Characteristics and transformations of dissolved organic nitrogen in municipal biological nitrogen removal wastewater treatment plants

Shouliang Huo, Beidou Xi, Honglei Yu, Yanwen Qin, Fengyu Zan and Jingtian Zhang

State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Science, Beijing 100012, People’s Republic of China

E-mail: huoshouliang@126.com and xibeidou@263.net

Received 15 July 2013
Accepted for publication 3 October 2013
Published 16 October 2013
Online at stacks.iop.org/ERL/8/044005

Abstract
Dissolved organic nitrogen (DON) represents most of the dissolved nitrogen in the effluent of biological nitrogen removal (BNR) wastewater treatment plants (WWTPs). The characteristics of wastewater-derived DON in two different WWTPs were investigated by several different methods. The major removals of DON and biodegradable dissolved organic nitrogen (BDON) along the treatment train were observed in the anaerobic process. Dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA) in the effluent accounted approximately for less than 4% and 1% of the effluent DON, respectively. Approximately half of wastewater-derived DON was capable of passing through a 1 kDa ultrafilter, and low MW DON cannot effectively be removed by BNR processes. More than 80% of effluent DON was composed of hydrophilic compounds, which stimulate algal growth. The study provided important information for future upgrading of WWTPs or the selection of DON removal systems to meet more demanding nitrogen discharge limits.

Keywords: dissolved organic nitrogen, biological nitrogen removal, molecular weight distribution, nitrogen fractionation

Nomenclature

| Acronym | Definition |
|---------|------------|
| DON     | Dissolved organic nitrogen |
| TDN     | Total dissolved nitrogen |
| DIN     | Dissolved inorganic nitrogen |
| BNR     | Biological nitrogen removal |
| BENR    | Biological enhanced nitrogen removal |
| WWTPs   | Wastewater treatment plants |
| BDON    | Biodegradable dissolved organic nitrogen |
| ABDON   | Bioavailable dissolved organic nitrogen |
| DCAA    | Dissolved combined amino acids |
| DFAA    | Dissolved free amino acids |
| DMA     | Dimethylamine |
| EDTA    | Ethylenediaminetetraacetic acid |
| MW      | Molecular weight |
| AS      | Activated sludge |
| TF      | Trickling filter |
| MBR     | Membrane bioreactor |
| AAO     | Anaerobic, anoxic, oxic process |
| COD     | Chemical oxygen demand |
| DOC     | Dissolved organic carbon |
| SMPs    | Soluble microbial products |

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
1. Introduction

The municipal wastewater effluent is a substantial source of anthropogenic nitrogen to surface waters and has adverse effects on the water quality, especially in effluent-dominated waters (Pagilla et al. 2008, Bronk et al. 2010, Liu et al. 2012). To reduce cultural eutrophication of the receiving waters, biological enhanced nitrogen removal (BENR) processes that remove most of the dissolved inorganic nitrogen are widely used in municipal wastewater treatment (Czerwionka et al. 2012). As a result of efficient BENR processing, the main fraction of the residual nitrogen in effluent discharged to certain sensitive surface waters consists of dissolved organic nitrogen (DON) (Pagilla et al. 2006, Liu et al. 2012). Previous studies indicated that effluent DON can be bioavailable to natural algae and plankton (Pehlivanoglu-Mantas and Sedlak 2004, Sattayatewa et al. 2009, Simsek et al. 2013). Thus, DON is drawing more and more attention in wastewater treatment plants (WWTPs) currently due to growing concerns such as stimulating algal growth of receiving waters (Pehlivanoglu-Mantas and Sedlak 2004) and forming nitrogenous disinfection by-product N-nitrosodimethylamine (Pehlivanoglu-Mantas and Sedlak 2006, 2008, Lee et al. 2007). The emerging concerns for wastewater-derived DON have increased the need to characterize their concentrations, structure and properties.

