

## Dissolved organic nitrogen (DON) in a full-scale drinking water treatment plant

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### ABSTRACT

Dissolved organic nitrogen (DON) is currently considered as one of the most important parameters in drinking water treatment due to its potential to form toxic nitrogenous disinfection by-products (N-DBPs). A comprehensive investigation was made in this study on the variation of DON in a full-scale drinking water treatment plant with a treatment train of coagulation/sedimentation, biofiltration and disinfection. The results showed that DON could be removed effectively by coagulation/sedimentation and disinfection, while biofiltration increased the DON concentration significantly. To determine the mechanism of DON increase, DON and other related parameters at different media depths of the biofilter were studied. The results showed that the DON concentration in the biofilter presented a rapid decrease from 0.73 to 0.44 mg l<sup>-1</sup> in the top media (0–10 cm), and a slow increase from 0.44 to 1.08 mg l<sup>-1</sup> in the bottom media (10–100 cm). Soluble microbial products (SMPs) released by bacterial metabolism might be a main source of the DON in the biofilter. These SMPs contained aromatic protein-like fractions, which were confirmed by EEM analysis.

**Key words** | biofiltration, dissolved organic nitrogen, drinking water, soluble microbial products

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### INTRODUCTION

During drinking water treatment, chlorine is frequently used to eliminate pathogenic microbes and ensure the microbiological safety of drinking water (Ates *et al.* 2007). However, undesirable disinfection by-products (DBPs) can be produced during chlorination. During the past decades, the halogenated DBPs have attracted most attention. The most frequent and hazardous species found and evaluated are trihalomethanes (THMs) and haloacetic acids (HAAs), which are considered as carcinogen or suspected carcinogens (Hrudey 2009). Recently, nitrogenous disinfection by-products (N-DBPs) with higher toxicity were found and reported (Westerhoff & Mash 2002; Lee *et al.* 2007). N-DBPs are formed during the reaction between chlorine and nitrogenous fractions of dissolved organic matter (DOM) (Plewa *et al.* 2004; Lee *et al.* 2007), and exhibit much higher mammalian cell genotoxicity than halogenated DBPs (Moudgal *et al.* 2000; Plewa *et al.* 2004). In view of this, the nitrogenous fractions of DOM, namely dissolved organic nitrogen

(DON), have received more and more attention in the field of drinking water.

DON is composed of a series of compounds including a variety of nitrogen-containing functional groups, consisting mainly of NH classes, amino category, nitrile, purine, pyrimidine, nitro compounds, and usually represents a low percentage (about 0.5–10.0% by weight) of natural organic matter (NOM) (Westerhoff & Mash 2002). Generally, soluble microbial products (SMPs) of bacterial metabolism, extracellular organic material (EOM) of algae, forest litter and agriculture fertilizers are considered as the main source of DON in surface water (Westerhoff & Mash 2002). It was reported that the median DON concentration in surface water was about 0.37 mg l<sup>-1</sup> (Westerhoff & Mash 2002) with the ratio of dissolved organic carbon (DOC)/DON averaged at 18.0 mg C/mg N (Lee *et al.* 2006). Nowadays, the studies on DON have focused on its analytical measurement, structural composition, occurrence and potential in N-DBPs formation (Chu *et al.* 2009), but its

profile during a conventional drinking water treatment process has rarely been reported. Due to the potential contribution of DON to the genotoxicity of drinking water, the study of DON transformation during drinking water treatment processes is of high significance to drinking water quality management.

Since SMPs have been regarded as a main source of DON, biological treatment processes deserve our concern. In drinking water treatment, biological treatment processes are widely used to remove organic matter and  $\text{NH}_4^+\text{-N}$  from source water. Biofiltration, as a typical example of such processes, has provided efficient removal of  $\text{NH}_4^+\text{-N}$  and DOC and increased the bio-stability of drinking water. It has been reported that SMPs can be released and its concentration can increase gradually with the microbial utilization of the substrates along the media depth of the biofilter (Carlson & Amy 2000).

In our work, the DON profile in a conventional drinking water treatment process consisting of coagulation/sedimentation, biofiltration and disinfection was investigated. Furthermore, the profile of DON along the media depth of the biofilter was studied, and the mechanism of DON formation in the biofilter was determined. This is the first time the phenomenon of a significant increase of the DON concentration during biofiltration has been reported, and the relationship between SMPs and DON in the biofilter established.

