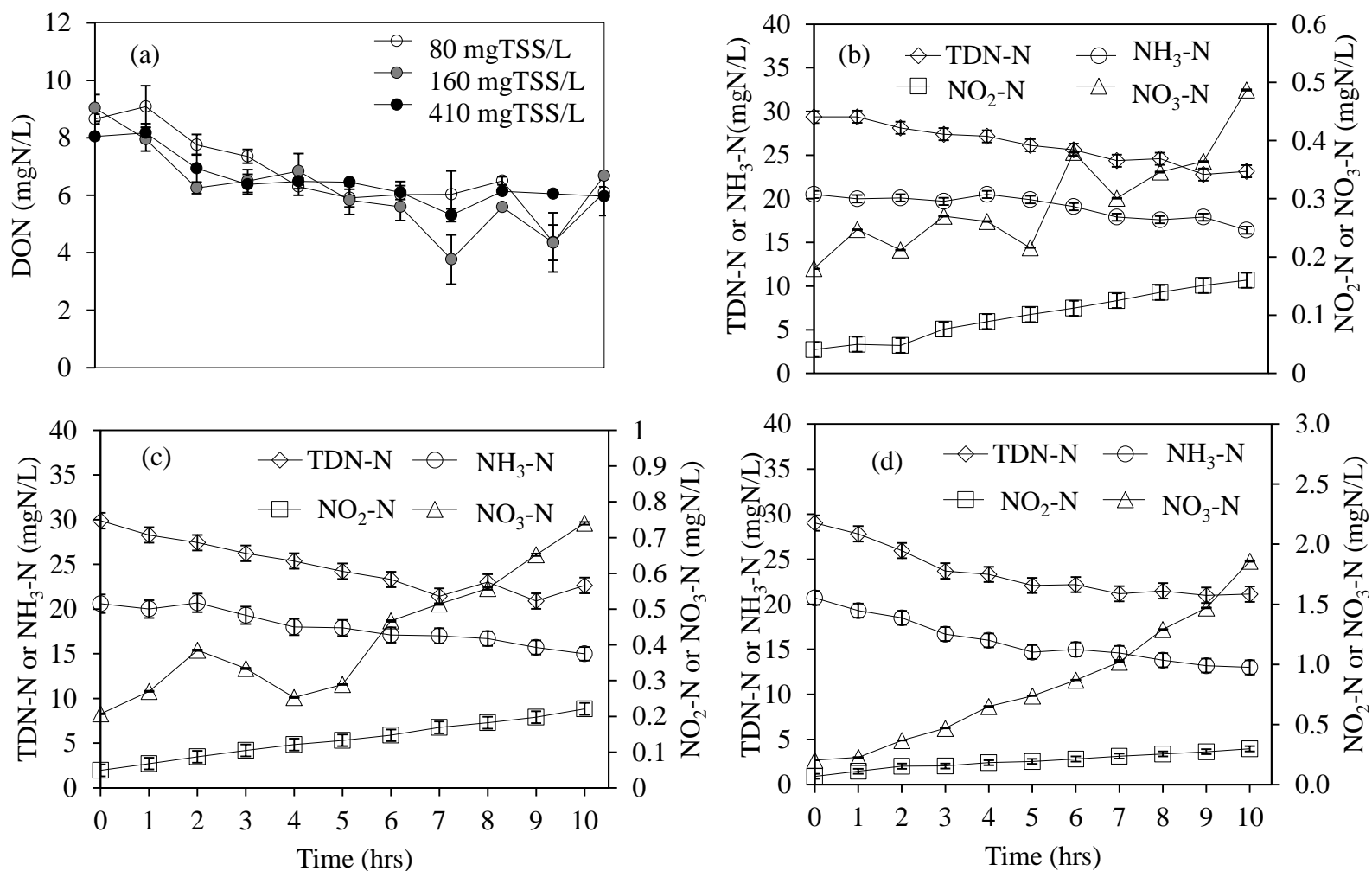


Figure 4.2. DON biodegradation batch test performed using PE and different concentrations of heterotrophic sludge. (a) DON biodegradation using 80, 160, and 410 mg TSS/L of sludge. Nitrogen species during the test with (b) 80 mg TSS/L of sludge, (c) 160 mg TSS/L of sludge, and (d) 410 mg TSS/L of sludge.

For nitrification sludge, the rate of DON biodegradation increased with the concentration of sludge added. For 80, 160, and 410 mg TSS/L of sludge added, the DON biodegradation rates were 0.158, 0.276, and 0.457 mg N/L/hr, respectively (Figure 4.3.). The amount of BDON exerted also increased with the sludge concentration, 12, 30, and 54% of DON for 80, 160, and 410 mg TSS/L of sludge, respectively. Very minimal $\text{NH}_3\text{-N}$ assimilation of 1.60-1.75 mg N/L was observed for all the sludge concentrations. The amount of $\text{NH}_3\text{-N}$ nitrified also increased from 2.5 to 13.4 mg N/L with sludge concentration.

The high concentration of MLSS used in the batch test expedites the quantification of BDON. Murthy et al. (2006) and Khan et al. (2009) conducted experiments using a very small concentration of MLSS allowing them to measure the amount of biodegradable DON in about 20 to 28 days of incubation. From Khan et al. (2009), DON biodegradation rate was 0.0009 to 0.001 mg/L/hr, a couple of magnitudes lower than those observed with the low F/M test in this study. Also, different concentrations of MLSS used by Khan et al. (2009) did not significantly impact DON biodegradation but higher concentrations were identified to be more reliable. A faster BDON method such as the one in this study will allow the operators to identify any upset in process possibly due to shock load. This may help prevent operators from exceeding the nutrient discharge limit. For instance in a hypothetical situation, an upset in process due to toxic shock load that impacts only biodegradation of DON can be identified with a low F/M test within 5 to 10 hrs as opposed to 20 or more days by the high F/M test.

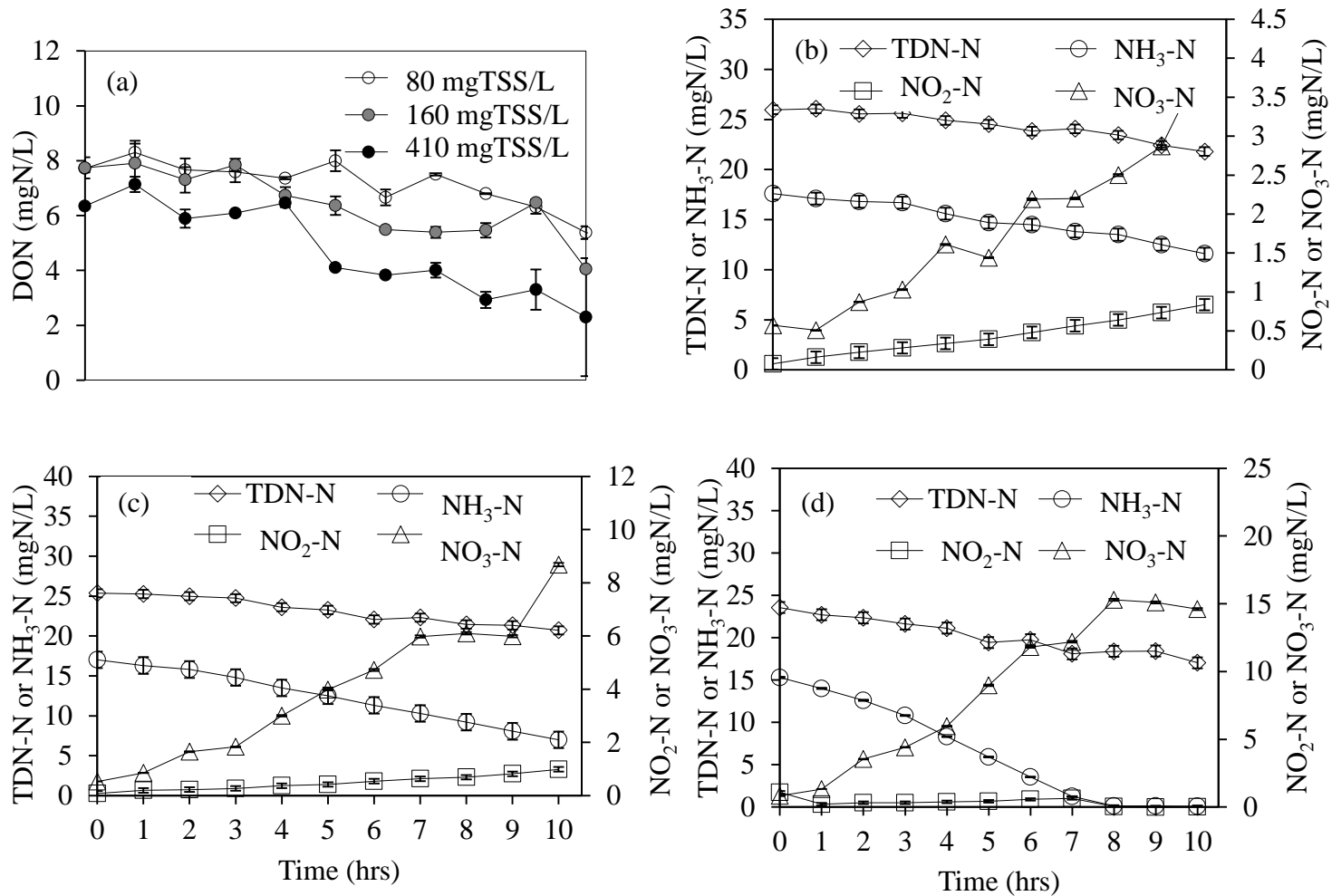


Figure 4.3. DON biodegradation batch test performed using PE and different concentrations of nitrification sludge. (a) DON biodegradation using 80, 160, and 410 mg TSS/L of sludge. Nitrogen species during the test with (b) 80 mg TSS/L of sludge, (c) 160 mg TSS/L of sludge, (d) 410 mg TSS/L of sludge.

4.3.2. Effect of COD:N Ratio on DON Biodegradation

DON degradation experiments were performed on wastewater with different total COD:N ratios and soluble COD:N ratios to identify the portion of DON and BDON that might be produced from PON.

4.3.2.1. Effect of Total COD:N Ratio on DON Biodegradation

The influent, PE, and SE samples were used to represent wastewater with high, medium and low COD:N ratios. The total COD:N ratios for influent, PE, and SE were 22, 6.2, and 1.5, respectively. The rate and amount of DON biodegradation can depend on the amount of COD present as it is the electron donor for energy and the carbon source for growth of microorganisms. Lack of COD might affect the ability of microorganisms to remove DON.

For both heterotrophic and nitrification sludge it was observed that the rate of DON biodegradation decreased with the decrease in total COD:N ratio. However, the rates observed by the nitrification sludge were still more than double of the rates provided by the heterotrophic sludge. For heterotrophic sludge (Figure 4.4.), the rates were 0.413, 0.297, and 0.101 mg N/L/hr for influent, PE, and SE, respectively. For nitrification sludge (Figure 4.5.), the rates were 0.993, 0.460, 0.400 mgN/L/hr for influent, PE, and SE, respectively. $\text{NH}_3\text{-N}$ assimilation of 1.5 to 5.6 mg N/L was observed for heterotrophic sludge while very minimal assimilation of less than 1.6 mg N/L was observed for nitrification sludge for all the sample types. The amount of BDON exerted also decreased with decreasing total COD:N ratio, percent of DON biodegraded was 38, 24, and 1.7% for influent, PE, and SE (Figure 4.4.). This may be because the plant was removing some of the BDON; however, the concentration of DON remained the same in PE and SE likely because rDON was being produced through hydrolysis of PON, and/or due to growth and decay of microorganisms. Similarly for nitrification sludge a decreasing trend of BDON exertion was

observed for decreasing total COD:N ratios. No statistical difference was observed between BDON exerted between PE and SE. For influent, PE, and SE with nitrification sludge 70, 54, and 57% of DON was biodegradable (Figure 4.5.). DON was more effectively removed by nitrification sludge compared to heterotrophic sludge.