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

Previous studies on wastewater-derived DON focused on two important research questions: where DON is removed or produced in BNR processes and what is the effect of BNR process on DON (Czerwionka et al. 2012, Simsek et al. 2013). Sattayatewa et al. (2009) reported that about 28–57% of the effluent DON was bioavailable or biodegradable by using different types of test species. Simsek et al. (2013) determined biodegradable dissolved organic nitrogen (BDON) and bioavailable dissolved organic nitrogen (ABDON) in activated sludge (AS) and trickling filter (TF) wastewater treatment processes. They reported that 65% BDON and 63% ABDON were removed in the TF facility and 68%, 56%, respectively in the AS facility, between primary clarification to final effluent. Moreover, they found that BDON and ABDON were 62% and 71% respectively of the effluent DON for the TF facility, while they were 26% and 47% respectively of the effluent DON for the AS WWTP. Knowledge on the behavior of DON along the biological processes within the WWTPs contributes to understanding the role of treatment units in the removal of DON. However, present data on the characteristics of DON in BNR WWTPs are still limited and insufficient to understand the fate or transformations of DON across the activated sludge process (Czerwionka et al. 2012).

The main objective of this study was to gain more information on wastewater-derived DON in WWTPs. DON, BDON and specific organic nitrogen compounds along the treatment units of a WWTP equipped with an AAO (anaerobic, anoxic, oxic) process and a WWTP equipped with an AO (anaerobic, oxic) and MBR (membrane bioreactor) process were measured to assess the effect of biological treatment on DON and its components. The MW distributions of DON compounds as measured by ultrafiltration and the fractionation of effluent DON by resin were investigated to provide a better understanding of the fate of the unidentified DON in WWTPs.

2. Materials and methods

2.1. Sample collection and preparation

The wastewater samples were collected from two WWTPs in Beijing which were designed and operated for biological nitrogen and phosphorus removal. Both of the WWTPs have to comply with the discharge limits for chemical oxygen demand (COD), ammonia, total nitrogen and total phosphorus. The Bxh WWTP has an anaerobic, oxic and MBR process with an average flow of 100000 m$^3$ day$^{-1}$. Grab samples were collected from four different locations (influent, anaerobic zone, oxic zone and MBR effluent) along the treatment train. The Xhm WWTP has an anaerobic, anoxic, oxic process (AAO) with an average flow of 600000 m$^3$ day$^{-1}$. Grab samples were collected from influent, primary clarifier effluent, anaerobic zone, anoxic zone, oxic zone and secondary effluent along the treatment process. The grab samples were collected from both of the WWTPs during four different months, specifically March 2012, June 2012, September 2012, and November 2012. All samples were collected in polyethylene containers which were acid-washed and rinsed with ultrapure water (Mill-Q, Millipore Corp. USA) before used, delivered to the laboratory on ice, filtered through 0.45 μm cellulose acetate membranes (BHL Co. Ltd, Beijing, China) upon arrival, and then stored at 4°C in the dark.

Environ. Res. Lett. 8 (2013) 044005
2.2. DON and BDON determination procedures

The method described by Xu et al (2010) and modified by Yu et al (2013), was adopted herein for DON measurement of wastewater samples. The experiments were conducted using two 400 ml stirred ultrafiltration cells (Model 8400, Millipore Corp., USA) in parallel at the same time. The BDON determination procedure described by Simsek et al (2012) was used to measure the BDON concentration in wastewater samples.

2.3. DFAA and DCAA determination procedures

A reversed-phase high performance liquid chromatography (RP-HPLC) combined with pre-column derivatization with ortho-phthaldialdehyde (OPA) and fluorescence detector (Agilent 1200 LC, USA) was used to measure dissolved free amino acids (DFAA) in unhydrolyzed wastewater samples and dissolved total amino acids (DTAA) after hydrolysis. The pretreatment method used for DFAA was modified from the procedure described by Confer et al (1995). A rapid vapor-phase hydrolysis step described by Keil and Kirchman (1991) was adopted for DTAA determination prior to derivatization and HPLC analysis. Dissolved combined amino acids (DCAA) were calculated as the difference between DTAA and DFAA. The recovery of 14 individual amino acids spiked into water prior to the hydrolysis step, ranged between was 92.1 and 101.3%. All analyses were carried out in triplicate for each sample.