## MATERIALS AND METHODS

### Drinking water treatment processes

This study was carried out at the Pinghu drinking water treatment plant ( $2.50 \times 10^4 \text{ m}^3 \text{ d}^{-1}$ ), Pinghu City (N  $30^\circ 35'$ , E  $120^\circ 57'$ ), Zhejiang Province, China. The treatment processes include coagulation/sedimentation, biofiltration and disinfection.

Polyaluminium chloride (PAC) is applied for coagulation at a high dosage of about  $75 \pm 25 \text{ mg l}^{-1}$ . Six granular activated carbon (GAC)-sand dual-media biofilters ( $6 \times 4 \text{ m}$ ) are employed with a filtration velocity of  $7.20 \text{ m h}^{-1}$ . The media depth is 100 cm, containing 70 cm of GAC and 30 cm of sand. The GAC (PJ-08, Xinhua Chemical Plant,

Taiyuan, China) is irregular in shape with an average diameter of 1 mm. Chlorine is used as disinfectant with the average dosage of  $3.20 \text{ mg l}^{-1}$ .

### Sampling

Samples of source water, effluent of sedimentation, effluent of biofilter, and effluent of disinfection were taken. To investigate the variation in DON in the biofilters, samples at different media depths were also taken. Before analysis, all water samples were filtered by using  $0.45 \mu\text{m}$  pore-sized membranes.

### Analytical methods

DOC was determined using Shimadzu TOC-VCHS analyzer. UV absorbance at 254 nm ( $\text{UV}_{254}$ ) was determined using a spectrophotometer (752N/UV-2101PC).  $\text{NH}_4^+\text{-N}$  was measured using a salicylate-hypochlorite method.  $\text{NO}_2^-\text{-N}$  was measured using a *N*-(1-naphthyl)-ethylenediamine photometric method.  $\text{NO}_3^-\text{-N}$  was determined using UV spectrophotometry. Total dissolved nitrogen (TDN), the sum of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and DON, was measured using a alkaline potassium persulfate digestion-UV spectrophotometric method. All determinations were done according to the Chinese National Standard Methods (SEPA of China 2002). DON was quantified as the difference between TDN and three dissolved inorganic nitrogen (DIN, including  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{NO}_2^-\text{-N}$ ). Biomass in the biofilter was measured using the phospholipid analysis method (Findlay *et al.* 1989).

Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy (F-4600 FL Spectrophotometer, Hitachi, Japan) was used to characterize DOM in water. Excitation (Ex) wavelength was set from 200 to 500 nm at 5 nm sampling intervals, corresponding to emission (Em) wavelengths from 280 to 500 nm at the same sampling intervals. The excitation and Em slits were set at 5 nm and the scanning speed was set at  $1,200 \text{ nm min}^{-1}$ . The spectrum of double distilled water was recorded as the blank. Software Origin 7.5 (OriginLab Inc, USA) was employed to process the data. The EEM spectra were plotted as elliptical contours. The *x*-axis represents the Em spectra from 280 to 500 nm, and the *y*-axis the excitation from 200 to 500 nm.

