Comparative assessment on the influences of effluents from conventional activated sludge and biological nutrient removal processes on algal bloom in receiving waters

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ABSTRACT

The goal of this study was to evaluate the effect of effluents from conventional activated sludge (CAS) and biological nutrient removal (BNR) processes on algal bloom in receiving waters. We made multiple effluent sampling from one CAS and two BNR facilities, characterized their effluents, and conducted bioassay using river and ocean water. The bioassay results showed that CAS effluents brought similar productivity in both river and ocean water, while BNR effluents were more reactive and productive in ocean water. Unexpectedly, nitrogen-based biomass yields in ocean water were up to six times larger for BNR effluents than CAS effluent. These results indicated that nitrogen in BNR effluents, although its total concentration is lower than that of CAS effluent, is more reactive and productive in ocean water. The ocean water bioassay further revealed that effluents of BNR and CAS led to considerably different phytoplankton community, indicating that different characteristics of effluents could also result in different types of algal bloom in receiving waters. The present study suggests that effects of upgrading CAS to BNR processes on algal bloom in receiving waters, especially in estuary and ocean, should be further examined.

Keywords: Algal bloom, BNR, CAS, Long Island Sound, Nitrogen, Organic Nitrogen

1. Introduction

Eutrophication, or increased algal blooms due to excessive nutrient loading to a body of water, is a problem for numerous water bodies throughout the world. One such affected water body is the Long Island Sound (LIS), an estuary bordered by the states of New York and Connecticut on three sides and open to the Atlantic Ocean on its fourth side. The LIS has been experiencing seasonal hypoxia and subsequent fish kills for years, especially in the bottom waters on its western border [1]. In order to improve conditions on the LIS, an agreement between the U.S. EPA and the state governments of New York and Connecticut was reached [2]. The affected states were required to first freeze nitrogen loadings to the LIS at 1990 levels and then eventually reduce N loadings (from human sources) to the LIS by 58.5% by the year 2014 [2]. One major step taken to achieve this goal was the upgrade of numerous wastewater treatment plants (WWTPs) from conventional activated sludge (CAS) processes to biological nutrient re-

moval (BNR) processes. As of approximately ten years after the initial implementation of the plan, around \$650 million had been spent on reducing N loadings from point sources [1]. Despite these efforts the LIS area affected by hypoxia in 2006 was actually larger than the area affected in 1991 [3], and eutrophication and hypoxia problems have continued to occur in LIS [4].

Traditionally, BNR processes remove dissolved inorganic N (DIN) by adopting extended solids retention time (SRT) with aerobic nitrification and anoxic denitrification in the main stream of wastewater treatment [5]. In spite of vigorous biological wastewater treatment involved in BNR processes, dissolved organic N (DON) remains persistent through treatment, leading to high DON/DIN in low TN effluents [6-8].

More studies have reported that phytoplankton can use DON as a source of N [7, 9-11] and it could actually lead to higher algal biomass yield than DIN [10]. The marine diatom *Thalassiosira* has been recently sequenced [12] and it was found to have genes for plasma membrane amino acid transporters

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Received December 9, 2015 Accepted April 21, 2016

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(reviewed by Bronk et al. [11]), providing genetic evidence that some phytoplankton can directly use amino acids, a pool of DON, for their metabolism. It can also be inferred that uptake of DON compared to DIN could lead to less energy expense for algal anabolism, possibly accounting for higher growth yields. Literature has also shown that DON as a primary N source could select different phytoplankton community than DIN. Berman and Chava [10] showed that N-fixing cyanobacteria proliferated in the media containing urea (DON) as sole N source than DIN. In a related context, Gilbert et al. [13] found that cyanobacterial bloom in Florida Bay has been associated with the highest concentration of DON. All this information implies that BNR effluents, with low TN but high DON/DIN, can render DON to become more readily available and spur the growth of certain phytoplankton community.