2.4. Molecular weight distribution

To gain more information of DON in wastewater, MW fractionations were conducted in 400 ml dead-end stirred cells (Model 8400, Millipore Corp., USA) mentioned above under nitrogen gas at 0.3–0.45 MPa, employing a series of regenerated cellulose UF membranes with MW cut-offs of 30 kDa, 10 kDa, 5 kDa, 3 kDa, and 1 kDa (76 mm; YM30, YM10, YM5, YM3, YM1, Millipore Corp., USA), respectively. The percentages of DON in each size range were calculated using the method described by Lee and Westerhoff (2006).

2.5. DON fractionation

Methods described by Liu et al (2012) were adopted herein for DON fractionation in samples. The DON in wastewater samples was fractionated into hydrophobic and hydrophilic fractions. Supelite DAX-8 resin and Dowex 1 × 8 chloride form resin (Sigma-Aldrich, Inc.) were cleaned by the method described in the literature (Leenheer 1981, Liu et al 2012). The concentrations of hydrophobic and hydrophilic fractions were represented by measuring DON concentration of samples.

2.6. Analytical methods

All samples were analyzed in triplicates. Total dissolved nitrogen (TDN) i.e. sum of \( \text{NH}_4^+ - \text{N} \), \( \text{NO}_3^- - \text{N} \), \( \text{NO}_2^- - \text{N} \) and DON, was determined using the alkaline persulfate digestion with ultra-violet light spectroscopy. \( \text{NH}_4^+ - \text{N} \) was quantified using the Nessler’s reagent colorimetric method on a UNICO 2100 UV spectrophotometer (Shanghai UNICO, China). \( \text{NO}_3^- - \text{N} \) and \( \text{NO}_2^- - \text{N} \) were measured using ion chromatography (Dionex ICS-2000, USA) with a conductivity detector. DOC was measured using a Shimadzu TOC-VCSH analyzer (Shimadzu, Japan). UV254 was measured at a wavelength of 254 nm by a UNICO 2100 UV spectrophotometer (Shanghai UNICO, China). A quality control procedure was applied throughout the different steps from sampling to preparation and analysis. Furthermore, blanks and standards were regularly run with each series of analyses in order to check the validity of the data. For every ten samples, a laboratory blank and a standard were incorporated in the analytical procedure.

3. Results and discussion

3.1. Dissolved organic nitrogen and biodegradable dissolved organic nitrogen

The variations of DON in each treatment process at the Bxh WWTP and Xhm WWTP are presented in figure 1. As shown in figure 1(a), average DON concentrations
in the Bxh WWTP influent and effluent were 5.05 and 1.02 mg l$^{-1}$ as N, respectively. The results were consistent with the concentration range previously reported in BNR plant effluents (Pagilla et al. 2006, Pehlivanoglu-Mantas and Sedlak 2008). Previous work on the DON in WWTPs has indicated that DON concentration in the secondary and nitrified/denitrified effluents ranged from 0.7 to 2.1 mg N l$^{-1}$ (Parkin and McCarty 1981, Qasim 1999, Pehlivanoglu-Mantas and Sedlak 2008). The Bxh WWTP removed approximately 80% of the influent DON. The major removal of DON along the treatment train was observed in the anaerobic process and the DON concentrations remained stable in the subsequent oxic and MBR processes.