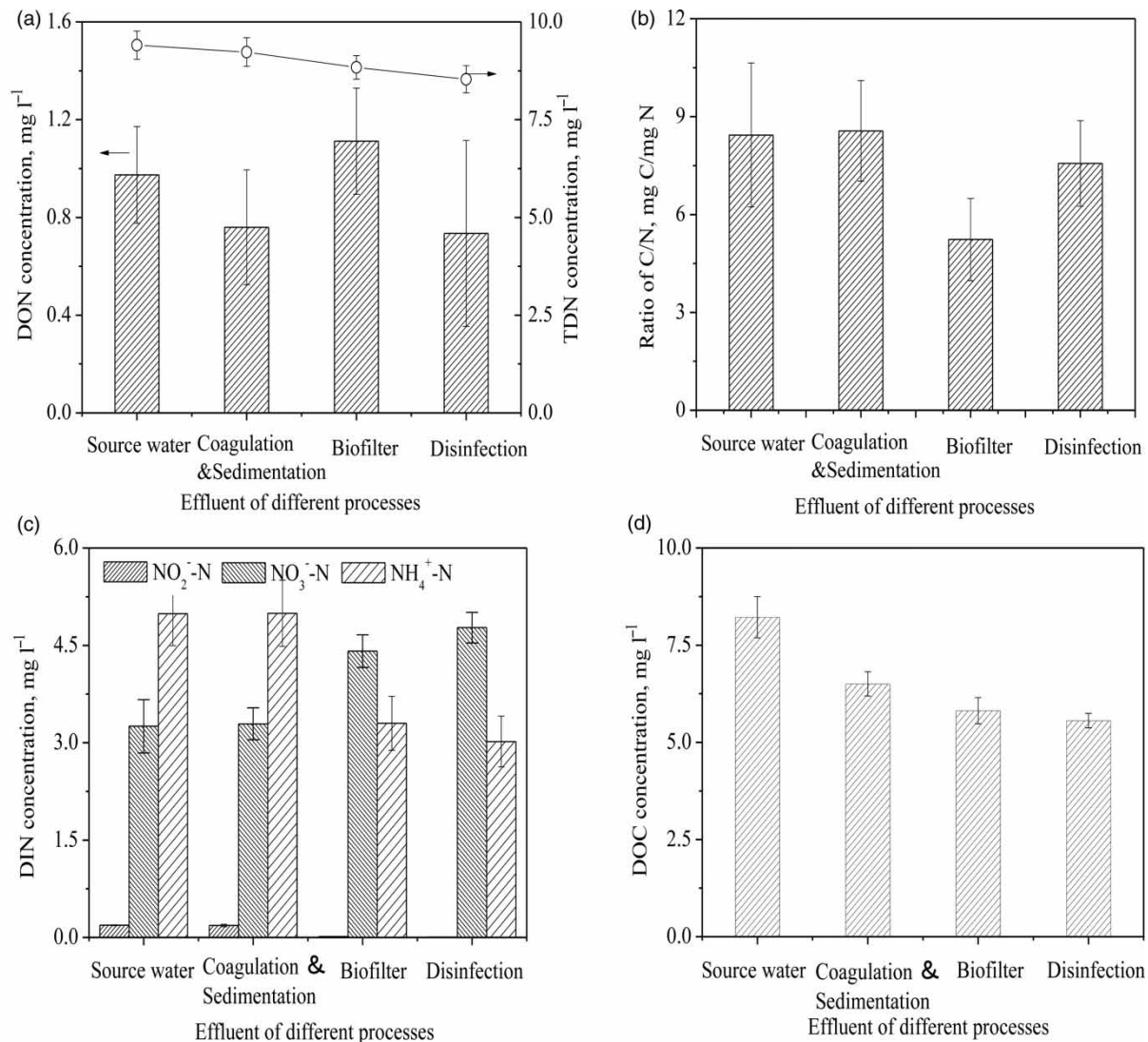
As the third dimension, a contour line is shown for each EEM spectra to express the fluorescence intensity at an interval of 5 nm.

## RESULTS

### DON and related species in the treatment train

The variations in the DON and TDN concentrations during the whole treatment train, from source water to finished water, as well as the ratio of C/N, are shown in Figures 1(a)

and (b). It could be seen that the DON concentration of source water was about  $1.0 \text{ mg l}^{-1}$  with the C/N ratio of about  $8.43 \text{ mg C/mg N}$ . About 20% of DON was removed in the process of coagulation/sedimentation ( $t$ -statistic = 3.12,  $p = 0.02$ ), and the DON concentration in its effluent was about  $0.76 \text{ mg l}^{-1}$  with the C/N ratio of  $8.56 \text{ mg C/mg N}$ . Subsequently, the water was subjected to biofiltration. During this process, the DON concentration increased significantly to  $1.11 \text{ mg l}^{-1}$  ( $t$ -statistic = 4.53,  $p = 0.003$ ) and the ratio of C/N decreased to  $5.23 \text{ mg C/mg N}$ . After disinfection, the DON concentration was lowered to  $0.73 \text{ mg l}^{-1}$  with the C/N ratio of about  $7.56 \text{ mg C/mg N}$ . During the



**Figure 1** | Variations in the DON, TDN, DIN and DOC concentrations and the C/N ratios in effluent of the different processes; (a) – DON and TDN concentrations; (b) – Ratio of C/N; (c) – DIN concentration; (d) – DOC concentration.

whole treatment train, the TDN concentration decreased gradually.