What might also be important to understand is the effect of types or characteristics (e.g., hydrophobicity, size, etc.) of DON on algal blooms in the receiving water. Berman and Chava [1999] made a proposition that different types of DON also affected the structure of the phytoplankton community. Our earlier study [8] investigated effluent proteins from three full-scale WWTPs (two CAS and one BNR facilities) and found that the type of proteins present in CAS and BNR effluents were different, indicating that the characteristics of DON in CAS and BNR effluents could also be considerably different. In addition, Westgate and Park [8] found that there was a difference for CAS and BNR effluents with respect to the profile of proteolytic enzymes. The protease profile was very similar for three plant influents but its profile in BNR effluent was more diverse than those in CAS effluents. These suggested that more complex proteases in BNR effluents, which would enter into the receiving water, resulted from the more complicated biological processes used in BNR systems. Consequently, it can be expected that when different DONs in BNR and CAS effluents enter into the receiving water they may also lead to different effects on nutrient cycling and algal growth.

The information provided in literature and findings from our previous research led us to develop a hypothesis that upgrading of WWTPs from CAS to BNR processes may not necessarily bring reduction in the events of algal blooms but cause different types of algal blooms, possibly with more abundant algal growth. To test this hypothesis, we have conducted laboratory bioassay experiments on field CAS and BNR effluents using both Connecticut River and LIS water; the bioassay on Connecticut River was included because it is the largest freshwater draining to LIS and also to learn the effect of same effluents on algal growth in both river and ocean water. The focus of the study was to find the biomass yield of CAS and BNR effluents, based on consumption of their nitrogen. During the study, we also characterized differences in the algal community that results from incubating BNR and CAS effluents in the same LIS water.

2. Materials and Methods

2.1. Sampling of Full-Scale Wastewater Treatment Effluents

Samples of secondary effluent were collected from three WWTPs

(one CAS and two BNR facilities) in winter, spring, and early summer period. CAS and BNR1 facilities are located in Western Massachusetts while the BNR2 facility is located farther downstream in Connecticut. All three facilities discharge their effluents to the Connecticut River permanently draining into LIS. The CAS facility (7.1 MGD design flow) uses a conventional activated sludge system with mechanical aeration at a solids retention time (SRT) in the range of 5-10 days. The BNR1 facility (67 MGD) uses the Ludzack-Ettinger (LE) process with a relatively long SRT, 25 days. The BNR2 facility (2.1 MGD) adopts a modified LE (MLE) process in the SRT of 15 days. Effluent samples were transported to the University of Massachusetts laboratory immediately after collection and were stored at 4°C (within two hours of collection). Within 24 hours of storage, whole effluent samples or effluents passed through a 0.45 μm filter (Millipore Express PLUS filter, Millipore, Billerica, MA, USA) were used for bioassay or transferred to separate 15 mL vials, frozen, and stored for later chemical analysis.

2.2. Sampling of Receiving Water

Receiving water samples for bioassay were collected from the Connecticut River and LIS. Samples from the Connecticut River were collected from a boat dock to avoid collecting organic matter from plants and other materials in the shallow water near the banks. The dock was located at a point along the Connecticut River in Hadley, MA and the travel time of the water from this point to LIS is approximately one day (C. Brown, personal communication). Water from LIS was collected from the White Sand Beach in Old Lyme, CT near the point where the Connecticut River drains into the Sound. Samples were collected away from the shore in order to avoid collecting large amounts of sand and other particles. Receiving water was transported to the laboratory immediately after collection and stored at 4°C (within 2 hours of collection). Samples of receiving water were then passed through a 100 μm Nylon Net filter for bioassay.