Average DON concentrations in the Xhm WWTP influent and effluent were 6.13 and 1.33 mg l$^{-1}$ as N, respectively. Approximately 10% of the influent DON was removed by primary clarifier process and 70% of the influent DON was removed by anaerobic, anoxic and oxic biological treatment process in this plant. Decreasing trend in DON concentration along the treatment train were seen in primary clarifier, anaerobic and anoxic processes and stayed almost constant in the last two stages (figure 1(b)). However, a slight increase of DON concentrations was observed in oxic zone. The possible reason is the release of DON due to either oxic biological activity or nitrification. Czerwionka et al. (2012) also found that the largest reductions of CON fractions occurred in the anaerobic and anoxic units of the studied bioreactors, whereas an increase of DON$_{0.1\mu m}$ concentrations was observed in the aerobic compartment. The result was not consistent with that of Sattayatewa et al. (2009). Their study found that DON concentration increased in anoxic zone for a full-scale four-stage Bardenpho bioreactor and the DON produced in the primary anoxic was not removed in subsequent zones such as primary aerobic, secondary anoxic and secondary aerobic zones (Sattayatewa et al. 2009).

Statistical analyses by the Kruskal–Wallis test indicated that there was no significant difference ($p > 0.05$) on DON concentrations in all the locations of the treatment train along the four sampling dates at the two studied WWTPs. The two WWTPs had also a similar DON removal efficiency, and the remaining DON may comprise either DON species difficult to be removed or DON by-products during the treatment. Gardner et al. (1996) suggested that the more labile and biologically available constituents are rapidly broken down and utilized, the bulk of the DON consist of compounds that are relatively recalcitrant to biological, chemical or physical degradation. Pehlivanoglu-Mantas and Sedlak (2006) reported that 10% of wastewater-derived DON was originally from drinking water source and 90% of effluent DON was produced by biological activity. The DON could be used as a surrogate for soluble microbial products (SMPs) produced in the biological treatment process because amino acids, proteins, and nucleic acids have been found as composition of SMP (Nam and Amy 2008). Therefore, the efforts to reduce SMP concentration by changing operating condition or adding additional treatment processes may contribute to DON minimization such as biofiltration process, biological treatment using soil systems, microbial fuel cells etc (Ieropoulos et al. 2013).

The BDON is an important variable to reveal the fraction of DON that can be mineralized by biological activity (Khan et al. 2009). The variation of BDON concentrations had a similar trend as that of DON along the treatment train in Bxh WWTP (figure 2(a)). The BDON concentrations decreased along the treatment train (figure 2(b)). Average BDON concentrations in the Bxh WWTP and Xhm WWTP influent and effluent were 3.25, 4.60 and 0.45, 0.56 mg l$^{-1}$ as N, respectively. More than 86% of the influent BDON was removed by BNR process and the major removal of BDON along the treatment train was found in the anaerobic process in the two studied WWTPs. The effluent BDON was observed to be between 36% and 54% of DON for the Bxh WWTP and between 35% and 55% of DON for Xhm WWTP. The results indicated that 46% or more of DON was not removed by each of the treatment processes. Moreover, there was no statistically significant difference ($p > 0.05$) on BDON concentrations in all the locations of the treatment train along the four sampling dates for the two studied WWTPs.

Similar trends of BDON concentrations along treatment train were also reported for some US WWTPs (Sattayatewa et al. 2009, Czerwionka et al. 2012, Simsek et al. 2012, 2013). These studies showed that a portion of effluent DON was biodegradable and/or bioavailable regardless of the type of wastewater treatment processes (Bronk et al. 2010, Filippino et al. 2011), and a significant portion of effluent DON was difficult to remove during biological treatment (Pehlivanoglu-Mantas and Sedlak 2008). The information of BDON profile along treatment train would help to understand the roles of wastewater treatment process in the removal of this fraction of DON (Simsek et al. 2013).
3.2. Dissolved free and combined amino acids

DFAA and DCAA concentrations were measured along the treatment train in Bxh and Xhm WWTPs to obtain information of the composition of effluent DON and the effect of treatment processes on DFAA and DCAA. DCAA concentrations were significantly larger than DFAA concentrations of samples from different treatment units in the studied WWTPs (figures 3(a)–(d)). DFAA and DCAA were measured in the influent, anaerobic zone, oxic zone and effluent in the Bxh WWTP (figures 3(a)–(d)). The DFAA and DCAA concentrations decreased along the treatment train by an average of 56% and 63%, respectively. The DCAA concentrations ranged from 7.35 to 16.6 µM in the influent and from 0.79 to 5.88 µM in the secondary effluent, accounting for 2.27–4.42% of the influent DON and 1.72–6.40% of the influent DON, respectively (figures 4(a)–(d)). The DFAA contributed to 0.1–0.62% of DON in the influent and to 0.61–0.97% of effluent DON.

