Investigation of Effluent Nitrogen Derived from Conventional Activated Sludge (CAS) and Biological Nutrient Removal (BNR) Systems and Its Impact on Algal Growth in Receiving Waters

Heonseop Eom

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INVESTIGATION OF EFFLUENT NITROGEN DERIVED FROM CONVENTIONAL ACTIVATED SLUDGE (CAS) AND BIOLOGICAL NUTRIENT REMOVAL (BNR) SYSTEMS AND ITS IMPACT ON ALGAL GROWTH IN RECEIVING WATERS

A Dissertation Presented

By

HEONSEOP EOM

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

February 2016

Department of Civil and Environmental Engineering
INVESTIGATION OF EFFLUENT NITROGEN DERIVED FROM CONVENTIONAL ACTIVATED SLUDGE (CAS) AND BIOLOGICAL NUTRIENT REMOVAL (BNR) SYSTEMS AND ITS IMPACT ON ALGAL GROWTH IN RECEIVING WATERS

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To my lovely parents and Hyeri
ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor Dr. Park for giving me the opportunity to study at UMass, and for his advice and guidance through my PhD study. I also like to thank my dissertation committee members, Dr. Tobiason, Dr. Butler, and Dr. Nüsslein, for serving on my committee and their valuable suggestion and comments.

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Last but not least, I really appreciate my parents and Heyri. They always encourage and support me when I am in difficulty.

Thank you all!
ABSTRACT
INVESTIGATION OF EFFLUENT NITROGEN DERIVED FROM CONVENTIONAL ACTIVATED SLUDGE (CAS) AND BIOLOGICAL NUTRIENT REMOVAL (BNR) SYSTEMS AND ITS IMPACT ON ALGAL GROWTH IN RECEIVING WATERS
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Directed by: Professor Chul Park

The main objective of this research was to evaluate the effects of effluent nitrogen from conventional activated sludge (CAS) and biological nutrient removal (BNR) processes on eutrophication in receiving estuaries.

To investigate differences in effluent nitrogen from CAS and BNR processes, lab-scale wastewater treatment systems having identical influent were operated under controlled conditions. This reactor study showed that the BNR system decreased nitrogen discharge by removing dissolved inorganic nitrogen (DIN) from wastewater but generated more effluent dissolved organic nitrogen (DON) than did the CAS system. The transition of treatment conditions from anoxic to oxic within the BNR process facilitated the production of low molecular weight dissolved organic nitrogen (LMW DON), causing the BNR effluent to contain more DON than the CAS effluent. Moreover, analysis of data from a local full-scale plant (the Amherst WWTP) confirmed that when the wastewater treatment mode was converted from CAS to BNR, the effluent DIN decreased but effluent DON increased.
Bioassays, incubated with effluent and natural estuary water (Long Island Sound water), were conducted to compare algal growth stimulated by CAS and BNR effluents. The results demonstrated that the BNR effluent, despite containing less dissolved total nitrogen (DTN), stimulated more algal biomass and higher nitrogen-based productivity than the CAS effluent. These unexpected outcomes were attributed to the greater potential of LMW DON to drive algal growth compared to DIN. Numerical analyses for algal growth yields for effluent nitrogen species illustrated that LMW DON had approximately 7 times higher yield than DIN.

In conclusion, this research revealed that the BNR system generated more effluent LMW DON, which showed greater algal growth yield, supporting for more algal biomass generation in the bioassays than did the CAS system. These findings indicate that simply decreasing the amounts of effluent nitrogen cannot ensure alleviating algal blooms in receiving estuaries. To evaluate the actual influence of effluents on eutrophication, not only amounts but also compositions of effluent nitrogen should be considered.
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<th>Full Form</th>
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<tbody>
<tr>
<td>BNR</td>
<td>Biological Nutrient Removal</td>
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<td>CAS</td>
<td>Conventional Activated Sludge</td>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
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<tr>
<td>DIN</td>
<td>Dissolved Inorganic Nitrogen</td>
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<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>DON</td>
<td>Dissolved Organic Nitrogen</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved Organic Carbon</td>
</tr>
<tr>
<td>DTN</td>
<td>Dissolved Total Nitrogen</td>
</tr>
<tr>
<td>HMW DON</td>
<td>High Molecular Weight Dissolved Organic Nitrogen</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic Retention Time</td>
</tr>
<tr>
<td>LIS</td>
<td>Long Island Sound</td>
</tr>
<tr>
<td>LMW DON</td>
<td>Low Molecular Weight Dissolved Organic Nitrogen</td>
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<tr>
<td>ON</td>
<td>Organic Nitrogen</td>
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<tr>
<td>SBR</td>
<td>Sequencing Batch Reactor</td>
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<tr>
<td>SRT</td>
<td>Solid Retention Time</td>
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<td>TKN</td>
<td>Total Kjeldahl Nitrogen</td>
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<tr>
<td>TSS</td>
<td>Total Suspended Solids</td>
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<tr>
<td>VSS</td>
<td>Volatile Suspended Solids</td>
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<td>WWTP</td>
<td>Wastewater Treatment Plan</td>
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CHAPTER 1

INTRODUCTION

1.1 Problem Statement

Eutrophication, the process by which a water body becomes enriched with nutrients and stimulates excessive algal growth, is responsible for numerous environmental problems. The most serious consequence of eutrophication is hypoxia: decomposition of dead algal biomass depletes dissolved oxygen, killing fish and other aquatic wildlife. Approximately two-thirds of estuaries and bays in the United States suffer from eutrophication (Pew Ocean Commission, 2003).

Long Island Sound (LIS), a semi-enclosed arm of the North Atlantic Ocean lying between the New York and Connecticut shores to the north and Long Island to the south, has been experiencing seasonal hypoxia for years. To improve the conditions of LIS, the US EPA and the bordering states reached an agreement to freeze nitrogen loading to LIS at 1990 levels and then eventually decrease it by 58.5% by 2014 (US EPA, 2011). One major step taken to achieve this goal was upgrading 39 out of 104 wastewater treatment plants (WWTPs) discharging into LIS from conventional activated sludge (CAS) to biological nutrient removal (BNR) processes (O’Shea and Brosnan 2000). Since 2004, total nitrogen loading to LIS decreased by approximately 20%; however, this result failed to improve the conditions of LIS. The area affected by hypoxia in 2006 actually became larger than in 1999 with longer periods of hypoxia (O’Shea and Brosnan 2000; Stelloh, 2007).
Wastewater-originated nitrogen provides a substantial nitrogen source to natural waters and contributes to eutrophication in receiving waters. Therefore, wastewater treatment plants (WWTPs) have been required to decrease their nitrogen discharge. Upgrading facilities from existing conventional process, CAS, to a more advanced system, BNR, is the common approach to comply with these requests. The CAS system is an aerobic process employing microbial aggregates to degrade organic substances in wastewater. The BNR systems include both anaerobic/anoxic and aerobic treatments to remove nitrogen, mainly dissolved inorganic nitrogen (DIN), as well as organic matter from wastewater through coupled nitrification and denitrification. This upgrading of CAS to BNR influences effluent nitrogen by decreasing the total nitrogen discharge and varying the major effluent nitrogen species from \( \text{NH}_4^+ \) to more oxidized DIN (\( \text{NO}_2^- \) and \( \text{NO}_3^- \)) and dissolved organic nitrogen (DON) (Grady et al., 2011).

Nitrogen is often the nutrient that limits algal growth in receiving waters especially in the nitrogen-sensitive aquatic environments such as estuaries and oceans. Algae in receiving waters metabolize effluent nitrogen as a nutrient source. Thus, it is reasonable to expect that changes in effluent nitrogen resulting from upgrading CAS to BNR affect algal growth in receiving estuaries. However, current evaluation of eutrophication caused by effluents focuses only on the quantitative aspect (i.e., the total amount of nitrogen discharge) without reflecting the effects from different effluent nitrogen species (Pehlivanoglu and Sedlak, 2004).

Our earlier bioassay studies (Sheppard 2011; Eom et al., 2013), evaluating the algal growth stimulated by effluents from several full-scale WWTPs, showed that decreased effluent nitrogen discharge did not ensure alleviating algal blooms in receiving estuaries. In these studies, contrary to the general consensus, effluents containing less dissolved total nitrogen (DTN) drove 1.3 ~ 6 times greater productivity than effluents having more DTN. These findings led us to
question whether decreased nitrogen discharge achieved by upgrading WWTPs from CAS to BNR actually alleviates eutrophication in receiving estuaries. Upgrading CAS to BNR effectively lowers nitrogen discharge, thus decreasing the amount of available nitrogen to algae in receiving estuaries. However, this upgrade can cause changes in effluent nitrogen species. These alterations may bring unexpected and undesirable results. Thus, to assess actual benefits of upgrading CAS to BNR on eutrophication, the effects from not only decreased nitrogen discharge but also changed effluent nitrogen species should be weighed.

1.2 Research Objectives

The overall objective of this research was to evaluate the impact of CAS and BNR effluents on eutrophication in receiving estuaries. To achieve this goal, we first studied differences in effluent nitrogen from CAS and BNR systems. Then, we attempted to quantify and compare algal growth stimulated by CAS and BNR effluents. In addition, algal growth yields of effluent nitrogen species, including DIN, high molecular weight DON (HMW DON), and low molecular weight DON (LMW DON), were estimated. Analysis of these results allowed investigation of the effects of changes in effluent nitrogen caused by upgrading CAS to BNR on algal growth in receiving estuaries.

1.3 Organization of Chapters

In this thesis, Chapter 1 introduces the research background and objectives. Chapter 2 is a literature review of DON, mechanisms of DON utilization, CAS and BNR treatment processes, and effluent DON. The main research results are described in Chapter 3 to 5. The last Chapter 6 presents overall research conclusions.
Chapter 3: Investigation of differences in effluent nitrogen derived from conventional activated sludge and biological nutrient removal wastewater treatment systems.

This study compared effluent nitrogen derived from CAS and BNR processes in both lab-scale systems and a local full-scale WWTP. In addition, the transformations of nitrogen during wastewater treatment processing were analyzed. The results showed that the BNR systems generated less effluent DIN but more effluent DON than the CAS systems. The transition of treatment conditions from anoxic to oxic within the BNR process produced LMW DON, which was principally responsible for rendering the BNR effluents to have more DON compared to the CAS effluents.

Chapter 4: A comparison of algal growth in receiving estuaries stimulated by effluents from conventional activated sludge and biological nutrient removal processes.

This study investigated algal growth in receiving estuaries stimulated by CAS and BNR effluents. For this goal, bioassays incubating either CAS or BNR effluents of lab-scale and full-scale systems with natural estuary receiving water (LIS water) were performed. The outcomes demonstrated that BNR effluents, in spite of containing less DTN, stimulated greater amounts of algal biomass than CAS effluents. Among effluent nitrogen species, only consumed LMW DON strongly correlated with algal biomass generation, suggesting that LMW DON has greater impact on algal growth than DIN.

Chapter 5: Evaluation of the impact of upgrading wastewater treatment processes from conventional activated sludge to biological nutrient removal on algal growth in receiving estuaries.
This study evaluated the effects of changes in effluent nitrogen species resulting from upgrading CAS to BNR on eutrophication in receiving estuaries. To achieve this aim, algal growth yields of effluent DIN, HMW DON, and LMW DON were experimentally and numerically estimated. It was revealed that that LMW DON had 7 times higher algal growth yield than DIN, allowing the BNR effluents to generate more algal biomass than the CAS effluents. This result suggests that not only amounts but also compositions of effluent nitrogen should be considered for actual evaluation of algal growth driven by effluents in receiving estuaries.
CHAPTER 2

LITERATURE REVIEW

2.1 Dissolved Organic Nitrogen

Nitrogen serves as one of the major nutrient sources for organisms. It is found in all living cells and is a main constituent of proteins and nucleic acids. Total nitrogen in a water environment is divided largely into two categories, inorganic and organic nitrogen, based on whether it contains carbon molecules. Inorganic nitrogen, lacking carbon molecules, may exist either as a gas (N\textsubscript{2}) or in the ionic forms, including ammonia (or ammonium) (NH\textsubscript{3} (or NH\textsubscript{4}\textsuperscript{+})), nitrate (NO\textsubscript{3}\textsuperscript{-}), and nitrite (NO\textsubscript{2}\textsuperscript{-}). Because the ionic forms of inorganic nitrogen are readily dissolved in water, they are regarded as dissolved inorganic nitrogen (DIN).

Traditionally, organic nitrogen, containing carbon molecules, has been classified into particulate organic nitrogen (PON) and dissolved organic nitrogen (DON) by a filter pore size of 0.45 µm. According to the IAWQ models, categorizing dissolved organic substances according to molecular weight and lability is an effective way to describe their behavior within wastewater treatment processes (Henze et al., 1987). In terms of molecular weight, DON is divided into two parts, high molecular weight DON (HMW DON) and low molecular weight DON (LMW DON), using an ultrafilter with a 1K Dalton (Da) cutoff (Benner et al., 1992; McCarthy et al., 1996; Bronk et al., 2007). Based on lability, DON has three components (Bronk et al., 2002). The first one is refractory DON consisting of complicated substances and persisting in the environment for hundreds of years. The second component is semi-labile DON including dissolved combined amino acids (DCAA) and amino polysaccharides which turnover on annual time scales. The last
one is highly labile DON such as urea, dissolved free amino acids (DFAA) and nucleic acids, having a turnover in timescales of hours or minutes.

2. 2 Bioavailability of DON

Bioavailability is defined as the extent to which a substance can be utilized by microorganisms for their metabolism. Historically, DON in ocean waters was regarded as inert and was thus non bioavailable for microorganisms, especially phytoplankton. Earlier oceanographic research provided support for this belief. Generally, the DIN concentration in open seas falls below the limits of analytical detection; in contrast, the DON concentration constantly exceeds a specific value, 56 µg N/L, leading to the traditional thought that DON does not serve as a significant nitrogen nutrient for bacteria and phytoplankton (Antia et al., 1980; Bronk, 2002). However, in the 1970s and 1980s, studies showed that uptake of ammonium and the production of amino acids were tightly coupled in an oceanic environment, explaining why a certain concentration of DON was maintained in oceans (Caperon et al., 1979; Glibert et al., 1982; Fuhrman et al., 1987). Moreover, due to advanced nitrogen tracer techniques, it was shown that DON is released during planktonic growth (Bronk et al., 1991; Bronk et al., 1994) and that the rates of DON uptake and release were similar in magnitude, thereby explaining the invariant nature of DON concentrations in oceanic systems (Bronk and Glibert, 1993; Bronk et al., 1998).

In addition, since the 1990s many studies have reported the utilization of DON by microorganisms. For example, Seitzinger and Sanders (1997) demonstrated that 40 ~ 72 % of DON in the Delaware and Hudson Rivers was utilized by heterotrophic bacteria during 10 to 15 day dark bioassays. Jorgensen et al. (1999) reported bioavailability of DON (13 %) to bacteria in their 7 to 8 day bioassay tests. Other dark and light bioassay studies found that 12 ~ 72 % of DON in river and saline waters was bioavailable by bacteria (reviewed in Bronk, 2002). Not only
for bacteria but also for phytoplankton, DON is a significant nitrogen source. Benner et al. (1997) found that 30 to 50% of the nitrogen demand by phytoplankton in the equatorial North Pacific is supplied by bioavailable DON pools. LaRoche et al. (1997) estimated that nitrate and DON can facilitate major sources for brown tides in Long Island Sound during drought years. Berg et al. (2001) showed that DON is an important contributing factor to eutrophication and algal blooms in the Gulf of Riga, Baltic Sea. Several algal growth bioassay studies also reported various ranges of bioavailability of effluent DON (18 ~ 100 %) to a specific species of algae or a mixture of alga and bacteria (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008; Filippino et al., 2011; Mesfioui et al., 2012). Based on the above and other literature, it is now widely accepted that DON provides a significant nitrogen source in the metabolism of bacteria and phytoplankton in natural water conditions.