This study identified that different percentages of DON in the raw influent are biodegradable by different processes. About 50 - 60% of DON was biodegradable during heterotrophic carbon removal. When exposed to nitrification sludge about 70% of DON was biodegraded. Increasing the SRT to promote the growth of nitrifiers might be a way to enhance DON biodegradation. Another alternative is to add an advanced treatment process such as powdered activated carbon to achieve higher biodegradation rates of DON (Parkin and McCarty, 1981a).

Even though increasing the SRT might help in lowering DON concentration in the final effluent of the plant, whatever DON is left behind might be further biodegradable in the environment. Parkin and McCarty (1981a, 1981b) showed DON production and excretion by bacteria during the activated sludge process and the excreted products might be refractory to the bacteria in short term but might become biodegradable over a period of time. Studies have shown that about 50% of the effluent DON is biodegradable by bacteria in long term high F/M tests (Murthy et al., 2006; Khan et al., 2009; Sattayatewa et al., 2009). DON and BDON in the effluent samples from four advanced treatment plants with nutrient removal technologies in the Chesapeake Bay region were quantified. About 25% to 33% of the effluent DON was identified to be biodegradable (Murthy et al., 2006). Seventy percent of DON in the effluent is produced during the wastewater treatment process but the produced DON has not been characterized (Pehlivanoglu-Mantas and Sedlak 2006, Parkin and McCarty, 1981a).

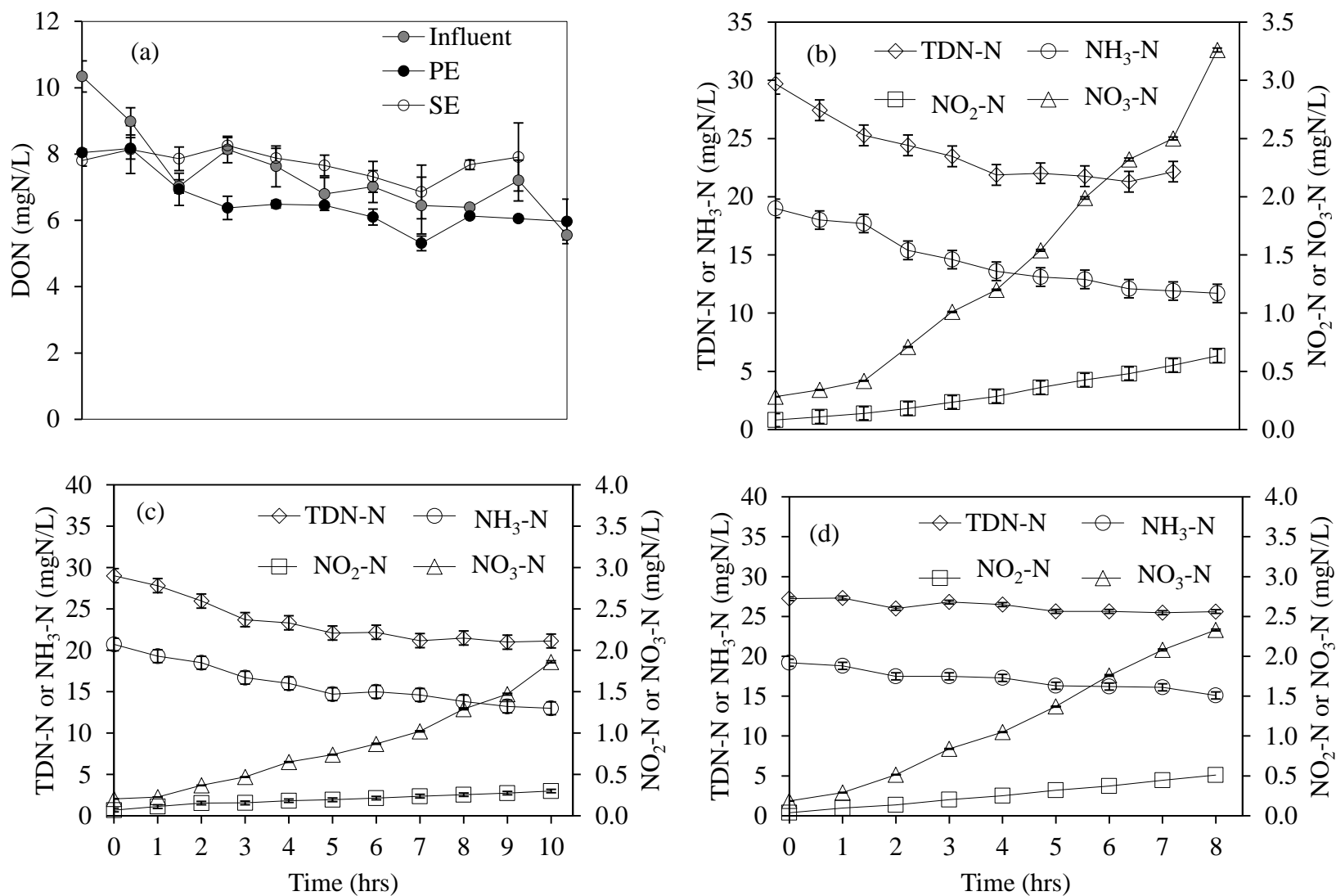


Figure 4.4. (a) DON biodegradation batch test performed on influent, PE, and SE using heterotrophic sludge. Nitrogen species during the test with (b) influent, (c) PE, and (d) SE.

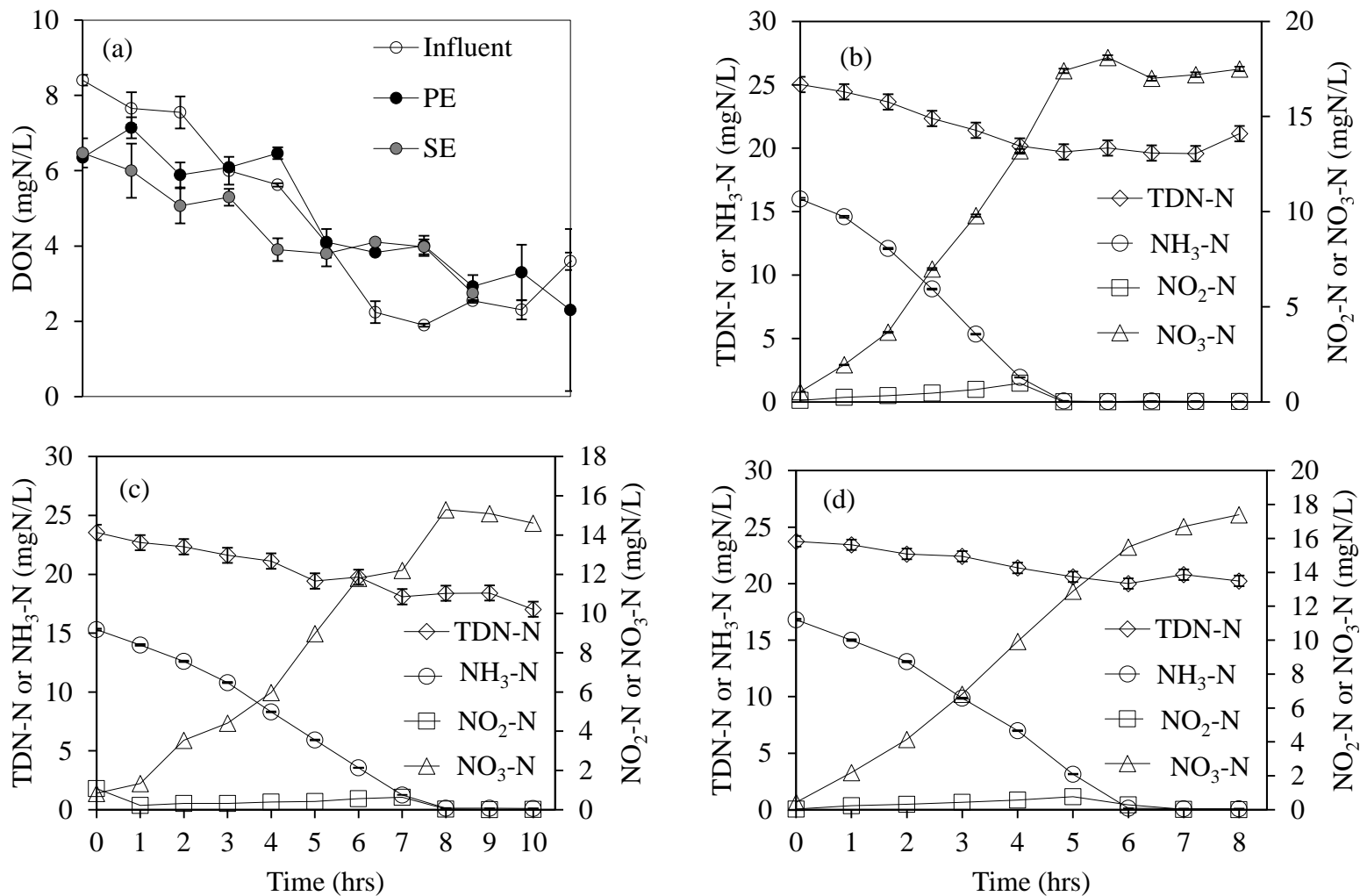


Figure 4.5. (a) DON biodegradation batch test performed on influent, PE, and SE using nitrification sludge. Nitrogen species during the test with (b) influent, (c) PE, and (d) SE.

Denitrification process removed DON only from the influent while a small percentage of DON increased in PE and SE samples. About 37% of DON in the influent was biodegradable under denitrification process. As COD is essential for denitrification, for conversion of $\text{NO}_3\text{-N}$ to N_2 , samples with high COD had higher denitrification compared to low COD samples. About 13.3, 11.8, and 1.0 mg N/L of $\text{NO}_3\text{-N}$ was removed from the influent (350 mg COD/L), PE (160 mg COD/L), and SE (45 mg COD/L), respectively. About 50% less biodegradation of DON concentration was observed under denitrification condition compared to nitrification condition in the influent samples.

Sattayatewa et al. (2009) investigated removal of DON through a 4-stage Bardenpho treatment process. The increase in organic nitrogen was also observed in the primary anoxic zone which could be due to either microbial activities or heterotrophic denitrification. They also reported that dilution from return activated sludge and internal recycling added DON in the treatment process. DON, produced in the primary anoxic zone, did not substantially change in the next three sections, which were primary aerobic, secondary anoxic, and secondary aerobic. Denitrification occurred in the secondary anoxic zone and nitrate decreased to about 0.5 mg N/L. Nitrite was in very low concentrations (< 0.03 mg N/L) through the entire treatment process. For deammonification process, except for SE where a slight DON was removed, the amount of DON increased in influent and PE samples. Higher COD or the type of COD in the influent and PE could have been the factors affecting DON biodegradation, by inhibiting the process (Güven et al., 2004). About 27% of DON in the SE was biologically removed by deammonification.