2.3. Set-up and Sampling of Bioassay Experiments

Bioassay experiments were conducted by mixing 1 L of effluent and 1 L of receiving water in a 2 L Pyrex bottle. This dilution rate does not mimic the natural dilution of effluents in the river and LIS, but was chosen to facilitate the study on the effects of different effluents on the same receiving waters. Note that a recent bioassay study [14], using higher dilution rates (4 and 10 times), still produced a similar trend of the data seen in the current study. The contents of the bottles were completely mixed using a stir bar and plate. The bioassay bottles were covered with 100 μm Nylon Net filter fabric to prevent dust from entering into the bottles. The bioassay took place on the bench top next to windows (windows making entire wall of the lab) for exposure to natural light and dark conditions. The laboratory room was air-conditioned and temperature was maintained at $21 \pm 2^\circ\text{C}$. Sample collection times were varied between incubation sets and were based on the reactions observed in each bioassay. The December and March bioassay sets consisted of a single bioassay bottle for each effluent. The May bioassay set consisted of duplicates for each effluent.

2.4. Chemical Analysis

Inorganic nitrogen species (ammonium, nitrate, and nitrite) were measured using a Metrohm ion chromatograph (IC) (Metrohm, Herisau, Sz) and dissolved ($< 0.45 \mu\text{m}$) total nitrogen (DTN) was measured using a Shimadzu TN/TOC analyzer (Shimadzu TOC-VCPH with TNM-1, Shimadzu North America, SSI Inc., Columbia, MD, USA). The TOC/TN machine detects concentrations of TOC down to 0.4 mg/L as carbon and 0.2 mg/L of TN as nitrogen. The experimental lower detection limit of the IC varies for each ion measured. Nitrite and nitrate can be effectively measured at concentrations down to 5 $\mu\text{g/L}$ and ammonium to 20 $\mu\text{g/L}$. DON was then calculated using the following equations:

$$\text{DON} = \text{DTN} - (\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-)$$

Phosphate (PO_4^{3-}) concentrations in effluents were measured using the same IC used for inorganic nitrogen and its detection limit was 20 $\mu\text{g/L}$. Total and volatile suspended solids (TSS and VSS) were measured according to Standard Methods [15]. Protein concentrations were determined using both the original Lowry method [16] and the Frølund modification of the Lowry method [17], as the revised method accounts for the interference of humic substances. Both values were compared and, based on other nitrogen concentrations obtained, the modified protein values were chosen for the current study. The absorbance of the protein assay was read on a Thermo Spectronic Genesys 10 UV Spectrophotometer (Thermo Spectronic, Madison, WI, USA) and the standard curve was determined using known concentrations of bovine serum albumin (BSA) (Fisherbrand Scientific, Pittsburg, PA, USA). All protein concentrations in this study are expressed as mg/L-N: N accounts for 16% of BSA molecule [8].

2.5. Microscopic Analysis

To study diversity and population of phytoplankton growing in each bioassay bottle, various microscopic analyses were adopted during this study. For bioassays conducted in December and March periods, a light microscope (Olympus CH2, Japan) was used. During the May bioassay, we also conducted phase contrast microscopy (Nikon Labophot Microscope, Japan). To obtain sufficient algal cell concentration for phase contrast microscopic analyses, 10 mL of bioassay sample was first filtered through a 0.45 μm filter and filtered algal cells were re-suspended in 2 mL of sample filtrate. Wet mounts of the concentrated sample solutions were prepared and images of phase contrast microscopes were taken using a digital camera (Sony HD digital camera, Japan).

3. Results and Discussion

The results of the study are presented in three effluent sampling events and subsequent receiving water bioassays.

• Effluent Sampling and Bioassay in December

The first effluent sampling and bioassay were conducted for one CAS and one BNR (BNR1) facility. As expected, BNR1 effluent showed much lower dissolved total nitrogen (DTN) in comparison to CAS effluent (Table 1). BNR effluent showed a relatively similar composition of ammonia, nitrate, nitrite, and dissolved organic N (DON), making up DTN at 2.23 mg/L-N. DON comprised almost 30% of DTN, some of which was actually protein at 0.48 mg/L-N. In contrast, DTN in CAS effluent was mostly ammonia, and the shorter SRT (5-10 days) used in this facility accounted for this high ammonia in the effluent. DON in CAS effluent was higher in comparison to BNR effluent, but its fractional composition was much smaller than the counterpart. Both Connecticut River and LIS water showed very similar DTN at around 0.3 mg/L-N. The PO_4^{3-} concentrations were similar between the two effluents, 0.72 mg/L-P for CAS and 1.04 mg/L-P for BNR.