The DFAA and DCAA concentrations measured at six locations at the Xhm WWTP were consistent with those measured at the Bxh WWTP (figures 3(e)–(h)). The DCAA concentrations ranged from 8.96 to 14.06 µM in the influent, and 0.71–2.57 µM in the secondary effluent. The DCAA concentrations ranged from 8.96 to 14.06 µM in the influent, and 0.71–2.57 µM in the secondary effluent. The DCAA concentrations...
concentration decreased by an average of 84% along the treatment train while the DFAA only decreased by average 48%. The DFAA accounted for about 10% of the dissolved total amino acids (DTAA) in the influent, and 30% of the DTAA in the effluent (figures 3(e)–(h)). The DCAA and DFAA concentrations in the effluent contribute to less than 4% and 1% of the effluent DON, respectively (figures 4(e)–(h)).

The concentrations of DFAA in untreated wastewater typically range between 0.3 and 2.4 µM while the concentrations of DFAA in wastewater effluents range between 0.04 and 2 µM (Confer et al. 1995, Pehlivanoglu-Mantas and Sedlak 2008). The concentrations of DFAA and DCAA in the effluents of both WWTPs were within ranges reported in previous studies (Parkin and McCarty 1981, Confer et al. 1995, Grohmann et al. 1998, Dignac et al. 2000). Assuming a typical DON concentration of 143 µM (∼2.0 mg N l⁻¹) in the wastewater effluent, the DFAA concentration accounted for between 0.3 and 3% of the DON, while the DCAA accounted for 1.5–13% of the DON (Parkin and McCarty 1981, Confer et al. 1995, Dignac et al. 2000). The relatively low concentrations of DCAA and DFAA in the effluent samples most likely were produced during biological treatment since amino acids and proteins are readily removed by bacteria (Confer et al. 1995, Pehlivanoglu-Mantas and Sedlak 2008).
3.3. Molecular weight distribution

Although the majority of the wastewater-derived DON cannot be identified with available methods until now (∼70%) (Pehlivanoglu-Mantas and Sedlak 2008), DON can be classified by its molecular weight (MW). To characterize the size distribution of DON species derived in different treatment units from the Bxh WWTP and Xhm WWTP, DON was measured after passing the wastewater samples through a series of UF membranes. Meanwhile, other related parameters both DOC and UV254 in each size fraction were also measured.

The profiles of DON MW distribution along the treatment train of the Bxh and Xhm WWTPs are shown in figure 5. Statistical analyses indicated that there is no significant difference ($p > 0.05$) on DON MW distribution in all sampling sites of the treatment units for both of the studied WWTPs between June and November 2013. According to the DON data, the average MW fraction of <3 kDa accounted for 64%, 62%, 70%, and 79% of the total DON concentration for the influent, anaerobic zone, oxic zone, and effluent at the Bxh WWTP, respectively. The MW fractions of <5 kDa ranged from 70 to 82% of the total DON in the different treatment units (figures 5(a)–(b)). It may be concluded that the majority of DON species in the Bxh WWTP was composed of small molecules. Similar trends were also found for DOC and UV254 in each size fraction (detailed data not shown). The results of the effluent are in accordance with those of Pehlivanoglu-Mantas and Sedlak (2008), who reported an average of 87 ± 4% DON compounds was in the <10 kDa fraction and low MW (<1 kDa) accounted for 67 ± 24% of DON in the TMWRF wastewater effluent. The MBR process at the Bxh WWTP could effectively remove the high MW fraction. Compared with the influent, the percentage of DON species in the effluent decreased by 77% in the MW fractions of >30 kDa, but increased by 23% in <3 kDa fraction. The <1 kDa fraction was not eliminated and accounted for 34% of DON in the effluent.

