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Formation of Low-Molecular-Weight Dissolved Organic Nitrogen in two-stage and four-stage Pre-denitrification Biological Nutrient Removal Processes

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**Formation of Low-Molecular-Weight Dissolved Organic
Nitrogen in two-stage and four-stage Pre-denitrification
Biological Nutrient Removal Processes**

by

Siwei Chen

**A project report submitted in partial fulfillment of the
requirements for the degree of**

Master of Science

**Department of Civil & Environmental Engineering University of
Massachusetts Amherst, MA 01003**

May 2018

**Formation of Low-Molecular-Weight Dissolved Organic Nitrogen
in Two-Stage and Four-Stage Pre-Denitrification
Biological Nutrient Removal Processes**

A Masters Project Presented

by

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Abstract

To alleviate the eutrophication caused by excessive loading of nutrients, the upgrading of conventional activated sludge (CAS) process to biological nutrients removal (BNR) process has been widely applied in USA. In this study, we found that the dissolved inorganic nitrogen (DIN) release can be effectively controlled by this upgrading, but dissolved organic nitrogen (DON) especially LMW-DON, which now is regarded as another important N source for supporting growth of phytoplankton in coastal water, cannot be removed effectively by BNR systems, especially by four-stage BNR systems. Different pre-denitrification BNR processes have different LMW-DON production rates. A four-stage pre-denitrification BNR releases more LMW-DON in effluent than two-stage pre-denitrification BNR. The higher DON production may be caused by longer anaerobic time. Also, the characteristics of influent influence the formation of LMW-DON in BNR system. Influent with acetate and higher COD concentration can stimulate more DON and LMW-DON release in a BNR process. This suggests that relative regulation should be established to prevent the release of DON. A post-treatment method should be added to remove DON produced by the BNR process.

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I. Introduction

1.1 Problem Statement

Eutrophication is occurring throughout the world and bringing substantial negative effects such as reduction of water quality, impairment of ecological structure and function of freshwaters and economic loss. Nitrogen, as the most essential nutrient for phytoplankton growth, is the main contributor to eutrophication and thus, the release of nitrogen is strictly regulated and controlled by legislation and strategies in the USA. Wastewater treatment plants (WWTPs) are employed as a necessary step to improve the quality of wastewater before it is discharged to surface or groundwater and re-enters water supplies. WWTPs are served as the primary method to prevent excessive nitrogen re-entering the receiving water and stimulates eutrophication.

In the past, the dissolved inorganic nitrogen (DIN) was regarded as the only nitrogen source that stimulated the growth of phytoplankton in estuarine and coastal environments (Wiegner et al, 2006). DON was believed to be unavailable as a source of N nutrients for phytoplankton or bacteria because it was regarded to be composed mainly of refractory compounds which are resistant to biological degradation (Wiegner et al., 2006). Nevertheless, later research found that algae (Lewitus et al., 2000), cyanobacteria (Berman 2001), bacteria (Antia et al., 1991), (Bronk et al., 2002), archaeobacteria (Ouverney et al., 2000) and perhaps even protists (Tranvik et al., 1993) can use various components of the DON pool as an N source either directly or after bacterial degradation. DON begin to be considered as an important nutrient source for phytoplankton and thus contributor to algal blooms in receiving waters.

The constituent of DON in wastewater effluent haven't been fully investigated, such that many DON species remain uncharacterized chemically. Operationally, components of the DON can be divided into high molecular weight (HMW, usually >1 kDa) and low molecular weight (LMW, usually<1kDa) compounds. HMW-DON includes proteins (such as enzymes, modified bacterial wall proteins, dissolved combined amino

acids [DCAA]), nucleic acids (DNA, RNA) and humic-like substances that have a relatively low N content. LMW-DON include urea, peptides (part of the DCAA pool), dissolved free amino acids (DFAA), amino sugars, purines, pyrimidines, pteridines, amides, methyl amides and others (Antia et al. 1991). Within these two forms of DON, LMW-DON has higher bioavailability which is more likely to stimulate phytoplankton blooms (Eom et al, 2017).

Conventional activated sludge (CAS) and biological nutrient removal (BNR) processes are two main strategies to remove nitrogen in WWTPs. CAS is the most typical wastewater treatment process which mainly uses biodegradation under aerobic conditions to remove organic matter and to transform some ammonia nitrogen to nitrate and nitrite by nitrification. Among the various types of BNR processes, single-sludge pre-denitrification system is most commonly used to remove N. This process puts the anoxic treatment ahead of the aerobic treatment to remove N by denitrification and decrease the demand for extra carbon source addition and production of biomass sludge. Currently, due to these benefits of a BNR system, many CAS systems have been upgraded to BNR systems. Previous studies have demonstrated that this kind of upgrade can significantly enhance DIN removal efficiency compared to a CAS system. However, the influence of upgrading a CAS system to a BNR system on DON removal has been studied by only a limited number of previous studies. Bronk et al. (2010) found that effluent from two typical WWTP comprised of a large fraction of DON and these DON can be assimilated by phytoplankton in bioassay. Sattayatewa et al. (2009) found organic nitrogen was released in a 4-stage Bardenpho nitrogen removal plant. Only the research of Eom et al. (2017) focused on a comparison of effluent DON from CAS process and two-stage pre-denitrification BNR process. They found that compared with the effluent of a CAS process, the effluent of a BNR system contained a much higher level of DON and LMW-DON, which has a much higher bioavailability than HMW-DON.

As for Effluent DON from some other BNR treatment processes, there is no research.

In this report, we studied the effluent DON from another commonly used BNR system, The four-stage pre-denitrification process. We investigated the removal of DON especially LMW-DON by four-stage pre-denitrification BNR system and compare it with CAS and two-stage BNR systems to explore DON release in a four-stage pre-denitrification BNR system.

1.2 Research Objectives:

This research characterized and compared bioavailable DON in effluents from three different kind of wastewater treatment technologies: conventional activated sludge (CAS), two-stage pre-denitrification biological nutrients removal (BNR1) and four-stage pre-denitrification biological nutrients removal (BNR2) to investigate

1. The influence of upgrading of CAS to BNR especially four-stage BNR process on DON especially LMW-DON release.
2. Discussing the source of LMW-DON in two-stage and four-stage pre-denitrification BNR systems
3. Discussing how the influent characteristic can influence LMW-DON release in BNR processes.

II. Methods and Material:

2.1 Operation of Lab-Scale CAS And Pre-denitrification BNRs

three lab-scale wastewater treatment systems which include one CAS, one two-stage pre-denitrification BNR (BNR1) and one four-stage pre-denitrification BNR (BNR2) were operated during 2017-2018. These systems were operated as sequencing batch reactors (4L volume) seeded with activated sludge collected from the Amherst (MA) WWTP. Each batch cycle last for 6 h, consisting of 10 min feeding, 4h 50 min treatment with mixing, 50 min settling and 10 min effluent decanting. The CAS system was entirely aerobic, the two-stage BNR system consisted of a first 2h anoxic phase and then a subsequent 2h 50 min aerobic phase, and the four-stage BNR system included a 1h first anoxic phase, a 1h 30min first aerobic phase, a 1h second anoxic phase and a

1h 20 min aerobic phase. In the two-stage BNR system, the anoxic phase include 1h 10 min nitrogen purging and 50 min mixing; in the four-stage BNR system, the anoxic phase included 40 min nitrogen purging and 20 min mixing (Fig. 1). The HRT and SRT for all three systems were 0.5 days and 20 days, respectively. All CAS and BNR systems were fed 8L per day of identical influent. To increase the total nitrogen and COD in the influent, these three reactors were feed with a 50/50 (v/v) mixture of real primary effluent from Amherst WWTP and synthetic wastewater. The synthetic wastewater was prepared in the laboratory and included for the purpose of increasing total nitrogen and chemical oxygen demand (COD) in the influent by adding NH_4Cl , NH_4HCO_3 , BactoPeptone and CH_3COONa . Other inorganic constituents included in the synthetic feed followed the composition used in Novak et al. (2007). The characteristics of primary effluent showed variation in COD and solids depending on the collection date. Table 2 represent the average values of various characteristics of the final influent (mixed) used in this study.

