Dissolved Organic Nitrogen (DON) in Full Scale Two-stage O₃-BAC with
Nitrate as Sole Inorganic Nitrogen Source

Fan, Z.H.¹, Zhang, H.N.¹, Xu, X.², Liu, B.³, Zhang, D.D.¹,⁴ and Yu, X.¹*

¹ Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
² Key Laboratory of Three Gorges Reservoir Region’s Eco-Environment, Ministry of Education, Chongqing University, Chongqing 400045, China
³ Xinyang Normal University, Xinyang 464000, China
⁴ School of Environment and Spatial Informatics, China University of Mining and Technology, Xuzhou 221116, China

ABSTRACT: Dissolved organic nitrogen (DON) can react with disinfectants to produce numerous disinfection byproducts (DBPs), particularly nitrogen-containing DBPs (N-DBPs), and produce serious adverse effects on public health. Widely used biological processes in drinking water treatment can increase DON in effluents, and enhance these ill effects. This study investigated DON in a full-scale two-stage ozonation-biological activated carbon (O₃-BAC) filtration system. DON concentrations generally increased as media depth increased. There was an ebb and flow pattern between DON and NO₃⁻_N along the media depth in the absence of NH₄⁺_N and NO₂⁻_N. This suggests that NO₃⁻_N is the nitrogen source for DON. Ozonation and nutrient availability significantly impacted microbial biomass and microbial activity. Microbial biomass and microbial activity were both very important to DON formation as they affected the release of soluble microbial products (SMPs). Typical SMPs such as tyrosine/tryptophan amino acids and proteins were found to be formed during biofiltration, and this formation correlated well with DON from the same sampling ports. In order to balance the mass difference between the increased DON and disappeared NO₃⁻_N, a hypothesis on the generation and consumption equilibrium of DON and NO₃⁻_N was posited. This hypothesis involves the existence of nitrogen in the influent, effluent, and backwashing water, and the synthesis of said nitrogen by microorganisms.

Key words: Dissolved organic nitrogen, Nitrate nitrogen, Ozone-biological activated carbon, Environment

INTRODUCTION

In order to eliminate pathogenic microorganisms and guarantee drinking water safety, disinfectants like chlorine, chlorine dioxide, and chloramine are widely used in drinking water treatment. However, these disinfectants can react with aquatic dissolved organic matters (DOM) and produce disinfection by-products (DBPs) that are toxic and have significant adverse effects on human health (Tabesh et al., 2011; Llopis-Gonzalez et al., 2011; Hudak, 2012; Li et al., 2012).

Dissolved organic nitrogen (DON), a large class of DOM, is an emerging issue in drinking water treatment because of its role as precursor of numerous nitrogen-containing disinfection byproducts (N-DBPs). N-DBPs have more genotoxic, mutagenic, and/or carcinogenic activity than traditional carbon-containing disinfection byproducts (C-DBPs) like trihalomethanes (THMs) and haloacetic acids (HAAs) (Plewa et al., 2004, 2008, Muellner et al., 2007). DON includes NH classes, the amino category, nitrile, purine, pyrimidine, and nitro compounds. Many studies have reported that DON levels depending on water sources and treatment processes. For example, Lee and Westerhoff reported that the average DON was 0.2 mg/L in raw water from 28 U.S water treatment plants (Lee et al., 2011). However, Liu et al. found that DON was about 1.0 mg/L in polluted source water in an eastern city in China (Liu et al., 2011).