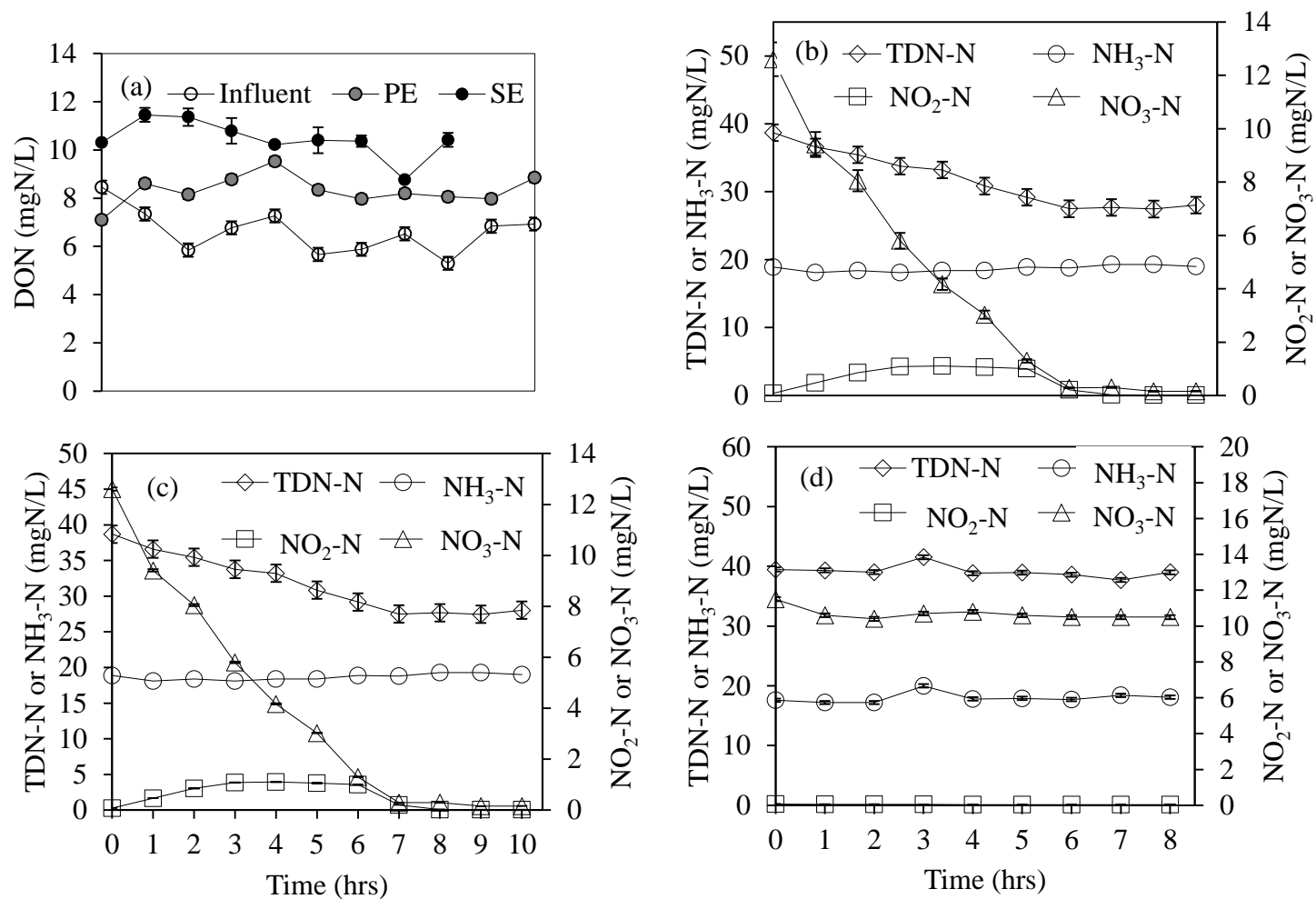


Figure 4.6. (a) DON biodegradation batch test performed on influent, PE, and SE using denitrification sludge. Nitrogen species during the test with (b) influent, (c) PE, and (d) SE.

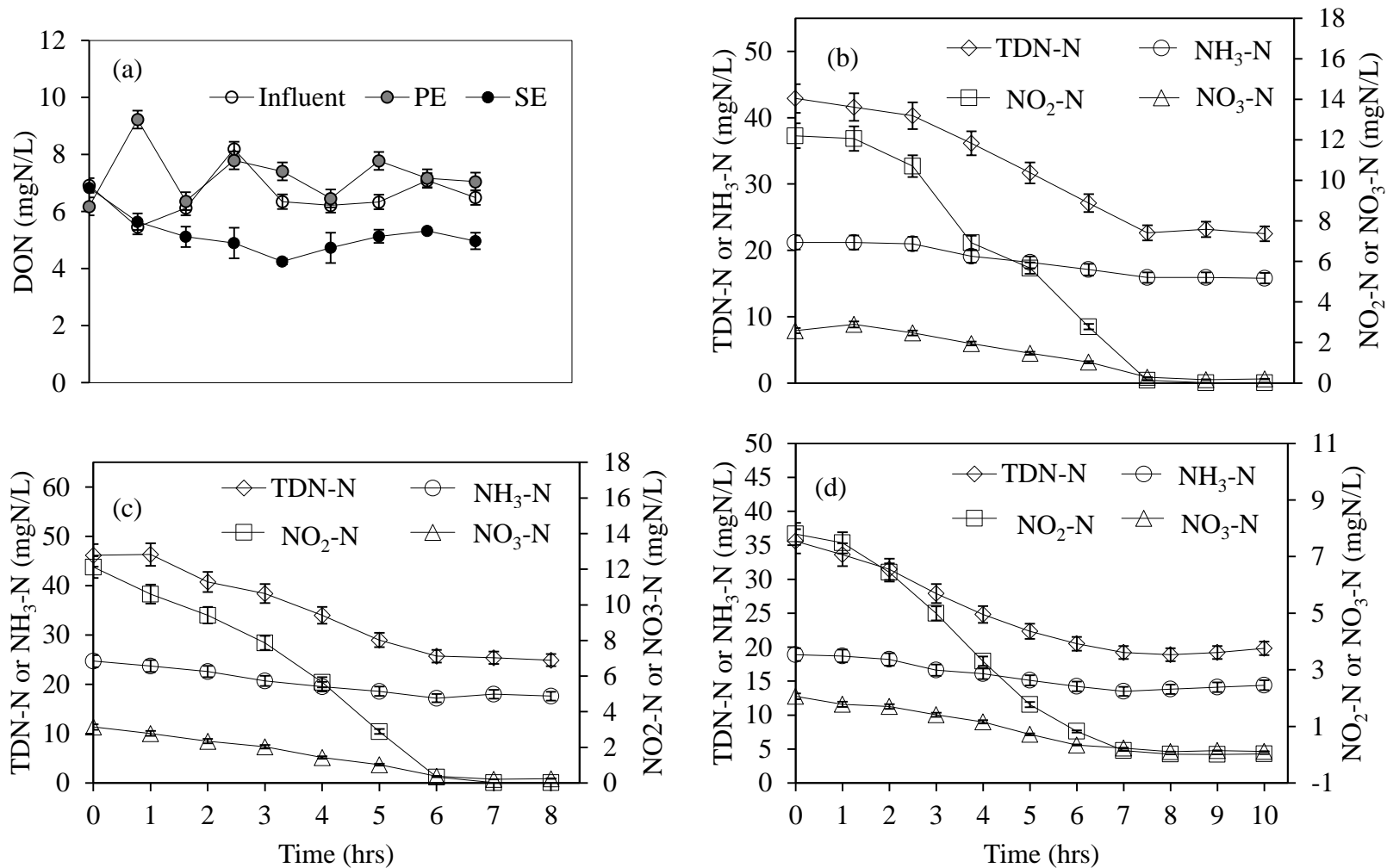


Figure 4.7. (a) DON biodegradation batch test performed on influent, PE, and SE using deammonification sludge. Nitrogen species during the test with (b) influent, (c) PE, and (d) SE.

4.3.2.2. Effect of Soluble COD:N Ratio on DON Biodegradation

Figure 4.8 shows DON biodegradation in filtered influent, PE, and SE samples evaluated with nitrification process. The soluble COD:N ratios for influent, PE, and SE were 10, 3.6, 1.4. A higher rate of DON biodegradation in the filtered influent sample (1.40 mg N/L/hr) compared to that of the unfiltered sample (0.99 mg N/L/hr) was observed. The lower DON removal rate in the unfiltered sample suggests that PON was likely present and its conversion to DON slowed down the net rate. Subtracting soluble COD from total COD provides particulate COD. In the influent, PE, and SE, the concentration of particulate COD ranged 200 to 250, 80 to 110, and 20 to 40 mg COD/L, respectively. A portion of the particulate COD is known to be biodegradable and it is estimated that 5% of the biodegradable portion is nitrogen (Metcalf and Eddy, 2003). Higher particulate COD in the influent samples indicates that PON might influence the rate of DON biodegradation. The rates of DON biodegradation in PE and SE were similar for the filtered and unfiltered samples, as there is very little or no PON present because of the solid separation processes before sampling location for PE and SE. The rates for DON biodegradation in filtered PE, and SE samples were 0.45 and 0.37 mg N/L/hr, respectively. However, the percentage of BDON exerted in the filtered samples was 17 to 20% less compared to that in the unfiltered samples. The decrease in DON biodegradation (BDON exertion) could be due to adsorption of BDON to the suspended particles that were removed during filtration and/or to the filter.