Table 1.
Composition of the synthetic wastewater

Ingredients	Concentration (mg/l)
Bacto Peptone	300
NaCH_3COOH	100
NH_4Cl	57
NH_4HCO_3	30
KH_2PO_4	60
KHSO_4	44
NaHCO_3	394
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	220
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	150
FeCl_3	20
$\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$	20

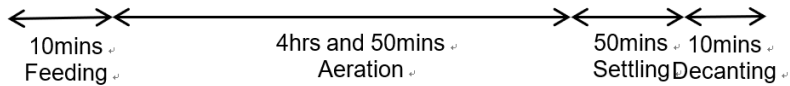
Table 2.
Characteristics of the mixed influent

Analyses	Concentration(mg/l)
TDN (n=26)	47.96±9.61
DIN (n=26)	34.89±4.19
DON (n=26)	13.07±1.47
NH ₄ ⁺ (n=26)	33.18±4.08
NO ₂ ⁻ (n=26)	0.28±0.19
NO ₃ ⁻ (n=26)	1.43±0.34
HMW-DON (n=26)	6.26±0.83
LMW-DON (n=26)	6.82±0.67
COD (n=26)	211.42±18.79
TSS	56±4.21
VSS	46±3.53

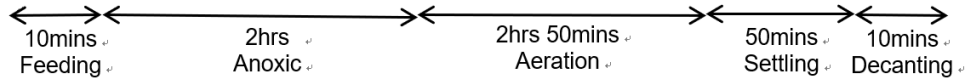
2.2 Size separation

Collected samples were immediately used to measure the total suspended solids (TSS), and volatile suspended solids (VSS). Samples were then filtered through 0.45 µm nitrocellulose membrane filters and 1 kDa ultrafilters and kept frozen at -20 °C until analysis to measure the dissolved N. The dissolved or soluble fraction of N was obtained by filtering samples through sterile 0.45 µm nitrocellulose membrane filters. The Amicon ultrafiltration cell (Millipore Corp.) with a 1 kDa cellulose membrane was used to separate the high-molecular-weight dissolved nitrogen from the 0.45 µm filtrate with the DIN and LMW remain in the 1kDa filtrate. The amount of LMW-DON can be determined by subtracting the DON in 1kDa filtrate from the TDN and the HMW-DON is the difference between DON and LMW-DON.

1) CAS

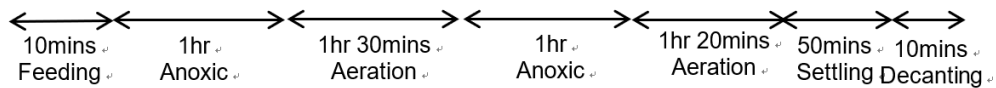


2) BNR 1 (2 steps)



- 2hrs Anoxic = 70 mins N purging + 50 mins Mixing

3) BNR 2 (4 steps)



- 1hr Anoxic (1st Anoxic) = 40mins N purging + 20 mins Mixing

- 1hr Anoxic (2nd Anoxic) = 40 mins N purging + 20 mins Mixing

Figure 1. Operational patterns of three lab reactors

2.3 Chemical analysis

Concentration of TDN was measured by the Shimadzu TOC/TN analyzer (Shimadzu North America, Columbia, MD); the detection limit is 5 µg N/L. Concentrations of DIN anion species including NO₂⁻, NO₃⁻ were measured by ion chromatography. The detection limits by IC for both NO₂⁻, NO₃⁻ in saline waters was 0.005 mg N/L. The NH₄⁺ was measured according to phenate method given in APHA (1998). Concentration of DON is the difference between TDN and DIN which can be calculated by the following equation:

$$\text{DON} = \text{TDN} - \text{NH}_4^+ - \text{NO}_2^- - \text{NO}_3^-$$

2.4 Statistical Analysis

Data were graphed using Microsoft Office Excel 2013. To examine the statistical significance between the results, p values were calculated based on the unpaired t test with unequal variance using the method reported in the study of Welch (1947).

III. Results and Discussion

3.1 The N removal performance of three lab-scale reactors

Table 3.

Pollutant concentrations (mean \pm standard deviation) for Influent and CAS, BNR1 and BNR2 effluent. n indicate the number of samples for pollutant analysis.

	Concentration(mg/l)		
	CAS	BNR1	BNR2
TDN (n=26)	44.40 \pm 4.72	19.60 \pm 5.51	20.50 \pm 3.91
DIN (n=26)	34.60 \pm 3.91	7.52 \pm 4.24	7.76 \pm 3.52
DON (n=26)	9.80 \pm 1.17	12.08 \pm 1.35	12.74 \pm 1.44
NH ₄ ⁺ (n=26)	1.52 \pm 0.78	0.03 \pm 0.02	0.04 \pm 0.09
NO ₂ ⁻ (n=26)	0.01 \pm 0.03	0.00 \pm 0.01	0.00 \pm 0.00
NO ₃ ⁻ (n=26)	33.07 \pm 4.21	7.49 \pm 4.25	7.72 \pm 3.54
HMW-DON (n=26)	4.28 \pm 0.50	2.82 \pm 0.41	2.76 \pm 0.46
LMW-DON (n=26)	5.53 \pm 0.75	9.26 \pm 1.03	9.98 \pm 1.09
COD (n=26)	43.31 \pm 4.42	42.17 \pm 6.85	43.84 \pm 5.63
TSS	8 \pm 1.32	18 \pm 5.13	22 \pm 4.25
VSS	4 \pm 1.57	14 \pm 6.78	18 \pm 5.31

As shown in Table 3, 26 groups of DIN, DON and TDN data were measured from January 20th to March 26th in 2018 (Table. 2). Both BNR reactors were able to substantially remove TDN (54.95% for BNR1 and 56.92% for BNR2), DIN (77.75% for BNR1 and 78.45% for BNR2). As for CAS, limited TDN (8.77%) and DIN (8.31%) were removed. Figure 2 shows average concentrations of total and different forms of N in the effluents of the three lab reactors. Both BNR systems show similar and much lower effluent DIN and TDN concentration than CAS. In both BNR systems, nitrate comprises the largest fraction of inorganic N in the effluent, ammonia and nitrate are nearly 0 in the effluents, which indicate that the nitrification process is completely performed. The CAS effluent contains higher concentration of ammonia and nitrate which indicate the relatively insufficient nitrification and the absence of denitrification.

CAS primarily complete BOD degradation and nitrification, it doesn't contain any denitrification process in principal. The assimilation of inorganic N by bacteria might be the only mechanism for inorganic N removal in CAS system. The DIN removal efficiency is low in CAS process.

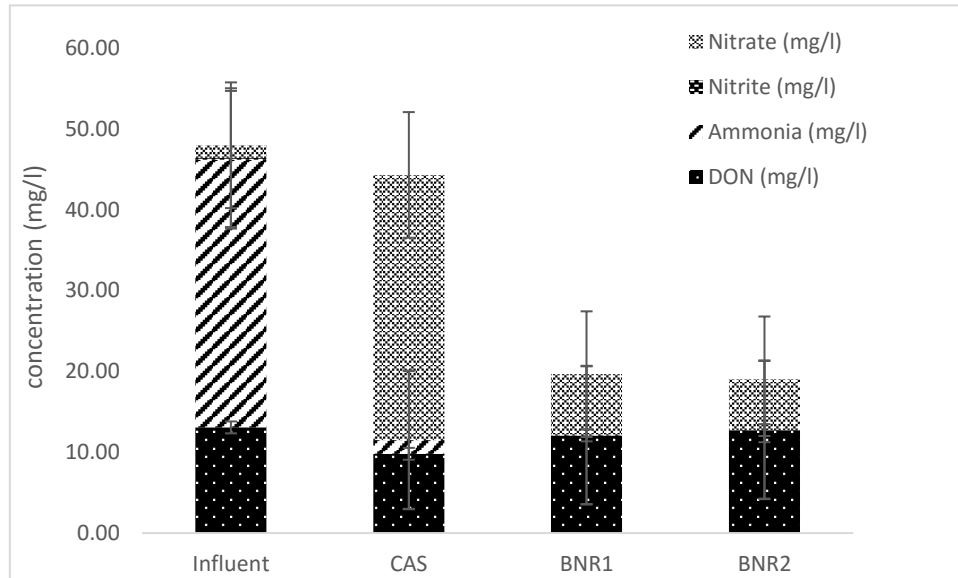


Figure 2. Concentrations of different DIN species in influent and CAS, BNR1 and BNR2 effluent. Error bars represent the standard deviations. Number of samples for different species of DIN analysis were all 5.