Also, related parameters including  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and DOC in the treatment train were evaluated as shown in Figures 1(c) and (d). The concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and DOC in source water were about 4.98, 0.19, 3.25 and  $8.22 \text{ mg l}^{-1}$ , respectively. After the coagulation/sedimentation process, the concentrations of DIN remained at about 4.99, 0.18,  $3.29 \text{ mg l}^{-1}$ , respectively, while the DOC concentration decreased to  $6.50 \text{ mg l}^{-1}$  corresponding to a removal efficiency of about 20%. In the biofiltration process, the concentration of  $\text{NH}_4^+\text{-N}$  decreased from 4.99 to  $3.30 \text{ mg l}^{-1}$ , and the concentration of  $\text{NO}_3^-\text{-N}$  increased from 3.29 to  $4.41 \text{ mg l}^{-1}$ . The DOC concentration decreased from 6.50 to  $5.81 \text{ mg l}^{-1}$ , and about 10% of DOC was removed. During the disinfection process, the  $\text{NH}_4^+\text{-N}$  concentration further decreased, while the  $\text{NO}_3^-\text{-N}$  concentration increased. The DOC concentration showed a slight decrease during the disinfection process.

### DON in the biofilter

As previously noted, an increase in the DON concentration was observed during biofiltration. Hence, to further investigate DON formation, the profiles of DON and other related parameters, including TDN, biomass, DIN, total dissolved inorganic nitrogen (TDIN), the sum of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ , and DOC along the media depth in the biofilter were evaluated.

As shown in Figure 2(a), with an increase in the media depth, the DON concentration initially decreased, and reached a minimum of  $0.44 \text{ mg l}^{-1}$  at a depth of 10 cm. After this rapid decrease, the DON concentration increased gradually to  $1.10 \text{ mg l}^{-1}$  at the bottom of the biofilter. In the treatment process of biofiltration, the DON concentration increased about  $0.35 \text{ mg l}^{-1}$ . Also, the TDN concentration decreased at the initial stage and was minimum at a depth of 20 cm, and then increased gradually. After biofiltration, about  $0.39 \text{ mg l}^{-1}$  of TDN was removed.

The variation in the biomass concentration along the media depth was also measured, as shown in Figure 2(b). It decreased continuously in the biofilter from 418.2 to

$101.3 \text{ nmol lipid P cm}^{-3}$  of media, and decreased quickest at the media depth of 0 to 10 cm from 418.2 to  $294.5 \text{ nmol lipid P cm}^{-3}$  of media.

Figure 2(c) shows that  $\text{NH}_4^+\text{-N}$  was rapidly removed from the media at a depth range of 0 to 20 cm. Its concentration decreased from 5.09 to  $3.36 \text{ mg l}^{-1}$ , corresponding to a fast increase of the  $\text{NO}_3^-\text{-N}$  concentration from 3.32 to  $4.48 \text{ mg l}^{-1}$ . Also, it was evident that the TDIN concentration rapidly decreased at the media depth of 0 to 20 cm, and about  $0.74 \text{ mg l}^{-1}$  of TDIN was reduced during biofiltration.