2.3 Mechanisms of DON Utilization by Phytoplankton

With increasing evidence of the bioavailability of DON, there have been increasing efforts to investigate the mechanisms of DON utilization by phytoplankton. Direct uptake of LMW DON can occur through active transport driven by a sodium ion pump or through facilitated diffusion (Mulholland and Lomas, 2008). However, this mechanism cannot be attributed to HMW DON because of its large and complex structure, showing that, unlike LMW DON, HMW DON is not directly bioavailable to phytoplankton. Therefore, phytoplankton must engage alternative mechanisms to degrade HMW DON to LMW DON or liberate inorganic nitrogen attached to HMW DON.

Enzymatic breakdown is one way to make HMW DON bioavailable. Proteolytic enzymes, which are usually attached to cell membranes or released from cells into the surrounding media, are able to break down large polymers to smaller ones. Palenik and Morel
(1991) found that several algal species possessed cell-surface enzymes, L-amino acid oxidases, which cleave off amino acids, producing free ammonium. Mulholland et al. (1998) estimated that approximately 20% of DON utilization in coastal and oceanic environments may result from amino acid oxidases. Peptide hydrolysis is another proteolytic process which can break the peptide bonds within proteins and liberate smaller peptides and amino acids (Mulholland et al. 2002). Christ (1991) reported that Leucine aminopeptidase (LAP) is capable of hydrolyzing peptide bonds. The author claimed that even if only bacteria produce this enzyme and phytoplankton cannot, phytoplankton still benefit from it released by bacteria. Jacobsen and Rai (1991) observed greater algal and bacterial growth where high aminopeptidase activity occurs but no algal growth without bacteria. Muholland et al. (2002) also found high rates of peptide hydrolysis in a mixture culture of bacteria and an algae species, *Aureococcus anophagefferens*.

Photochemical decomposition has been proposed as another mechanism for the consumption of HMW DON. Due to their aromatic nature, humic-substances are photo-reactive to ultraviolet light. They release inorganic nitrogen and DON under light source (Kieber et al., 1999). Among released compounds, NH$_4^+$ is most frequently reported nitrogen species (Bushaw et al., 1996). This photochemical decomposition has been observed in several places including an estuary in Georgia, US (Bushaw et al., 1996; Gao and Zepp, 1998) and a humic-rich lake in Venezuela (Gardner et al., 1998). In addition, humic-substances can be reactive with salinity. Humic acids contain a number of cation exchange sites, allowing them to retain ammonium (Rashid, 1969). In river or estuaries with low salinity conditions, ammonium can be adsorbed to humic-substances and then released when exposed to increased salinity conditions such as in seas or oceans (See and Bronk, 2005), demonstrating that humic-substances can behave like an inorganic nitrogen transport shuttle. Alberts and Takacs (1999) estimated that approximately up
to 77 tons of nitrogen can be delivered to Altamaha estuary over a year through this humic shuttle.

2.4 CAS and BNR Wastewater Treatment Processes

A biological wastewater treatment process using microorganisms to treat wastewater is robust and offers economic advantages both in terms of capital investment and operating costs over other treatment processes (Stricker and Beland, 2006). CAS and BNR are currently the most widely used biological treatment processes. In the CAS process, primary effluent from the primary clarifiers enters an aeration basin. Microbial aggregates or activated sludge flocs consume and oxidize organic matter (electron donors) collectively called carbonaceous biological oxygen demand (BOD). When the slurry of treated wastewater and microbial flocs move to the secondary clarifier, the flocs are removed from the treated wastewater by settling and returned to the aeration tank or wasted to control the SRT. The treated effluent is discharged to receiving waters or sent on for further treatment. The BNR process is designed to eliminate nitrogen and phosphorus from raw sewage water. Removal of nitrogen is primarily achieved by biochemical oxidation/reduction processes that convert inorganic nitrogen to nitrogen gas through two-step processes, consisting of nitrification and denitrification. During nitrification, ammonia is oxidized to nitrite by one group of autotrophic bacteria, most commonly Nitrosomonas. Nitrite is, in turn, oxidized to nitrate by another autotrophic bacteria group, the most common being Nitrobacter. Denitrification involves the biological reduction of nitrate to nitrogen gas. The most widely distributed denitrifying bacteria are the gram negative proteobacteria such as Pseudomonas, Alcaligens, Paracoccus, and Thiobacillus, which can use hydrogen, methanol, carbohydrates, organic acids, alcohols, benzoates, and other aromatic
compounds as electron donors. Under anoxic conditions, these microorganisms utilize nitrate as an electron acceptor, allowing nitrate to be reduced to nitrogen gas.

There are a number of configurations for BNR processes. Some BNR systems are designed to remove either nitrogen or phosphorus, while others remove both. The configurations of the BNR processes can be classified based on sequence of treatment conditions (i.e., aerobic, anaerobic, and anoxic) and timing (Jeyanayagam, 2005). Common BNR system configurations are the following: 1) the Ludzack-Ettinger (LE) process or modified Ludzack-Ettinger (MLE) process: a continuous flow suspended growth process with an initial anoxic treatment followed by an aerobic treatment; 2) an A2O process: an LE process preceded by an initial anaerobic stage; 3) the Bardenpho or modified Bardenpho process: a continuous flow suspended growth process with 3 or 4 stages of alternating anoxic and aerobic treatment; 4) a sequencing batch reactor (SBR) process: a fill-and-draw suspended growth batch process repeating anoxic and aerobic treatment. Although configurations for each BNR system differ, common and distinguishing features of BNR processes, unlike the CAS process, are having both aerobic and anaerobic/anoxic treatments, as well as relatively long SRTs to maintain favorable conditions for nitrifying and denitrifying microorganisms.

2.5 Fate of DON during Wastewater Treatment Processing

In the early 1980s, Parkin and McCarty (Parkin and McCarty, 1981a, b) pioneered research describing the removal and production of DON during wastewater treatment. They reported that DON in wastewater effluent includes: 1) inert DON which initially resides in raw wastewater and resists biological treatment, 2) biodegradable DON which originates from influent but cannot be completely removed by treatment, and 3) DON which is produced during treatment and accounts for approximately 35% of total DON in the effluent. Sattayatewa et al.
(2009) investigated the occurrence of organic nitrogen within a Bardenpho process plant consisting four serial compartments including the primary anoxic and aerobic and the secondary anoxic and aerobic processes. They pointed out that DON is produced in the first anoxic zone possibly due to the DON released from microbial activity. This produced DON was not removed or increased during the subsequent treatments. However, Czerwionka et al. (2012) and Huo et al. (2013) showed results inconsistent with Sattayatewa et al. (2009). Czerwionka et al. (2012) analyzed the conversion of colloidal organic nitrogen (CON) and DON across full-scale and lab-scale BNR reactors consisting of the first anoxic and subsequent aerobic treatment processes. They observed that CON and DON were removed under anoxic treatment; however, DON was generated during aerobic treatment in both full-scale and lab-scale BNR processes. Huo et al. (2013) measured DON concentrations along the treatment units of two BNR full-scale plants. They also found that most removal of DON occurred in the anaerobic process and an increase in DON was observed under the subsequent aerobic treatment.

2. 6 DON in Wastewater Treatment Effluents

Organic nitrogen in wastewater is comprised of highly heterogeneous molecules ranging from simple compounds (Henze, 1992). Depending on the operating conditions of a wastewater treatment process, the composition and concentration of effluent DON can vary. Earlier studies evaluated the impacts of influent carbon loading, SRTs, temperature, and reactor hydraulics on effluent DON. For example, Aquino and Stuckey (2003) illustrated that the amounts of carbon in influents have a strong linear correlation with soluble microbial products (SMPs) and contribute to production of effluent DON. Parkin and McCarty (1981a) noted that increased SRT could further degrade influent DON but generate more effluent DON. They found that the lowest effluent DON occurred when the aeration time was 6 ~ 9 hours in their bench-scale reactor,
which corresponded to 6 ~ 10 days of SRT at the local WWTP in Palo Alto, CA. Barker and Stuckey (1999) also reported that 2 ~ 15 days of SRTs can minimize effluent DON in aerobic biological wastewater processing. Sharp et al. (2009) showed the effects of temperature on effluent DON. They observed that as temperature increased from 17 to 25 °C, concentrations of effluent DON decreases from 1.4 mg N/L to 0.8 mg N/L in a BNR full-scale plant. Furthermore, O’Shaughnessy et al. (2006) demonstrated that reactors in parallel produced greater amounts of effluent DON than reactors in series during 2 years of full-scale plant operations.

Typically, DON concentration in secondary treated effluent ranges from 1 to 5 mg N/L, accounting for 25 ~ 50 % of overall effluent DTN (reviewed by Pehlivanoglu and Sedlak, 2008). There has been efforts to quantify specific components of DON such as urea, amino acids and protein. In raw wastewater, urea accounts for 80 % of organic nitrogen and is generally converted to ammonia in the wastewater collection system and primary treatment; thus, it is rarely identified in effluents (Hanson and Lee, 1971). The concentrations of total amino acids in effluents are reported to be 0.017 ~ 0.084 mg N/L (Scully et al., 1988; Grohmann et al., 1998). Dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA) account for 0.05 ~ 3% and 0.6 ~ 13 % of effluent DON, respectively (Parkin and McCarthy, 1981a; Confer et al., 1995; Dignac et al., 2000b; Pehlivanoglu and Sedlak, 2008). Furthermore, Manka and Rebhun (1982) estimated that 14 ~ 25 % of soluble organic compounds in wastewater effluents are protein. Westgate and Park (2010) showed that amounts of protein-nitrogen in effluents of field full-scale plants were 0.6 ~ 1.9 mg N/L, accounting for approximately 60 % of effluent DON. Hwang et al. (1995) demonstrated that the amounts of aliphatic amine, which contributes to odor problems, in effluents ranged between less than 0.14 and 60 µg/L. In wastewater effluents, nitrogen-containing synthetic chelating agents were also found. EDTA, one of the most
studied synthetic chelating agents in effluents, has been detected at levels between 0.14 and 14 µg/L (Alder et al., 1990). Pehlivanoglu and Sedlak (2008) concluded that only approximately 30% of effluent DON is identifiable: DCAA and DFAA, 10-20%; EDTA, less than 5%; and humic-substances, 10%; the remaining 70% represents an uncharacterizable fraction, considered to be a complex combination of partially metabolized compounds of biogenic origin.

Analyzing the molecular weight and hydrophilicity of effluent DON is another way to investigate its chemical properties. Several studies found that most effluent DON passes through a 10K Da filter and at least 50% of DON is less than 1K Da, suggesting that effluent DON is largely composed of LMW DON (Keller et al., 1978; Parkin and McCarty, 1981a; Bratby et al. 2008; Pehlivanoglu and Sedlak, 2008). Liu et al. (2008) and Huo et al. (2013) demonstrated that more than 80% of effluent DON is hydrophilic compounds. They explained that hydrophobic compounds in wastewater would be easier removed by adsorption by activated sludge and other particles; however, hydrophilic compounds have a low affinity for the surface of organic particles and thus DON having this characteristic, hydrophilicity, can stay in effluents.

2. 7 Bioassay Tests Evaluating Bioavailability of Effluent DON

In the 1970s and 1980s, Parkin and McCarty measured the bioavailability of effluent DON for microorganisms, but with conflicting results. In their 1975 study, DON released from activated sludge failed to support the growth of algal species. However, in a 1981 study, they found up to 60% of DON in wastewater effluents was utilized by bacteria over a period of 60 days. Based on these outcomes, the authors concluded that effluent DON can serve as a nitrogen source for not algae but bacteria.
Since the Parkin and McCarty research, a series of bioassay tests have been conducted to evaluate bioavailability of effluent DON under different conditions. In a study by Pehlivanoglu and Sedlak (2004), the bioavailability of effluent DON from a full-scale BNR treatment plant was investigated using bioassays incubating a single species of algae, *Selenastrum capricornutum*, with or without bacteria inoculum which had been obtained from river receiving waters. Effluent DON was not bioavailable in the absence of bacteria; however, 60% of the effluent DON was used up in the presence of bacteria over 2 weeks. Moreover, they found that chlorophyll a production was nearly identical between bioassays incubated with unfractionated and 1K Da ultrafiltrated effluents, suggesting that algal growth is not dependent on HMW DON.

Urgun-Demirtas et al. (2008) incubated nitrified or denitrified wastewater effluents which had been passed through a 1.2 µm filter with an algae species, *Selenastrum capricornutum*, or bacteria inoculum, or both in 8 different bioassay conditions for 14 days. They observed the highest DON consumption (61%) from the bioassay employing bacteria, algae, and additional nitrate together, compared to the lowest DON utilization (18%) from the bioassays containing algae only. Furthermore, they observed that the higher decrease in DON corresponded to the greater algal biomass generation in their bioassays. From these results, they claimed that low total nitrogen wastewater effluent having a higher percentage of DON is a good nitrogen source to stimulate algal growth in receiving waters.

Bronk et al. (2010) designed a more realistic experimental set-up to examine the fate of effluent DON along the receiving water. Their bioassays contained receiving waters of varying salinities and a small volume of wastewater effluent, which had been concentrated using rotary evaporation, from two full-scale BNR WWTPs. The increasing salinity of the receiving water was intended to describe the passage of effluent from freshwater to more saline water. They
showed that a larger percentage of DON is removed in the higher salinity bioassays (12% in lower salinity vs. 23% in higher salinity). Also, they found that release of amines from DON during exposure to sunlight or higher salinities, showing that DON is both photo and saline-reactive.

Liu et al. (2011) assessed the bioavailability of DON depending on its hydrophilicity. Before they incubated effluents from BNR full-scale plants with a mixture of algae and bacteria inoculum in bioassays, effluent DON was treated by Amberite XAD-8 resin to separate hydrophobic and hydrophilic DON. The hydrophilic DON stimulated algal growth (0.5 mg N/L of hydrophilic DON caused 0.22 mg/L of chlorophyll a during 14 days of bioassay operation), but hydrophobic DON did not drive algal growth. They estimated that hydrophilic DON is protein enriched DON, whereas, hydrophobic DON is humic enriched DON based on the C/N ratios of dissolved organic substance.

Filippino et al. (2011) performed similar study to Bronk et al. (2010). In this bioassay study, effluents from full-scale BNR WWTPs were mixed with three receiving waters having different salinity to describe the travel of the effluent from freshwater to saline water. They reported that all of the inorganic nitrogen and 31% to 96% of DON were removed within the first 2 days. In addition, they found that the stimulated dominant algal species in bioassays varied depending upon the salinity. In low salinity, specific algal species was not dominant. However, in middle salinity, dinoflagellates were stimulated a lot; in high salinity, cyanobacteria was predominant.

Mesfioui et al. (2012) attempted to determine bioavailability of effluent DON using fourier transform ion cyclotron resonance mass spectrometry. Their bioassays were conducted by employing effluents from full-scale BNR plants and natural river receiving waters. Their results
showed that the lignin-like fraction of effluent DON was almost conserved but a large portion (79 ~ 100 %) of aliphatic and aromatic fraction of effluent DON was removed and supported growth of a mixture of algae and bacteria inoculum.