Despite lower concentrations of TDN and in BNR effluents, the BNR processes exhibited lower DON removal efficiency (around 7.57% for BNR1 and 2.52% for BNR2) than CAS system (around 25.02%). BNR1 effluent contains 12.08 ± 1.35 mg/l DON and they account for 61.63% of effluent TDN, 12.74 mg/l DON can be found in BNR2 effluent and they account for 62.01% of effluent TDN. CAS effluent contains nearly 9.80 ± 1.17 mg/l DON and only around 23.61% of effluent TDN is DON which is significantly lower than those in BNR effluents. By comparing these two kinds of BNR processes, more DON can be found in four-stage pre-denitrification BNR process, the DON removal efficiency of the BNR2 system is lower than that of the BNR1 system.

3.2 LMW-DON in effluent of three reactors

The BNR effluents contain not only larger amounts of DON but also higher fractions of LMW-DON in comparison to CAS (Fig. 3). Over 75% of effluent DON in the BNR systems are LMW-DON ($76.72 \pm 1.89\%$ for BNR1 and $78.39 \pm 2.08\%$ for BNR2) and it only accounts for $56.31 \pm 2.21\%$ of effluent DON for the CAS reactor. BNR effluents contain even higher concentration of LMW-DON than influent. BNR effluents have over 9 mg/l LMW-DON (9.26 ± 1.03 for BNR1 and 9.98 ± 1.09 for BNR2) and influents only contains 6.82 ± 0.67 mg/l LMW-DON. This result shows that large amount of LMW-DON is produced in BNR systems. A previous study also found a release of LMW-DON, and the increase of LMW-DON may be caused by microbial activity as a form of soluble microbial product (SMP) (Eom et al., 2017). Eom et al. (2017) speculate that hydrolysis and degradation of HMW-DON may contributed to the partial formation of LMW-DON. They also speculate that the formation of SMP may be another major source for LMW-DON formation as SMP's characteristics are very similar to those of LMW-DON released during the post-aerobic period in BNR system.

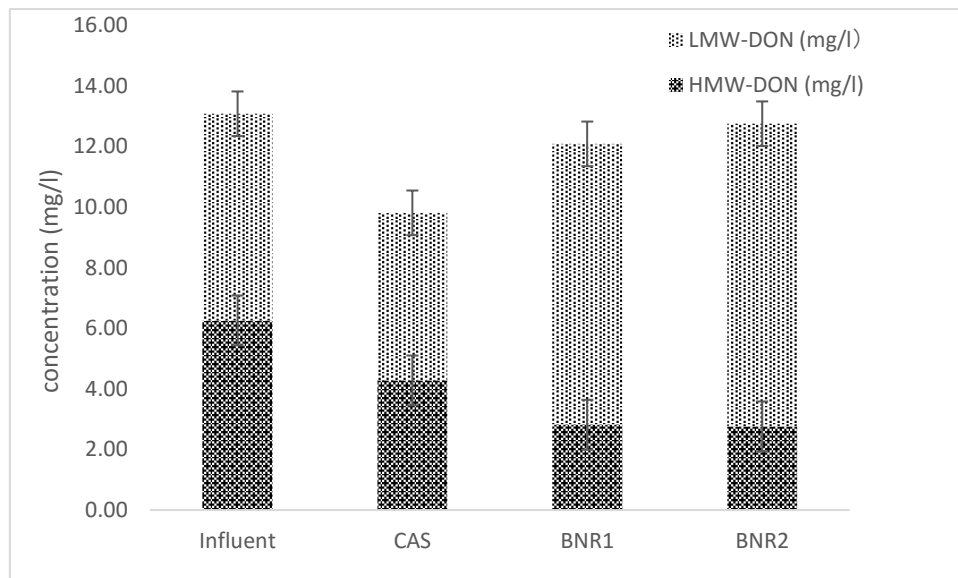


Figure 3. Concentration of LMW-DON and HMW-DON in DON for influent and CAS, BNR1 and BNR2 effluents. Error bars represent the standard deviations.

By comparing the two different pre-denitrification BNR processes, we found that BNR2 effluent contains a higher concentration and fraction of LMW-DON in effluent.

This result indicates that the four-stage pre-denitrification BNR system has a higher potential to produce the LMW-DON.

We speculated that longer anaerobic time may be the main factor to lead to the higher LMW-DON in effluent. In the research of Eom et al. (2017) LMW-DON continuously decreased during the aerobic period in the CAS reactor but in the two-stage pre-denitrification BNR reactor, the LMW-DON decreased during the nitrogen purging period which can expel oxygen in water and cause an anaerobic condition. LMW-DON kept constant during the remain anoxic period, and then it increased during the following aerobic period. So the anaerobic period may be an important stage to stimulate the formation of LMW-DON in the subsequent aerobic time in the BNR process. In this study, BNR2 contains a longer nitrogen purging time (80 min) than BNR1 (70 min), and the LMW-DON in the BNR2 effluent is a little higher than that in the BNR1 effluent.

From my perspective, there are some mechanisms may explain the formation of LMW-DON in th aerobic stage of BNR. The first one is the hydrolysis of LMW-DON to inorganic N, especially ammonia, in the anaerobic period. Some research found that under anaerobic condition, LMW-DON can be degraded by some bacteria and be transformed to some inorganic forms. For example, ammonification can transform organic nitrogen to ammonia under anaerobic condition. Some researchers found that the formation of ammonia in anoxic basin may due to the ammonification. (Kasi et al, 2017) During the hydrolysis of LMW-DON, some substances may be formed and activate the formation of some LMW-DON in microbial activity during the following aerobic period with the presence of oxygen.

Another mechanism may be that anaerobic stage may influence the microbial activities of autotroph or heterotroph bacteria. Autotroph and heterotroph bacteria coexist in nitrifying system. The autotroph bacteria can convert inorganic compound into organic compound, including SMP, to support the growth of heterotrophs. Furthermore, the

heterotrophs return inorganic substances to the autotrophs from SMP oxidation. (Ni et al., 2011) Maybe some specific substances released during the anoxic period, especially the anaerobic period, can inhibit LMW-DON assimilation by heterotrophs. Less LMW-DON can be transformed to inorganic nitrogen which lead to more LMW-DON in the BNR effluent.

Also, the degradation of LMW-DON may be another mechanism for increasing LMW-DON. In CAS reactor, the concentration of HMW-DON didn't change significantly under aerobic condition, and in BNR reactor the HMW-DON keep constant during anoxic period and then decreased during aerobic condition, The microbial activity in anoxic time may transform some unbiodegradable HMW-DON to biodegradable HMW-DON and this form of HMW-DON may be degraded in next aerobic period and form some LMW-DON. To verify this speculation, we suggest to operate a post denitrification BNR reactor in future study and compare it with a pre-denitrification BNR.

3.3 Influence of influent characteristic:

The experiment can be divided into two stages. The first stage was from May, 2017 to Dec, 2017, we used real wastewater collected from the Amherst WWTP for influent. The second stage was from Jan, 2018 to March, 2019 we used a mixture of real wastewater and synthetic wastewater for influent, their characteristic is shown in Table 4. The data during this period were useful to investigate the influence of influent characteristic on LMW-DON release. Figure 4 represents the fraction of LMW-DON in TDN from the influent and effluents of three reactors. The fraction of LMW-DON in TDNs from influent and effluents of CAS, BNR1 and BNR2 after Jan, 2018 increased by 50.65%, 58.28%, 148.45%, 165.05% in comparison to those before Jan, 2018 respectively. The increase of LMW-DON in BNR systems is much higher than that in CAS and influent which indicate that the mixture influent stimulates more LMW-DON production in BNR processes.