Until now, there have been few reports on the removal efficiency and removal mechanisms. Provide a detailed legend (without abbreviations) to each figure, refer to the figure in the text and note its approximate location in the margin of DON using conventional drinking water treatment methods.
to low molecular weight and strong hydrophilicity, DON removal via coagulation is difficult, and coagulation is the main unit used to reduce DOM levels in drinking water. The survey conducted by Lee and Westerhoff showed that DON removal reached only 9% with conventional coagulation, and this percentage increased to 23% when cationic polymer flocculation was applied (Lee et al., 2006). Tomaszewska and Mozia found that DON reduction increased to 45% using the combined process of powder activated carbon-ultra filtration (PAC/UF) in 12 WTPs in Europe (Tomaszewska et al., 2002). Yeomin and his colleagues used reverse osmosis (RO) and UF membranes to deal with DON in raw water. This method raised the average removal using 6 RO membranes to 65%, higher than the 50% removal achieved by a method using 2 UF membranes (Yeomin et al., 2005). In addition, some advanced oxidation processes (AOPs) such as UV, O3, and H2O2 were studied. All seemed less useful in DON removal than in the removal of other organic contaminants. Currently, O3-BAC is regarded as the most widely used advanced process in drinking water treatment. Many studies have investigated its full-scale performance and mechanisms used in reducing organic matters. Due to the very strong oxidation ability of ozone, refractory organic matters and those with high molecular weights, such as humic acid, can be decomposed into organic matters with lower molecular weights and higher biodegradability. Activated carbon has a large specific surface area and great porosity, which allows it to absorb contaminants and contribute to microorganism growth (Seredynska et al., 2006). O3-BAC is very powerful at eliminating DOM and bioavailability contaminants like NH4+-N (Su et al., 2005; Wang et al., 2005). Yang and his colleagues reported that the O3-BAC process enhanced NPDOC removal efficiency by about 30%, as compared to the rapid sand filtration process that showed a removal of 33.9% (Yang et al., 2010). Comparatively less research has been conducted on DON removal by the process of O3-BAC. Some researchers have thought the O3-BAC process to be effective at DON reduction because of its effectiveness in reducing other organic matters. However, our recent work found that DON increased rather than decreased during biological processes like O3-BAC. Further, this finding challenged the viewpoint that O3-BAC was genotoxicologically safe, since DON is an important precursor for N-DBPs, as mentioned above.

Nevertheless, how DON varies in the O3-BAC biofilters, and what the connections between DON and inorganic nitrogen are remain unknown. And these unknown factors are important for understanding the formation and control of DON. In this study, these questions will be investigated and discussed, and the information provided will be useful for further DON treatment applications.

MATERIALS & METHODS

Samples were collected in a full-scale drinking water treatment plant with a production capability of 2.50×10^4 m³ drinking water per day, located in Pinghu city, Zhejiang province, PR China. Because the source water is severely polluted, this plant has a long treatment train, consisting of biological fluidized bed pretreatment, coagulation, sedimentation, sand filtration, the first stage O3-BAC, the second stage O3-BAC, and disinfection with a mixture of Cl2 and ClO2. Our study focused on the influents and effluents of the dual stages of O3-BAC, whose parameters are listed in Table 1.

During this study, three different types of water samples were collected. The first one was along the target processes, i.e. the influents and effluents of the each process unit of the dual stages of O3-BAC. The second and third types were samples along the first and second BAC media depth. The sampling profiles are shown in Fig. 1. Each water sample had a volume of at least 500 mL. The activated carbon samples of both BAC were taken along the media depth at the points as sampling water. Each sample was about 15-20 g in wet weight. All of the water samples were firstly pretreated by filtration through 0.45 µm cellulose acetate membrane to remove particles in field (BD, Shanghai, PR China). Two aliquots were prepared for each of the filtrated samples. One aliquot was used for the immediate in-field analysis of routine parameters including total nitrogen (TN), ammonia nitrogen (NH4+-

<table>
<thead>
<tr>
<th>Height (m)</th>
<th>Hydraulic retaining time (min)</th>
<th>Backwashing cycle (d)</th>
<th>Dosage of ozone (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAC</td>
<td>Sand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The primary BAC</td>
<td>1.75 0.25 12</td>
<td>7</td>
<td>2.0</td>
</tr>
<tr>
<td>The secondary BAC</td>
<td>1.70 0.20 12</td>
<td>8</td>
<td>2.0</td>
</tr>
</tbody>
</table>
N), nitrite nitrogen (NO₂⁻-N), nitrate nitrogen (NO₃⁻-N), and ultraviolet absorbance at 254 nm (UV254). The other aliquot was adjusted to pH<2 with sulfuric acid. It was then stored in a PTFE bottle for later analysis of dissolved organic carbon (DOC) and fluorescence excitation emission matrix (EEM). The samples were stored for less than 7 days before these parameters were determined. Activated carbon samples were stored in 50 ml beakers, kept wet, and were tested for microbial biomass and activity in the plant lab as soon as possible. About 10 g activated carbon was taken for testing of microbial activity, and about 4 g was taken for the determination of biomass.

TN, NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N were determined according to the Chinese National Standard Methods (SEPA of China, 2002). DON was determined by subtracting NO₂⁻-N, NO₃⁻-N, and NH₄⁺-N from TN. DOC was measured with a TOC analyzer (TOC-VCHS, Shimadzu) and UV254 was measured with a spectrometer (752N/UV-2101PC). SUVA254 was determined by the ratio of UV254 (in m⁻¹) to DOC in each of the samples.