Qin et al. (2015) investigated the impact of hydrophilicity of effluent DON on bioavailability, similar to Liu et al. (2011). Tertiary effluents from two full-scale BNR plants were separated into two fractions, hydrophilic and hydrophobic DON, using XAD-8 resin. Their study demonstrated that although overall bioavailability of DON varies (28 ~ 61 %) among the bioassays, most of the bioavailable effluent DON was hydrophilic DON, which was consistent with Liu et al. (2011).
2.8 References


Bronk, DA. (2002). Dynamics of DON. *In Biogeochemistry of Marine Dissolved Organic Matter*


CHAPTER 3

INVESTIGATION OF DIFFERENCES IN EFFlUENT NITROGEN DERIVED FROM
CONVENTIONAL ACTIVATED SLUDGE AND BIOLOGICAL NUTRIENT
REMOVAL WASTEWATER TREATMENT SYSTEMS

To be submitted to Water Research

3.1 Abstract

The objective of this study was to investigate differences in effluent nitrogen generated from conventional activated sludge (CAS) and biological nutrient removal (BNR) systems. For this purpose, we compared effluent nitrogen in lab-scale CAS and BNR sequencing batch reactors (SBRs) treating identical wastewater and also in a local wastewater treatment plant (WWTP) before and after upgrading its process from CAS to BNR. In the lab-scale systems, the BNR process achieved lower concentrations of effluent dissolved total nitrogen (DTN) than the CAS processes mainly due to decrease in amounts of effluent dissolved inorganic nitrogen (DIN). However, the BNR system generated more effluent dissolved organic nitrogen (DON) than the CAS systems, particularly low molecular weight DON (LMW DON) and proteinaceous DON. Analysis of effluent nitrogen data from the local WWTP showed similar results as in the lab-scale systems. When the local WWTP was retrofitted from CAS to BNR, effluent DIN decreased but effluent DON increased. To understand causes for these variations in CAS and BNR effluent nitrogen, nitrogen profiles during wastewater treatment processing were analyzed. The results demonstrated that the transition from anoxic to aerobic conditions within the BNR process caused the production of LMW DON, leading the BNR effluents to contain greater amounts of DON than the CAS effluents.
3. 2 Introduction

Wastewater-originated nitrogen leads to substantial nitrogen loading in natural water systems, accounting for 12 ~ 33 % of nitrogen pollution in rivers world-wide and contributing to eutrophication in receiving waters (Howarth, 2004). Wastewater treatment plants (WWTPs) are accordingly required to decrease their nitrogen discharge. Complying with these regulations has meant upgrading WWTPs’ processes from conventional activated sludge (CAS) to biological nutrient removal (BNR). The CAS process utilizes microbial aggregates (activated sludge flocs) in an aeration tank to degrade soluble organic matter in wastewater. The BNR processes not only treat organic substances but also remove nitrogen, mainly dissolved inorganic nitrogen (DIN), from wastewater through alternating aerobic nitrification and anaerobic denitrification with extended solid retention times (SRTs).

The general consensus is that decreasing amounts of nitrogen discharge from BNR processes alleviate eutrophication in receiving waters compared to that from CAS process. However, our earlier bioassay study (Sheppard, 2011), investigating algal growth caused by effluents, demonstrated results contrary to this belief. In this earlier study, BNR effluents with lowered concentrations of effluent dissolved total nitrogen (DTN) stimulated 1.3 to 6 times greater productivity than CAS effluents. This finding suggests that a decrease in nitrogen discharge achieved by upgrading CAS to BNR cannot ensure alleviating algal blooms in receiving waters.

Upgrading CAS to BNR not only lowers amounts of effluent nitrogen but also changes the composition of effluent nitrogen (Grady et al. 2011). Basically, microorganisms expend unequal energy costs in assimilating diverse of nitrogen sources, causing different microbial growth (Rittmann and McCarty, 2001). Thus, the changes in effluent nitrogen species resulting
from upgrading CAS to BNR can affect algal growth in receiving waters. To evaluate this influence, understanding of variations in CAS and BNR effluent nitrogen is necessary. It is generally known that the major nitrogen species in the CAS effluent is NH$_4^+$, whereas, in BNR effluent, NO$_3^-$ and dissolved organic nitrogen (DON) constitute the dominant nitrogen forms (Grady et al. 2011). However, no research, to date, has directly compared effluent nitrogen species, especially effluent DON, of CAS and BNR processes from identical sources of wastewater.

This study investigated differences in effluent nitrogen derived from CAS and BNR processes. In addition, transformations of nitrogen during wastewater treatment processing were analyzed to identify the causes for variations in CAS and BNR effluent nitrogen. We assumed that making these comparisons at the full-scale plant level would be challenging. Field WWTPs have different characteristics of influents and operating conditions, thus not isolating the impact of the treatment process on effluent nitrogen. In this study, we employed lab-scale CAS and BNR systems treating the same wastewater, the primary effluent from a local WWTP (the Amherst WWTP, MA, US), to generate CAS and BNR effluents under controlled conditions. In addition, during the period of this study (in early 2013), the local WWTP changed their operating mode from CAS to BNR to comply with a new nitrogen regulation. This provided us with the unique opportunity to study changes in effluent nitrogen before and after upgrading CAS to BNR within the same full-scale plant.
3. Materials and Methods

3.1 Lab-scale CAS and BNR Systems and Local WWTP

A total of three lab-scale (two CAS and one BNR) wastewater treatment systems, seeded with identical activated sludge from the local WWTP, were operated in a sequencing batch reactor (SBR) mode. One sequencing cycle lasted for a total of 6 hours, consisting of 10 minutes of influent feeding, 4 hours and 50 minutes of treatment, 50 minutes of settling, and 10 minutes of effluent decanting. The CAS processes contained only aerobic treatment, whereas, the BNR process included first anoxic (2 hours and 20 minutes) and subsequent aerobic (2 hours and 30 minutes) treatments (The detailed operation schematic layout is presented in Appendix, Figure A.1). The aerobic conditions in both the CAS and BNR systems were maintained by aerating the same house air. The anoxic condition in the BNR system was created by sparging nitrogen gas. All the CAS and BNR systems were fed 6 L of identical influent, which was the primary effluent collected from the local WWTP, with 0.75 days of hydraulic retention time (HRT). One CAS system (CAS 1) had 6 days of solid retention time (SRT); the other CAS (CAS 2) and BNR systems each had 20 days of SRT. The CAS 1 and BNR reactors started operation on September 15th, 2012; the CAS 2, on July 5th, 2013. These three lab reactors were continuously operated until January 12th, 2015. At least once per week, concentrations of soluble chemical oxygen demand (sCOD), total suspended solids (TSS), volatile suspended solids (VSS), dissolved organic carbon (DOC), dissolved total nitrogen (DTN), DIN species, DON, and PO$_4^{3-}$ in influents and all effluents were analyzed. Average values of the 124 analyses for the CAS 1 and BNR systems and the 98 analyses for the CAS 2 system during two years (2013 and 2014) are presented in the results.
The local WWTP is located in a college town in the Amherst, Massachusetts, US. With nearly one-third of the loading originating from colleges, this plant has an influent flow rate, on average, of 4 million gallons per day. Two of three aeration trains are operated routinely with 6 hours of HRT. This local WWTP retrofitted its treatment process from CAS to BNR in early 2013 by introducing intermittent aeration without expanding the main basin. Before upgrading, it employed mechanical aeration with approximately 10 days of SRT; after upgrading, it adopted a modified Ludzack-Ettinger (MLE) process with 15 ~ 20 days of SRT. To analyze effluent nitrogen of this plant, we received data about the concentrations of effluent DTN, DIN species, and total kjehldahl nitrogen (TKN) from the plant. The local WWTP measured effluent nitrogen concentrations every 4 or 5 days. A total of 79, 172, and 98 data sets in 2012, 2013, and 2014, respectively, were used for determining average values presented in the results. Except for the concentrations of effluent nitrogen, we measured sCOD, TSS, VSS, DOC, and PO$_4^{3-}$ in effluents (22, 27, and 19 times in 2012, 2013, and 2014, respectively) of this plant.

3. 3. 2 Nitrogen Profile Analysis

To understand the causes creating variations in effluent nitrogen of CAS and BNR processes, nitrogen profiles were analyzed within the lab-scale systems. In the CAS reactors, a total of three samples were collected at the beginning, middle, and near the end point of one aeration cycle; in the BNR reactor, two samples each under anoxic and aerobic treatment conditions were collected. Then, concentrations of nitrogen species in the collected samples were determined. A total of 5 of nitrogen profile analyses in 2014 (April 17$^{th}$, June 2$^{nd}$, August 26$^{th}$, October 10$^{th}$, and December 6$^{th}$) were performed; the average values are presented in the results.

Additionally, to evaluate the effects on effluent nitrogen of orders of treatment conditions within wastewater treatment processes, the four SBRs, Anoxic(A)/Oxic(O), O/A, A/O/A, and
A/O/A/O, were operated in 2015 (Figure A.2 depicts detailed treatment sequencings of these SBRs). All four SBRs were seeded and fed activated sludge and primary effluents from the local WWTP. They had influent flow rates of 6 L/one sequencing cycle with 0.75 days and 20 days of HRT and SRT, respectively. The A/O and O/A reactors were begun on August 1st, 2015 and were allowed a seven day stable period. Nitrogen profiles were then analyzed on August 8th, 9th, 10th, 2015. Similarly, the A/O/A and A/O/A/O reactors were operated in a stable mode from August 11th through 17th, 2015, and then nitrogen profiles were investigated for the next three days.

3.3.3 Chemical Analysis

In this study, the dissolved and low molecular weight fractions were the substances passing through a 0.45 µm nitrocellulose membrane and a 1K Dalton (Da) ultrafilter, respectively. For the low molecular weight fractions, an Amicon stirred cell ultrafiltration device (Millipore Corp., USA) was employed with a 1K Da molecular weight cut-off cellulose membrane (76 mm; Millipore Corp., USA). The concentrations of DTN and DOC were measured by a Shimadzu TN analyzer (Shimadzu TOC-VCPH with TNM-1, Shimadzu North America, SSI Inc., Columbia, MD). All DIN species (NH₄⁺, NO₂⁻, NO₃⁻) and PO₄³⁻ were determined using a Metrohm ion chromatograph (Metrohm, Herisau, Sz). The amount of DON was calculated by subtracting the amount of DIN from the amount of DTN. Low molecular weight DON (LMW DON) was the difference between LMW DTN and DIN. High molecular weight DON (HMW DON) was determined by the difference between DON and LMW DON. Proteinaceous nitrogen was measured based on the modification of Lowery method described in Frølund et al (1995) with a calibration curve generated with bovine serum albumin (Fisherbrand
Scientific, Pittsburg, PA, USA). The concentrations of TSS, VSS, sCOD were analyzed according to Standard Method 2540 D, E and 5220 B, respectively (APHA, 2005).

3. 3. 4 Statistics

Data were graphed using Microsoft Office Excel 2013 and Sigma Plot 10. To evaluate the statistical significance between the results of CAS and BNR effluent DON in the lab-scale systems and local WWTP, p-values were calculated based on unpaired t-test with unequal variance (Welch’s t-test) using the method proposed in the study of Welch (1947).

3. 4 Results and Discussions

3. 4. 1 Removal of Carbon and Phosphorus in Lab-scale Systems and Local Full-scale WWTP

Over a two year period (2013 and 2014), the average concentrations of TSS and VSS in the influents for the lab-scale systems were 42.3 and 35.3 mg/L, respectively. The lab-scale reactors showed 57 ~ 65 % solids removal efficiency; their effluents contained, on average, 15 ~ 18 mg/L of TSS and 12 ~ 15 mg/L of VSS. The average concentrations of sCOD and DOC in the influents for the lab-scale systems were 105.7 and 33.5 mg/L, respectively. All the lab-scale systems demonstrated similar sCOD and DOC removal efficiency: 77 ~ 80 % of sCOD and 65 ~ 69 % of DOC. Their average effluent sCOD and DOC concentrations were 20.9 ~ 24.1 mg/L and 10.5 ~ 11.8 mg/L, respectively. This removal of organic matter in the lab-scale reactors was comparable to the local WWTP. During two years (2013 and 2014), the average amounts of solids and sCOD in the effluents of the local WWTP were 10 ~ 12 mg/L and 21.2 mg/L, respectively.
The concentration of PO$_4^{3-}$ in the influents for the lab-scale systems was, on average, 3.7 mg P/L. The effluents from the lab-scale CAS 1 and 2 reactors contained similar amounts of PO$_4^{3-}$, 3.3 and 3.1 mg P/L, respectively. The lab-scale BNR system removed approximately 27% of influent PO$_4^{3-}$; it showed an average of 2.7 mg P/L effluent PO$_4^{3-}$ concentration. During two years (2013 and 2014), the effluents from the local WWTP had an average of 2.4 mg P/L PO$_4^{3-}$ concentration, not substantially different from that of the lab-scale BNR reactor. Basically, our lab-scale BNR was a simple anoxic/oxic (A/O) process and the local WWTP was a modified Ludzack-Ettinger (MLE) process. These systems were not designed to optimize removing phosphorus, thereby resulting in insignificant PO$_4^{3-}$ removal.

### 3.4.2 Comparisons of Concentrations of CAS and BNR Effluent Nitrogen Species in Lab-scale Systems and Local Full-scale WWTP

Table 3.1 presents average concentrations of DTN, DIN, and DON in the influents and effluents during operation of lab-scale reactors. In the influents, the average amount of DTN was 31.5 mg N/L consisting of 26.1 mg N/L of DIN, which was entirely NH$_4^+$-N, and 5.4 mg N/L of DON. Both CAS systems showed a very similar nitrogen removal efficiency of 22%; their effluents contained, on average, 25.1 mg N/L of DTN. The BNR system eliminated 50% of influent DTN; its average effluent DTN concentration was 16.3 mg N/L.

<table>
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<th>DTN (mg N/L)</th>
<th>DIN (mg N/L)</th>
<th>DON (mg N/L)</th>
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<td>NO$_3^-$N</td>
<td>NH$_4^+$-N</td>
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<td>BNR effluent</td>
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</table>

Table 3.1 Average concentrations of DTN, DIN, and DON in influent and effluents during operations of lab-scale reactors in 2013 and 2014. (Unit: mg N/L, Standard deviation is in the parenthesis)
This nitrogen removal efficiency of our lab-scale BNR reactor was comparable to that of a previously evaluated lab-scale BNR system, sharing similar working conditions as ours and treating real domestic wastewater, in Zhao et al. (2008). They investigated the impact of varying influent COD/TKN ratios on nitrogen removal in a lab-scale SBR reactor. In their study, when the COD/TKN ratio was increased from 2.8 to 5.7, the nitrogen removal efficiency increased from 44% to 81%. In our lab-scale BNR reactor, the average COD/TKN value of influents was 3.2 and the nitrogen removal efficiency was 50%, falling within the ranges reported by Zhao et al. (2008). However, the local WWTP eliminated more DTN than our BNR system. In 2013 and 2014, the average effluent DTN concentrations of the local WWTP were 11.4 and 10.4 mg N/L, respectively. This greater DTN removal might have resulted from the local WWTP’s ability to control aerator speeds and cycles by monitoring dissolved oxygen (DO) in real time to optimize nitrification and denitrification processes, thereby achieving lower levels of effluent DTN.