Table 4

Pollutant concentrations (mean \pm standard deviation) for influent before and after Jan, 2018

	Concentration(mg/l)	
	Before Jan,2018	After Jan, 2018
TDN	25.53 \pm 10.71	45.5 \pm 9.61
DIN	22.34 \pm 8.58	34.91 \pm 4.21
DON	4.36 \pm 0.99	13.07 \pm 1.47
NH ₄ ⁺	20.55 \pm 7.95	33.14 \pm 4.07
NO ₂ ⁻	0.71 \pm 0.94	0.27 \pm 0.19
NO ₃ ⁻	1.08 \pm 9.61	1.53 \pm 0.38
HMW-DON	1.95 \pm 0.64	6.26 \pm 0.83
LMW-DON	2.41 \pm 0.57	6.82 \pm 0.67
COD	82.09 \pm 14.04	211.42 \pm 18.79
TSS	68 \pm 3.56	56 \pm 4.21
VSS	53 \pm 4.74	46 \pm 3.53

Higher COD and the presence of acetate may lead to a higher fraction of LMW-DON in effluent TDN in experiments after Jan, 2018 in comparison to the experiments before Jan, 2018. LMW-DON production is highly related to the production of SMP during microbial activity. The COD loading rate can increase the release of SMP, especially utilization-associated products (UAP). UAP, which mainly comprises of low molecular weight substance including LMW-DON production increases with increasing organic substrate volumetric loading because the formation of UAP is proportional to the concentration of organic substrate removed. For the experiment before Jan, 2018 we used real wastewater collected from the Amherst WWTP, its organic substance loading rate is around 328 mg COD/(l*day) which is lower than the organic substance loading rate 844 mg/(l*day) after Jan, 2018 which used a mixture of real wastewater and synthetic wastewater. The higher COD loading rate increased the formation of LMW-DON in the BNR system. Also, different substances may influence the DON releasing.

Some substances may stimulate the formation of UAP. Jiang et al. (2008) found that UAP which included protein-like biopolymers and LMW humic-like compounds generally increased after acetate addition. The synthetic wastewater in influent after Jan, 2018 contains sodium acetate and it may stimulate the formation of LMW-DON.

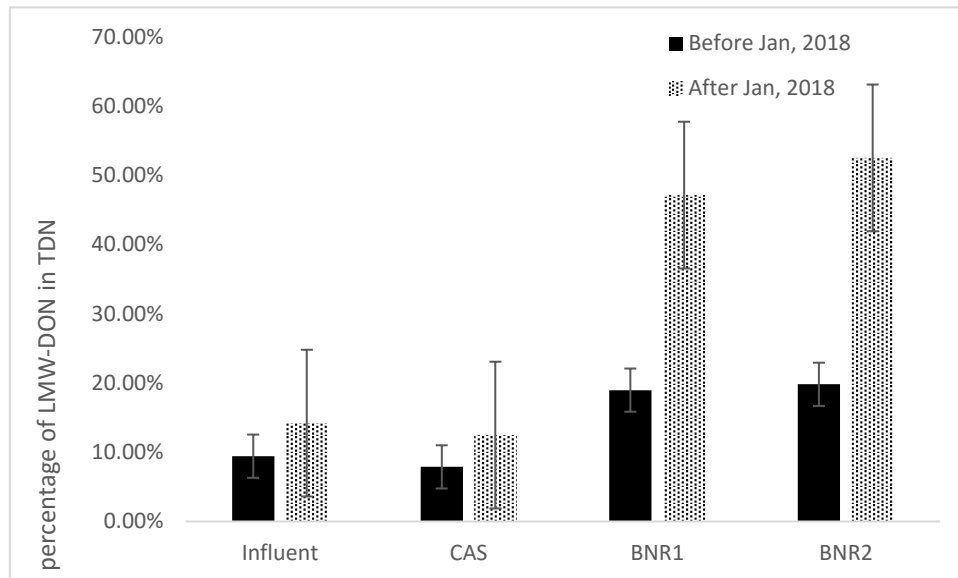


Figure 4. Fraction of LMW-DON in TDN for influent and CAS, BNR1 and BNR2 effluents. Error bars represent the standard deviations.

IV. Implication:

The upgrading of WWTP from CAS to BNR system is inevitable due to more-stringent N-discharge requirements. The pre-denitrification BNR system has been widely applied due to its operational and economic advantages. This upgrading has been proved to remove total and inorganic nitrogen effectively. However, there is a high probability that pre-denitrification BNR systems generates larger amounts of DON, especially LMW-DON, than CAS. LMW-DON showed greater potential to stimulate phytoplankton growth than DIN in coastal waters. This may explain why the upgrading of a WWTP cannot effectively control eutrophication.

The introduction of Total Maximum Daily Loads (TMDLs) for various water bodies to 1987 Clean Water Act (CWA) amendment and the proposed nutrient criteria guidelines

by the U.S. Environmental Protection Agency (USEPA) lead to widespread use of various nutrient removal process, however, these regulation or criteria only regulate the release of concentration of TDN and some species of DIN to a water body. No regulation restricts the release of DON has been established. Due to the higher potential to cause the algal growth in water, regulations should be established to control the release of DON.

Also, in this research, we found that the BNR treatment type may also influence DON release. The four-stage BNR effluent contained higher concentration and fraction of DON especially LMW-DON, which is more bioavailable than two-stage BNR effluent. The reason for the higher DON release may be the longer pre-anoxic especially pre-anaerobic time which may play important role in activating LMW-DON releasing during following aerobic period. In future, LMW-DON release in post-anoxic BNR process can be studied. If formation of LMW-DON can be controlled in post-anoxic BNR process, the upgrading from CAS to post-anoxic may be widely applied in future WWTP upgrading.

For wastewater treatment plants discharging into nitrogen-limited waters, such as estuaries or terminal lakes, post-treatment processes are needed to remove the highly bioavailable DON, especially the LMW-DON. HMW-DON can be removed by some physical-chemical method such as oxidation or coagulation. Lee et al, (2006) found that cationic polymer coagulation can increase the removal of all molecular weight fractions of DON with the highest molecular weight fraction (>10,000 Da) being preferentially removed., Dwyer et al, (2007) used advanced oxidation process to remove DON in water and caused a partial reduction of the DON and DOC associated with the large molecular weight fraction (>10 kDa). Arnaldos et al, (2010) used enhanced coagulation using alum (at doses commonly employed in tertiary phosphorus removal) followed by microfiltration (using 0.22 μm pore size filters) can simultaneous remove effluent DON and dissolved phosphorus (DP) effectively. However, as for the removal of more bioavailable LMW-DON, there are still limited researches to investigate it. Bio-

flocculation caused by adding multivalent cations may be a potential method to remove the LMW-DON (Eom et al., 2017).

The operation condition can also be studied to decrease the production of DON. DON is regarded to be generated from microbial activity as a form of SMP. Thus, the control of SMP may be an effective way to control DON. The formation of SMP is highly influenced by the PH, temperature, organic carbon loading and substance type (Barker et al., 1999). As a speculation in this study, the anaerobic time may also influence DON releasing. A future study can focus on finding an optimum operation condition to achieve both high inorganic nitrogen removal efficiency and lower LMW-DON production.

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VI. Appendices

Appendix A. Dissolved Total Nitrogen Measurement (mg/l)

Before, 2018

	Dissolved Total Nitrogen			
	Influent	CAS	BNR 1	BNR 2
4/28/2017	36.27	33.91	28.44	27.85
5/3/2017	31.62	29.61	24.08	23.33
5/10/2017	27.32	24.75	18.09	18.87
5/17/2017	24.785	23.11	17.035	14.045
5/24/2017	26.775	24.555	15.205	13.5
5/30/2017	25.945	23.58	13.335	12.105
6/8/2017	22.75	20.4	11.6	11.37
6/14/2017	14.67	13.59	10.65	10.13
6/22/2017	12.94	13.54	12.24	12.07
6/30/2017	15.04	12.07	13.25	11.43
7/5/2017	13.57	12.32	11.94	12.65
7/14/2017	11.48	12.71	12.54	11.79
7/19/2017	12.96	11.33	10.48	10.83
7/26/2017	17.03	14.56	13.2	14.97
8/4/2017	12.79	12.39	12.01	11.66
8/9/2017	10.31	10.89	11.21	10.72
8/18/2017	16.94	14.72	10.33	9.68
8/23/2017	22.4	21.12	15.39	15.07
8/30/2017	22.83	21.64	14.2	13.29
9/6/2017	29.63	27.04	19.33	18.85
9/13/2017	31.08	30.97	20.33	15.83
9/20/2017	26.52	25.52	18.25	19.08
10/7/2017	43.97	29.94	12.13	14.72
10/15/2017	40.62	29.71	23.59	20.58
10/20/2017	48.81	33.36	24.23	25.58
10/25/2017	40.81	34.09	25.76	26.89
10/30/2017	36.7	35.42	22.54	25.56
11/1/2017	26.93	21.2	18.48	21.74
11/2/2017	25.2	22.18	16.77	16.2
11/6/2017	33.33	24.02	19.22	19.5
11/9/2017	37.14	29.19	22.04	22.43
11/10/2017	35.12	22.29	11.52	11.43
11/14/2017	20.41	17.32	9.20	12.52
11/17/2017	30.95	8.74	1.35	7.49
11/25/2017	11.68	7.72	7.98	5.52
11/26/2017	14.93	7.52	5.96	2.66
11/28/2017	33.13	11.43	10.05	7.05
11/29/2017	6.10	5.09	4.68	2.21
11/30/2017	12.49	6.51	4.89	5.88
12/1/2017	15.00	11.95	8.76	11.81
12/5/2017	31.45	25.78	22.42	10.94
12/6/2017	26.45	15.18	14.26	10.5