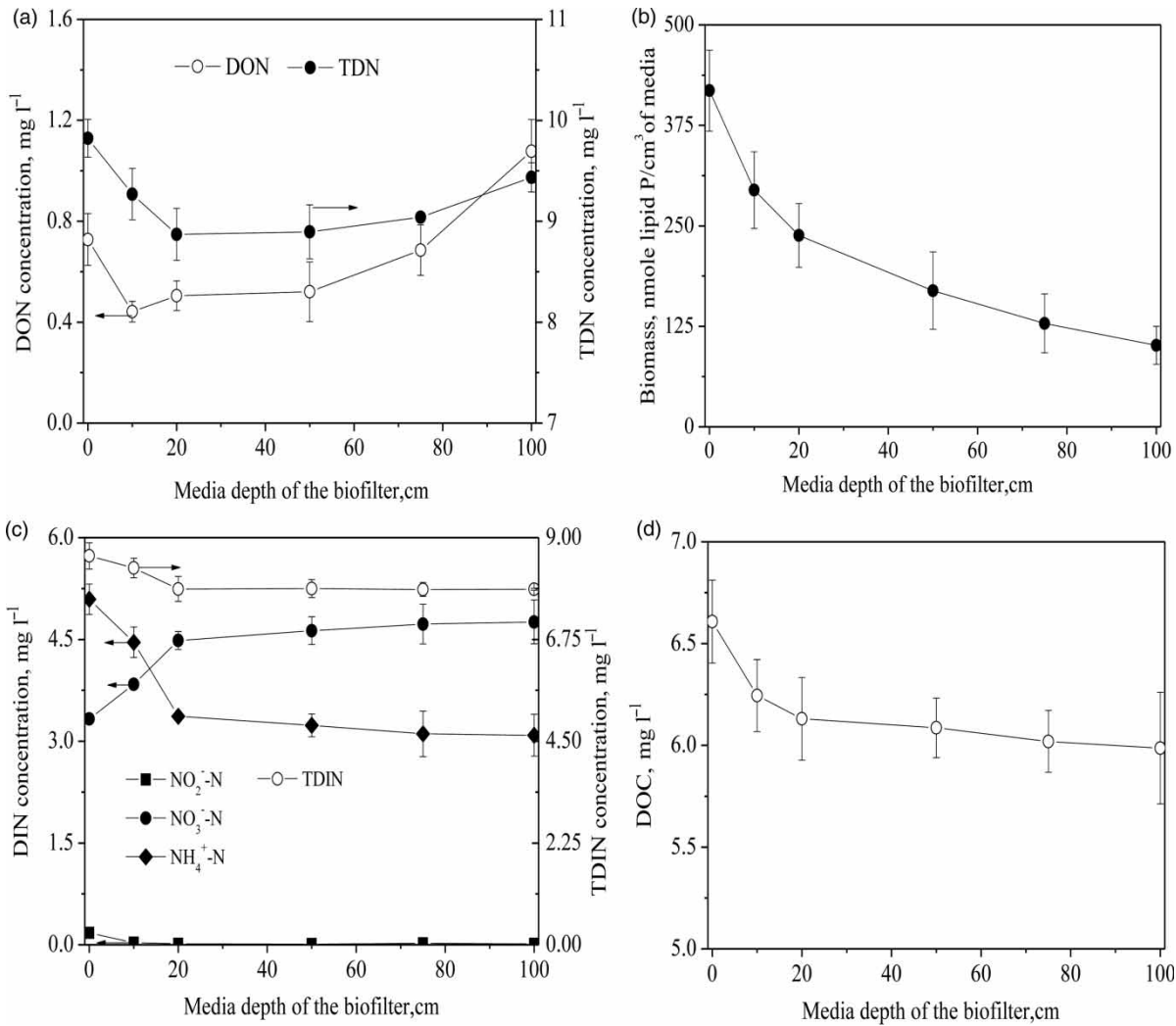
The concentration of DOC along the media depth is shown in Figure 2(d). The DOC concentration decreased continuously in the biofilter from 6.61 to  $5.98 \text{ mg l}^{-1}$ . In addition, DOC was removed much faster at the media depth of 0 to 10 cm where its concentration reduced from 6.61 to  $6.24 \text{ mg l}^{-1}$ .

### Characterization of DON

The increased DON concentration in the biofilter suggests that new nitrogen enriched compounds might be formed. To find evidence of DON formation, EEM was applied to characterize dissolved organic substances in water. Figure 3 shows the EEM fluorescence spectra of the different samples, including source water, effluent of sedimentation, water at the media depth of 50 cm in the biofilter and effluent of the biofilter.

From the EEM spectra, two main peaks (Peak A and Peak B) could be identified. It is worth noting that there were still many peaks with relatively low intensity which could not be clearly seen. The first main peak (Peak A) was located at the excitation/emission (Ex/Em) wavelengths of 230/340 nm. The other main peak (Peak B) was located at the Ex/Em wavelengths of 275/310 nm. These two main peaks, Peak A and Peak B, were reported to be protein-like compounds, associated with aromatic protein-like substances and tryptophan protein-like substances, respectively (Chen *et al.* 2003; Aryal *et al.* 2009).