The CAS 1 effluent DTN consisted of 23.0 mg N/L of DIN (92% of DTN) and 2.1 mg N/L of DON (8% of DTN); the CAS 2 effluent DTN, 22.6 mg N/L of DIN (90% of DTN) and 2.5 mg N/L of DON (10% of DTN). Significant differences in effluent nitrogen species between the CAS 1 and 2 effluents were NO$_3^-$ and DON. The proportion of NO$_3^-$ to DIN in the CAS 1 effluent was 77%; in the CAS 2 effluent, 93%. These values reflect the degree of nitrification in wastewater treatment processes. Since the growth rate of nitrifying bacteria is slow, long SRTs are required to achieve full-nitrification, conversion from NH$_4^+$ to NO$_3^-$, in wastewater treatment (Rittmann and McCarty, 2001). The lab-scale CAS 1 reactor had a relatively short SRT (6 days) which did not allow full-nitrification. Subsequently, it contained more NH$_4^+$ and NO$_2^-$ than did the lab-scale CAS 2 reactors. Furthermore, the SRTs influenced the effluent DON in our CAS systems. The lab-scale CAS 2 reactor generated approximately 20% more effluent DON than
the lab-scale CAS 1 reactor. Parkin and McCarthy (1981) concluded that increased SRTs could further degrade influent DON but produce more effluent DON in an aerobic biological wastewater treatment process. They suggested that the lowest effluent DON occurred at 6 ~ 10 days of SRTs. Similar to their observations, 6 days of SRT resulted in less effluent DON than 20 days of SRT in our CAS systems.

The BNR effluent DTN was comprised of 12.5 mg N/L (77 % of DTN) of DIN, which was almost entirely NO$_3^-$-N, and 3.8 mg N/L (23 % of DTN) of DON, showing that the BNR effluent had less DIN but more DON compared to the CAS effluents. The difference in effluent DIN between the CAS and BNR effluents was about 10 mg N/L, accounting for 110 % of the variation in effluent DTN. This result confirms that the main cause of decreased nitrogen discharge achieved by the BNR process was attributed to removal of DIN from wastewater. The BNR effluent contained 81 % and 52 % of more effluent DON than did the CAS 1 and 2 effluents, respectively (CAS 1: 2.1 mg N/L vs. CAS 2: 2.5 mg N/L vs. BNR: 3.8 mg N/L). To evaluate whether these differences in effluent DON of CAS and BNR systems were statistically significant, unpaired t-tests with unequal variance were conducted on data sets of effluent DON of the CAS 1, 2 and BNR systems. The calculated p-value between the CAS 1 and BNR systems was 0.014; between the CAS 2 and BNR systems, 0.027. Both these values were less than 0.05, indicating that the differences in effluent DON were statistically significant. To the best of our knowledge, this is the first report to directly compare absolute amounts of effluent DON in CAS and BNR processes from identical sources of wastewater.

These decrease in effluent DIN and increase in effluent DON between the CAS and BNR processes were also observed in the local WWTP. Table 3.2 illustrates the average
concentrations of effluent DTN, DIN, and DON when the WWTP was operated in CAS (2012) and BNR (2013 and 2014).

**Table 3.2** Average concentrations of DTN, DIN, and DON in effluents of the local WWTP in 2012, 2013, and 2014. (Unit: mg N/L, Standard deviation is in the parenthesis)

<table>
<thead>
<tr>
<th>Year</th>
<th>DTN</th>
<th>DIN</th>
<th>DON</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>16.6 (5.1)</td>
<td>15.6 (4.2)</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>2013</td>
<td>11.4 (4.7)</td>
<td>9.8 (4.6)</td>
<td>1.6 (0.3)</td>
</tr>
<tr>
<td>2014</td>
<td>10.4 (5.3)</td>
<td>8.6 (4.8)</td>
<td>1.8 (0.3)</td>
</tr>
</tbody>
</table>

As the plant was changed from CAS to BNR, effluent DTN decreased (from 16.6 mg N/L to 11.4 and 10.4 mg N/L) mainly by reducing DIN (from 15.6 mg N/L to 9.8 and 8.6 mg N/L); however, effluent DON (from 1.0 mg N/L to 1.6 and 1.8 mg N/L) increased. The calculated p-value based on the unpaired t-tests with unequal variance between effluent DON in 2012 and 2013 was 0.041; between in 2012 and 2014, 0.044, showing statistical significance. Although further research is needed to generalize the impact of CAS and BNR processes on effluent DON, outcomes from our lab-scale systems and data analysis for the local full-scale WWTP suggest that the BNR process not only has a greater proportion of effluent DON to effluent DTN but also generates more absolute amounts of effluent DON than does the CAS process.

**3. 4. 3 Comparisons of Characteristics of Effluent DON in Lab-scale Systems and Local full-scale WWTP**

Influent and effluents in our lab-scale systems was subjected to a size fractionation intended to separate HMW DON and LMW DON. In the influents, average concentration of LMW DON was 4.0 mg N/L, which was 74% of total influent DON. The CAS 1, 2, and BNR
The ratio of carbon to nitrogen (DOC/DON) of 0.45 µm filtered dissolved organic fractions in the lab-scale effluents was analyzed to investigate the lability of effluent DON. Earlier studies suggested that the C/N ratio in organic matter can serve as an indicator for its source (Stepanauskas et al., 1999; Westerhoff and Marsh, 2002; Leenheer et al., 2007). They proposed that a high C/N ratio, 15 ~ 30, is a humic-like DON and a low C/N ratio, 3 ~ 6, is a proteinaceous-like DON. Moreover, it was claimed that organic substances having a high C/N ratio tend to resist biodegradation due to their complex molecular structures. Based on the
DOC/DON ratios, all the influent and effluent DON in our lab-scale systems were proteinaceous-like DON. In the influents, the average DOC/DON ratio was 6.2, which was decreased to 5.0 and 4.7 by the CAS 1 and 2 processes, respectively, and to 3.0 by the BNR process. Compared to the CAS 1 and 2 effluents, the BNR effluent showed a lower DOC/DON value, suggesting that the BNR effluent DON was more protein-enriched and could be more biodegradable than the CAS effluent DON. This was confirmed by quantification of effluent protein measured by the Frølund adaptation of the Lowery method. In the CAS 1 and 2 effluents, the average concentrations of protein were 0.42 and 0.52 mg N/L (both 20% of effluent DON), respectively; the BNR effluent contained approximately twice as much amounts of protein, 0.92 mg N/L (24% of effluent DON). Unlike the DOC/DON values, the C/N ratio (LMW DOC/LMW DON) in 1K Da ultrafiltered LMW organic fractions of the CAS and BNR effluents was comparable. In the CAS 1 effluent, its average value was 3.2; in the CAS 2 effluent, 3.1; in the BNR effluent, 2.8. This finding demonstrates that LMW DON in the CAS 1, 2, and BNR effluents had similar characteristics despite being derived from different treatment processes. In the local WWTP, CAS and BNR effluents showed similar DOC/DON trends to our lab-scale reactors. In 2012, when the WWPT operating mode was CAS, the average DOC/DON ratio in effluent was 5.3. After the WWTP had been converted to BNR, it decreased to 3.4 in 2013 and 3.1 in 2014. Furthermore, the LMW DON/LMW DON value in effluents in 2014 was 2.9.

The two variables, LMW DON/DON and DOC/DON, in the effluents are basically related with each other. As LMW DON/DON increases and DOC/DON decreases, bioavailability of effluent DON is expected to become greater. Figure 3.1 illustrates the inverse relationship of these two ratios in our lab-scale reactors. Compared to the lab-scale CAS systems, the lab-scale BNR system generated more amounts of LMW DON and proteinaceous-like DON.
These results suggest that the type of treatment process can affect characteristics of effluent DON, possibly resulting in different bioavailability of effluent DON when it releases to receiving waters.

![Figure 3.1](image-url)

**Figure 3.1** Relationship between effluent LMW DON/DON and DOC/DON in lab-scale systems.

### 3.4.4 Transformation of Nitrogen within Lab-scale CAS and BNR Systems

To understand how different effluent nitrogen species are derived from the CAS and BNR processes, nitrogen profiles showing the transformations of nitrogen within the lab-scale CAS and BNR systems were investigated. During this analysis, the average influent DTN concentration was 23.7 mg N/L, consisting of 18.2 mg N/L of DIN and 5.5 mg N/L DON. All the influent DIN was NH$_4^+$-N and 78% of influent DON (4.3 mg N/L) was LMW DON. The transformations of DIN species are illustrated in Figure 3.2.
Figure 3.2 DIN profiles in lab-scale systems. (A) CAS 1 reactor; (B) CAS 2 reactor; (C) BNR reactor (Error bars represent standard deviation of five measurement).
In the CAS systems, as aeration was maintained, $\text{NH}_4^+$ decreased and $\text{NO}_3^-$ increased. In the CAS 1 reactor, an increase of $\text{NO}_3^-$-N accounted for 64 % of the decrease in $\text{NH}_4^+$-N; in the CAS 2 reactor, 73 %. These changes suggest that some of $\text{NH}_4^+$ was oxidized to $\text{NO}_3^-$ possibly due to nitrification. In the BNR system, during the first anoxic treatment, influent $\text{NH}_4^+$-N fell 11.0 mg N/L; however the production of $\text{NO}_3^-$-N was only 2.9 mg N/L. Some of this difference might be accounted for by elimination of DIN from wastewater through nitrification and denitrification processes. DIN removal under the first anoxic condition in the BNR system was 8.0 mg N/L, representing 80 % of overall DTN removal, again confirming that lower DTN discharge of BNR was based on the removal of DIN. During the subsequent aerobic treatment, the remaining 7.2 mg N/L of $\text{NH}_4^+$-N fell to zero; $\text{NO}_3^-$-N increased by 5.0 mg N/L. It was most likely that $\text{NH}_4^+$ was converted to $\text{NO}_3^-$ through nitrification.

Figure 3.3 depicts the DON profile showing the fate of DON from the influent to the effluents. In the two CAS reactors, DON (HMW DON and LMW DON) continuously decreased as aeration proceeded (CAS 1: from 5.5 to 3.4 mg N/L; CAS 2: from 5.5 to 4.1 mg N/L). Removal of LMW DON in CAS 1 and CAS 2 accounted for 87 % and 77 % of the decrease in DON, respectively. In the BNR reactor, during the first anoxic treatment, DON decreased from 5.5 to 3.9 mg N/L; removal of LMW DON contributed 91 % of the decrease in DON. However, under the subsequent aerobic treatment, DON increased from 3.9 to 5 mg N/L (p-value: 0.009); generation of LMW DON accounted for 100 % of this increase in DON, which was the main cause for the BNR effluents to contain more DON than the CAS effluents.
Figure 3.3 DON profiles in lab-scale systems. (A) CAS 1 reactor; (B) CAS 2 reactor; (C) BNR reactor (Error bars represent standard deviation of five measurement).
It is thought that hydrolysis of HMW DON and cell lysis occurring during the transition of the treatment process in BNR contributed to the formation of LMW DON. In the BNR process, the amount of HMW DON remained almost unchanged under the first anoxic condition; to the contrary, 43% of HMW DON was degraded under the subsequent aerobic condition, a possible indication that HMW DON was hydrolyzed to LMW DON. In addition, cell lysis can release LMW DON. Foladori et al. (2015) investigated cell lysis of return sludge exposed to alternating aerobic and anaerobic conditions. Their study was unable to identify a significant reduction in the total amount of cells in anaerobic conditions; however, after moving to aerobic conditions, the total number of cells substantially decreased. They attributed this observation to cells initially damaged under anaerobic conditions undergoing cell lysis when exposed to aerobic conditions. This implies that cell lysis can occur in the aerobic treatment following anoxic treatment in BNR, resulting in possible generation of LMW DON. These DON profile analyses demonstrate that different behaviors of LMW DON under aerobic treatment in the CAS (removal of LMW DON) and BNR (generation of LMW DON) caused variations in DON between the CAS and BNR effluents. There have been previous studies reporting similar results as ours. Czerwionka et al. (2012) found that most DON removal occurred in anaerobic and anoxic units, whereas DON was increased in the aerobic unit during operation of their batch bioreactors. Huo et al. (2013) also observed a major removal of DON under anaerobic/anoxic compartments and a slight increase in DON in the oxic zone in full-scale BNR plants.

3. 4. 5 Effects of Orders of Sequencing Treatment Conditions on Effluent DON

To evaluate the effects of orders of sequencing treatment conditions on effluent DON, DON profiles within a total of four sets of SBR configurations, A/O, O/A, A/O/A, and A/O/A/O, were investigated. Figure 3.4 compares the results in the A/O and O/A processes.
Figure 3.4 DON profiles in A/O and O/A systems. (A) A/O reactor; (B) O/A reactor. (Error bars represent standard deviation of three measurement).

Under anoxic conditions in both the A/O and O/A processes, regardless of treatment order, changes in DON were similar: HMW DON was persistent and significant removal of LMW DON occurred (1.2 mg N/L in A/O and 0.8 mg N/L in O/A). However, under oxic conditions, behavior of DON in these two processes differed depending on treatment order. When oxic followed anoxic (A/O), which was the same configuration as the lab-scale BNR system, HMW DON (0.5 mg N/L) was removed but LMW DON (0.6 mg N/L) was generated. On the other
hand, when oxic precede anoxic (O/A), both HMW DON (0.15 mg N/L) and LMW DON (0.16 mg N/L) were eliminated. In other words, the transition from anoxic to oxic produced LMW DON; in contrast, the transition from oxic to anoxic did not generate LMW DON. This is possibly due to differences in the extent of hydrolysis of HMW DON and cell lysis. The removal of HMW DON under oxic condition in the A/O process (0.5 mg N/L) was greater than that in the O/A process (0.15 mg N/L). Moreover, cell lysis may vary depending on the order of treatment conditions. Foladori et al. (2015) found that returned sludge, having already undergone anaerobic treatment, showed a much greater cell decay rate under aerobic conditions than did activated sludge, not having experienced anaerobic treatment. These observations imply a greater tendency of hydrolysis of HMW DON and cell lysis under oxic conditions in the A/O process than in the O/A process.

Conversions of DON in the A/O/A and A/O/A/O processes illustrate in Figure 3.5. These systems were combinations of the A/O and O/A processes. The findings in the A/O and O/A processes were also observed in the A/O/A and A/O/A/O systems. In the A/O/A process, under the anoxic periods, HMW DON remained unchanged and LMW DON decreased by 1.1 and 0.4 mg N/L. In the oxic condition, 0.4 mg N/L of HMW DON was removed but 0.4 mg N/L of LMW DON was generated. In the A/O/A/O process, during the anoxic treatments, LMW DON decreased by 0.5 and 0.6 mg N/L. However, under the oxic conditions, LMW DON increased by 0.2 and 0.4 mg N/L with the removal of 0.1 and 0.3 mg N/L of HMW DON. The consistent finding during operations of the four configurations of SBR was that transition from anoxic to oxic conditions removed HMW DON but generated LMW DON. However, during conversion from oxic to anoxic conditions, HMW DON was persist and LMW DON was removed.
Among the four configurations, the A/O/A process showed the greatest DTN, DIN, and DON removal efficiencies (64 % for DTN; 70 % for DIN; 44 % for DON) possibly due to its containing two anoxic treatments. The A/O/A/O process also demonstrated high DTN and DIN removal efficiency (60 % for DTN; 70 % for DIN). However, this system eliminated only 26 % of DON with 15 % of LMW DON removal efficiency, which were the lowest values among the four systems. Compared to the O/A process, the A/O process demonstrated high DTN and DIN
removal efficiency (57 % for DTN and 61 % for DIN in the A/O process VS. 29 % for DTN and 27 % for DIN in the O/A process). The low DIN removal efficiency in the O/A process can be related to deficit carbon substrate for denitrifying bacteria. Due to oxidation of most carbon substrates under the first oxic condition in the O/A process, activity of denitrifying bacteria in the subsequent anoxic condition can be constrained, causing less DIN removal through denitrification. Yet, the O/A system showed the highest LMW DON removal efficiency (46 %) among four systems because its order of treatment conditions in this system (O/A) did not produce LMW DON.