12/19/2017	19.29	12.7	11.22	10.03
12/24/2017	43.25	22.73	14.23	13.51
12/26/2017	38.62	22.7	12.11	10.57
12/30/2017	36.14	23.82	10.16	11.36
Average	25.29	19.80	14.59	14.10
Stdev	10.71	8.41	6.02	6.12

After Jan, 2018

Date	Dissolved Total Nitrogen			
	Influent	CAS	BNR1	BNR2
1/20/2018	53.86	47.73	34.22	32.45
1/21/2018	41.51	37.35	26.11	26.10
1/24/2018	49.52	43.88	25.23	25.67
1/26/2018	52.9	46.91	24.25	26.11
1/28/2018	43.97	39.91	21.26	18.82
1/30/2018	47.1	40.31	18.61	20.90
2/2/2018	51.87	47.74	22.27	19.32
2/3/2018	39.24	38.18	18.86	14.94
2/4/2018	39.81	39.8	19.00	16.84
2/5/2018	39.27	37.25	18.77	16.17
2/6/2018	42.53	36.22	20.75	18.26
2/13/2018	45.75	41.71	22.71	19.81
2/14/2018	53.77	47.34	20.43	21.90
2/19/2018	53.09	50.05	22.36	20.02
2/21/2018	54.91	49.44	23.74	24.35
2/25/2018	51.64	51.03	24.27	19.30
3/1/2018	44.73	42.1	17.93	16.98
3/3/2018	53.85	47.4	25.53	21.90
3/7/2018	54.16	48.86	21.36	20.62
3/10/2018	47.29	46.95	22.88	17.72
3/12/2018	52.89	48.25	25.04	19.71
3/17/2018	42.02	39.29	20.55	18.48
3/18/2018	47.42	45.37	23.16	18.74
3/20/2018	42.74	42.27	20.46	16.23
3/21/2018	47.52	44.37	23.66	18.73
3/24/2018	54.11	52.18	26.24	23.36
Average	45.50	41.51	21.39	19.48
Stdev	9.61	4.72	5.15	3.91

Appendix B. Dissolved Inorganic Nitrogen Species Measurement (mg/l)

	Inf				CAS				BNR1				BNR2			
	Total	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	Total	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	Total	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	Total	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻
4/28/2017	28.5	27.52	0.22	0.76	28.61	4.38	1.78	22.45	22.54	0	0	22.54	21.73	0	0	21.73

5/3/2017	28.13	25.48	1.08	1.57	27.31	2.15	0.55	24.61	20.14	0.72	0.69	18.73	18.81	0	0	18.81
5/10/2017	22.52	20.03	1.12	1.37	21.42	3.77	0.29	17.36	13.59	0	0	13.59	14.14	0	0	14.14
5/17/2017	20.74	19.43	0	1.31	19.87	0.20	0.17	19.5	12.67	0.08	0.01	12.6	8.988	0.11	0.04	8.84
5/24/2017	22.45	21.63	0.08	0.74	21.08	0.33	0	20.75	10.23	0	0	10.23	8.43	0	0	8.43
5/30/2017	22.52	21.86	0	0.66	20.53	0.48	0	20.05	9.65	0	0	9.65	8.17	0	0	8.17
6/8/2017	19.13	18.52	0.48	0.13	17.08	0	0	17.08	7.46	0	0	7.46	6.95	0	0	6.95
6/14/2017	11.24	9.28	1.06	0.9	10.84	2.07	1.33	7.44	7.2	0	0	7.2	6.52	0	0	6.52
6/22/2017																
6/30/2017																
7/5/2017																
7/14/2017																
7/19/2017																
7/26/2017																
8/4/2017																
8/9/2017																
8/18/2017	13.29	11.86	0.59	0.84	11.76	0.76	0.44	10.56	6.91	0.32	0	6.59	6.34	0	0	6.34
8/23/2017	16.86	14.48	1.32	1.06	17.54	0	0	17.54	11.08	0	0	11.08	10.52	0	0	10.52
8/30/2017	18.45	18.45	0	0	18.33	0	0	18.33	10.07	0	0	10.07	8.6	0	0	8.6
9/6/2017	24.75	24.05	0.29	0.41	23.72	0.93	0.68	22.11	15.73	0	0	15.73	15.01	0	0	15.01
9/13/2017	26.97	25.57	0.63	0.77	28.06	0	0	28.06	16.9	0	0	16.9	13.93	0	0	13.93
9/20/2017	23.09	22.18	0.3	0.61	22.24	0	0	22.24	15.04	0	0	15.04	15.93	0	0	15.93
10/7/2017																
10/15/2017																
10/20/2017																
10/25/2017	34.93	33.88	1.05	0.00	30.17	3.62	1.09	25.47	20.83	0.00	0.00	20.83	22.21	0.00	0.00	22.21
10/30/2017	32.13	27.95	1.29	2.89	32.01	3.20	0.77	28.04	18.63	0.00	0.00	18.63	21.08	0.00	0.00	21.08
11/1/2017	22.27	19.82	1.11	1.34	18.49	1.85	0.30	16.34	14.66	0.00	0.00	14.66	17.42	0.00	0.00	17.42
11/2/2017	20.66	20.45	0.03	0.18	19.79	1.78	0.45	17.56	13.70	0.68	0.14	12.88	12.96	0.00	0.00	12.96
11/6/2017	29.59	26.63	1.48	1.48	21.32	1.49	0.19	19.64	15.21	0.61	0.30	14.30	15.16	0.30	0.15	14.71
11/9/2017	32.21	31.89	0.00	0.32	26.03	2.86	0.57	22.59	17.37	0.00	0.00	17.37	17.04	0.00	0.34	16.69
11/10/2017	31.63	31.00	0.08	0.55	19.85	2.78	0.56	16.52	8.43	0.00	0.00	8.43	7.84	0.00	0.00	7.84
11/14/2017	15.96	14.04	0.16	1.76	14.09	1.55	0.28	12.26	4.78	0.00	0.00	4.78	7.79	0.00	0.00	7.79
11/17/2017	27.40	25.20	0.27	1.92	6.32	0.95	0.18	5.19	1.01	0.00	0.00	1.01	3.93	0.00	0.00	3.93
11/25/2017	8.46	7.53	0.08	0.85	5.73	0.29	0.04	5.40	4.65	0.00	0.00	4.65	2.03	0.00	0.00	2.03
11/26/2017	11.10	10.66	0.11	0.33	4.59	0.69	0.07	3.83	2.20	0.00	0.00	2.20	0.50	0.00	0.00	0.50
11/28/2017	29.71	29.12	0.00	0.59	8.67	0.61	0.11	7.95	6.65	0.00	0.00	6.65	2.76	0.00	0.00	2.76
11/29/2017	2.59	2.43	0.05	0.10	2.39	0.36	0.05	1.98	1.03	0.00	0.00	1.03	0.42	0.00	0.00	0.42
11/30/2017	8.52	8.27	0.24	0.01	4.55	0.41	0.12	4.02	1.20	0.00	0.00	1.20	2.27	0.00	0.00	2.27
12/1/2017	10.48	10.06	0.10	0.31	9.27	1.02	0.27	7.99	4.83	0.15	0.10	4.59	7.42	0.00	0.00	7.42
12/5/2017	27.09	25.74	0.00	1.35	23.29	1.16	0.16	21.96	18.26	0.55	0.73	16.98	6.63	0.00	0.07	6.56
12/6/2017	21.86	19.46	0.44	1.97	11.71	0.70	0.17	10.84	10.31	0.52	0.21	9.59	6.46	0.26	0.13	6.07
12/19/2017	15.04	12.48	2.56	0.00	8.86	2.75	0.22	1.09	7.35	0.11	0.06	7.18	6.12899	0.06	0.14	5.93
12/24/2017	36.42	32.78	2.56	1.08	19.05	1.59	0.30	2.09	8.17	0.25	0.08	7.84	7.44	0.29	0.08	7.07
12/26/2017	33.19	24.23	1.99	6.97	18.91	0.31	0.02	3.48	7.58	0.18	0.20	7.20	6.02	0.09	0.04	5.90