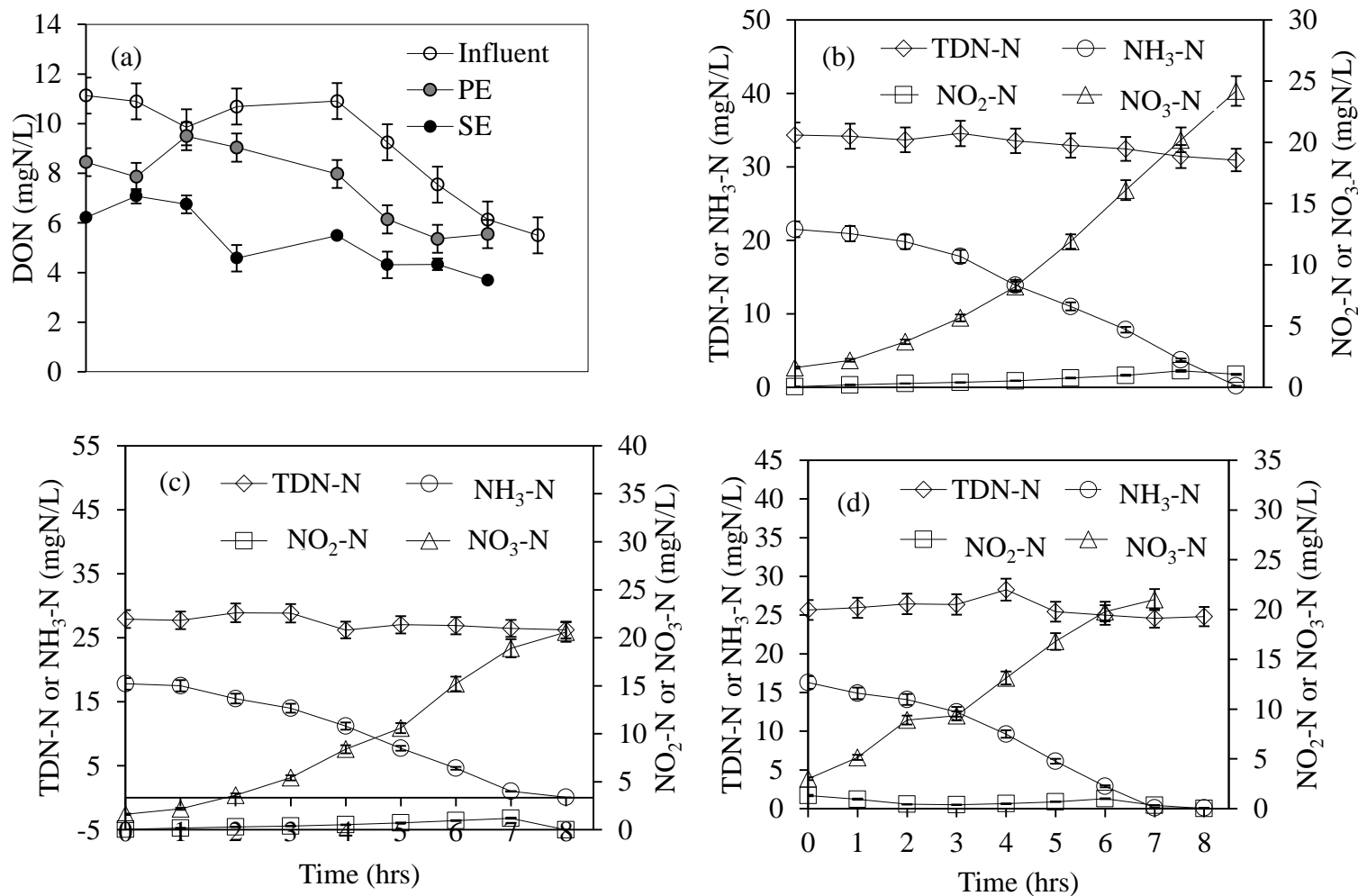


Figure 4.8. (a) DON biodegradation batch test performed on influent, PE, and SE samples (filtered through 0.45 μ m) using nitrification sludge. Nitrogen species during the test with (b) influent, (c) PE, and (d) SE.

4.3.3. Role of Nitrification Bacteria in DON Biodegradation

DON production rather than DON removal was observed when spiked with ATU or ATU and NO_2 compared to control (Figure 4.9 a and b).

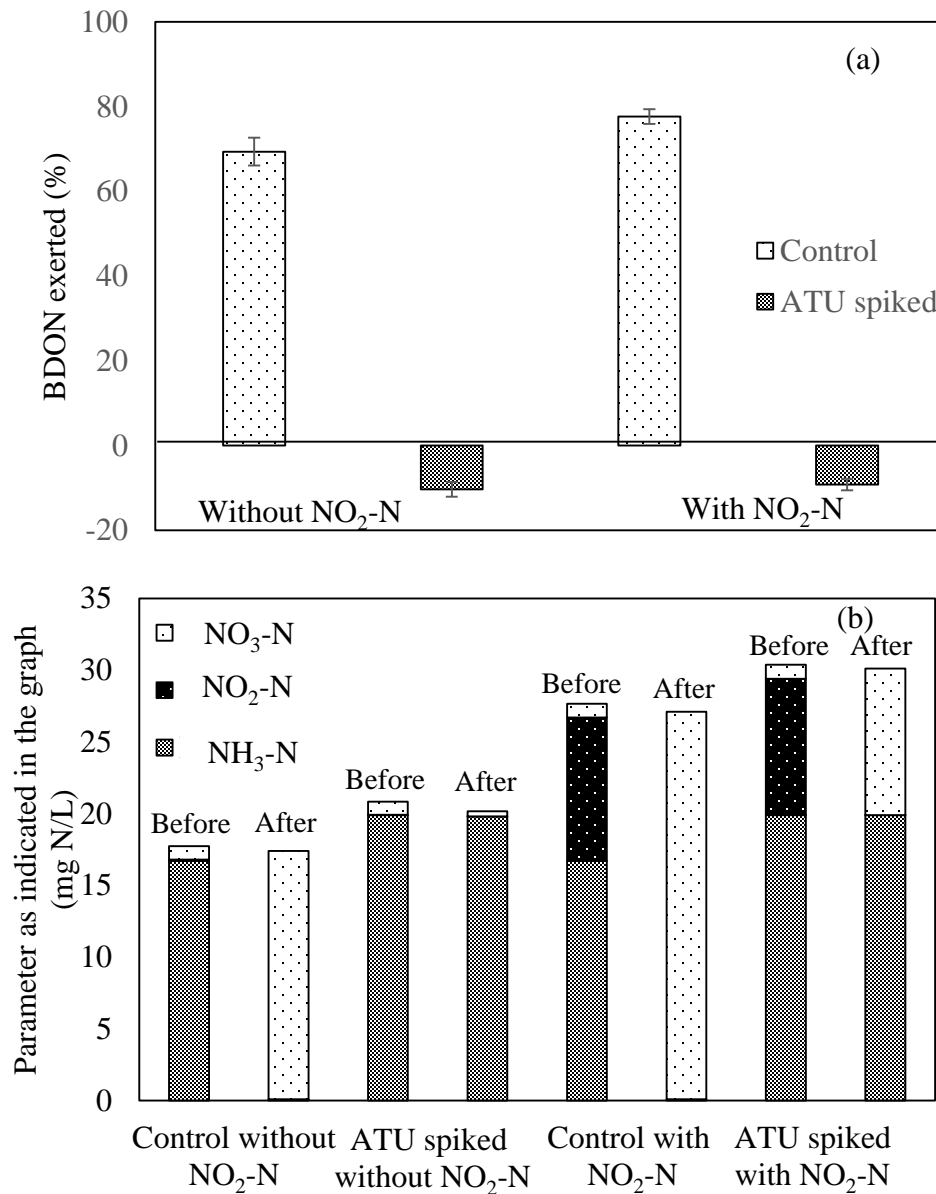


Figure 4.9. (a) BDON exertion with and without ATU using nitrification sludge. (b) Concentrations of DIN species with and without ATU.

This confirms the role of nitrifying bacteria in DON removal; however, it does not distinguish whether AOBs or NOBs were responsible for DON biodegradation in the absence of ATU. Although ATU only inhibited AOBs, NOBs would not be active as NO_2^- , the energy source for NOBs, was not present in the sample. For the samples spiked with ATU and NO_2^- together, complete conversion of NO_2^- to NO_3^- was observed; however, DON was not removed (Figure 4.9 b) suggesting NOBs were active but was not involved in DON biodegradation.

4.3.4. DON and BDON Profiles

The DON and BDON profiles for the three sampling locations help evaluate DON and BDON biodegradation by the primary and secondary treatment. The percentage of BDON exerted by each process was different in different types of sample. For the influent sample, BDON varied from 65.6 to 82.3% of DON for the nitrification sludge while for the heterotrophic sludge BDON was only 30 to 44% of DON (Figure 4.10 a). For the denitrification process, only 13.5 to 31.3 % of DON was biodegradable although complete denitrification ($\text{NO}_3\text{-N}$ to N_2 gas) for influent (Figure 4.10 a) and PE was observed (Figure 4.10 b). Deammonification on the other hand produced DON in the influent, DON increased by 1.3 to 30% (Figure 4.10 a). For PE, the nitrification sludge removed DON the most, the amount of BDON exerted was 47.8 to 60% of the DON (Figure 4.10 b). The heterotrophic sludge only removed 3 to 27% of DON while both denitrification and deammonification increased the concentration of DON.

Based on the nitrification and heterotrophic sludge, the primary treatment removed 17.8 to 22% and 17 to 27% of BDON, respectively. These BDON removal percentages are based on BDON values in the influent (Figure 4.10 a) and PE (Figure 4.10 b). The primary treatment is a chemically enhanced phosphorus treatment process using iron oxide to remove particulate matter. Iron oxide such as ferric oxide was previously used to remove DON from water (Carr

and Erickson 1988). Sattayatewa et al (2009) reported that primary treatment reduced about 20% of organic nitrogen. For SE, 38.4 to 65% of the DON was biodegradable for the nitrification sludge and only 3 to 4.5 % of DON was biodegradable to the heterotrophic sludge (except in one case where DON was produced). The secondary treatment removed 17.3 to 27.2% and 27 to 39.5% of BDON from the influent when evaluated using nitrification and heterotrophic sludge, respectively. Denitrification and deammonification conditions are not involved in DON biodegradation in primary and secondary treatment. Denitrification seemed to increase DON in SE and very minimal denitrification was observed because the COD was very low. Deammonification removed 8.75 to 11.7 % of DON.

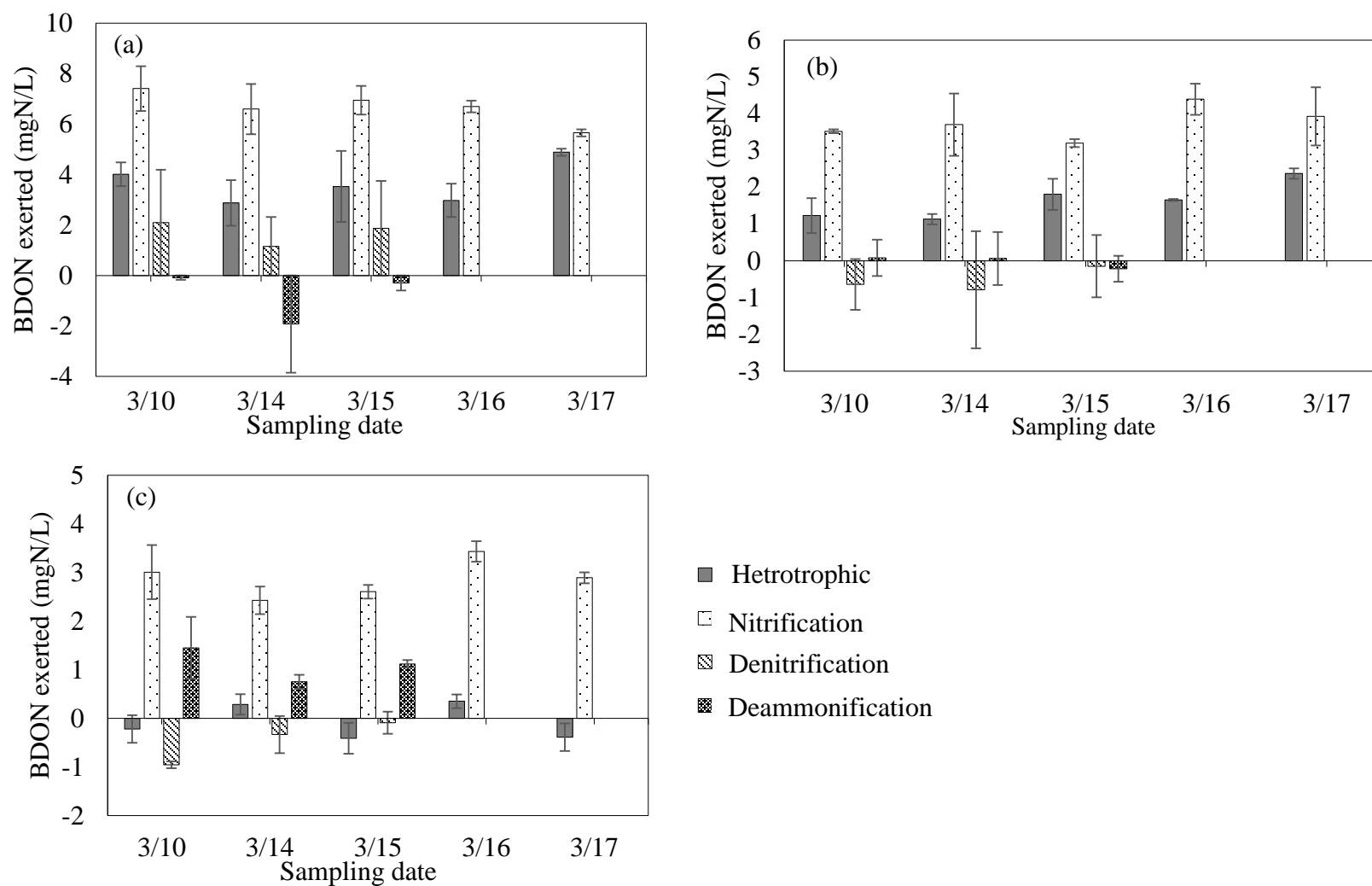


Figure 4.10. Exerted BDON in (a) influent, (b) PE, and (c) SE samples by different biological processes.