The fluorescence parameters of the spectra, including peak locations, fluorescence intensity and peak intensity ratios, are summarized in Table 1. After the coagulation/sedimentation process, the intensity of Peaks A and B



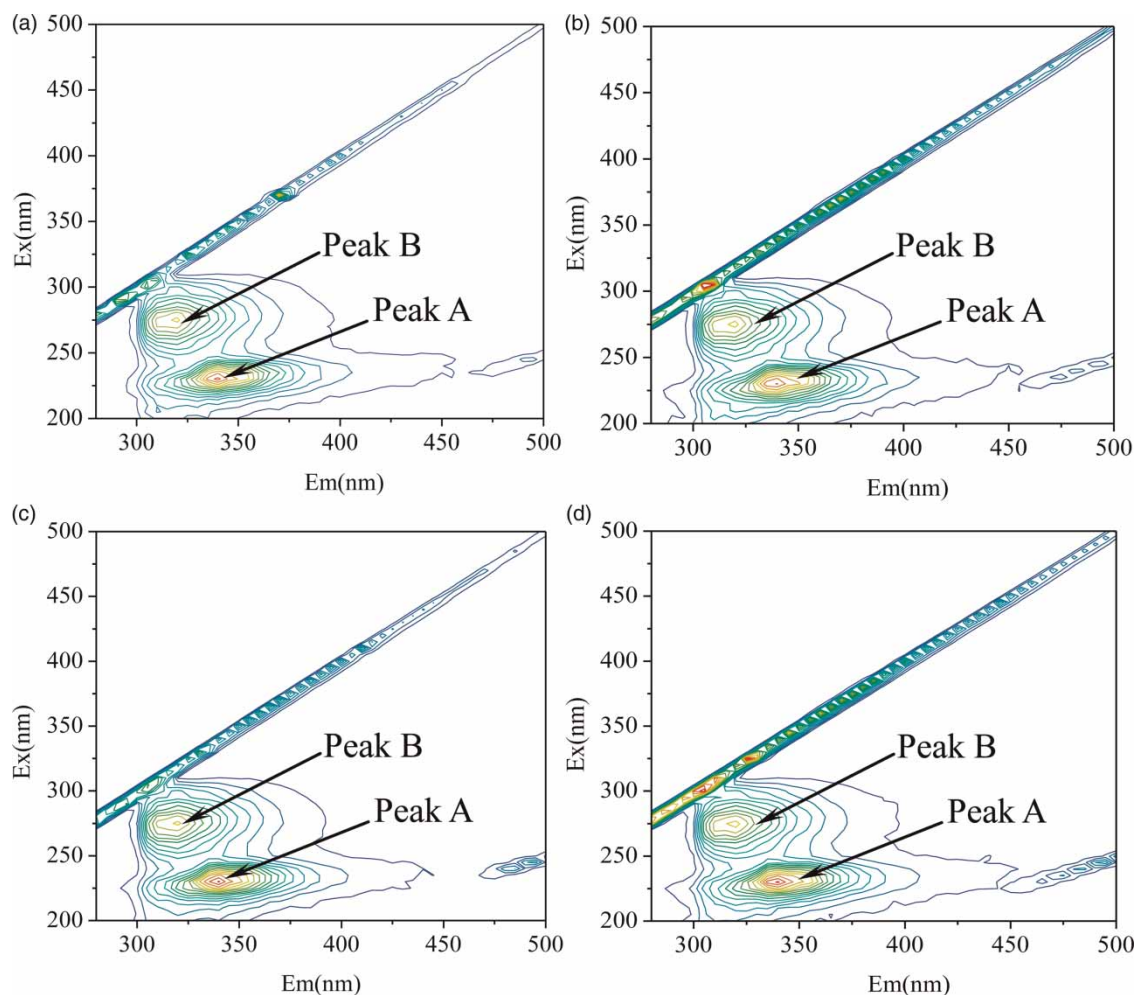
**Figure 2** | Profiles of DON and other related parameters along the media depth of the biofilter; (a) – DON and TDN concentrations; (b) – biomass concentration; (c) – DIN and TDIN concentrations; (d) – DOC concentration.

decreased, while the intensity ratios of Peak A/Peak B decreased from 1.25 to 1.22. For the fluorescence spectral parameters of water at 50 cm media depth of the biofilter and effluent of the biofilter, the intensity of Peak A increased, but that of Peak B decreased. In addition, the intensity ratios of Peak A/Peak B increased from 1.22 in effluent of coagulation/sedimentation to 1.27 and 1.32, suggesting that the molecular structure of organic compounds in water changed in the biofilter. After the disinfection process, the intensity of Peaks A and B decreased, while the intensity ratios of Peak A/Peak B decreased from 1.32 to 1.22.