3. 5 Conclusions

This study investigated differences in effluent nitrogen of CAS and BNR systems treating identical sources of wastewater. In addition, transformations of nitrogen within wastewater treatment processes were analyzed to determine the causes of the variations in CAS and BNR effluent nitrogen. The conclusions drawn from this study are listed as follows:

- The BNR process generated lower concentrations of effluent DTN through removing DIN from wastewater but greater amounts of effluent DON, particularly LMW DON and proteinaceous DON, than the CAS process.

- The treatment condition, a transition from anoxic to aerobic, in the BNR process was more prone to generate LMW DON than the purely aerobic conditions of the CAS process, causing the BNR effluent to contain more DON than the CAS effluent.

- The order of treatment conditions impacts the behavior of DON within wastewater treatment processing. A transition of treatment conditions from anoxic
to oxic produced LMW DON; in contrast, a transition from oxic to anoxic did not generate LMW DON.
3.6 References


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CHAPTER 4

A COMPARISON OF ALGAL GROWTH IN RECEIVING ESTUARIES STIMULATED BY EFFLUENTS FROM CONVENTIONAL ACTIVATED SLUDGE AND BIOLOGICAL NUTRIENT REMOVAL PROCESSES

To be submitted to *Water Environment Research*

4.1 Abstract

This study investigated algal growth in receiving estuaries caused by effluents from conventional activated sludge (CAS) and biological nutrient removal (BNR) processes. For this purpose, bioassays incubating effluents of lab-scale and field full-scale systems with natural estuary water were conducted. The results showed that the BNR effluents, despite containing less amounts of dissolved total nitrogen (DTN), stimulated greater algal biomass generation than the CAS effluents. Analyses of nitrogen utilization and algal growth in bioassays demonstrated low molecular weight dissolved organic nitrogen (LMW DON) has greater impact on algal growth than dissolved inorganic nitrogen (DIN), possibly causing greater productivity of BNR effluents compared to CAS effluents. This bioassay study suggests that a decrease simply in the amounts of effluent nitrogen cannot ensure alleviating algal blooms in receiving estuaries. For actual evaluation of eutrophication stimulated by effluents, not only amounts of effluent nitrogen but also the effects from compositions of effluent nitrogen should be recognized.
4.2 Introduction

Algal blooms and subsequent hypoxia are one of the most critical environmental issues threatening many water bodies. One such place is Long Island Sound (LIS), an estuary bordered by the states of New York and Connecticut and open to the Atlantic Ocean. In order to improve the conditions of LIS, the federal government and the states of New York and Connecticut reached an agreement to decrease the amount of nitrogen entering to LIS by 58.5% by the year 2014 (US EPA, 2011). To achieve this goal, many wastewater treatment plants (WWTPs) in the affected area have been upgraded from conventional activated sludge (CAS) to biological nutrient removal (BNR) processes at a cost exceeding $600 million over the last two decades (O’Shea and Brosnan, 2000). Although these efforts have contributed to reducing total nitrogen loading to LIS by approximately 20% since 2004, the area suffered from hypoxia in 2006 actually expanded compared to that in 1991 (O’Shea and Brosnan 2000; Stelloh, 2007).

Retrofitting CAS to BNR is one common countermeasure to requests for decreasing total nitrogen discharge from wastewater treatment facilities. However, this retrofitting event causes changes in effluent nitrogen. Our earlier lab-scale reactor study (Eom and Park, Chapter 3) showed that upgrading CAS to BNR decreased effluent dissolved inorganic nitrogen (DIN) but increased effluent dissolved organic nitrogen (DON). Particularly, the BNR process produced more low molecular weight DON (LMW DON) and proteinaceous-like DON, which are known to be bioavailable DON, than did the CAS process. Effluent discharged from WWTPs provides nutrient sources for algae in receiving waters. Thus, it is reasonable to expect that these changes in effluent nitrogen caused by upgrading CAS to BNR systems alter the nitrogen source for algae, leading to different algal growth in receiving waters, especially in nitrogen-limited aquatic environments such as estuaries and marine areas.
Despite importance of this issue, there has been no research yet to directly compare the effects of CAS and BNR effluents on eutrophication in the same receiving estuaries. This is probably due to the long-believed assumption that BNR effluent with its lowered total nitrogen discharge causes less eutrophication than CAS effluent. However, as has occurred in LIS, lowered amounts of nitrogen loading cannot ensure alleviating algal blooms in receiving estuaries. In addition, earlier bioassay studies (Urgun-Demirtas et al., 2008; Sheppard, 2011; Eom et al., 2013) also demonstrated that effluents containing more nitrogen did not stimulate greater algal generation than effluents having less nitrogen in receiving waters.

The objective of this study was to evaluate the influences of CAS and BNR effluent on algal blooms in receiving estuaries. For this goal, we performed bioassays incubating effluent and natural estuary water (LIS water). In the bioassays, effluents from lab-scale CAS and BNR systems were employed. These lab-scale systems treated identical wastewater and were operated under controlled conditions; thus, their effluents can entirely reflect the effects from treatment process (i.e., CAS vs. BNR). Furthermore, effluents from field full-scale CAS and BNR WWTPs were also tested. Particularly, effluents from a local WWTP (the Amherst WWTP) before and after upgrading CAS to BNR provided an opportunity to investigate the impact on algal growth of field CAS and BNR effluents generated from the same source of wastewater. During operations of these lab and field effluent bioassays, we measured utilization of effluent nitrogen and the corresponding algal biomass generation to quantify and compare the impact of CAS and BNR effluents on algal growth.
4. 3 Materials and Methods

4. 3. 1 Lab-scale CAS and BNR Systems

Lab-scale CAS and BNR sequencing batch reactors (SBRs), seeded with the same activated sludge from the local WWTP, were operated for one year to generate CAS and BNR effluents. These two lab-scale reactors fed 6 L of identical influent (primary effluent from the local WWTP) with 0.75 days of hydraulic retention time (HRT). The operation schematic layout for the CAS and BNR systems is depicted in Figure A.1. The CAS system included only aerobic treatment, whereas the BNR system had alternating anoxic and aerobic treatments. House air was supplied for sustaining the aerobic period in both systems; nitrogen gas was used to create anoxic conditions in the BNR system. Solid retention time (SRT) in the CAS and BNR reactor was 6 and 20 days, respectively. During the reactor operation, dissolved total nitrogen (DTN), DIN species, and DON of influent and effluents were measured regularly. These results are presented in Table 4.1.

<table>
<thead>
<tr>
<th></th>
<th>DTN (mg N/L)</th>
<th>DIN (mg N/L)</th>
<th>DON (mg N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent</td>
<td>Total</td>
<td>NO$_2$-N</td>
</tr>
<tr>
<td></td>
<td>28.7 (7.3)</td>
<td>24.2 (7.7)</td>
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</tr>
<tr>
<td>CAS effluent</td>
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<td>0.1</td>
</tr>
<tr>
<td>BNR effluent</td>
<td>15.7 (3.8)</td>
<td>13.1 (4.1)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

4. 3. 2 Bioassay Test

Bioassays were performed by combining, either the CAS or BNR effluent, with natural estuary receiving water, LIS water, in a 2 L Pyrex glass bottle. The LIS water was collected from
the White Sand Beach in Old Lyme at Connecticut. As effluent provides a source of dissolved nutrients, a 0.45 µm nitrocellulose membrane was used to filter it. To remove large particles but retain the phytoplankton and heterotrophic bacteria employed as the inoculum, a nylon net filter with a pore size of 100 µm was chosen to filter the receiving water. The dilution rate for effluent to receiving water in the bioassays was 1:4. The bioassay bottles were completely mixed using a magnetic stirrer and a stir bar and placed in front of the lab windows (four windows: 2.0 m length x 1.9 m height; four windows: 0.9 m length x 1.9 m height). There was no shaded area, ensuring very similar sun light conditions for all the bioassay bottles. Each bioassay was operated in duplicate at ambient temperature (20 ~ 22 ºC). To confirm that algal biomass generated in the bioassays was stimulated by nutrients in the effluent, a control bioassay incubating only estuary receiving water without effluent was also run. On designated days, samples were taken from each bottle and then analyzed directly for volatile suspended solids (VSS) and chlorophyll a or were filtered by a 0.45 µm nitrocellulose membrane and then frozen for later determination of concentrations of nitrogen species.

During the entire experiments, a total of three sets of lab effluent bioassays (November 2012, March 2013, and September 2013) and two sets of field effluent bioassays (September 2014 and local WWTP) were conducted.

4. 3. 3 Field Effluents from Full-scale WWTPs

For the September 2014 bioassays, the field effluents were collected from secondary clarifier outfalls in four different full-scale WWTPs (two CAS plants and two BNR plants). One CAS plant (Plant A) treats, on average, 2.4 million gallons per day (MGD) of domestic wastewater with a mechanically aerated sludge process. The second CAS plant (Plant B) has approximately 380 MGD of mixed domestic and industrial wastewater with a pure oxygen-
activated sludge process. One of the BNR plants (Plant C) adopts a modified Ludzack-Ettinger (MLE) process with 27 MGD of influent flow rates. The other BNR plant (Plant D) treats around 45 MGD of wastewater and employs a general Ludzack-Ettinger (LE) process.

During this study, we also had a chance to collect and study effluents from a local WWTP which changed its operation mode from CAS to BNR in early 2013 to comply with new nitrogen discharge regulation. Therefore, the effluent collected in March 2011 was generated under CAS processing, whereas, the effluent collected in March 2013 was produced under BNR processing. When this local WWTP was a CAS process, it adopted mechanical aeration with approximately 10 days of SRT. After upgrading to BNR, it employed a MLE process with 15 ~ 20 days of SRT.

4.3.4 Laboratory Analysis

The term “dissolved” in this study indicates substances that pass through a 0.45 µm membrane filter. The concentration of DTN was measured by a Shimadzu TN analyzer (Shimadzu TOC-VCPH with TNM-1, Shimadzu North America, SSI Inc., Columbia, MD). The concentrations of DIN species (NH$_4^+$, NO$_2^-$, NO$_3^-$) and PO$_4^{3-}$-P in effluents were determined using a Metrohm ion chromatograph (Metrohm, Herisau, Sz). Since DIN species in saline waters cannot be measured by ion chromatography due to high salinity, we analyzed DIN in LIS water and bioassay samples using traditional wet-chemical based methods. NH$_4^+$-N in saline waters was determined by the phenol hypochlorite colorimetric method (Koroleff, 1983). The concentrations of NO$_2^-$-N and NO$_3^-$-N in saline waters were measured by the technique proposed by Strickland and Parson (1968) and Zhang and Fischer (2006), respectively. For evaluation of the amount of PO$_4^{3-}$-P in saline waters, the method in Strickland and Parson (1968) was employed.
Organic nitrogen (ON) was classified based on its molecular weight. A criteria of 0.45 \( \mu \text{m} \) was chosen to divide ON into particulate organic nitrogen (PON) or DON. The DON fraction was then separated into two groups: high molecular weight DON (HMW DON, larger than 1K Dalton) and low molecular weight DON (LMW DON, smaller than 1K Dalton). Ultrafiltration was conducted with an Amicon stirred cell device (Millipore Corp., USA) with a 1K Dalton (Da) cellulose membrane (Millipore Corp., USA). As DON cannot be directly measured, it was determined by subtracting sum of DIN from DTN. LMW DON was the difference between LMW DTN and DIN. The amount of HMW DON was obtained by subtracting the amount of LMW DON from the amount of DON. Algal biomass generation was quantified by volatile suspended solids (VSS) and chlorophyll a using the methods in *Standard Methods*. For VSS measurement, *Standard Methods* 2540 D, E was used; for Chlorophyll a, *Standard Methods* 10200 H.

### 4.4 Results and Discussion

#### 4.4.1 Comparisons of Algal Growth Stimulated by Lab-scale CAS and BNR Effluents

Table 4.2 presents the concentrations of nitrogen species and \( \text{PO}_4^{3-} \) in the lab-scale CAS and BNR effluents employed in the March and September 2013 bioassays.

<table>
<thead>
<tr>
<th></th>
<th>DTN (mg N/L)</th>
<th>DIN (mg N/L)</th>
<th>DON (mg N/L)</th>
<th>( \text{PO}_4^{3-} ) (mg P/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>( \text{NO}_2^-\text{-N} )</td>
<td>( \text{NO}_3^-\text{-N} )</td>
<td>( \text{NH}_4^+\text{-N} )</td>
</tr>
<tr>
<td>November 2012</td>
<td>CAS</td>
<td>23.0</td>
<td>16.4</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>17.4</td>
<td>8.8</td>
<td>0.0</td>
</tr>
<tr>
<td>March 2013</td>
<td>CAS</td>
<td>18.5</td>
<td>17.4</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>10.4</td>
<td>7.7</td>
<td>0.5</td>
</tr>
<tr>
<td>September 2013</td>
<td>CAS</td>
<td>25.5</td>
<td>21.7</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>18.8</td>
<td>12.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>
In the both bioassay sets, the CAS effluents contained more DTN with greater amounts of DIN, whereas, the BNR effluents had more DON with higher concentrations of LMW DON. The concentrations of \( \text{PO}_4^{3-} \) in effluents did not vary much between the CAS and BNR effluents: only 0.4 and 0.3 mg P/L differences in the March and September bioassays, respectively.

Figure 4.1 illustrates the utilization of nitrogen species and biomass generation during operation of the March CAS and BNR 2013 bioassays.

**Figure 4.1** Consumption of nitrogen species and generation of biomass in March 2013 bioassays. (A) CAS bioassay; (B) BNR bioassay.
Up to day 4, nitrogen consumption and biomass generation were insignificant. From day 4 to day 8, 86 and 105 mg/L of VSS were generated with 4.8 and 2.8 mg N/L of DTN consumption in the CAS and BNR bioassays, respectively. After peak on day 8, VSS started decreasing most likely due to the limit of nitrogen in bioassays. The September 2013 bioassays also showed similar results as the March 2013 bioassays. As Figure 4.2 depicts, in the September 2013 bioassays, biomass generation peaked on day 7 at 135 and 158 mg/L in the CAS and BNR bioassays, respectively.

**Figure 4.2** Consumption of nitrogen species and generation of biomass in September 2013 bioassays. (A) CAS bioassay; (B) BNR bioassay.
After day 7, biomass was no longer increased. In both the March and September 2013 bioassays, VSS and chlorophyll a generation were strongly correlated ($R^2$: 0.98 ~ 0.99, Figure 4.3), demonstrating that the VSS value can be a proxy for algal biomass. The control bioassays, incubated only with estuary receiving water without effluents, generated no algal biomass, indicating that algal growth was driven by nutrients in the effluents.