For bioassay experiments, filtered (0.45 μm) effluents were incubated with the river water and LIS ocean water. It was visually observed that the ocean bioassay for BNR effluent led to the fastest algal growth. Light brownish algal biomass (mainly diatoms) started blooming on day 5, which was about four days earlier than all other bioassays. This observation can be further explained with the analysis of chemical data. Fig. 1 shows changes in DTN measured during the first bioassay. Consistent with the visual observation, it was the ocean bioassay for BNR effluent that showed the fastest N uptake. The N consumption in all other bioassays, including the river bioassay for BNR effluents, was much slower and similar to each other. These data indicated that N in BNR effluent was more reactive or more bioavailable in the ocean water.

We also measured soluble ($< 0.45 \mu\text{m}$) proteins, a significant fraction of DON [8], during the bioassay (Fig. 2). The fate of proteins in BNR-ocean bioassay was dynamic, showing early degradation, re-generation, and re-degradation. Depletion of soluble proteins occurred on day 4, which was a day prior to the first algal bloom observed, along with a sudden decrease in DTN (Fig. 1). This indicates that the bioavailability and degradation of proteins played an important role in the first algal bloom in the BNR-ocean bioassay. The dramatic increase and decrease of soluble proteins over the later period indicates that proteins were also released during phytoplankton growth and became bioavailable for further microbiological

Table 1. Concentration and Composition of Nitrogen in Cas Effluent, Bnr Effluent, and Receiving Waters for the December Bioassay

	DTN (mg/L)	NO ₃ -N (mg/L)	NO ₂ -N (mg/L)	NH ₄ -N (mg/L)	DON (mg/L)	DON/DTN (%)	Soluble Protein-N (mg/L)
CAS	16.97	0.25	BDL	15.44	1.27	7.5	0.79
BNR1	2.23	0.50	0.28	0.80	0.66	29.4	0.48
CT River	0.32	0.28	BDL	BDL	0.04	12.0	0.45
LIS	0.31	—	—	—	—	—	—

BDL) below detection limit

—) not measured

Fig. 1. Changes in dissolved total nitrogen (DTN) in the ocean and river bioassay for 0.45 μ m filtered CAS and BNR effluent; the December bioassay.

Fig. 2. Changes in soluble protein in the ocean and river bioassay for CAS and BNR effluent; the December bioassay.

reactions. In contrast, ocean bioassay for CAS effluent and river bioassays for both CAS and BNR effluents did not show such change in soluble proteins throughout the incubation period. Furthermore, reduction of DTN in these sets was only seen after eight days of incubation (Fig. 1). These results suggest that high salinity in ocean water helped BNR-protein become bioavailable, which might be relevant to the results of Bronk et al. [18] who reported that degradation of effluent organic N in an estuary occurred via the salinity-mediated N release. The salinity-induced degradation, however, should not be a sole factor for our observation, because then a similar pattern should have also been

Table 2. Results from the December River and Ocean Water Bioassay

		CAS filtered effluent	BNR1 filtered effluent
River	Maximum VSS produced (mg/L)	40	16
	N consumed (mg/L-N)	6.6	1.1
	Yield (mg VSS/mg N)	6	14
Ocean	Maximum VSS produced (mg/L)	32	32
	N consumed (mg/L-N)	5.8	0.9
	Yield (mg VSS/mg N)	6	36

observed in CAS-ocean bioassay. Therefore, it is likely that different characteristics of proteins and organic N in BNR effluent in comparison to CAS effluent [8] also account for the degradation of BNR-proteins in ocean water and early algal bloom in that particular bioassay.