## DISCUSSION

The DON concentration in source water studied here was much higher than the levels reported in source water of other countries, and the ratio of C/N was lower than those reported in the literature (Westerhoff & Mash 2002). Since the river flows through a heavily populated area and an industrial park, the high DON concentration in source water could be due to water pollution caused by inadequate treatment and illegal disposal of domestic or industrial wastewater in this area.





**Figure 3** | EEM spectra of the different samples; (a) – source water; (b) – effluent of sedimentation; (c) – water at 50 cm media depth of the biofilter; (d) – effluent of the biofilter.

**Table 1** | Fluorescence spectral parameters of DOM samples

Samples	Peak A		Peak B		A/B
	Ex/Em	Intensity	Ex/Em	Intensity	
Source water	230/340	3,888	275/310	3,121	1.25
Coagulation/ sedimentation	230/340	3,809	275/310	3,119	1.22
50 cm in the biofilter	230/340	3,940	275/310	3,114	1.27
Effluent of the biofilter	230/340	4,090	275/310	3,101	1.32
Disinfection	230/340	3,566	275/310	2,925	1.22

Source water was initially treated by the process of coagulation/sedimentation. Though this process was applied to remove suspended particles and colloid materials

from source water, it could also act as an effective method to remove dissolved organic substances (Lee & Westerhoff 2006). It has been reported that flocs formed during coagulant hydrolysis have a positive charge, possessing a strong ability to absorb negatively charged dissolved organic substances (He et al. 2006). From Figure 1(d), it can be seen that 20.7% of DOC was removed in the process of coagulation/sedimentation. Some removed substances contain nitrogenous functional groups, and can thus be classified as DON. DON in source water was co-removed, and the removal percentage (about 20%) was very close to that of DOC. Therefore, the C/N ratio remained almost unchanged. The concentrations of DIN remained constant after the coagulation/sedimentation process. In addition, it should be noted that the amount of DON removed was almost

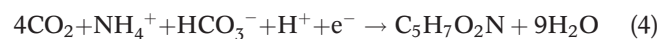
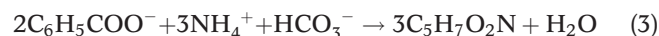
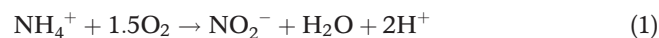
equal to that of TDN removed. During disinfection, it is possible that DON was transferred to DIN or nitrogen gas by the oxidation, and therefore its concentration reduced.

Generally, biofiltration is designed to remove  $\text{NH}_4^+\text{-N}$  and DOC. And in this study, the concentrations of these two species were reduced. However, the DON concentration increased after the treatment of biofiltration (Figure 1(a)). During the process of substrate metabolism with biomass growth in biochemical processes, a variety of organic compounds (SMPs) can be formed and released into the extracellular solution. SMPs, containing a series of organic compounds such as polysaccharides, proteins, nucleic acids, amino acids and steroids, are nitrogen-enriched compounds (Rittmann *et al.* 1987). It has been reported that these SMPs present a lower biodegradability and C/N ratio (Ciner & San 2001). Therefore, the release of SMPs due to biological activity might be the reason for the increased DON concentration during biofiltration. To confirm this, a further investigation on the mechanism of DON formation in the biofilter, as well as the concentrations of DON, biomass, TDN, DIN, TDIN and DOC at different media depths of the biofilter was made (results shown in Figure 2).