12/30/2017 | 32.15 25.40 4.18 2.57 20.85 1.00 0.07 1.97 6.58 0.09 0.10 6.40 7.55 0.24 0.05 7.26

After Jan, 2018

	Total Dissolved Inorganic Nitrogen															
	Influent				CAS				BNR1				BNR2			
	Total	NH4+	NO2-	NO3-	Total	NH4+	NO2-	NO3-	Total	NH4+	NO2-	NO3-	Total	NH4+	NO2-	NO3-
1/20/2018	40.79	38.66	0.23	1.90	37.80	2.82	0.00	34.98	22.00	0.03	0.00	21.97	19.88	0.00	0.00	19.88
1/21/2018	29.11	27.49	0.22	1.40	28.60	1.62	0.00	26.98	14.55	0.01	0.00	14.54	14.21	0.00	0.00	14.21
1/24/2018	36.72	35.18	0.16	1.37	34.75	1.04	0.00	33.70	13.20	0.03	0.00	13.17	13.27	0.00	0.00	13.27
1/26/2018	37.84	36.09	0.29	1.46	36.02	1.01	0.00	35.02	10.14	0.00	0.00	10.13	11.61	0.00	0.00	11.61
1/28/2018	32.24	31.08	0.3	0.85	30.91	0.67	0.00	30.24	6.28	0.01	0.00	6.27	7.46	0.00	0.00	7.46
1/30/2018	35.03	32.76	0.65	1.62	31.49	1.75	0.00	29.74	3.30	0.00	0.00	3.30	8.88	0.00	0.00	8.88
2/2/2018	38.82	36.73	0.27	1.82	38.59	2.43	0.05	36.11	8.89	0.00	0.00	8.89	6.82	0.00	0.00	6.82
2/3/2018	27.95	26.68	0.19	1.08	29.97	2.70	0.00	27.27	5.16	0.00	0.00	5.16	3.81	0.00	0.00	3.81
2/4/2018	27.91	25.97	0.28	1.67	30.69	0.63	0.00	30.06	3.48	0.06	0.00	3.42	5.49	0.00	0.00	5.49
2/5/2018	29.10	27.09	0.29	1.72	29.91	2.93	0.03	26.95	3.79	0.05	0.00	3.74	6.24	0.15	0.00	6.09
2/6/2018	30.66	29.45	0.21	1.00	26.75	2.32	0.09	24.34	2.43	0.04	0.00	2.39	6.49	0.28	0.00	6.21
2/13/2018	33.46	32.00	0.14	1.31	32.85	1.75	0.00	31.10	9.29	0.04	0.00	9.25	7.96	0.07	0.00	7.89
2/14/2018	39.85	37.73	0.26	1.86	36.79	0.92	0.00	35.87	9.61	0.00	0.00	9.61	8.31	0.00	0.00	8.31
2/19/2018	39.49	38.16	0.22	1.11	39.33	0.92	0.00	38.41	4.29	0.06	0.01	4.21	6.89	0.00	0.00	6.89
2/21/2018	38.90	37.10	0.26	1.55	37.32	2.19	0.00	35.13	6.56	0.05	0.00	6.51	8.54	0.00	0.00	8.54
2/25/2018	38.01	35.47	0.46	2.08	41.01	1.46	0.10	39.45	8.68	0.04	0.00	8.64	6.03	0.28	0.00	5.75
3/1/2018	32.38	30.99	0.13	1.26	33.16	0.63	0.00	32.53	7.83	0.00	0.00	7.83	5.19	0.02	0.00	5.17
3/3/2018	38.92	37.65	0.11	1.16	35.95	1.26	0.00	34.68	5.57	0.00	0.00	5.57	7.26	0.00	0.00	7.26
3/7/2018	40.40	38.37	1.01	1.02	39.17	0.52	0.00	38.65	9.28	0.03	0.03	9.22	7.15	0.00	0.00	7.15
3/10/2018	33.76	31.68	0.21	1.87	36.25	0.86	0.00	35.39	8.31	0.00	0.00	8.31	4.36	0.00	0.00	4.36
3/12/2018	37.67	36.34	0.23	1.10	37.13	1.45	0.02	35.66	6.83	0.02	0.00	6.81	5.09	0.00	0.00	5.09
3/17/2018	29.95	28.38	0.29	1.28	30.73	2.96	0.00	27.77	6.28	0.06	0.00	6.23	6.67	0.00	0.00	6.67
3/18/2018	34.06	32.48	0.1	1.47	35.65	1.73	0.00	33.92	7.39	0.02	0.00	7.37	5.41	0.00	0.00	5.41
3/20/2018	31.96	30.24	0.21	1.51	33.67	0.82	0.00	32.85	4.22	0.05	0.00	4.17	5.77	0.00	0.00	5.77
3/21/2018	33.26	32.01	0.27	0.99	34.00	1.28	0.00	32.72	2.77	0.07	0.00	2.70	4.83	0.16	0.00	4.67
3/24/2018	38.88	36.82	0.29	1.77	41.23	0.79	0.00	40.43	5.32	0.03	0.00	5.29	8.16	0.16	0.00	8.00
Average	34.89	33.18	0.28	1.43	34.60	1.52	0.01	33.07	7.52	0.03	0.00	7.49	7.76	0.04	0.00	7.72
Stdev	4.19	4.08	0.19	0.34	3.91	0.78	0.03	4.21	4.24	0.02	0.01	4.25	3.52	0.09	0.00	3.54

Appendix C. Dissolved organic Nitrogen Species Measurement (mg/l)

Before Jan, 2018

	Dissolved Organic Nitrogen											
	Influent			CAS			BNR1			BNR2		
	Total	HMW	LMW	Total	HMW	LMW	Total	HMW	LMW	Total	HMW	LMW