The maximum biomass (VSS) growth, consumed N, and N-based biomass yields are summarized in Table 2. BNR effluent led to higher VSS yield for both river and LIS bioassays. The difference was particularly prominent for the ocean bioassay. The ocean bioassay with BNR effluent resulted in N-based VSS yield to be approximately six times greater than the CAS-ocean bioassay. These results indicate that N in BNR effluent was not only more reactive (Fig. 1, 2) but also more efficient in supporting biomass growth compared to N present in CAS effluent.

The VSS generation potential can be obtained by multiplying these N-based VSS yields with actual N concentration in each effluent. In this way, ocean bioassays with filtered BNR effluent would result in values of 80 mg VSS/L. The same approach would generate 93 mg VSS/L with CAS effluent. The difference is not that huge despite the fact that CAS effluent contained approximately eight times greater N than BNR effluent. These results imply that the type of DON present in BNR effluent is more potent to cause algal growth than DIN as well as DON in CAS effluent.

• Effluent Sampling and Bioassay in March

For the second set of bioassay, we included effluent from one more BNR facility (BNR2). This time, bioassays were conducted with only LIS ocean water and also whole (unfiltered) effluent. The summary of N composition in three effluents and receiving waters is shown in Table 3. The N data for CAS and BNR1 effluents were very similar with those from the December sampling event. Again, DON in BNR1 effluent was comprised of a large fraction, 38%, of DTN. DTN from the BNR2 facility was larger than we originally expected, since this facility usually achieves the effluent N in the range of 5-8 mg/L-N. Later, we learned that the higher effluent N from BNR2 facility was due to spring runoff that had occurred the day before sampling; the plant turned off their pre-anoxic operation and internal mixed liquor recirculation to deal with the higher influent flow rate. Nevertheless, DTN concentration was lower than that of CAS and was also mostly composed of nitrate and relatively high DON. For phosphate concentrations, BNR2 showed the highest concentration at 2.12 mg/L, followed by CAS at 1.74 mg/L, and by BNR1 at 1.16 mg/L.

Table 3. Concentration and Composition of Nitrogen in CAS Effluent, Two BNR Effluents, and Receiving Waters for the March Bioassay

	DTN (mg/L)	NO ₃ -N (mg/L)	NO ₂ -N (mg/L)	NH ₄ -N (mg/L)	DON (mg/L)	DON/DTN (%)	Soluble Protein-N (mg/L)
CAS	17.75	0.18	0.08	16.29	1.20	6.8	0.54
BNR1	2.61	0.68	0.15	0.79	0.99	38.0	0.28
BNR2	12.57	9.23	0.11	0.35	2.88	22.9	0.42
CT River	0.55	0.32	BDL	BDL	0.24	42.8	0.04
LIS	0.19	—	—	—	—	—	—

BDL) below detection limit

-) not measured

Table 4. Results from the March Bioassay

		CAS whole effluent	BNR1 whole effluent	BNR2 whole effluent
	Maximum VSS produced (mg/L)	80	75	141
Ocean	N consumed (mg/L-N)	3.3	0.7	5.6
	Yield (mg VSS/mg N)	25	101	25

Fig. 3. Changes in total VSS and DTN in the ocean bioassay for whole effluent of CAS (top), BNR1 (middle), and BNR2 (bottom); the March bioassay.

Fig. 3 shows changes in total VSS and DTN that occurred during the second bioassay. In all three bioassays, almost all biomass growth and DTN consumption took place before 12 days of incubation. Nevertheless, the reaction patterns were considerably different among the bioassays. Consistent with the results from the December bioassay, effluent from BNR1 facility caused much faster growth reaction, which happened simultaneously with faster N uptake. In bioassay with BNR1 effluent, maximum VSS growth and N uptake were achieved within five days of incubation, which was seven days faster than the bioassay with CAS effluent. This result supports the earlier observation that N in BNR effluent, most likely DON in BNR effluent, is more reactive than DIN and DON in CAS effluent. Interestingly, in BNR2 bioassay, N was consumed fast but the growth of biomass was more steady and slower than BNR1 bioassay. Since the current BNR2 effluent contained high NO₃⁻, this result was likely attributed to the fast uptake of NO₃⁻; however, biomass growth was more similar to CAS effluent bioassay.