The performance of the biofilter in removing bioavailable materials depends on microbial biomass and activity on the media of the biofilter. At the media depth of 0 ~ 10 cm, microbial biomass and activity were much higher than that at the media depth of 10–100 cm, corresponding to a fast removal of DOC including DON. Though SMPs could also be formed and released, the rate of DON utilization was much higher than that of SMPs generation, resulting in the reduction of the DON concentration. Furthermore, SMPs are composed of growth related utilization associated products (UAPs) and non-growth related biomass associated products (BAPs) (Carlson & Amy 2000). The formation of UAPs is directly related to the substrate utilization rate, while the formation of BAPs is related to the endogenous respiration of the cell. Therefore, at the media depth of 0 ~ 10 cm, much more UAPs could be produced than BAPs. It was also reported that UAPs presented much better biodegradability than BAPs (Carlson & Amy 2000). This characteristic of UAPs might lead to more degradation of SMPs and contribute to a reduction of DON at this stage of media.

Along with the increased media depth, the levels of biodegradable organic matter and other nutrients such as  $\text{NH}_4^+\text{-N}$  in water decreased, while the concentration of biomass and the rate of DOC and  $\text{NH}_4^+\text{-N}$  removal were lowered. Hence, the behavior of the microbes transformed gradually from substrate utilization to endogenous respiration, leading to formation of more residual dead cells. At the latter stage of the biofilter, more BAPs were produced, leading to a poor biodegradability of water. So, the rate of SMPs generation tended to be higher than that of substrate utilization, and SMPs were accumulated gradually along the media depth of the biofilter, resulting in an increase of the DON concentration at the media depth of 10–100 cm.

In the biofilter, nitrifying bacteria oxidize  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  via the process of nitrification, i.e.  $\text{NH}_4^+\text{-N} \rightarrow \text{NO}_2^-\text{-N} \rightarrow \text{NO}_3^-\text{-N}$  (Equations (1) and (2)). Other pathways, such as microbial assimilation, may be involved in the transformation of  $\text{NH}_4^+\text{-N}$ , too (Yu *et al.* 2007). Equations (3) and (4) present cell synthesis of heterotrophic and nitrifying bacteria. In addition, about  $0.74 \text{ mg l}^{-1}$  of N in inorganic form was consumed, while about  $0.35 \text{ mg l}^{-1}$  of N in organic form was generated. These phenomena suggested that at least part of  $\text{NH}_4^+\text{-N}$  could be assimilated to form cell components and then become SMPs, and finally released into water as DON.



As seen from Figure 3 and Table 1, the EEM parameters from source water to disinfected water obviously show that there was a good relationship between the DON concentration and the intensity of Peak A, revealing that the variation in DON during the different treatment processes depended intimately on the amount of aromatic protein-like substances. Previous studies confirmed that aromatic protein-like substances had direct relationship with

microbial activity, and they might be transported into the system (allochthonous) or be created by microbial activity within the system (autochthonous) (Coble 1996; Gone et al. 2009). Therefore, aromatic protein-like substances generated during biofiltration should be SMPs. This indicates that SMPs account for an increase of DON during biofiltration.

## CONCLUSIONS

The DON concentration in source water for a full-scale drinking water treatment plant in southeast China reached about  $1.0 \text{ mg l}^{-1}$ , which was much higher than previously reported values.

In the conventional drinking water treatment processes used in this study, coagulation/sedimentation and disinfection presented considerable efficiency in DON removal. About 20% of DON was removed in coagulation/sedimentation and 30% was removed in disinfection. However, a marked increase of DON occurred during biofiltration.

At the initial stage of biofiltration, DON was rapidly utilized, leading to a marked reduction of the DON concentration. However, with decreased biodegradability of DOM, SMPs began to accumulate along the media depth, and BAPs became predominant in SMPs, resulting in a substantial increase of the DON concentration in effluent of biofiltration. EEM results confirmed that nitrogen-enriched aromatic protein-like fractions of SMPs contributed to an increase of DON in the biofilter.

From the point of view of water quality management, the usage of biological drinking water treatment should be reconsidered for its potential contribution to N-DPBs formation from the increased DON in water after biofiltration.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support for this work provided by National Natural Science Foundation of China (NSFC) (No. 51078343), Fujian Provincial Natural Science Foundation (No. 2009J06028 and 2011J05144), Xiamen Municipal Bureau of Science and Technology (No. 3502Z20116007 and

3502Z20101010), the Hundred Talents Program, Chinese Academy of Sciences and Xinyang Normal University PhD Research Fund (No. 91231).

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First received 12 December 2011; accepted in revised form 28 December 2011