The DON MW distribution along treatment train at the Xhm WWTP was similar to that measured at Bxh WWTP. In the influent, the average >30 kDa fraction contributed to 16% of DON, and the highest contribution (57% of DON) was by the <1 kDa fraction (figures 5(c)–(d)). The majority of the DON compounds were associated with compounds with MW less than 1 kDa and contributed to 65% of the DON in the Xhm WWTP effluent. Compared with the Bxh WWTP MBR effluent, the removal of high MW fractions (>10 kDa) in the secondary effluent from the Xhm WWTP was not satisfactory, indicating that the MBR process was more effective in removing the large MW molecules. Overall, similar to the size distribution of DON at the Bxh WWTP, the dominant fraction of DON was found to be in the <5 kDa fraction along the treatment train at the Xhm WWTP (72%, 68%, 76%, 77%, 71%, and 82% for the influent, primary clarifier, anaerobic zone, anoxic zone, oxic zone, and effluent, respectively) (figures 5(c)–(d)).

The previous studies reported that the low MW DON fractions with <1 kDa are urea, amino acids, DNA, peptides and various synthetic compounds, and the DON group with >1 kDa are composed of fulvic acids and humic substances (Pagilla et al 2008). Biological nitrogen removal processes, such as the activated sludge process, have been considered effective in removing low MW DON, while high MW DON is considered refractory to this kind of treatment (Dignac et al 2000, Pagilla et al 2008). However, the results of DON MW distributions at the two studied WWTPs in this study indicated that approximately half of the DON was capable of passing through a 1 kDa ultrafilter, and low MW DON cannot effectively be removed by the biological nitrogen removal processes or the MBR process. Shon et al (2005) also found that microfiltration or nanofiltration may not be very useful in removing wastewater-derived DON due to the fouling problems often encountered in micro and/or nanofiltration. According to the present studies, the low MW DON fractions were most likely produced during biological treatment. The
bench-scale experiments investigated by Czerwionka et al. (2012) indicated that DON$_{0.1\mu m}$ has been explicitly produced in the aerobic unit of the anaerobic/anoxic/aerobic process. Moreover, the increase of DON$_{0.1\mu m}$ concentrations in the aerobic compartment was also observed for the full-scale activated sludge process (Czerwionka et al. 2012). This study also found that the DON concentrations increased in the oxic zone as described above. Therefore, the conditions and processes responsible for low MW DON production should be better clarified and appropriate operating conditions or treatment processes should be employed to reduce effluent low MW DON to lower levels.

### 3.4. Hydrophobicity and hydrophilicity of effluent DON

The effluent DON was separated into hydrophobic and hydrophilic fractions by DAX-8 resin. The overall recovery of DON in the two fractions ranged from 95.3% to 114.1%, with an average of 104.3 ± 6.9% (mean ± std. deviation). The hydrophobic DON accounted for an average of 15.5 ± 3.0% of the total DON in the effluent samples of Bxh WWTP (figure 6(a)), and an average of 18.1 ± 3.6% of the total DON in the effluent samples of Xhm WWTP (figure 6(b)). The results suggested that the majority of DON species in both of the studied WWTPs was composed of hydrophilic compounds, which was consistent with the previous studies (Pehlivanoglu-Mantas and Sedlak 2008, Liu et al. 2012). The possible reasons are that hydrophobic fractions would be much more easily removed by adsorption of activated sludge in the biological treatment systems, whereas the hydrophilic compounds have a low affinity for the surfaces of organic particles (Pehlivanoglu-Mantas and Sedlak 2008). Moreover, the C:N ratios of hydrophobic fraction and hydrophilic fraction were 15.4 ± 4.2 and 5.7 ± 2.3 in this study. The hydrophilic fractions have low C:N ratios indicating the presence of amino acids and proteins, which result in N-rich hydrophilic fractions (Leenheer et al. 2007). Liu et al. (2012) assessed bioavailability of hydrophobic and hydrophilic DON by the algal bioassay tests. The results showed that hydrophilic DON, which accounted for approximately 80% of the effluent DON, stimulated algal growth, whereas the remaining DON as hydrophobic DON had no effect on algal growth during a 14-day incubation period. Thus, the hydrophobic DON may be considered to exclude from the effluent total nitrogen regulations, while hydrophilic DON in WWTPs with different BNR processes might be reduced by using alternative biological treatment systems or physical systems such as reverse osmosis, activated carbon adsorption (Krasner et al. 2009).