4.4. Summary

Low F/M batch tests were performed to examine DON biodegradation under different biological wastewater treatment process conditions. Nitrification process biodegraded more DON in wastewater compared to heterotrophic process. For the influent, PE, and SE with nitrification sludge, 70, 54, and 57% of DON was biodegraded. Denitrification and deammonification played a minimal role in DON biodegradation and in most cases increased the amount of DON in the samples. For nitrification process, AOBs rather than NOBs are responsible for DON biodegradation. This study is the first to elucidate the role of nitrification in DON degradation. The low F/M test was applied to examine the fate of BDON through an advanced WWTP. The primary and secondary treatment removed 17.8 to 22% and 27.2 to 17.3% of BDON when evaluated using nitrification sludge. As nitrification sludge removed higher concentrations of DON compared to heterotrophic sludge, WWTPs looking to achieve high DON removal should include or focus on nitrification process. This study identifies AOBs as a key player in DON biodegradation. This new knowledge could lead to an updated nitrogen cycle and suggests that nitrification particularly nitrification may be important for DON biodegradation in the natural environment.

CHAPTER 5. INVESTIGATING BIODEGRADABILITY OF DON IN THE EFFLUENT OF A SIDESTREAM DEAMMONIFICATION PROCESS

5.1. Introduction

WWTPs are required to stabilize waste activated sludge (WAS), produced during biological processes, before disposal to landfills. Most of the WWTPs use anaerobic digestion (AD) to stabilize the sludge that also provides additional benefits. AD leads to sludge reduction, minimizes greenhouse gas emission from landfills, and provides a potential for biogas production. Successful application of AD as a source for clean energy has been recognized as a possible alternative to non-renewable sources of energy such as coal. One of the major disadvantages of AD is that its effluent can be high in dissolved Total Kjeldahl Nitrogen (dTKN) concentrations which is a sum of DON and $\text{DNH}_3\text{-N}$. WWTPs that are required to meet a low TN discharge limits are cautious in implementing AD process. A major retrofit of their design to achieve removal of dTKN produced during AD will be required.

There are a number of biological pathways taking place during an AD process. The sludge contains complex organic material which first undergoes disintegration, hydrolysis, acidogenesis, acetogenesis, and the final step with is methanogenesis. Disintegration and hydrolysis are two major pathways that are involved in releasing dTKN from the sludge during digestion. Disintegration breaks down the cells to release cell lysate that is rich in proteins and amino acids. Extracellular enzymes are involved in hydrolysis of proteins or non-protein nitrogenous compounds into $\text{NH}_3\text{-N}$. The concentration of $\text{NH}_3\text{-N}$ usually increases while the concentration of DON decreases with increasing SRT. At lower SRT of ≤ 5 days the $\text{NH}_3\text{-N}$ concentration can range 800-1,000 mgN/L and at SRTs of ≥ 30 days the $\text{NH}_3\text{-N}$ is about 1,800-2,000 mgN/L (Hindin and Dunstan, 1960; Cacho Rivero 2005). The dTKN concentration is not

necessarily impacted by the changes in SRT and remain relatively constant as DON is converted to $\text{NH}_3\text{-N}$. Very minimal change in dTKN due to loss of $\text{NH}_3\text{-N}$ during assimilation by microbial cells and stripping of free ammonia ($\text{NH}_3\text{-N}_{(g)}$) is expected.

Conventional AD processes do not completely remove organic matter or hydrolyze DON to $\text{NH}_3\text{-N}$ from the sludge and digester effluent can contain different concentrations of inorganic material bound to carbon and organic material which is mostly inert biomass. During digestion only about 50 % of the organic matter fed is destructed to form methane and a major portion is TKN. Digestion process can be improved to produce higher amounts of biogas if the inert biomass can be made more available for digestion. The intercellular content of WAS cells is biodegradable and has a potential for producing biogas. However, the cell wall that protects the cellular content from physical and chemical stress is made up of D-amino acid cross linked polysaccharide chains that is resistant to biodegradation. Thermal hydrolysis pretreatment (THP) can be used to disintegrate the WAS sludge excreting cellular biodegradable content for digestion. THP can help intensify and accelerate the AD process by increasing the rates of disintegration and hydrolysis.

THP allows to run digesters at lower SRTs to achieve same or increased amount of gas compared to conventional AD process. However, it also increases the concentration of dTKN especially $\text{NH}_3\text{-N}$ in the effluent. The average dTKN concentrations of a 15 day SRT THP-AD process is greater than 2,000 mg N/L (Phothilangka et al., 2008). Also, there are speculations that during heating at high pressure might result in conversion of organic nitrogen matter to rDON which goes through the AD system without being hydrolyzed to $\text{NH}_3\text{-N}$. The rDON in the effluent of AD process can be very problematic for WWTPs with nutrient removal requirements.

Currently, there are sidestream processes that are capable of removing high concentrations of $\text{NH}_3\text{-N}$ (≥ 500 mgN/L). One such process is deammonification, the N removal during this process is carried out by anammox bacteria (See Chapter 2 for more details). However, deammonification is an autotrophic process and it is not known if DON is being removed during the process (Constantine et al., 2006). Also, the effluent from sidestream is usually recycled back to the first treatment stage of a WWTP. If the DON is refractory to the main treatment train, then it can result in high TN in the effluent of a plant leading to problems in meeting discharge N limits. Quantification of biodegradability of effluent DON of sidestream processes is therefore important for WWTPs. This study investigates the biodegradability of effluent DON of a sidestream process receiving feeds from a THP-AD and conventional AD (without THP).

5.2. Materials and Methods

5.2.1. Sample Sources and Pilot Deammonification Unit Description

Two lab-scale sidestream treatment reactors (D1 and D2) with deammonification processes were operated. The first deammonification reactor (D1) received effluent from a conventional full-scale mesophilic digester (MAD) as a feed while the second deammonification reactor (D2) received filtrate from a pilot scale THP-AD. The full scale MAD is operated by the City of Alexandria Advanced WWTP, Virginia. The pilot scale THP-AD was operated by the Blue Plains AWTP. The two 10 L sidestream deammonification reactors were operated using sludge obtained from the Zillertal WWTP, Strass, Austria. The reactors were operated as a sequencing batch reactor under anoxic conditions with a SRT of about 25 days.

5.2.2. Experimental Design

As described in Chapter 3, DON determination involved measurements of DIN and TDN, and subtraction of the concentrations of the two parameters. The measurements of the three DIN species and TDN have analytical errors. The errors add up to give a larger error in DON calculation. Lee and Westerhoff (2005) show that in the case of high DIN/TDN ratios (>0.6), the analytical error might be greater than the calculated DON concentration. The impact of DIN/TDN on DON determination was experimentally evaluated. The ratio of DIN/TDN in an anaerobic digester effluent and influent of a sidestream can be as high as 0.99 (Phothilangka et al., 2008). Different ratios of DIN/TDN were prepared using NH_4Cl or KNO_3 for DIN and glutamic acid as a standard DON compound. The concentration of glutamic acid was kept constant while the concentrations of NH_4Cl or KNO_3 were varied to attain different DIN/TDN ratios.

The experiments to quantify BDON in the effluents of two sidestream reactors were performed using nitrification sludge obtained from the Blue Plains AWTP. The detailed information on the BDON test is the same as described above in Chapter 4 except that the MLSS concentration used in the BDON measurement was higher (1,500 mg TSS/L). The reason for using a higher concentration was to represent DON biodegradation under a full scale plant condition. Parkin and McCarty (1981b) suggested that higher MLSS might contribute to more DON release during the test, so a control experiment using inocula and dechlorinated tap water was also performed to account for biodegradation of DON that might be released by the MLSS during the test. The control experiments were spiked with 20 mg N/L of NH_4^+ and 150 mg/L of bicarbonate alkalinity.

5.2.3. Analytical Methods

The analytical techniques are presented in Chapter 4, Section 4.1.4.

5.2.4. Statistical Analysis

The statistical analysis performed is the same as that presented in Chapter 4, Section 4.1.5.

5.3. Results and Discussion

Figure 5.1. shows the percentages of DON recovered under different DIN/TDN ratios. The percentage of DON recovered was calculated by dividing DON calculated (from subtracting measured DIN from measured TDN) by DON added and multiplying by 100%. At lower DIN/TDN ratios of 0.5 to 0.7, the DON recovery percentage varied from 106 to 123%. For DIN/TDN of 0.7 to 0.9, DON recovery was about 112 to 183%. DON recovery of 138 to 1795% was observed for samples with greater than DIN/TDN of 0.9. DON recovery increases linearly for DIN/TDN of 0.5 to 0.9 while it increases exponentially after DIN/TDN of 0.9. This suggests that for high DIN/TDN samples such as the anaerobic digester effluent and sidestream influent (0.95 to 0.99), the concentration of DON determined will not be accurate and will have a large error. The data presented by Lee and Westerhoff (2005) and Graeber et al. (2012) showed that for freshwater samples where the DIN/TDN ratio > 0.6 but less than 0.9, a large error can be expected for the determined DON concentration. However, for samples with DIN/TDN ratios greater than 0.9 and closer to 0.98 the error in DON determination is even larger. Determination of BDON in the samples with high DIN/TDN will be very difficult and inaccurate as BDON is a portion of DON.

Lee and Westerhoff (2005) recommended reducing the DIN concentration using dialysis and other catalysts to improve the DON determination. However, these methods have two

drawbacks. First they do not work for DIN/TDN of greater than 0.9 and second DON adsorbs onto the dialysis membrane and catalyst interfering with accurate DON determination. As deammonification is an autotrophic process and does not consume organic including DON. In this work, the sidestream deammonification process helps reduce the DIN/TDN from 0.99 to < 0.9. Due to this reduction in DIN/TDN in sidestream effluent samples, determining DON and BDON accurately was possible.