4/28/2017	7.77	3.91	3.86	5.30	3.43	1.87	5.90	1.78	4.12	6.12	1.75	4.37
5/3/2017	3.49	1.80	1.69	2.30	1.38	0.92	3.94	1.19	2.75	4.52	1.06	3.46
5/10/2017	4.80	2.66	2.14	3.33	2.12	1.21	4.50	1.97	2.53	4.73	2.12	2.61
5/17/2017	4.05	1.69	2.36	3.24	1.62	1.62	4.35	1.48	2.87	5.06	1.53	3.53
5/24/2017	4.33	2.15	2.18	3.47	1.90	1.57	4.98	1.57	3.41	5.07	1.38	3.69
5/30/2017	3.42	1.44	1.98	3.05	1.22	1.83	3.69	1.02	2.67	3.94	1.06	2.88
6/8/2017	3.62	1.89	1.73	3.32	1.71	1.61	4.14	1.08	3.06	4.42	1.30	3.12
6/14/2017	3.43	1.49	1.94	2.75	1.21	1.54	3.45	0.99	2.46	3.61	1.04	2.57
6/22/2017												
6/30/2017												
7/5/2017												
7/14/2017												
7/19/2017												
7/26/2017												
8/4/2017												
8/9/2017												
8/18/2017	3.65	1.92	1.73	2.96	1.49	1.47	3.42	1.08	2.34	3.34	1.19	2.15
8/23/2017	5.54	2.63	2.91	3.58	1.56	2.02	4.31	1.09	3.22	4.55	1.27	3.28
8/30/2017	4.38	2.10	2.28	3.31	1.82	1.49	4.13	1.00	3.13	4.69	0.97	3.72
9/6/2017	4.88	1.73	3.15	3.32	1.55	1.77	3.60	1.31	2.29	3.84	1.18	2.66
9/13/2017	4.11	1.99	2.12	2.91	1.43	1.48	3.43	1.22	2.21	1.90	0.85	1.05
9/20/2017	3.43	1.25	2.18	3.28	1.55	1.73	3.21	1.56	1.65	3.15	1.29	1.86
10/7/2017												
10/15/2017												
10/20/2017												
10/25/2017	5.88	3.60	2.28	3.92	2.16	1.76	4.93	2.08	2.85	4.68	1.46	3.22
10/30/2017	4.57	2.19	2.38	3.41	1.89	1.52	3.91	1.08	2.83	4.48	0.97	3.51
11/1/2017	4.66	2.35	2.31	2.72	1.56	1.16	3.82	1.30	2.52	4.32	1.27	3.05
11/2/2017	4.54	2.73	1.81	2.40	1.49	0.91	3.07	0.72	2.35	3.24	0.63	2.61
11/6/2017	3.74	1.63	2.11	2.70	1.43	1.27	4.01	1.29	2.72	4.34	1.21	3.13
11/9/2017	4.93	1.95	2.98	3.16	1.79	1.37	4.67	1.51	3.16	5.39	1.32	4.07
11/10/2017	3.49	1.49	2.00	2.44	1.36	1.08	3.09	0.91	2.18	3.59	1.48	2.11
11/14/2017	4.45	1.71	2.74	3.23	1.20	2.03	4.42	0.97	3.45	4.74	1.01	3.73
11/17/2017	3.55	1.41	2.14	2.42	0.92	1.50	0.34	0.11	0.23	3.56	0.90	2.66
11/25/2017	3.22	1.25	1.97	1.98	0.96	1.02	3.33	0.90	2.42	3.48	0.94	2.54
11/26/2017	3.83	1.15	2.68	2.94	0.98	1.96	3.77	0.82	2.95	2.16	0.72	1.44
11/28/2017	3.42	0.73	2.69	2.76	0.72	2.04	3.40	0.52	2.88	4.29	0.61	3.68
11/29/2017	3.51	1.17	2.34	2.70	1.06	1.64	3.66	0.92	2.74	1.79	0.56	1.23
11/30/2017	3.97	1.92	2.05	1.96	1.04	0.92	3.69	1.19	2.50	3.61	1.23	2.38
12/1/2017	4.52	2.15	2.37	2.68	1.59	1.09	3.93	1.25	2.68	4.39	1.26	3.13
12/5/2017	4.36	1.69	2.67	2.49	1.29	1.20	4.16	1.17	2.99	4.31	1.20	3.11
12/6/2017	4.59	1.93	2.66	3.47	1.69	1.78	3.95	1.02	2.93	4.04	1.17	2.87
12/19/2017	4.25	2.14	2.11	3.84	1.67	2.17	3.87	1.39	2.48	3.90	1.30	2.60
12/24/2017	6.83	2.47	4.36	3.68	1.61	2.07	6.06	1.48	4.59	6.07	1.41	4.65

12/26/2017	5.43	2.41	3.02	3.79	1.49	2.30	4.53	1.23	3.31	4.55	1.14	3.41
12/30/2017	3.99	1.66	2.33	2.97	1.00	1.97	3.58	0.82	2.77	3.81	0.87	2.95
Average	4.36	1.95	2.41	3.08	1.51	1.57	3.92	1.17	2.75	4.11	1.16	2.94
Stdev	0.99	0.64	0.57	0.63	0.48	0.39	0.93	0.38	0.70	0.97	0.32	0.81

After Jan, 2018

	Dissolved Organic Nitrogen											
	Influent			CAS			BNR1			BNR2		
	Total	HMW	LMW	Total	HMW	LMW	Total	HMW	LMW	Total	HMW	LMW
1/20/2018	12.65	5.75	6.90	9.63	4.48	5.15	11.94	2.84	9.10	12.47	2.84	9.63
1/21/2018	12.40	6.03	6.37	8.75	3.63	5.12	11.40	2.75	8.65	11.92	2.73	9.18
1/24/2018	12.80	6.05	6.75	10.19	4.18	6.01	11.77	2.96	8.81	12.29	2.34	9.95
1/26/2018	15.06	7.46	7.59	10.95	4.53	6.43	13.76	3.65	10.11	14.43	3.36	11.06
1/28/2018	11.73	5.72	6.01	9.27	4.34	4.94	11.13	2.56	8.57	11.45	2.14	9.30
1/30/2018	12.07	5.87	6.20	9.27	3.99	5.28	11.08	2.45	8.63	11.98	2.96	9.02
2/2/2018	13.05	5.89	7.16	10.12	4.61	5.51	11.84	2.58	9.26	12.47	3.03	9.43
2/3/2018	11.29	5.39	5.90	8.02	3.63	4.39	10.71	2.50	8.20	10.81	2.24	8.56
2/4/2018	11.90	5.5	6.40	8.53	3.83	4.70	10.83	2.48	8.35	11.46	2.55	8.91
2/5/2018	10.17	4.59	5.58	7.78	3.51	4.27	9.27	1.95	7.32	9.96	2.02	7.94
2/6/2018	11.87	5.43	6.44	9.36	3.93	5.43	11.14	2.99	8.14	11.35	2.55	8.80
2/13/2018	12.29	5.97	6.32	9.08	4.20	4.89	11.08	2.35	8.73	11.98	2.43	9.55
2/14/2018	13.92	6.72	7.19	10.26	4.31	5.95	12.80	2.85	9.94	13.91	3.39	10.52
2/19/2018	13.60	6.75	6.86	9.60	4.19	5.41	12.30	3.30	9.01	13.56	2.74	10.82
2/21/2018	16.01	7.91	8.10	11.89	5.38	6.52	14.81	3.66	11.15	15.41	3.76	11.65
2/25/2018	13.63	6.46	7.17	10.70	4.38	6.32	12.39	2.54	9.85	13.31	2.54	10.77
3/1/2018	12.35	5.66	6.69	9.42	3.86	5.56	11.36	2.46	8.90	12.13	2.56	9.57
3/3/2018	14.93	7.41	7.52	11.41	5.35	6.06	13.88	3.44	10.44	14.69	3.57	11.12
3/7/2018	13.76	6.70	7.05	10.36	4.17	6.19	12.97	3.02	9.94	13.26	2.40	10.87
3/10/2018	13.53	6.27	7.26	10.33	4.55	5.77	12.56	2.86	9.70	13.34	3.07	10.28
3/12/2018	15.22	7.27	7.94	11.75	4.73	7.02	14.18	3.13	11.06	15.18	3.50	11.68
3/17/2018	12.07	5.65	6.42	8.82	3.97	4.84	11.22	2.52	8.70	12.01	2.57	9.43
3/18/2018	13.36	6.31	7.05	9.52	4.02	5.50	12.65	2.68	9.97	13.09	2.37	10.71
3/20/2018	10.78	5.22	5.57	7.72	3.61	4.11	9.98	2.59	7.39	10.62	2.38	8.23
3/21/2018	14.26	7.12	7.14	11.37	5.04	6.33	13.14	2.75	10.39	13.67	2.82	10.85
3/24/2018	15.23	7.59	7.63	10.82	4.77	6.05	13.89	3.30	10.59	14.61	2.86	11.74
Average	13.07	6.26	6.82	9.80	4.28	5.53	12.08	2.82	9.26	12.74	2.76	9.98
Stdev	1.47	0.83	0.67	1.17	0.50	0.75	1.35	0.41	1.03	1.44	0.46	1.09

Appendix D. COD Measurement (mg/l)