### 4. Conclusions

Our study provides important insights into the occurrence and treatment of DON in WWTPs with different BNR processes. The major removals of DON were observed in the anaerobic process and a slight increase of DON concentrations occurred in oxic zone. The low MW DON fractions most likely were produced during biological treatment. Thus, the removal of low MW DON from WWTPs via AAO and MBR processes, as part of receiving water protection, may be difficult. Moreover, more than 80% of effluent DON was composed of hydrophilic compounds, which indicated the effluent DON is difficult to be removed by physical adsorption treatment processes. In situ biological treatment by changing operating condition (i.e. hydraulic retention time, sludge retention time and oxygen concentration) or adding additional treatment processes such as trickling filter process, soil systems, and constructed wetland may remove part of the DON in WWTPs.

### Acknowledgments

This study is supported by the Mega-projects of Science Research for Water Environment Improvement (Program No. 2012ZZX07101-002), the China Environmental Public Welfare Program (201209014) and the National Natural Science Foundation of China (No. 41303085).

### References

Bronk D A, Roberts Q N, Canuel E A, Mesfinou R, Filippino K C, Mulholland M R and Love N G 2010 Effluent organic nitrogen bioavailability and photochemical and salinity-mediated release Environ. Sci. Technol. 44 5830–5

Confer D R, Logan B E, Aiken B S and Kirchman D L 1995 Measurement of dissolved free and combined amino acids in unconcentrated wastewaters using high performance liquid chromatography Water Environ. Res. 67 118–1256
Czerwionka K, Makinia J, Pagilla K R and Stensel H D 2012 Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants Water Res. 46 2057–66
Dignac M F, Ginestet P, Ryback D, Bruchet A, Urbain V and Scribe P 2000 Fate of wastewater organic pollution during activated sludge treatment: nature of residual organic matter Water Res. 34 4185–94
Filippino K C, Mulholland M R, Bernhardt P W, Boniello G, Morse R, Semchecki M, Marshall H, Love N G, Roberts Q and Brook D A 2011 The bioavailability of effluent-derived organic nitrogen along an estuarine salinity gradient Estuaries Coasts 34 269–80
Gardner W S, Bennet R, Amon R, Cotner J, Caveletto J and Johnson J 1996 Effects of high molecular weight dissolved organic matter on the nitrogen dynamics on the Mississippi River plume Mar. Ecol. Prog. Ser. 133 287–97
Grohmann K, Gilbert E and Eberle S H 1998 Identification of nitrogen-containing compounds of low molecular weight in effluents of biologically treated municipal wastewater Acta Hydrochim. Hydrobiol. 26 20–30
Ieropoulos I A, Ledezma P, Stinchcombe A, Papaharalabos G, Krasner S W, Westerhoff P, Chen B, Rittmann B E, Nam S and Grohmann K, Gilbert E and Eberle S H 1998 Identification of soluble organic nitrogen in untreated and activated-sludge treated wastewaters Water Res. 32 139–49
Lee W and Westerhoff P 2006 Dissolved organic nitrogen removal in treated wastewater Water Environ. Res. 78 779–87
Lee W and Westerhoff P 2005 Dissolved organic nitrogen measurement using dialysis pretreatment Environ. Sci. Technol. 39 2911–8
Lee W and Westerhoff P 2006 Dissolved organic nitrogen removal during water treatment by aluminum sulfate and cationic polymer coagulation Water Res. 40 3767–74