![Graph showing correlation between VSS and chlorophyll a for March and September bioassays](image)

**Figure 4.3** Comparison of generation of VSS and chlorophyll a in bioassays. (A) March 2013 bioassays; (B) September 2013 bioassays.
Table 4.3 shows the nitrogen consumed and VSS generated in the March and September 2013 bioassays. Approximately 92 ~ 96% of effluent DTN was utilized in the bioassays. Nearly all the DIN and LMW DON were used up, whereas, 50 ~ 60% of HMW DON was removed. Overall, 63 ~ 85% of effluent DON was consumed. These values are within the range of reported bioavailability of DON (31 ~ 100%) from previous bioassay studies that incubated effluent and a mixture of algae and bacteria (Filippino et al., 2011; Mesfioui et al., 2012).

Table 4.3 Consumed nitrogen species, generated maximum VSS, and nitrogen-based productivity in lab effluent bioassays.

<table>
<thead>
<tr>
<th>Set</th>
<th>Bioassay</th>
<th>Consumed nitrogen (mg N/L)</th>
<th>Maximum VSS (mg VSS/L)</th>
<th>Productivity (mg VSS/mg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>November 2012</td>
<td>CAS</td>
<td>3.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>March 2013</td>
<td>CAS</td>
<td>4.91</td>
<td>4.55</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.98</td>
<td>2.09</td>
<td>0.19</td>
</tr>
<tr>
<td>September 2013</td>
<td>CAS</td>
<td>5.11</td>
<td>4.44</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>3.78</td>
<td>2.64</td>
<td>0.39</td>
</tr>
</tbody>
</table>

In our earlier lab-scale reactor study (Eom and Park, Chapter 3), it was expected that as effluent LMW DON/DON increases and effluent DOC/DON decreases, bioavailability of effluent DON could become greater. This expectation was supported by the results in the March and September 2013 bioassays. Figure 4.4 illustrates that effluents with higher LMW DON/DON and lower DOC/DON showed greater bioavailability of effluent DON in the bioassays. While most of the effluent nitrogen (92 ~ 96%) was consumed during the bioassay, only 65 ~ 71% of effluent PO$_4^{3-}$ was consumed in the bioassays, indicating that the limiting nutrient to drive algal growth was not phosphorus, but nitrogen.
Figure 4.4 Relationship between bioavailability of effluent DON and ratios of DON/DOC and LMW DON/DON in March and September 2013 bioassays.

The sets of bioassay that generated greater algal biomass were those that incubated BNR effluents. Even though the BNR effluents contained less DTN than the CAS effluents (39% and 26% less DTN in the March and September bioassays, respectively), the BNR effluents produced more algal biomass (32% and 15% more maximum VSS in the March and September, respectively) than the CAS effluents. To the best of our knowledge, this is the first report to directly compare algal growth stimulated by CAS and BNR effluents, which generated from the same sources of wastewater, in identical estuary receiving waters. Based on the results of consumed nitrogen and generated maximum VSS, productivity of the effluents nitrogen was analyzed. Generally, productivity represents the average measurement of the efficiency of production. In this study, productivity caused by effluent nitrogen was assessed by the maximum algal biomass generation yield of consumed effluent DTN: the ratio of the mass of maximum generated algal biomass (VSS) to the mass of consumed DTN during the bioassay. In the March bioassays, the CAS effluent nitrogen drove 17.9 mg VSS/ mg N of productivity; the BNR
effluent nitrogen, 38.6 g VSS/g N. In the September bioassays, the CAS and BNR effluent
nitrogen showed 19.2 and 31.8 mg VSS/ mg N of productivity, respectively. In both the March
and September 2013 bioassay sets, the BNR effluents caused approximately twice greater
productivity than the CAS effluents, illustrating that the BNR effluents was more efficient in
driving algal growth than the CAS effluents. Urgun-Demirtas et al. (2008) also reported a similar
observation. They analyzed nitrogen-based algal growth yield in bioassays incubated with
nitrified WWTP effluents or denitrified WWTP effluents. Similar to our results, this earlier study
found that the denitrified effluent having lower amounts of DTN but a higher percentage of DON
stimulated the greatest yield value (38 mg VSS/mg N) among eight different bioassays.

4. 4. 2 Consumed Effluent Nitrogen Species and Algal Growth in Lab Effluent Bioassays

The consistent finding in the lab effluent bioassays was that the BNR effluents stimulated
larger amounts of algal biomass and higher nitrogen-based productivity than the CAS effluents
(Table 4.3). In the March and September 2013 bioassays, among nitrogen species, only the
amounts of consumed LMW DON had a direct correlation with maximum algal biomass
generation (Figure 4.5).

![Figure 4.5](image.png)

**Figure 4.5** Relationship between consumed LMW DON and generated maximum algal biomass
in March and September 2013 bioassays.
Moreover, as the proportion of consumed LMW DON to consumed DTN increased, generated maximum algal biomass and effluent productivity increased (Figure 4.6 A). In contrast, as the proportion of consumed DIN to consumed DTN increased, generated maximum algal biomass and effluent productivity decreased. (Figure 4.6 B). These analyses clearly show that LMW DON had more significant influence on algal growth than did DIN, accounting for the observation that the BNR effluents containing more LMW DON generated greater algal growth than the CAS effluents having less LMW DON, in the bioassays.

Figure 4.6 Relationship between the proportions of consumed effluent nitrogen species to consumed effluent DTN and algal growth in March and September 2013 bioassays. (A) Consumed LMW DON/Consumed DTN vs. Generated maximum VSS and Productivity; (B) Consumed DIN/Consumed DTN vs. Generated maximum VSS and Productivity.
4.4.3 Comparisons of Algal Growth Stimulated by Full-scale CAS and BNR Effluents

The concentrations of nitrogen species and \( \text{PO}_4^{3-} \) in effluents incubated in the field effluent bioassays (September 2014 and local WWTP bioassays) are shown in Table 4.4. In the September 2014 bioassays, the field CAS effluents contained not only larger amounts of DTN but DON as well compared to the field BNR effluents, which was inconsistent with the results in the lab-scale reactors. Generally, field WWTPs treated different wastewater and had different operating conditions, substantially affecting effluent nitrogen. Due to these uncontrolled conditions, the results of effluent nitrogen from the field WWTPs cannot show meaningful comparisons of CAS and BNR effluent nitrogen.

Table 4.4 Concentrations of nitrogen species and \( \text{PO}_4^{3-} \) in effluents which were incubated in field effluent bioassays.

<table>
<thead>
<tr>
<th></th>
<th>DTN (mg N/L)</th>
<th>DI (mg N/L)</th>
<th>DON (mg N/L)</th>
<th>PO(_4^{3-}) (mg P/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>( \text{NO}_2^-\text{N} )</td>
<td>( \text{NO}_3^-\text{N} )</td>
<td>( \text{NH}_4^+\text{N} )</td>
</tr>
<tr>
<td>September 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant A (CAS)</td>
<td>42.2</td>
<td>36.3</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Plant B (CAS)</td>
<td>49.2</td>
<td>43.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Plant C (BNR)</td>
<td>6.8</td>
<td>4.2</td>
<td>0.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Plant D (BNR)</td>
<td>4.3</td>
<td>3.1</td>
<td>0.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Local WWTP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 2011 (CAS)</td>
<td>19.8</td>
<td>19.4</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>March 2013 (BNR)</td>
<td>10.5</td>
<td>8.8</td>
<td>0.2</td>
<td>8.6</td>
</tr>
</tbody>
</table>

On the other hand, in the local WWTP bioassays, the outcomes of effluent nitrogen generated under CAS and BNR processing were comparable to those in the lab-scale systems. When the local WWTP was operated in CAS (March 2011), the effluent contained 19.8 mg N/L of DTN consisting of 19.4 mg N/L of DIN and 0.4 mg N/L of DON. After it changed to BNR (March 2013), the effluent had only 10.5 mg N/L of DTN; the concentration of DIN decreased to 8.8 mg N/L but the amount of DON increased to 1.7 mg N/L. As these CAS (March 2011) and
BNR (March 2013) effluents were produced from identical full-scale WWTP based on the same influents, they can better reflect the impact of the treatment process compared to those from different field WWTPs.

Table 4.5 presents the details about consumed nitrogen species, generated maximum VSS, and nitrogen-based productivity during the operation of the September 2014 and the local WWTP bioassays. In the September 2014 bioassays, the field CAS effluents (Plant A: 106 mg VSS/L; Plant B: 126 mg VSS/L) stimulated greater algal biomass than the field BNR effluents (Plant C: 66 mg VSS/L; Plant D: 40 mg VSS/L), which was contrary to the findings in the lab effluent bioassays.

Table 4.5 Consumed nitrogen species, generated maximum VSS, and nitrogen-based productivity in field effluent bioassays.

<table>
<thead>
<tr>
<th>Set</th>
<th>Bioassay</th>
<th>Consumed nitrogen (mg N/L)</th>
<th>Maximum VSS (mg VSS/L)</th>
<th>Productivity (mg VSS/mg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>September 2014</td>
<td>Plant A (CAS)</td>
<td>4.55</td>
<td>3.77</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Plant B (CAS)</td>
<td>5.25</td>
<td>4.57</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Plant C (BNR)</td>
<td>1.01</td>
<td>0.56</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Plant D (BNR)</td>
<td>0.76</td>
<td>0.45</td>
<td>0.10</td>
</tr>
<tr>
<td>Local WWTP</td>
<td>March 2011 (CAS)</td>
<td>4.31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>March 2013 (BNR)</td>
<td>2.12</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

One possible explanation for this result is that the field CAS effluent bioassays consumed larger amounts of LMW DON than the field BNR effluent bioassays. In the September 2014 bioassays, among effluent nitrogen species, both the consumed DIN and LMW DON had strong relationship with generated algal biomass. However, slope function of their relationship was different. Between consumed DIN and generated maximum VSS ($y=17.5x+43.7$, $R^2$: 0.93), the
slope function of was 17.5, while, between consumed LMW DON and generated maximum VSS (y=354x-37, \( R^2: 0.93 \)), the value was 354. This clear difference indicates that the impact of LMW DON on algal growth was substantially greater than the effect of DIN in the September 2014 bioassays. Thus, the field CAS effluent bioassays that consumed more LMW DON can stimulate greater algal growth compared to the field BNR effluent bioassays. Moreover, the concentrations of DIN in the field CAS effluents were much higher (32 ~ 40 mg N/L higher) than those in the field BNR effluents, contributing the field CAS bioassays to cause more algal biomass than the field BNR bioassays.

In contrast, in the local WWTP bioassays, the results of algal biomass generation were consistent with in the lab effluent bioassays. Maximum VSS generations stimulated by CAS and BNR effluents of the local WWTP were 121 mg VSS/L and 142 mg VSS/L, respectively, showing that the effluent under BNR processing stimulated more algal biomass than under CAS processing. Furthermore, the BNR effluent demonstrated greater nitrogen-based productivity than the CAS effluent (CAS: 28 mg VSS/mg N vs. BNR: 67 mg VSS/mg N), indicating the greater efficiency of the BNR effluent in driving algal growth compared to the CAS effluent.

4. 4. 4 Implication

The bioassay study on the effluents from the lab-scale systems and the local WWTP illustrated that the BNR effluents with lowered amounts of DTN caused greater amounts of algal biomass and nitrogen-based productivity than did the CAS effluents, which is contradictory to the general belief. To date, previous research has applied only quantitative analysis considering amounts of effluent nitrogen to evaluate eutrophication in receiving waters with assumption that all effluent nitrogen species have the same ability to drive algal growth. The limitations of this approach have led to upgrading wastewater treatment processes from CAS to BNR without
investigating possible unintended outcomes in receiving waters. Our bioassays showed that simple reduction in effluent nitrogen does not alleviate algal blooms in receiving estuaries. In addition, we found that effluent nitrogen species do not have identical potential to cause algal growth: LMW DON was more significant nitrogen species influencing algal generation than DIN.

Upgrading WWTPs from CAS to BNR can efficiently achieve the goal of decreasing total nitrogen discharges, thus reducing the amount of available nitrogen to algae in receiving waters. However, this upgrade also causes changes in effluent nitrogen, especially with increase of LMW DON. These changes may cause to unexpected and undesirable effects on the downstream ecosystem by stimulating more algal growth. Thus, for evaluation of eutrophication caused by CAS and BNR effluents, the impacts not only from amounts of effluent nitrogen but also from differences in compositions of effluent nitrogen should be considered.

4.5 Conclusions

In this study, algal biomass generation caused by CAS and BNR effluents were quantified and compared to evaluate the impacts of differences in CAS and BNR effluent nitrogen on algal growth in receiving estuaries. The main conclusions of this study are as follows:

- BNR effluent with greater amounts of LMW DON stimulated more algal biomass compared to CAS effluent in bioassays.
- BNR effluent showed higher nitrogen-based productivity than CAS effluent in bioassays, indicating that BNR effluent nitrogen was more efficient in causing algal growth than CAS effluent nitrogen.
- LMW DON showed more significant impact on algal growth than DIN, allowing BNR effluent to cause larger amounts of algal biomass than CAS effluent in bioassays.
- For evaluation of algal growth driven by effluent, the effects from compositions of effluent nitrogen as well as amounts of effluent nitrogen should be weighed.
4. 6 References


Eom, H.; Park, C. Investigation of differences in effluent nitrogen derived from conventional activated sludge and biological nutrient removal wastewater treatment systems. *In process.*


5.1 Abstract

This study evaluated whether lower nitrogen discharge from biological nutrient removal (BNR) processes, compared to nitrogen discharge from conventional activated sludge (CAS) processes, can alleviate eutrophication in receiving estuaries. Bioassays which investigated algal growth caused by CAS and BNR effluents in estuary water demonstrated that BNR effluents stimulated more algal growth than CAS effluents although dissolved total nitrogen (DTN) in BNR effluents was less than in CAS effluents. This unexpected result was attributed to the different potential of effluent nitrogen species to stimulate algal growth. Numerical analysis of bioassay data determined that low molecular weight dissolved organic nitrogen (LMW DON) had approximately 7 times greater algal growth yield compared to dissolved inorganic nitrogen (DIN). These findings suggest that considering not only the amount but also the composition of effluent nitrogen is necessary to assess the actual impact of upgrading CAS to BNR on eutrophication in receiving estuaries.
5. 2 Introduction

Nitrogen is typically the limiting nutrient for algal growth in estuarine and marine environments. Biological nutrient removal (BNR) processes have been employed for wastewater treatment plants (WWTPs) discharging effluent to these nitrogen-sensitive water bodies with the goal of alleviating eutrophication. However, our earlier bioassay study (Eom and Park, Chapter 4), which evaluated algal growth stimulated by conventional activated sludge (CAS) and BNR effluents, showed that upgrading WWTPs from CAS to BNR can cause results contrary to general belief. In this earlier study, the BNR effluents, in spite of having lower dissolved total nitrogen (DTN) concentrations, drove greater amounts of algal biomass in receiving estuaries than did the CAS effluents. Moreover, among effluent nitrogen species, it was only low molecular weight dissolved organic nitrogen (LMW DON) that showed strong correlation with generated algal biomass, suggesting that LMW DON had more significant impact on algal growth than dissolved inorganic nitrogen (DIN).