Before Jan, 2018

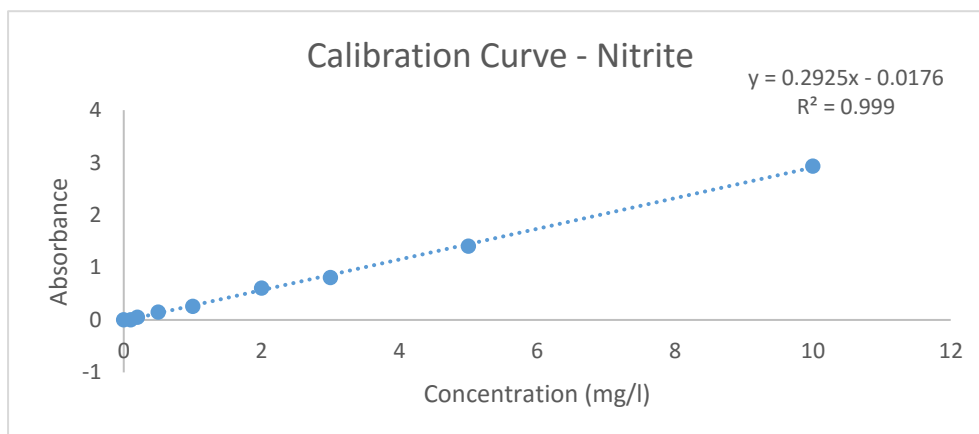
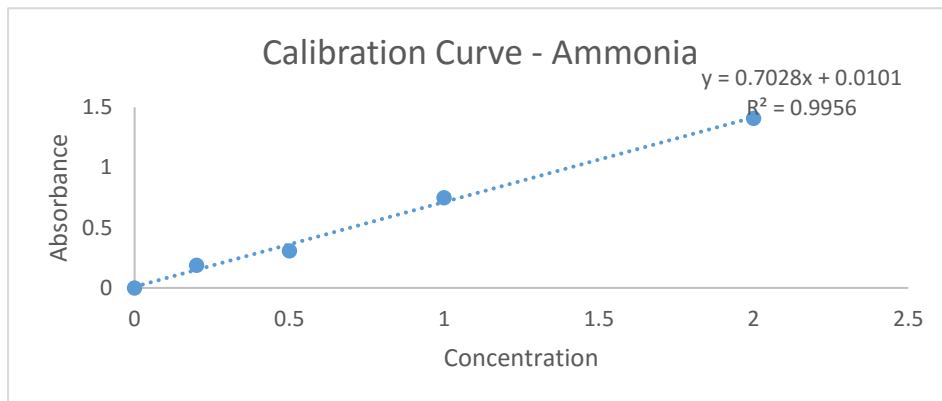
	COD			
	Influent	CAS	BNR1	BNR2
10/7/2017	84.84	53.30	24.04	31.07
10/15/2017	91.34	9.78	21.96	8.04
10/20/2017	97.83	24.57	4.57	1.89
10/25/2017	67.61	1.52	19.78	7.61
10/30/2017	94.96	53.30	24.04	31.07
11/1/2017	59.78	20.89	25.52	13.67
11/2/2017	54.41	13.48	14.22	5.33
11/6/2017	78.27	10.77	24.23	7.50
11/9/2017	74.81	20.58	10.96	6.35
11/10/2017	72.88	3.85	12.88	3.65
11/14/2017	90.48	20.88	19.68	3.68
11/17/2017	108.68	12.08	19.68	25.28
11/25/2017	76.88	9.19	6.88	29.58
11/26/2017	70.73	2.65	3.50	12.65
11/28/2017	56.50	6.58	11.12	14.58
11/29/2017	88.42	26.50	34.58	23.04
11/30/2017	96.85	14.82	26.11	22.31
12/1/2017	85.45	16.47	17.78	25.30
12/5/2017	84.49	14.58	15.43	14.66
12/6/2017	91.31	11.93	24.38	22.25
12/19/2017	90.68	18.32	15.92	23.03
12/24/2017	80.45	14.49	23.97	21.73
12/26/2017	99.43	11.95	25.64	25.16
12/30/2017	73.14	15.23	20.66	14.55
Average	82.09	16.99	18.65	16.42
Stdev	14.05	12.88	7.60	9.42

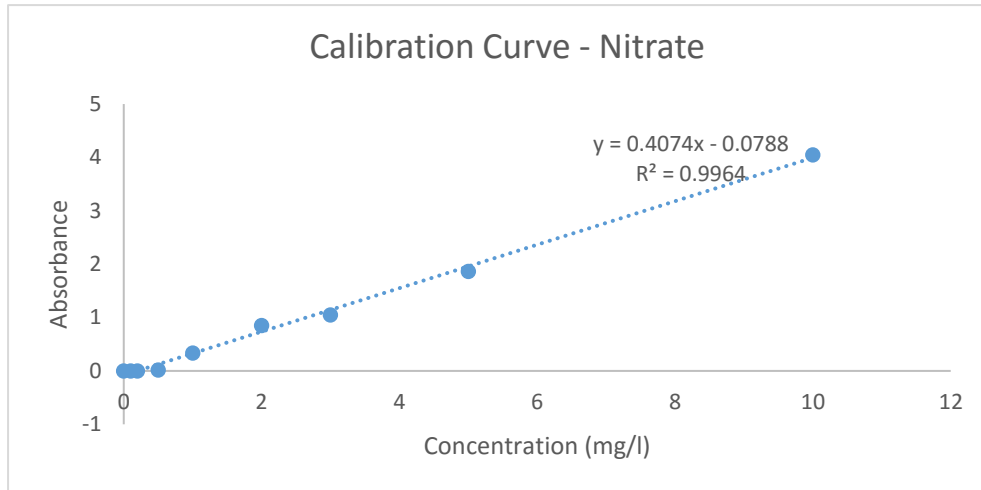
After Jan, 2018

	COD			
	Influent	CAS	BNR1	BNR2
1/20/2018	231.86	24.44	33.41	14.91
1/21/2018	203.35	21.86	29.73	17.73
1/24/2018	231.66	21.94	31.07	21.10
1/26/2018	191.31	24.49	16.70	16.57
1/28/2018	210.05	20.58	30.79	18.69
1/30/2018	213.21	31.13	19.91	30.17
2/2/2018	234.43	24.26	21.83	33.50
2/3/2018	192.86	21.02	21.79	27.69

2/4/2018	233.51	33.37	24.59	25.10
2/5/2018	227.72	16.28	22.61	15.83
2/6/2018	188.82	27.49	23.66	26.17
2/13/2018	185.56	20.24	16.70	20.24
2/14/2018	183.27	21.00	17.17	19.72
2/19/2018	212.26	19.99	17.34	17.19
2/21/2018	211.91	21.53	28.16	31.07
2/25/2018	196.82	24.41	16.99	25.98
3/1/2018	235.31	27.44	31.41	32.43
3/3/2018	236.10	24.06	16.17	20.89
3/7/2018	201.50	20.86	13.80	20.86
3/10/2018	223.44	33.07	14.48	28.35
3/12/2018	185.37	27.77	15.59	21.22
3/17/2018	193.13	20.66	13.73	21.28
3/18/2018	216.71	20.57	28.84	28.52
3/20/2018	239.22	18.30	24.78	31.79
3/21/2018	223.90	18.18	33.43	23.89
3/24/2018	193.60	21.04	11.73	29.04
Average	211.42	23.31	22.17	23.84
Stdev	18.789	4.423973	6.851803	5.626766

Appendix E. Calibration Curve





Appendix F. unpaired t test

	<i>BNR1 TDN</i>	<i>BNR2 TDN</i>
Mean	19.65	19.01
Variance	22.41	15.32
Observations	26	26
Hypothesized Mean Difference	0	
df	48	
t Stat	0.53	
P(T<=t) one-tail	0.29	
t Critical one-tail	1.67	
P(T<=t) two-tail	0.59	
t Critical two-tail	2.01	

	<i>BNR1 DIN</i>	<i>BNR2 DIN</i>
Mean	7.58	6.27
Variance	19.00	12.71
Observations	26	26
Hypothesized Mean Difference	0	
Df	48	
t Stat	1.18	
P(T<=t) one-tail	0.12	
t Critical one-tail	1.68	
P(T<=t) two-tail	0.24	
t Critical two-tail	2.01	

	<i>BNR1 DON</i>	<i>BNR2 DON</i>
Mean	12.08	12.74
Variance	1.83	2.06
Observations	26	26
Hypothesized Mean Difference	0	
df	50	
t Stat	-1.7174054	
P(T<=t) one-tail	0.05	
t Critical one-tail	1.68	
P(T<=t) two-tail	0.04	
t Critical two-tail	2.01	

	<i>BNR1 LMW-DON</i>	<i>BNR2 LMW-DON</i>
Mean	9.26	9.98
Variance	1.07	1.20
Observations	26	26
Hypothesized Mean Difference	0	
df	50	
t Stat	-2.43925	
P(T<=t) one-tail	0.01	
t Critical one-tail	1.68	
P(T<=t) two-tail	0.02	
t Critical two-tail	2.01	