Biomass yield based on N consumption (Table 4) showed that BNR1 effluent led to much higher VSS yield in the ocean water in comparison to any other bioassay sets. The clear difference between VSS yield for CAS and BNR1 was consistent with the December bioassay set. This time, the COD yield of BNR1 was four times larger than that from CAS bioassay. The COD yield of BNR2 bioassay was not much different from the CAS bioassay. This result again implies that nitrate, the major N species in BNR2 effluent, led to faster algal growth in the ocean water, but the overall biomass yield, measured by N-based COD yield, was similar to the bioassay for CAS effluent which had ammonia as a dominant N species.

• Effluent sampling and bioassay in May

In May, we conducted our third effluent sampling and laboratory bioassay experiments. We collected effluents from the same facilities sampled in March. Effluent N concentrations for CAS and BNR1 were consistent for the May sample set as well (Table 5). It was noticeable that BNR2 effluent showed much lower DTN this time due to a normal MLE process occurring in the facility. It is also worth noting that DON in BNR2 effluent now comprised up to 38% of DTN.

Table 5. Concentration and Composition of Nitrogen in CAS Effluent, Two BNR Effluents, and Receiving Waters for the May Bioassay

	DTN (mg/L)	NO ₃ -N (mg/L)	NO ₂ -N (mg/L)	NH ₄ -N (mg/L)	DON (mg/L)	DON/DTN (%)	Soluble Protein-N (mg/L)
CAS	16.40	0.25	0.377	13.85	1.93	11.8	0.76
BNR1	3.22	0.09	0.06	2.43	0.64	19.8	0.28
BNR2	3.60	1.40	0.17	0.65	1.37	38.1	0.31
LIS	0.43	–	–	–	–	–	–

BDL) below detection limit

-) not measured

Table 6. Results from the May Bioassay

		CAS whole effluent	BNR1 whole effluent	BNR2 whole effluent
	Maximum VSS produced (mg/L)	106	74	74
Ocean	N consumed (mg/L-N)	2.5	0.6	0.9
	Yield (mg VSS/mg N)	42	123	87

Fig. 4. Changes in total biomass (VSS) in the ocean bioassay set for whole effluent of CAS, BNR1, and BNR2; the May bioassay. Results are the average of the duplicate bioassays. Error bars show the range of the duplicate bioassays. **Fig. 5.** *Thalassiosira* and *Skeltonema* blooming in the ocean bioassay for BNR1 effluent (day 4), May 2011: X200 magnification phase contrast microscopy.

Fig. 4 shows that BNR1 effluent led to the fastest VSS production, indicating more reactivity of BNR1 effluent in LIS water than any other effluents. In this bioassay, almost all biomass growth was achieved in four days of incubation. CAS effluent set led to a slower VSS growth but the maximum VSS value was greater than that of BNR1. The BNR2 effluent set showed the slowest VSS increase but there was sudden bloom on day 8, which is similar to the pattern in BNR1 bioassay.

Phase contrast microscopic analysis revealed that the first algal bloom that occurred in BNR1 set (on day 4) was caused by dominant growth of marine diatom *Thalassiosira* sp. and *Skeletonema* sp. (Fig. 5). *Thalassiosira* has been recently found to possess the genes that can express transporter proteins to uptake amino acids (Armbrust et al., 2004), indicating that they are able to directly uptake DON from the surrounding environment. A combination of their genetic capability and the characteristics of BNR1 effluent in ocean water likely caused the early *Thalassiosira* bloom in this bioassay. Since *Thalassiosira* and *Skeletonema* are among dominant species of algae found in LIS blooms on a biomass basis [1], our results emphasize the importance of DON in BNR effluents and its role in algal bloom in the receiving ocean water.