Even after reducing the DIN/TDN ratio, the pattern of DON biodegradation was not very clear in the experiments performed. DON biodegradation was observed in the control, as well as D1 and D2 effluents. In the control, the percentage of DON biodegraded varied from about 70 to 86% (Figures 5.2, 5.3, and 5.4) while DON biodegradation in D1 and D2 effluents varied from 43 to 95% and 35 to 99%, respectively. DON biodegradation in the control suggests that the MLSS had adsorbed DON which is biodegradable, as observed by Parkin and McCarthy (1981a). Irrespective of DON biodegradation observed in the control, the experiments were useful in identifying that DON in D1 and D2 effluents were biodegradable. However, no trend between DON biodegradation and time was observed and consequently the DON biodegradation rate could not be determined. Specific nitrification and nitrification rates shown in Table 5.1 suggest that the effluents of the two sidestream reactors (D1 and D2) did not affect the activities of AOBs and NOBs in the nitrification sludge, indicating that effluent from sidestream can be treated by the mainstream process. It is interesting to find that during the BDON tests on the effluents of D1 and D2, the activities of the AOBs and NOBs slightly increased rather than decreased. Both the effluents of D1 and D2 had higher COD, compared to the control, including by-products of AD such as higher free ammonia and recalcitrant organic material which are known to inhibit AOBs and NOBs (Phothilangka et al., 2008). The higher rates can be explained

by higher alkalinity in the effluents of sidestream reactors which can impart higher rates for $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ removal.

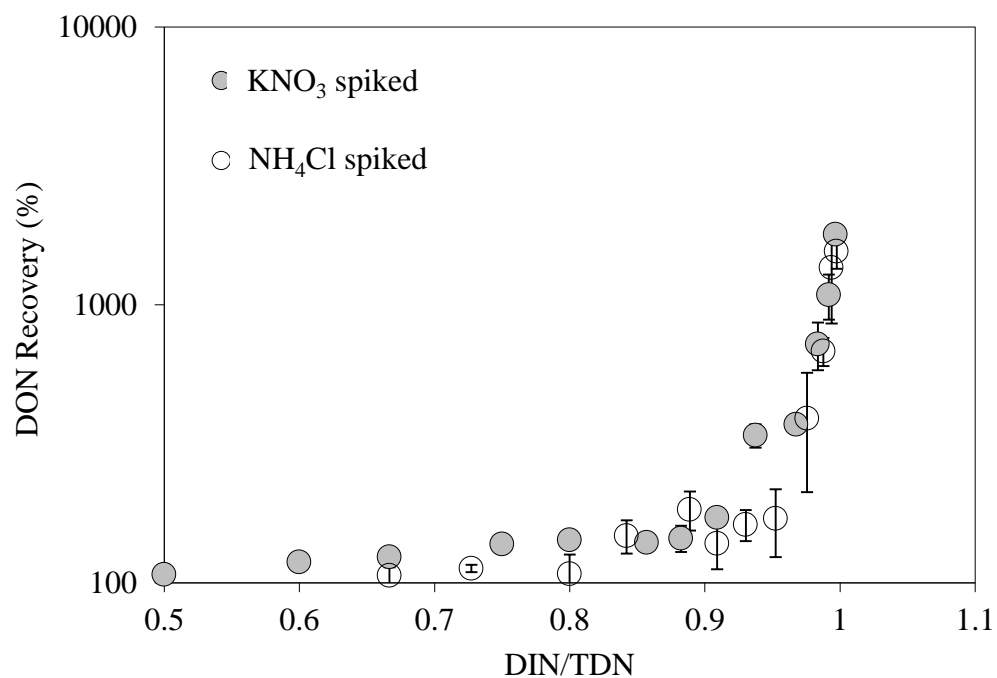


Figure 5.1. Percentages of DON recovery under different DIN/TDN ratios.

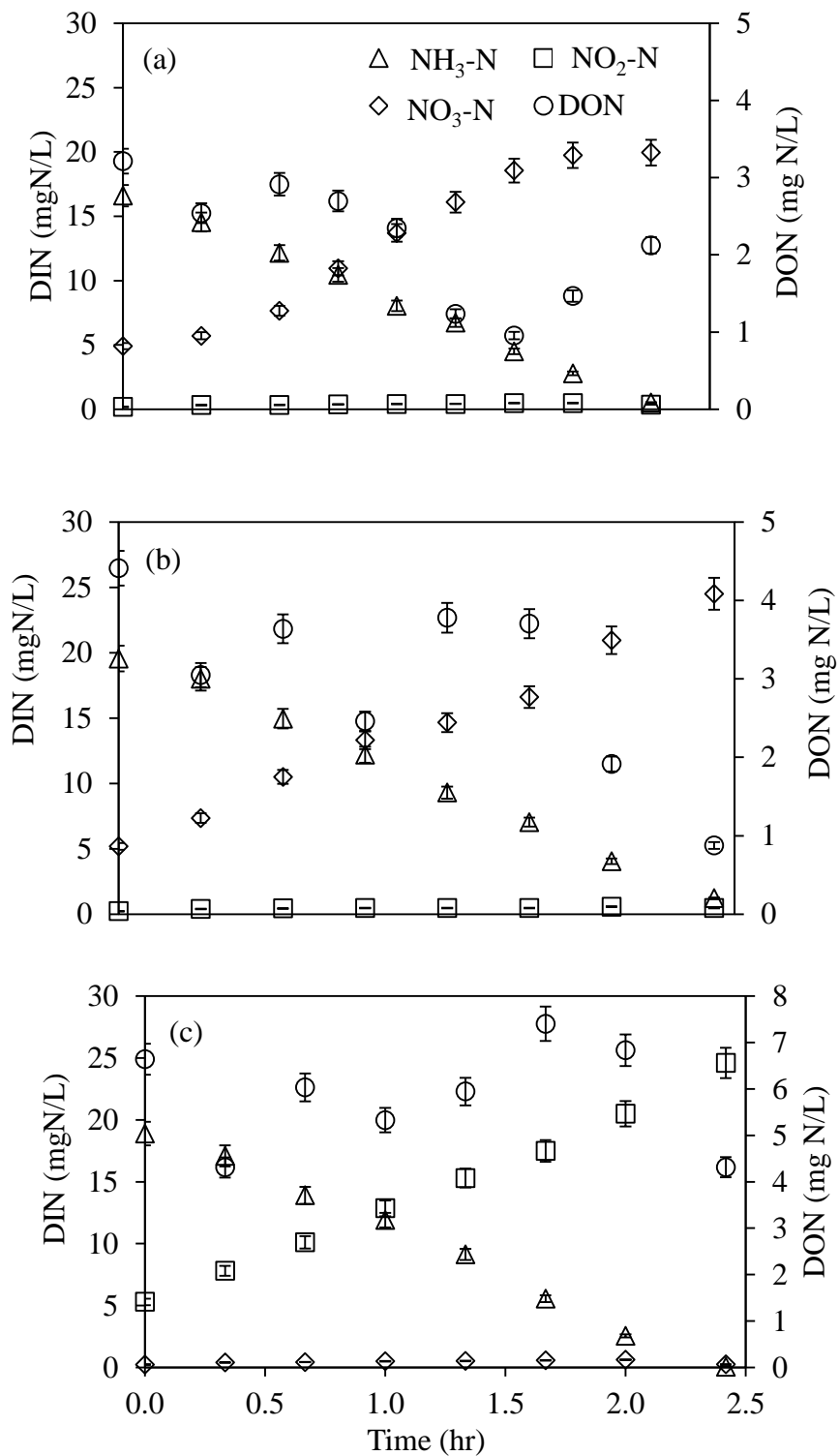


Figure 5.2. DON biodegradation by nitrification sludge: (a) Control, (b) D1 deammonification reactor, and (c) D2 deammonification reactor on 4/7/2014.

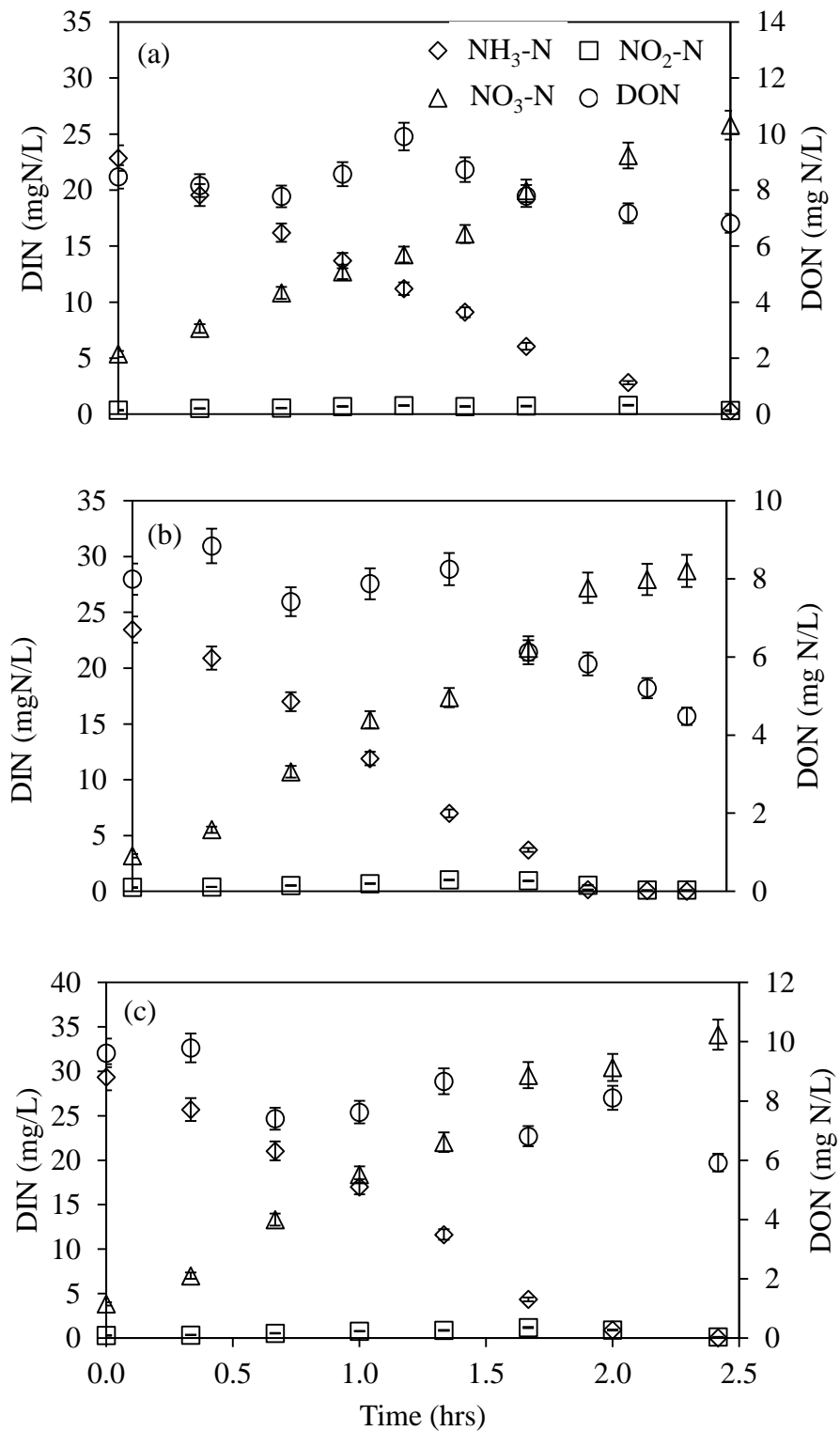


Figure 5.3. DON biodegradation by nitrification sludge using (a) Control, (b) D1 deammonification reactor, and (c) D2 deammonification reactor on 4/8/2014.