It is generally agreed that BNR processes decrease nitrogen discharge by removing DIN from wastewater through coupled aerobic nitrification and anoxic denitrification (Grady et al., 2011). However, these alternating anoxic and aerobic treatment conditions within the BNR process can produce LMW DON, which does not occur under CAS system with aerobic conditions only. Our previous lab-scale reactor study (Eom and Park, Chapter 3) demonstrated that during the transition of treatment from anoxic to aerobic conditions, LMW DON was generated while high molecular weight dissolved organic nitrogen (HMW DON) was decreased, causing BNR effluents to contain more LMW DON than CAS effluents. Moreover, Foladori et al. (2015) observed significant cell lysis of returned sludge upon being exposed to the switchover
from anoxic to aerobic conditions, indicating that the treatment processes within the BNR system are more prone to generate DON than CAS system.

These results from our earlier bioassay (Eom and Park, Chapter 4) and lab-scale reactor (Eom and Park, Chapter 3) studies led us to question the long assumed benefit of upgrading wastewater treatment processes from CAS to BNR to alleviate algal blooms in receiving estuaries. Upgrading CAS to BNR can, on the one hand, alleviate eutrophication by decreasing effluent DIN but, on the other hand, may exacerbate eutrophication by changing effluent nitrogen species, particularly by increasing effluent LMW DON in receiving waters. However, current evaluation of eutrophication caused by effluents considers only the total amount of nitrogen discharge but neglects influences from effluent nitrogen species. To evaluate the true impact of upgrading WWTPs from CAS to BNR on algal growth in receiving waters, the effects from both concentration and composition of effluent nitrogen should be recognized.

This study investigated the impact of CAS and BNR effluents on eutrophication in receiving estuaries. To achieve this goal, we conducted bioassay tests, using natural estuary water, to experimentally and numerically determine algal growth yields of effluent nitrogen species. Based on the results, we evaluated whether upgrading CAS to BNR actually brings benefits for reducing algal growth in receiving estuaries.

5.3 Materials and Methods

5.3.1 Operation of Lab-scale CAS and BNR Reactors

To generate CAS and BNR effluents under controlled conditions, a total of three lab-scale wastewater treatment systems (two CAS and one BNR) were operated in a sequencing batch reactor (SBR) mode during a year, 2014. These three systems were seeded with identical
activated sludge from a local WWTP (the Amherst WWT). The CAS 1 and 2 systems had only an aerobic treatment (4 hours and 50 minutes) with 6 days and 20 days of solid retention time (SRT), respectively. On the other hand, the BNR system had repeating anoxic (2 hours and 50 minutes) and aerobic treatments (2 hours) with 20 days of SRT (The configurations of treatment processes within the CAS and BNR reactors are illustrated in Figure A.1). All the CAS and BNR reactors were fed 6 L of identical influents, which was primary effluent from the local WWTP, for one sequencing cycle with 0.75 days of hydraulic retention time (HRT). The same house air was employed to sustain the aerobic conditions in both CAS and BNR systems; nitrogen gas was used for making anoxic condition in the BNR system. During operations of the lab-scale reactors, the concentrations of nitrogen species in the influents and effluents were measured regularly; their average values are presented in Table 5.1 (More detailed information about the lab-scale reactor study can be found in Chapter 3).

### Table 5.1 Average concentrations of nitrogen species in influents and effluents during operations of lab-scale systems in 2014. (Standard deviation is in the parenthesis)

<table>
<thead>
<tr>
<th></th>
<th>DTN (mg N/L)</th>
<th>DIN (mg N/L)</th>
<th>DON (mg N/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>NO$_2^-$N</td>
<td>NO$_3^-$N</td>
</tr>
<tr>
<td>Influent</td>
<td>34.3 (8.6)</td>
<td>27.9 (8.9)</td>
<td>0</td>
</tr>
<tr>
<td>CAS 1 effluent</td>
<td>26.5 (6.3)</td>
<td>23.4 (6.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>CAS 2 effluent</td>
<td>25.9 (5.9)</td>
<td>22.2 (6.1)</td>
<td>0.6</td>
</tr>
<tr>
<td>BNR effluent</td>
<td>16.8 (4.2)</td>
<td>11.7 (4.7)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

### 5.3.2 Estuary Receiving Water Bioassay Tests

Bioassay tests were conducted by incubating effluent and estuary receiving water in a two liter of glass bottle. Before use, the glass bottle was placed into an acid bath (20% sulfuric acid) over night and then rinsed with deionized water for several times. The effluents were taken
from our lab-scale CAS 1, 2, and BNR reactors and filtered using a 0.45 µm nitrocellulose membrane (except for the third sub-group in the December 2014 bioassays, which used 1K Dalton ultrafilter). The estuary receiving water was collected at the White Sand Beach in Old Lyme, CT where the Connecticut River drains into Long Island Sound. The collected receiving water sample was stored at 4°C and transported to the laboratory within 3 hours. A nylon net filter with a pore size of 100 µm was used for the receiving waters to remove large particles but to retain phytoplankton and bacteria as inoculum of bioassays. The mixing ratios of the effluent to the receiving water in the bioassays was 1:10. Each bioassay was operated in duplicate at ambient temperature in air-conditioned laboratory (21 ~ 23 ºC) and its content was thoroughly mixed using a magnetic stirrer and a stir bar. The bioassays were placed parallel to the front of the laboratory windows (four windows: 2.0 m length x 1.9 m height; four windows: 0.9 m length x 1.9 m height) and received very similar light conditions due to no shading area and identical natural day and night cycles. To confirm that the growth of biomass during the bioassays was supported by nutrients originating from effluents, a control bioassay was also run using only receiving waters without the addition of effluents. During operation of each bioassay, samples were collected with algal biomass generation (VSS and chlorophyll a) being directly measured. For analysis of nitrogen species, the collected samples were frozen after filtration via a 0.45 µm nitrocellulose membrane.

In this current study, two sets of lab effluent bioassays were conducted (one set each in March and December 2014). The March 2014 set had CAS 1, 2, and BNR bioassays. The December 2014 set contained three subgroups. Each of three subgroups also had CAS 1, 2, and BNR bioassays and used identical CAS 1, 2, and BNR effluents. However, these effluents were differently pre-treated. The first subgroup employed effluents filtered by a 0.45 µm
nitrocellulose membrane, whereas, in the second subgroup, 1 mg N/L of DIN (NaNO₃) was added to the 0.45 µm filtered effluents. The third subgroup utilized the 1K Dalton (Da) ultrafiltered effluents.

5.3.3 Analytical Measurement

The dissolved fractions in this study were defined as the matter that passes through filters with a pore size of 0.45 µm. The concentrations of dissolved total nitrogen (DTN) in all collected samples were determined by a Shimadzu TN analyzer (Shimadzu TOC- VCPH with TNM-1, Shimadzu North America, SSI Inc., Columbia, MD). In effluents, amounts of DIN species (NH₄⁺, NO₂⁻, NO₃⁻) and PO₄³⁻ were determined by a Metrohm ion chromatograph (Metrohm, Herisau, Sz), but in saline waters such as estuary receiving waters and samples from bioassays, they were analyzed using chemical-based methods. NH₄⁺ in saline waters was determined by the phenol hypochlorite colorimetric method (Koroleff, 1983). NO₂⁻ and NO₃⁻ concentrations in saline waters were measured by using the method described in Strickland and Parson (1968) and Zhang and Fischer (2006), respectively. PO₄³⁻ concentration in saline waters was analyzed based on the method proposed by Strickland and Parson (1968).

Historically, a size of 0.45 µm has been used to classify organic nitrogen into either particulate organic nitrogen (PON) or DON (Parkin and McCarty, 1981). We also followed this traditional approach in this study. In addition, the DON was divided into two groups: HMW DON (larger than 1K Da) and LMW DON (smaller than 1K Da). For this molecular weight fractionation of DON, ultrafiltration was conducted using an Amicon stirred cell ultrafiltration device (Millipore Corp., USA) with a molecular weight cut-off of a 1K Da of a cellulose membrane (Millipore Corp., USA). DON concentration was calculated by subtracting DIN.
concentration from DTN concentration. LMW DON was the difference between LMW DTN and DIN. HMW DON was determined by subtracting LMW DON from DON.

To quantify generated algal biomass, both VSS and chlorophyll a were measured. The VSS value represents the overall concentration of microorganisms including bacteria and phytoplankton while the chlorophyll a value is for algal biomass. Tests of VSS were performed according to *Standard Methods* 2540 D, E. Chlorophyll a was measured by the spectrophotometric method as described in *Standard Methods* 10200 H.

### 5. 3. 4 Numerical Analysis for Evaluating Algal Growth Yield of Effluent Nitrogen Species

Based on the results of amounts of consumed effluent nitrogen and generated maximum algal biomass in the bioassays, algal growth yields of effluent DIN, HMW DON, and LMW DON were numerically analyzed. The yields of effluent nitrogen species were assigned as the variables. Then, equations describing that the sum of VSS stimulated by DIN, HMW DON, and LMW DON is equal to the actual generated VSS were established at every bioassay (Eq. (1)).

$$\sum \text{Amount of Consumed N Species} \times \text{Algal Growth Yield of N species} = \text{Actual Generated Algal Biomass in Bioassay}$$

(1)

As the number of equations were more than the number of variables, solutions were calculated by a least square numerical method, which is a mathematical procedure to find the best-fitting to a given set of point by minimizing the sum of the square of the residuals from the fitting.
5. 4 Results and Discussion

5. 4. 1 Algal Growth Stimulated by Lab-scale CAS and BNR Effluents

Table 5.2 demonstrates concentrations of nitrogen species and PO$_4^{3-}$ in the effluents incubated in the March and December 2014 bioassays. In both bioassay sets, the CAS effluents had greater amounts of DIN; however, the BNR effluents showed higher concentrations of LMW DON. PO$_4^{3-}$ concentration was similar among three effluents: it ranged between 2.3 and 2.6 mg P/L in the March 2014 bioassays, while, it ranged from 2.1 to 2.4 mg P/L in the December 2014 bioassays.

Table 5.2 Concentrations of nitrogen species and PO$_4^{3-}$ in effluents which were incubated in bioassays.

<table>
<thead>
<tr>
<th></th>
<th>DTN (mg N/L)</th>
<th>DIN (mg N/L)</th>
<th>DON (mg N/L)</th>
<th>PO$_4^{3-}$ (mg P/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAS 1</td>
<td>29.5</td>
<td>23.4</td>
<td>0</td>
<td>16.9</td>
</tr>
<tr>
<td>CAS 2</td>
<td>29.8</td>
<td>22.9</td>
<td>0</td>
<td>16.3</td>
</tr>
<tr>
<td>BNR</td>
<td>18.7</td>
<td>9.1</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td>December 2014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAS 1</td>
<td>15.5</td>
<td>13.0</td>
<td>0.4</td>
<td>6.3</td>
</tr>
<tr>
<td>CAS 2</td>
<td>16.9</td>
<td>13.6</td>
<td>0</td>
<td>9.6</td>
</tr>
<tr>
<td>BNR</td>
<td>9.0</td>
<td>4.0</td>
<td>0</td>
<td>9.0</td>
</tr>
<tr>
<td>January 2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAS 1</td>
<td>12.7</td>
<td>11.6</td>
<td>0</td>
<td>8.1</td>
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<tr>
<td>CAS 2</td>
<td>11.6</td>
<td>9.7</td>
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<td>9.7</td>
</tr>
<tr>
<td>BNR</td>
<td>6.7</td>
<td>4.3</td>
<td>0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Figure 5.1 illustrates changes in concentrations of nitrogen species and biomass (VSS) in the March 2014 bioassay set. The CAS 1, 2, and BNR bioassays had comparable trends of nitrogen consumption and biomass production. VSS generation peaked on day 8 at 93, 129, and 161 mg VSS/L in the CAS 1, 2, and BNR bioassays, respectively. After day 8, biomass no longer increased.
Figure 5.1 Consumption of nitrogen species and generation of biomass in March 2014 bioassays. (A) CAS 1 bioassay; (B) CAS 2 bioassay; (C) BNR bioassay.
Figure 5.2 shows utilization of nitrogen and generation of biomass (VSS) in the first subgroup of the December 2014 bioassay set.

**Figure 5.2** Consumption of nitrogen species and generation of biomass in the first set of December 2014 bioassays. (A) CAS 1 bioassay; (B) CAS 2 bioassay; (C) BNR bioassay.
In the CAS 1 and 2 bioassays, the greatest VSS production was 63 and 70 mg VSS/L, respectively, on day 14. The BNR bioassay generated 82 mg VSS/L of the highest biomass concentration on day 12. After attaining the peak, VSS values started decreasing in the bioassays. In both the March and December 2014 bioassay sets, generation of VSS and chlorophyll a demonstrated a strong linear correlation with each other (Figure 5.3, $R^2$: 0.97 ~ 0.98), suggesting that the VSS concentration can serve as a proxy for algal biomass. The control bioassay, employed with only receiving water without addition of effluent, did not produce biomass, showing that the algal biomass generation was supported by nutrients in the effluents.

![Comparison of generation of VSS and chlorophyll a in bioassays. (A) March 2014 bioassays; (B) The first subgroup of December 2014 bioassays.](image)

**Figure 5.3** Comparison of generation of VSS and chlorophyll a in bioassays. (A) March 2014 bioassays; (B) The first subgroup of December 2014 bioassays.
The details of consumed nitrogen species and generated maximum VSS in the March 2014 bioassays and the first subgroup of the December 2014 bioassays are presented in Table 5.3 and 5.4, respectively. In both bioassay sets, the greatest algal biomass was generated in the BNR bioassays. In the March 2014 set, although the BNR effluent contained approximately 10 mg N/L less DTN compared to the CAS 1 and 2 effluents, the BNR effluent stimulated 68 and 33 mg VSS/L more algal biomass in the bioassays.

### Table 5.3

Consumed nitrogen species, generated maximum VSS, and nitrogen-based productivity in March 2014 bioassays.

<table>
<thead>
<tr>
<th>Set</th>
<th>Bioassay</th>
<th>Consumed nitrogen (mg N/L)</th>
<th>Maximum VSS (mg VSS/L)</th>
<th>Productivity (mg VSS/mg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>March 2014</td>
<td>CAS 1</td>
<td>3.46</td>
<td>2.58</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>CAS 2</td>
<td>3.43</td>
<td>2.50</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.52</td>
<td>1.13</td>
<td>0.30</td>
</tr>
</tbody>
</table>

In the first subgroup of the December 2014 set, the DTN concentration in the BNR effluent was 6 ~ 7 mg N/L less than those in the CAS 1 and 2 effluents. Nevertheless, the BNR effluent drove 19 and 11 mg VSS/L greater algal biomass than the CAS 1 and 2 effluents in the bioassays.

These results demonstrate that the BNR effluents were more productive in stimulating algal growth than the CAS effluents, which was consistent with the findings in our earlier lab effluent bioassays (Eom and Park, Chapter 4).
Table 5.4 Consumed nitrogen species, generated maximum VSS, and nitrogen-based productivity in December 2014 bioassays.