We continued algal image analysis during the current bioassay and additional results are presented in Fig. 6. Samples obtained from the BNR1 bioassay on days 7 and 10 showed mostly diatoms and a variety of diatoms in comparison to day 4. Samples from the CAS bioassay were dominated by diatom *Cyclotella* on day 7, and by a larger number of green algae than *Cyclotella* on day 12. This data indicates that the original algal communities that developed in BNR1 and CAS bioassays were substantially different due to different characteristics of the effluents. BNR2 bioassay was also observed to have a very different phytoplankton community. The microscopic images of the samples from BNR2 bioassay taken on day 7 and 11 showed a diatom bloom dominated by *Chaetoceros*. Overall, these results clearly suggest that different inputs of wastewater effluents select for different types of phytoplankton community in the same LIS water. This observation supports the proposition that different N species have significant effects on regulating the structure of the microalgal community [10].

Finally, N-based VSS yield for the May bioassay set is summarized

Fig. 6. Bloom of different phytoplankton community in the LIS ocean bioassay: whole effluent of CAS (left column), BNR 1 (middle), and BNR 2 (right). The bioassay set for CAS effluent showed bloom of diatom *Cyclotella* on day 7 and green algae on day 12. The bioassay set for BNR 1 effluent on day 7 and day 10 showed growth and death of several species of diatoms including *Pinnularia*, *Skeletonema*, and *Thalassiosira*. The bioassay set for BNR 2 effluent showed growth of diatom *Chaetoceros* on day 7 and their bloom on day 11. Images were taken from phase contrast microscopy: the magnification for each image is included within the figure. The same LIS ocean water was used for incubating these three different wastewater effluents in May 2011.

in Table 6. BNR1 bioassay led to a higher biomass yield in comparison to the CAS bioassay, which has been a consistent finding throughout the current study. It is also worth noting that the bioassay for BNR2 effluent also caused higher VSS yield than CAS effluent, this time, indicating that BNR effluents led to effective VSS production.

3.1. Implications

The current study employed simple bioassay-based experiments to evaluate the effects of CAS and BNR effluents on algal blooms in the same receiving water. Although bioassays have limit in mimicking the natural processes, the results of this study support that N in BNR effluents is more productive than N in CAS effluents. The results seen in the December set for filtered effluents and the recent study [14] suggest that not only N-based biomass yields but total biomass generation could also be higher for BNR effluents than CAS effluents. These are certainly unintended results because many WWTPs are upgrading from CAS to BNR processes to comply with more stringent N regulations. We postulate that these unexpected results are caused because DON in BNR effluents is readily bioavailable, compared to DON in CAS effluents, and also more effective for supporting algal biomass than DIN. These results are not likely caused by different influent sources because our ongoing research using CAS and BNR effluents generated from the same source of wastewater also showed very similar results [14]. The effects of upgrading CAS to BNR processes on eutrophica-

tion in receiving ocean waters needs to be considered and more research warranted. Finally, the knowledge obtained from current and future research should be taken into consideration when establishing a permit-based effluent quality solution.

4. Conclusions

The specific conclusions that were drawn from this study are summarized as follows:

- The bioassay adopted in the current study showed that BNR effluents, compared to CAS effluents, produced up to six times higher N-based biomass yields in Long Island Sound water.
- BNR effluents resulted in faster N uptake and biomass growth in ocean water than river water, while CAS effluents behaved similarly in both river and ocean water.
- Both salinity and characteristics of BNR-originated DON should be responsible for faster reactions and increased biomass yield for BNR effluents in ocean water.
- Species of N and characteristics of DON input to an ocean receiving water play a key role in regulating the structure of phytoplankton community.
- Discharging BNR effluents may still cause algal bloom issues and associated hypoxic conditions in the N-limited ocean environment.

Acknowledgements

This work was supported by the Springfield Water and Sewer Commission under 109-1698, 111-0016, 111-1388 and by the Massachusetts Water Resource Research Center under 2009MA186B. We gratefully acknowledge the wastewater treatment facility employees who have assisted us in sample collection and provided us with any data we sought.

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