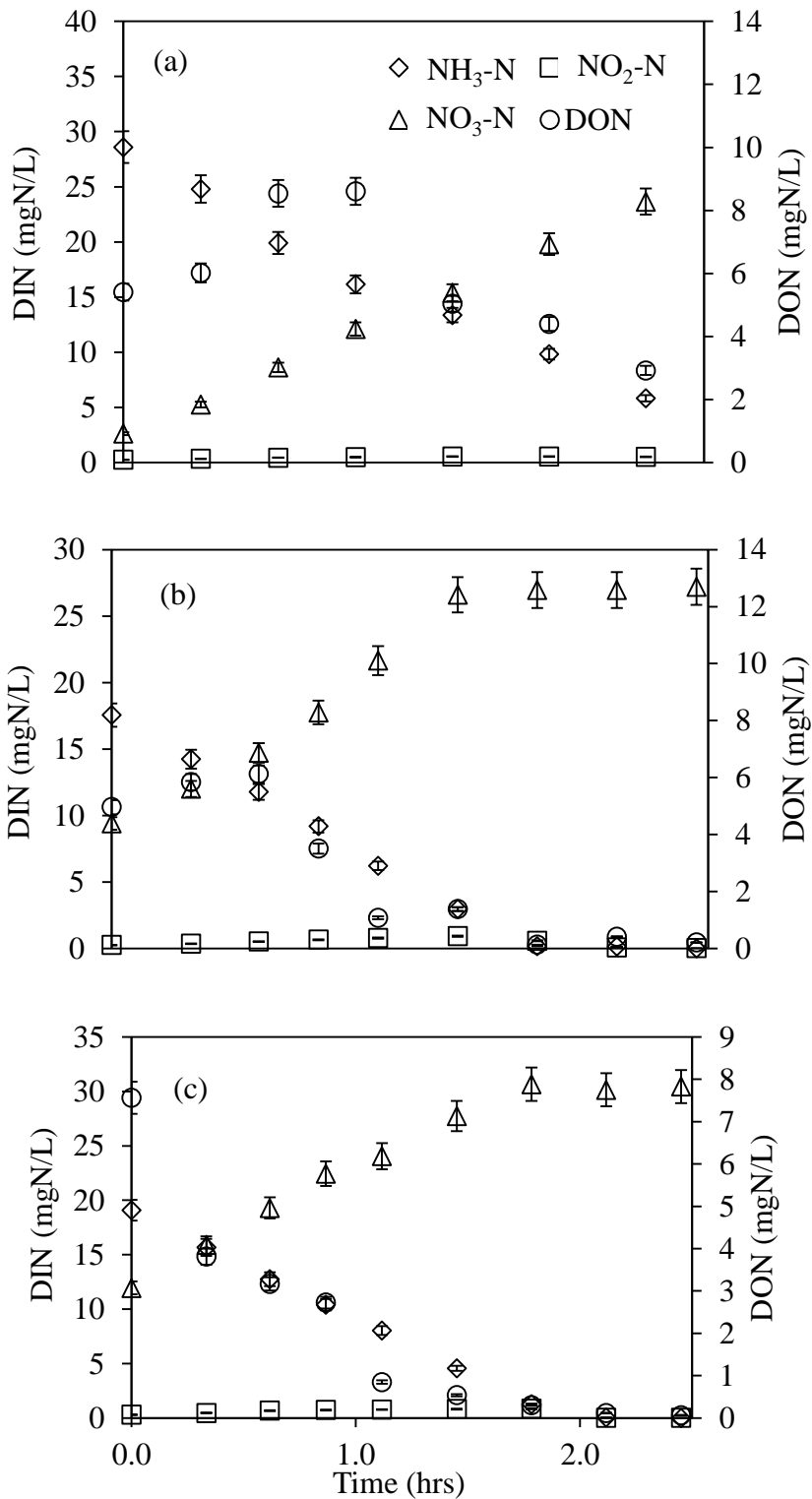


Figure 5.4. DON biodegradation by nitrification sludge using (a) Control, (b) D1 deammonification reactor, and (c) D2 deammonification reactor on 4/17/2014.

Table 5.1. Specific nitritation and nitrataion rates during the BDON batch tests.

	Specific Nitritation Rate (mg N/g VSS/hr)	Specific Nitrataion Rate (mg N/g VSS/hr)
Control	5.47 – 6.66	5.92 – 6.05
D1 Effluent	6.14 – 7.42	5.92 – 6.86
D2 Effluent	6.13 – 8.27	6.12 – 7.03

5.4. Summary

DON biodegradation batch tests using nitrification sludge as an inoculum were performed on the effluents of two sidestream deammonification pilot reactors that were fed with centrate from THP-AD and conventional AD. As DON is calculated from measured DIN and subtracting from measured TDN, any errors associated with DIN and TDN measurements lead to an error in DON determination. DON recovery from high DIN/TDN solutions prepared using standard solutions were evaluated and the results showed that the DIN/TDN ratio of > 0.9 results in the DON recovery ranging from 138 to 1750%. This suggests that determining DON and BDON in the centrate which had extremely high DIN/TDN (0.99) would give inaccurate results. The sidestream reduced DIN/TDN to below 0.9. The effluent of the two sidestream deammonification reactors did not affect the activities of AOBs and NOBs in the nitrification sludge, suggesting that the effluents from sidestream reactors can be treated by the mainstream to get further TN removal. The amount of DON biodegraded varied from 43 to 95% for D1 effluent while for the D2 effluent it was 35 to 99%. The study concludes that DON in the effluents of the sidestream processes is biodegradable; however, a rate at which DON is biodegraded by nitrification sludge could not be identified as no trend of DON biodegradation with time was observed.

CHAPTER 6. OVERALL CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1. Conclusions

DON in water and wastewater is a major concern. DON in drinking water is gaining attention because DON in water prior to and after chlorination may promote formation of carcinogenic nitrogenous disinfectant by-products and growth of microorganisms in the distribution system. While DON removal from wastewater is very important as DON and BDON in treated wastewater can promote algal growth in receiving waters causing oxygen depletion and/or eutrophication. To minimize impact of TN species including DON on receiving waters, WWTPs are subject to more stringent effluent total nitrogen limits. Understanding DON and its biodegradability is very important for improving the treatment efficiency of DON from both water and wastewater. A comprehensive study was conducted to investigate the fate and biodegradability of DON in water and wastewater processes.

To determine the fate and biodegradability of DON, first a bioassay for determining BDON in water was developed and optimized. Following which the bioassay was used to determine the fate of BDON through the Moorhead WTP which has four treatment stages including ozonation and biologically active filtration. For studying the removal of DON in different wastewater processes a modified low F/M method was used. Biodegradability of DON in the influent, PE, SE, and effluent of a sidestream deammonification process removing $\text{NH}_3\text{-N}$ from filtrate of AD was determined.

During the development of the bioassay for determination of BDON in water, an inoculum size of 2 ml of 150 mg TSS/L MLSS in 150 ml of sample and incubation time of 14 days were sufficient to completely exert BDON in the water samples. The bioassay was tested for its sensitivity in determining low concentration of BDON, as seen in drinking water, using

DON standards including glutamic acid and glycine. About 60% of glutamic acid was mineralized to $\text{NH}_3\text{-N}$, while almost complete BDON exertion of 50 and 100 $\mu\text{g L}^{-1}$ as N of glycine was observed. DON in glycine and glutamic acid had different sensitivities to biodegradation due to molecular structure differences. Glycine was more sensitive to complete DON biodegradation. The amount of BDON determined in water samples varied from 17-275 $\mu\text{g L}^{-1}$ as N. During the bioassay incubation, DON was biodegraded (ammonified) and partially nitrified. Coagulation removed BDON while ozonation increased BDON. BDON decreased through biologically active filters (immediately after ozonation). The studied WTP on average removed 68% of BDON. The optimized bioassay can serve as a simple tool to determine the fate of BDON through water treatment plants.

The low F:M BDON test was optimized and performed to identify the wastewater treatment processes which play a major role in the biodegradation of DON in wastewater. Four commonly used processes were evaluated during the study, heterotrophic carbon removing, nitrification, denitrification, and deammonification. Nitrification sludge biodegraded more DON in wastewater compared to heterotrophic sludge. For nitrification sludge, the rate of DON biodegradation increased with the concentration of sludge inoculum, 0.158, 0.276, and 0.457 mg N/L/hr for 80, 160, and 410 mg TSS/L of sludge inoculum added, respectively. Assimilation of $\text{NH}_3\text{-N}$ was a major reason for TN reduction for the heterotrophic sludge while very minimal of it was observed for the nitrification sludge. For influent, PE, and SE with nitrification sludge, 70, 54, and 57% of DON was biodegradable.

The biodegradability of DON in the effluent of side stream deammonification process receiving centrate from a THP-AD and a non-THP process was evaluated using nitrification sludge. Sidestream effluents are known to inhibit nitrifying bacteria. However, the effluent of the

two sidestream units did not affect the activities of AOBs and NOBs in the nitrification sludge, indicating that effluent from sidestream can be treated by the mainstream. DON biodegradation in the effluent from THP-AD receiving side stream varied from 43 to 95 % while for the AD centrate receiving side stream it was 35 to 99%.

6.2. Recommendations for Future Work

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

1. To further understand the nature and treatability of BDON, a future study on chemical characterizations of BDON in water through different treatment processes based on differences in chemical compositions of DON before and after incubation is recommended.
2. Microbial community in the biologically active filters involved in removal of DON in drinking water should be evaluated.
3. Bench scale studies to quantify the rate of DON production by bacteria should be conducted. This can be achieved by growing bacteria on heavy nitrogen labelled $\text{NH}_3\text{-N}$ and a carbon substrate.
4. The roles of temperature and pH on biodegradability of DON should be determined. pH can result in protonated or unprotonated states of DON which may affect its bioavailability.
5. Molecular work including quantitative polymerase chain reactions and sequencing to identify genes and pathways involved in removal of DON in heterotrophic sludge and nitrification sludge is recommended.

6. Enzymes in AOBs that are responsible in DON removal in the nitrification sludge should be investigated.
7. Effect of micronutrients in DON biodegradation during the BDON batch tests should be examined.