<table>
<thead>
<tr>
<th>Set</th>
<th>Bioassay</th>
<th>Consumed nitrogen (mg N/L)</th>
<th>Maximum VSS (mg VSS/L)</th>
<th>Productivity (mg VSS/mg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>December 2014 Subgroup 1</td>
<td>CAS 1</td>
<td>1.99</td>
<td>1.39</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>CAS 2</td>
<td>1.77</td>
<td>1.43</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>1.01</td>
<td>0.46</td>
<td>0.05</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td>CAS 1</td>
<td>3.01</td>
<td>2.47</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>CAS 2</td>
<td>2.80</td>
<td>2.47</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.02</td>
<td>1.51</td>
<td>0.05</td>
</tr>
<tr>
<td>Subgroup 3</td>
<td>CAS 1</td>
<td>1.53</td>
<td>1.36</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>CAS 2</td>
<td>1.71</td>
<td>1.44</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>0.98</td>
<td>0.47</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Overall, 96 ~ 98 % and 91 ~ 96 % of DTN were consumed in the March 2014 and the first subgroup of the December 2014 bioassays, respectively. All DIN was used up in all the bioassays. In terms of bioavailability of DON, the BNR effluents showed greater values than the CAS effluents. In the March 2014 set, the bioavailability of DON in the CAS 1, 2, and BNR bioassays were 89, 91, and 97 %, respectively. In the first subgroup of the December 2014 set, the CAS 1, 2, and BNR bioassays showed 74, 81, and 91 % of bioavailability of DON. As BNR effluents contained more LMW DON, which is generally regarded as bioavailable DON, than CAS effluents (Table 5.2), BNR effluent DON demonstrated greater bioavailability compared to CAS effluent DON. Unlike most of the nitrogen (91 ~ 98 %) being used, only 65 ~ 75 % of PO4\(^{3-}\) was consumed in the two sets of bioassays, suggesting that the limiting nutrient for algal growth was not phosphorus but nitrogen.

5.4.2 Experimental Analysis of Algal Growth Yields of Effluent Nitrogen Species

In the December 2014 bioassays, the first and second subgroups incubated identical CAS 1, 2, and BNR effluents filtered by 0.45 µm nitrocellulose membrane; however, 1 mg N/L of DIN (NaNO\(_3\)) was added to each effluent in the second subgroup. Thus, a comparison of these
two subgroups of algal biomass generation can demonstrate the influence of DIN on algal growth. As Table 5.4 presents, the difference of DIN consumption in the CAS 1, 2, and BNR bioassays between the first and second subgroup was 1.1, 1.0, and 1.1 mg N/L, respectively, with very similar DON utilization. These differences caused a variation of 15, 17, and 16 mg VSS/L of maximum algal growth in the CAS 1, 2, and BNR bioassays between the two subgroups. Thus, these results indicate that 1 mg N/L of DIN has the yield to stimulate 14 ~ 16 mg VSS/L of algal biomass. Urgun-Demirtas et al. (2008) also showed algal growth yield of DIN similar to our estimation. They observed that the additions of 1 mg N/L of DIN to bioassay resulted in 12 mg VSS/L increase in algal biomass. This outcome suggests that 1 mg N/L of DIN possesses ability to drive 12 mg VSS/L of algal biomass, which was comparable to our estimation (14 ~ 16 mg VSS/L).

As different size of filtrations were employed for the first (0.45 µm) and third (1K Da) subgroup in the December 2014 bioassays, their effluents had different components of DON. In the effluents in the first subgroup, the effluents contained HMW DON and LMW DON; on the other hand, the effluents in the third subgroup had only LMW DON. Thus, a comparison of the first and third subgroup can show the ability of HMW DON to cause algal growth. Table 5.4 presents the details of algal biomass generation in these two subgroups of bioassays. Between the first and third subgroup, maximum algal biomass generation was almost the same with very similar utilization of DIN and LMW DON, suggesting that algal growth was not dependent on HMW DON. It appears that removal of HMW DON was not caused by algal growth but by different mechanisms such as adsorption to biomass of algae and bacteria in the bioassays. Pehivanoglu and Sedlak (2004) also reported similar results with ours. They found that chlorophyll a production was nearly identical between algal growth bioassays incubated with
unfractionated and 1K Da ultrafiltered wastewater effluents, supporting that HMW DON has a minimal direct effect on algal growth.

5.4.3 Numerical Analysis of Algal Growth Yields of Effluent Nitrogen Species

Using data of consumed effluent nitrogen species and generated maximum algal biomass in the bioassays, algal growth yields of effluent nitrogen species were numerically analyzed. For this analysis, the results from not only the bioassays in this current study (a total of 12 bioassays in March and December 2014 sets) but also those from an earlier study (a total of 4 bioassays in March and September 2013 sets, Table 4.4 in Chapter 4) were utilized. As discussed in the methodology, equations showing that the sum of VSS stimulated by each nitrogen species is equal to the measured VSS in the bioassays were established and solved through a least square method. Since the December 2014 bioassay set demonstrated that HMW DON did not support algal biomass generation, only DIN and LMW DON were designated as variables. As a result, algal growth yields of DIN and LMW DON were calculated to be 15 and 108 mg VSS/mg N, respectively. This algal growth yield of DIN was very similar to the experimentally estimated values (14 ~ 16 mg VSS/mg N).

As expected, LMW DON showed much higher (approximately 7 times greater) algal growth yield than DIN, which was the main cause for BNR effluents to stimulate more algal biomass in the bioassays compared to CAS effluents. Figure 5.4 confirms this greater ability of LMW DON in the March and December 2014 bioassay sets. Among nitrogen species, only consumed LMW DON was strongly correlated with generated maximum algal biomass (Figure 5.4A). In addition, as the ratio of consumed LMW DON to consumed DTN increased, nitrogen-based productivity increased (Figure 5.4B). Some earlier studies also support that DON can have greater potential to stimulate algal growth than DIN. For example, Urgun-Demirtas et al.
(2008) reported that the higher decrease in effluent DON corresponded to the more algal biomass generation in their bioassays. Moreover, Berman and Chava (1999) found that algal growth yields in lake water stimulated by organic nitrogen including hypoxanthine, guanine, and urea was up to five times greater than by inorganic nitrogen such as NH$_4^+$ and NO$_3^-$.

Figure 5.4 Relationship between consumed LMW DON and algal growth in March and December 2014 bioassays. (A) Consumed LMW DON vs. Generated maximum VSS; (B) Proportion of consumed LMW DON in consumed DTN vs. Nitrogen-based productivity.
Based on the numerically calculated algal growth yields of DIN and LMW DON, algal biomass generation was estimated and compared to actual generated VSS in the current (March and December 2014) and earlier (March and September 2013) bioassays. Figure 5.5 illustrates the relationship between our projection and the actual growth of biomass occurred. As the figure shows, the estimated VSS production closely approximates the actual amount of maximum VSS generation. This result strongly supports that our numerical analysis of algal growth yield is reasonable to describe actual algal biomass generation in the bioassays.

**Figure 5.5** Relationship between estimated VSS and actually generated VSS in current (March and December 2014) and earlier (March and September 2013, Eom and Park, Chapter 4) bioassays.

### 5. 4. 4 Implication

Current regulations and efforts to alleviate eutrophication in receiving waters focus only on reducing total nitrogen loading to receiving waters with the assumption that all nitrogen
species contribute identically to eutrophication. This has served as a basis for the need to upgrade wastewater treatment systems to BNR processes so the total discharge of nitrogen from WWTPs can be decreased. However, the results from the current study demonstrated that effluent nitrogen species, specifically LMW DON vs. DIN, have substantially different potential for algal growth in receiving estuaries.

Upgrading CAS to BNR efficiently removes DIN from wastewater. However, previous studies showed that the BNR process is more prone to generate LMW DON than the CAS process (Czerwionka et al., 2012; Huo et al., 2013; Eom and Park, Chapter 3). Thus, to evaluate algal growth supported by CAS and BNR effluent nitrogen, both influences from a reduction in effluent DIN and an increase of effluent LMW DON should be recognized. In particular, as LMW DON has much greater algal growth yield than DIN, algal generation stimulated by increased LMW DON should not be ignored even though an increase of LMW DON is not substantial. For example, in the March 2014 set, compared to the CAS 1 system, the BNR system generated 21.7 mg VSS/L less algal biomass by effluent DIN but produced 57.5 mg VSS/L more algal biomass by effluent LMW DON, demonstrating that the net change was an increase of 35.8 mg VSS/L. Furthermore, between CAS 1 and BNR bioassays in the first subgroup of December 2014 set, the decreased algal biomass by lowered effluent DIN was 13 mg VSS/L; the increased algal biomass via raised effluent LMW DON was 33 mg VSS/L, showing that the net change in algal growth was an increase of 20 mg VSS/L. These analyses indicate that simple reduction of effluent DIN through upgrading CAS to BNR does not ensure alleviating algal blooms in receiving estuaries.

Because algal growth in real receiving waters is a complicated phenomenon affected by many factors, including nutrients, organisms, and some abiotic characteristics such as geological
features, the results from our bioassay tests may not apply to all natural environments. Nevertheless, the significance of our study is that it for the revealed different abilities of effluent nitrogen species to drive algal growth in receiving estuaries. For actual evaluation of the eutrophication caused by effluents in receiving waters, not only the amount but also composition of effluent nitrogen should be weighed.

5.5 Conclusions

This study experimentally and numerically evaluated algal growth yields of effluent nitrogen species including DIN, HMW DON, and LMW DON. From these results, the effects of CAS and BNR effluents on eutrophication in receiving estuaries were evaluated. The main conclusions are:

- BNR effluent having less DTN but more LMW DON stimulated greater amounts of algal growth in bioassays compared to CAS effluent containing more DTN but less LMW DON.
- Experimental analyses illustrated that DIN had 14 ~ 16 mg VSS/mg N of algal growth yield and HMW DON cannot drive algal growth.
- Numerical method calculated the algal growth yield of DIN and LMW DON to be 15 and 108 mg VSS/mg N, respectively.
- For true evaluation of the impact of upgrading CAS to BNR on eutrophication, both amounts and changes in composition of effluent nitrogen should be considered.
5.6 References


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CHAPTER 6

RESEARCH CONCLUSIONS

The purpose of this research was to assess whether the decreased nitrogen discharge achieved by upgrading WWTPs from CAS to BNR alleviate eutrophication in receiving estuaries. For this research question, the lab-scale reactors treating identical wastewater and subsequent bioassays incubating the nature receiving estuary water were employed. The results of effluent nitrogen in the lab-scale reactor and the local WWTP demonstrated that the BNR system removed more DIN in wastewater but produced more DON than the CAS system. The treatment condition of the BNR system, a transition from anoxic to aerobic, was prone to generate LMW DON, contributing the BNR effluent to contain greater amounts of DON than the CAS effluent. The lab effluent and local WWTP bioassays showed that the BNR effluent, in spite of containing lowered DTN, drove greater algal biomass and nitrogen-based productivity in comparison to the CAS effluent. This unexpected occurrence was due to a higher algal growth yield of LMW DON. The experimental and numerical evaluation for algal growth yields of effluent nitrogen species showed that LMW DON had much greater yield (6 ~ 10 times) than did DIN. Overall, this research illustrated that the BNR process produced more effluent LMW DON having greater algal growth yield, which contributed to drive larger amounts of algal biomass than the CAS process.
APPENDIX A

SCHEMATIC LAYOUT OF TREATMENT PROCESSES IN LAB-SCALE SYSTEMS AND FOUR SBRs HAVING DIFFERENT ORDERS OF TREATMENT CONDITIONS

Figure A. 1 Schematic layout of treatment processes in lab-scale systems. (A) CAS 1 and 2 reactors; (B) BNR reactor.

Figure A. 2 Schematic layout of treatment processes in four SBRs. (A) A/O process; (B) O/A process; (C) A/O/A process; (D) A/O/A/O process.
APPENDIX B

A COMPARISON OF ALGAL GROWTH STIMULATED BY EFFLUENTS IN RIVER AND ESTUARY RECEIVING WATERS

This study compared algal growth caused by effluents in river and estuary receiving waters. In March 2014, effluents from lab-scale CAS 1, 2, and BNR systems were incubated with either Connecticut River or LIS estuary receiving water in bioassays. Table 5.2 presents the concentrations of effluent nitrogen species and $\text{PO}_4^{3-}$ employed in these tests. Figures B. 1 illustrates the results of the river bioassays; Figures 4. 1 presents the outcomes of the estuary bioassays. As these figures show, the trends of nitrogen utilization and algal biomass generation in the bioassays were similar. During the initial period, DIN was used first and then the DON was consumed later with greater algal biomass generation than earlier. However, the times when algal biomass reached at a maximum were different. The river bioassays attained it on day 14; the estuary bioassays, on day 8. Table B.1 shows generated maximum algal biomass and nitrogen-based productivity in the bioassays. Compared to the river bioassays, the estuary bioassays caused greater algal growth and productivity. One possible explanation for this result is microbial synergistic effects. Initially, the estuary receiving water contained greater VSS and chlorophyll a concentrations, showing the existence of more bacteria and algae than the river receiving water. Therefore, greater synergistic effects between microorganisms may occur in the estuary bioassays, causing greater VSS generation. Moreover, Table A1 illustrates that the variations between the CAS and BNR bioassays in the estuary set were greater than those in the river set. This outcome may result from the different characteristics of receiving waters. In general, freshwater environments such as rivers are more P-sensitive while saline-water environments including estuaries are more N-sensitive (Doering et al., 1995; Fisher et al., 1999).
Thus, the estuary bioassays reacted more sensitively to differences in effluent nitrogen than the river bioassays.

**Table B. 1** Consumed nitrogen species, generated maximum VSS, and nitrogen based productivity in March 2014 River and Estuary bioassays.

<table>
<thead>
<tr>
<th>Set</th>
<th>Bioassay</th>
<th>Consumed nitrogen (mg N/L)</th>
<th>Maximum VSS (mg VSS/L)</th>
<th>Productivity (mg VSS/mg N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2014</td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>River</td>
<td>CAS 1</td>
<td>3.74</td>
<td>3.15</td>
<td>0.07</td>
</tr>
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<td></td>
<td>CAS 2</td>
<td>3.77</td>
<td>3.06</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.73</td>
<td>1.72</td>
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</tr>
<tr>
<td>March 2014</td>
<td></td>
<td>DTN</td>
<td>DIN</td>
<td>HMW DON</td>
</tr>
<tr>
<td>Estuary</td>
<td>CAS 1</td>
<td>3.46</td>
<td>2.58</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>CAS 2</td>
<td>3.43</td>
<td>2.50</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>BNR</td>
<td>2.52</td>
<td>1.13</td>
<td>0.30</td>
</tr>
</tbody>
</table>
Figure B.1 Consumption of nitrogen species and generation of biomass in March 2014 River bioassays. (A) CAS 1 bioassay; (B) CAS 2 bioassay; (C) BNR bioassay.
APPENDIX C
ALGAL GROWTH YIELDS OF EFFLUENT NITROGEN SPECIES IN FIELD EFFLUENT BIOASSAYS

Numerical analysis of algal growth yields of effluent nitrogen in the field effluent bioassays (the September 2014 bioassays) was conducted with the same protocols employed for the lab effluent bioassays. Concentrations of consumed effluent nitrogen species and generated maximum algal biomass in this bioassay set are presented in Table 4.4. The algal growth yields of effluent DIN and effluent LMW DON were calculated to be 11 and 161 mg VSS/mg N, respectively, which was comparable to the values in the lab effluent bioassays. Still in the field effluent bioassays, LMW DON possessed much greater algal growth yield than DIN. Figure C.1 shows the relationship between the estimated algal biomass generation based on the above yield factors and the actual generation of algal growth in the bioassays. Our estimation closely approximated the actual occurrence of algal generation.

![Graph showing relationship between estimated VSS and generated VSS](image)

**Figure C.1** Relationship between estimated VSS and actually generated maximum VSS in September 2014 bioassays.
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