Lee W, Westerhoff P and Crues J P 2007 Dissolved organic nitrogen as a precursor for chloroform, dichloroacetonitrile, N-nitrosodimethylamine, and trichloronitromethane Environ. Sci. Technol. 41 5485–90
Leenheer J A 1981 Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters Environ. Sci. Technol. 15 578–87
Leenheer J A, Dotson A and Westerhoff P 2007 Dissolved organic nitrogen fractionation Ann. Environ. Sci. 1 45–56
Liu H Z, Jeong J, Gray H, Smith S and Sedlak D L 2012 Algal uptake of hydrophobic and hydrophilic dissolved organic nitrogen in effluent from biological nutrient removal municipal wastewater treatment systems Environ. Sci. Technol. 46 713–21
Nam S N and Amy G 2008 Differentiation of wastewater effluent organic matter (EfOM) from natural organic matter (NOM) using multiple analytical techniques Water Sci. Technol. 57 1009–15
Pagilla K R, Czerwionka K, Urgun-Demirtas M and Makinia J 2008 Nitrogen species in wastewater treatment plant influents and effluents—the US and Polish case studies Water Sci. Technol. 57 1511–7
Pagilla K R, Urgun-Demirtas M and Ramani R 2006 Low effluent nutrient technologies for wastewater treatment Water Sci. Technol. 53 165–72
Parkin G F and McCarty P L 1981 A comparison of the characteristics of soluble organic nitrogen in untreated and activated-sludge treated wastewaters Water Res. 15 139–49
Pehlivanoglu-Mantas E and Sedlak D L 2004 Bioavailability of wastewater-derived organic nitrogen to the alga Selenastrum Capricornutum Water Res. 38 3189–96
Pehlivanoglu-Mantas E and Sedlak D L 2006 The fate of wastewater-derived NDMA precursors in the aquatic environment Water Res. 40 1287–93
Pehlivanoglu-Mantas E and Sedlak D L 2008 Measurement of dissolved organic nitrogen forms in wastewater effluents: concentrations, size distribution and NDMA formation potential Water Res. 42 3890–8
Qasim S R 1999 Wastewater Treatment Plants: Planning, Design, and Operation (Lancaster, PA: Technomic Publishing Company)
Sattayawet C, Pagilla K, Pitt P, Selock K and Bruton T 2009 Organic nitrogen transformations in a four-stage Bardenpho nitrogen removal plant and bioavailability/biodegradability of effluent Don Water Res. 43 4507–16
Shon H K, Vigneswaran S, Ben Aim R, Ngo H H, Kim I S and Cho J 2005 Influence of flocculation and adsorption as pretreatment on the fouling of ultrafiltration and nanofiltration membranes: application with biologically treated sewage effluent Environ. Sci. Technol. 39 3864–71
Simsek H, Kasi M, Ohm J B, Blonigen M and Khan E 2013 Bioavailable and biodegradable dissolved organic nitrogen in activated sludge and trickling filter wastewater treatment plants Water Res. 47 3201–10
Simsek H, Kasi M, Ohm J B, Wadhawan T, Bye C, Blonigen M and Khan E 2012 Fate of dissolved organic nitrogen in two stage trickling filter process Water Res. 46 5115–26
Vandenbruwane J, Neve S D, Qualls R G, Salomez J and Hofman G 2007 Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations Sci. Total Environ. 386 103–13
Xu B, Li D P, Li W, Xia S J, Lin Y L, Hu C Y, Zhang C J and Gao N Y 2010 Measurements of dissolved organic nitrogen (DON) in water samples with nanofiltration pretreatment Water Res. 44 5376–84
Yu H L, Huo S L, Yang Z S, Xi B D, Zan F Y and Zhang J T 2013 Measurement of dissolved organic nitrogen (DON) with nanofiltration pretreatment and its distribution characteristics in landscape water Environ. Sci. 34 192–8 (in Chinese)