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APPENDIX

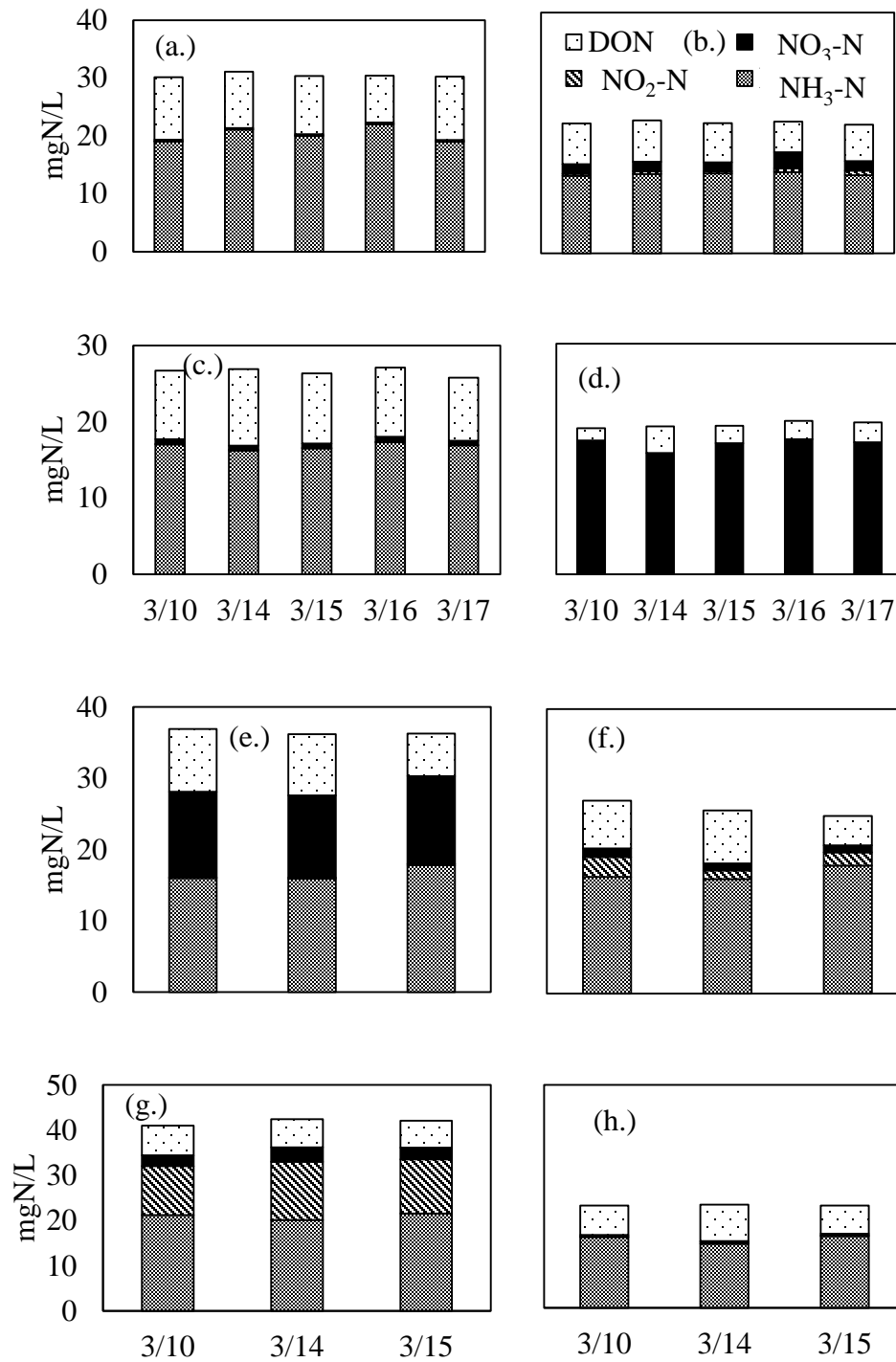


Figure A. 1. A stacked graph with DON and DIN concentrations for influent samples collected on different dates. The data is from before and after BDON batch test performed using heterotrophic (a, b), nitrification (c, d), denitrification (e, f), deammonification (c, d).

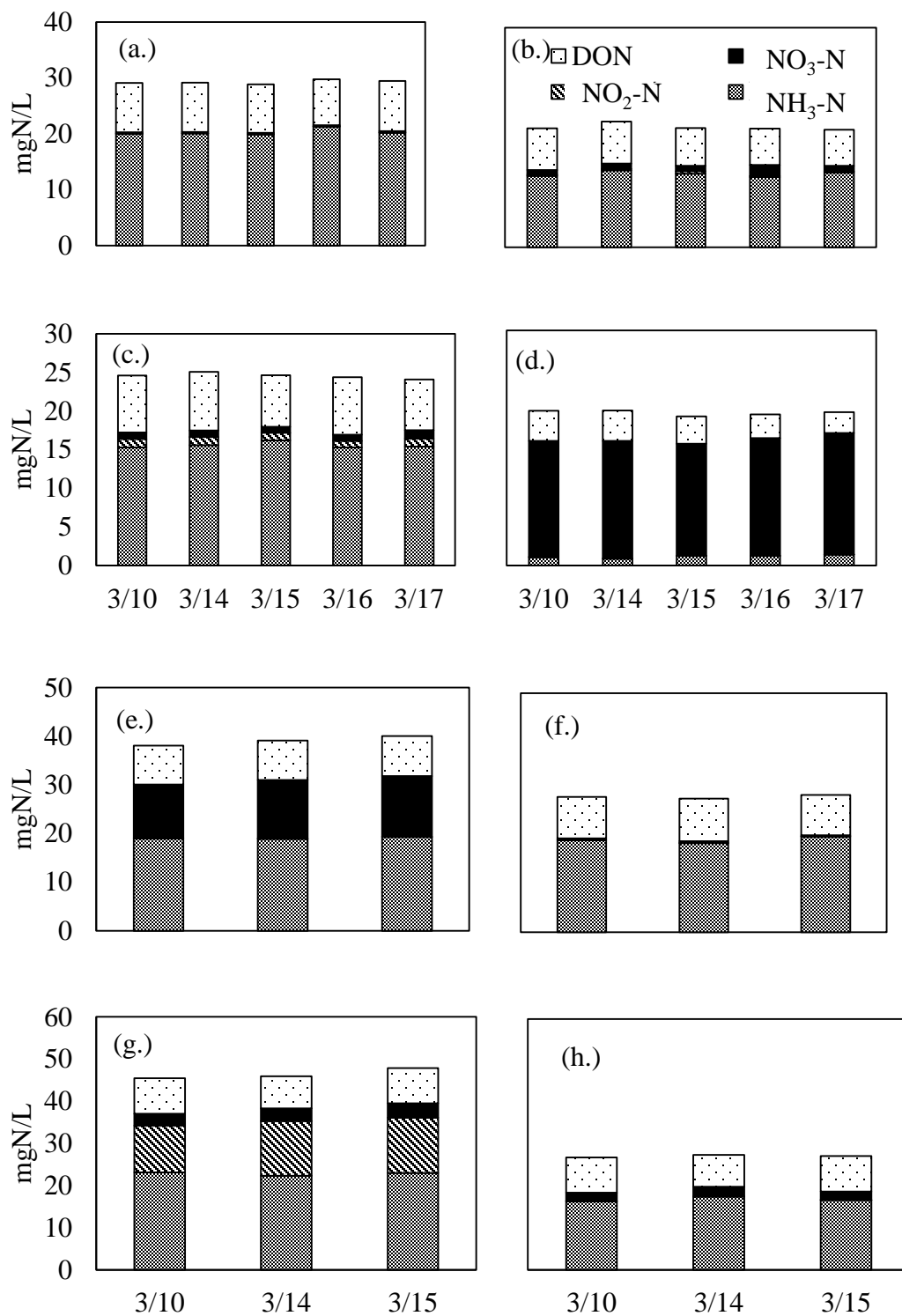


Figure A. 2. A stacked graph with DON and DIN concentrations for PE samples collected on different dates. The data is from before and after BDON batch test performed using heterotrophic (a, b), nitrification (c, d), denitrification (e, f), deammonification (g, h).

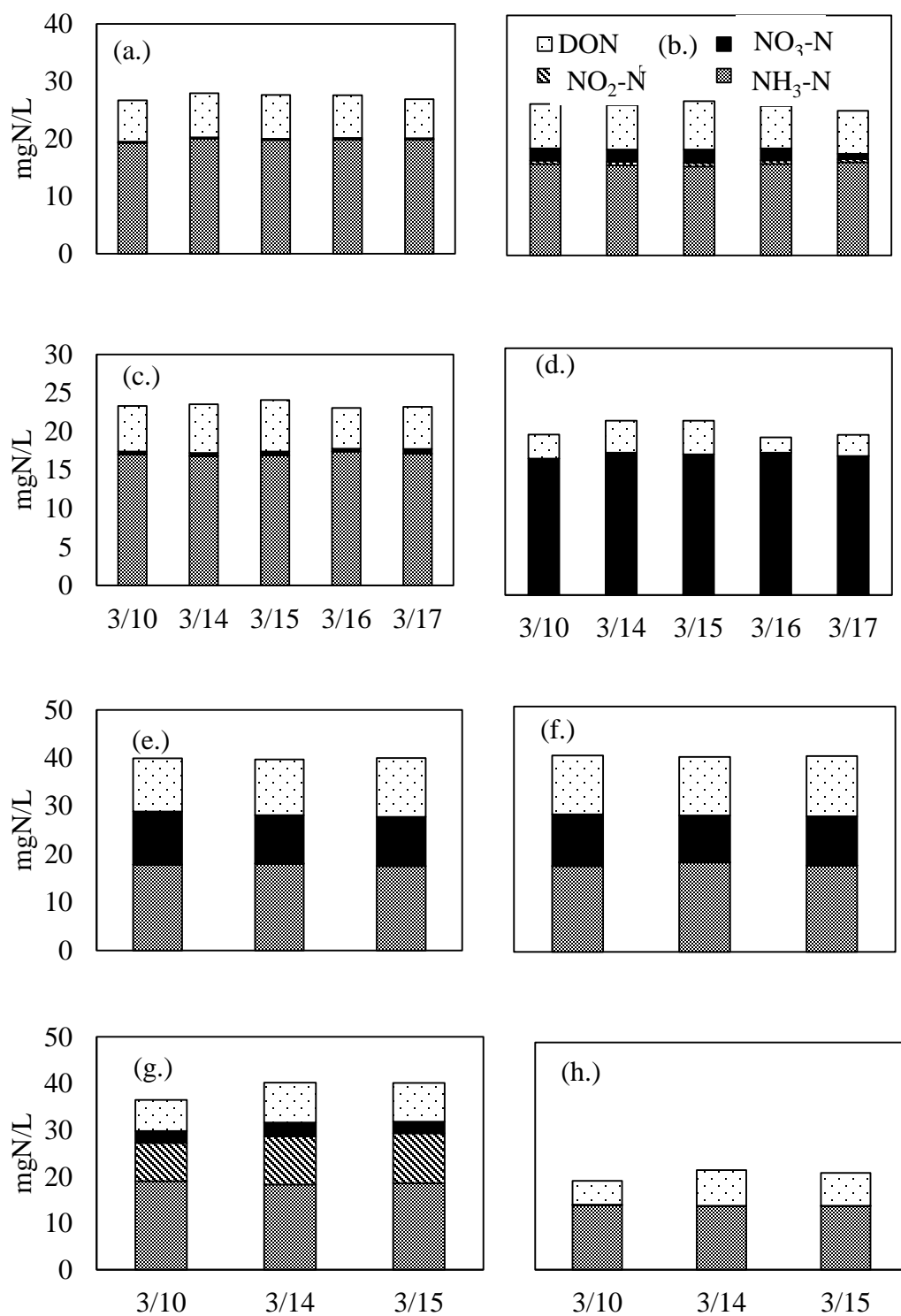


Figure A. 3. A stacked graph with DON and DIN concentrations for SE samples collected on different dates. The data is from before and after BDON batch test performed using heterotrophic (a, b), nitrification (c, d), denitrification (e, f), deammonification (c, d).