Biomass in activated carbon was calculated using P using the phospholipids method (Yu et al., 2002; Findlay et al., 1989). 1nmol P was equivalent to about 10⁸ cells with the size of E. coli. Specific oxygen uptake rate (SOUR) of microorganisms was determined using a respirometric method (Surmacz et al., 1996; Urfer et al., 2001; Wu et al., 2010) to indicate microbial activity with forms of SOUR per biomass and SOUR per volume of activated carbon (AC) particles. The former form presents the average activity of unit biomass and the latter presents the general activity of the AC unit volume.

Five typical water samples in the depths of 0, 0.3, 1.0, 1.75, and 2.0 m and 0, 0.3, 1.0, 1.70, and 1.9 m for both BAC filters, respectively, were characterized by fluorescence excitation emission matrix (EEM). EEM was conducted with a fluorescence spectrophotometer (Hitachi F-4600, Japan) at a scan rate of 2400 nm/min and an excitation/emission slit bandwidth of 5 nm. The scanning field was set with emission spectra from 280 to 500 nm and excitation from 200 to 450 nm. The three-dimensional plots and contour maps were produced using the Origin Pro 7.5 program. All contour maps were plotted using the same scale range of fluorescence intensities and the same number of contours.

RESULTS & DISCUSSION
As mentioned above, the raw water was treated in succession by biological fluidized bed pretreatment, coagulation, sedimentation, and sand filtration. The treated water was then pumped into the first stage ozonation process. Because of the oxidation of O3, the effluent has a slightly lower DON of 0.12 mg/L in comparison to the influent DON of 0.26 mg/L. This level increased to 0.56 mg/L with the following primary BAC treatment. The DON levels in the second stage O₃-BAC had the same trend, but with a lower increase to 0.20 mg/L, and an increase to 0.31 mg/L (Fig. 2).

The DON shifts along the media depth were measured in order to investigate the track of their generation and degradation during biofiltration. Three batches of samples were collected during two backwash cycles for the primary BAC, and they showed the same DON shift trends. The results and average is shown in Fig. 3. Although there were fluctuations in DON levels...
Fig. 2. DON levels in the effluent of each stage of O3-BAC

Fig. 3. DON variations along the media depth of each stage of BAC
as media depth increased, the DON concentrations generally increased as media depth increased. During the first 0.30 m, DON was 1.33 mg/L in the influent and increased to 1.36 mg/L at the 0.30 m sampling port. In the deeper media, DON once reached 1.44 mg/L at a depth of 1.75 m. The concentration finally dropped to 1.38 mg/L in the effluent, a level that was still significantly higher than that seen in the influent. The secondary BAC showed almost the same DON variation trend as the primary BAC (Fig. 3). During the first 0.30 m depth, DON levels increased sharply. At the points close to the filter bottom, the highest concentration appeared at a depth of 1.50 m. But this concentration was not higher than the concentration in the 0.30 m depth. Finally, the DON level decreased in the effluent, but the level still remained higher than that in the influent. The samples in Figs 2 and 3 were taken on different days, but the results were in agreement with DON trends before and after the biofiltration treatment. Summarily, the DON levels lowered through ozonation, and then were elevated by the backwash, which was consistent with the previous report by Gu et al. (Gu et al., 2010).

Our previous studies suggested that there was an obvious link connecting NH₄⁺-N loss in the influent with DON generation during biofiltration (Lee et al., 2006, Yu et al., 2007). In this study, NH₄⁺-N was lower than the detectable limit (0.01 mg/L) in the O₃-BAC influent, because NH₄⁺-N was removed by preceding processes like biological pretreatment and sand filtration. For this reason the NO₂⁻-N concentration was also very low (less than 0.003 mg/L). Interestingly, an ebb and flow pattern was observed between NO₂⁻-N and DON with the absence of NH₄⁺-N and NO₂⁻-N along the media depth (Fig. 4). In the primary BAC, the complementary trends between NO₂⁻-N and DON were obviously observed from the samplings from below 0.50 m. For example, DON decreased to 1.33 mg/L at the depth of 1.00 m as shown in Figure 3, while NO₂⁻-N increased from 4.84 mg/L at a 0.5 m depth, to 4.90 mg/L at a 1.0 m depth. When DON reached 1.44 mg/L at the depth of 1.75 m, NO₂⁻-N correspondingly lowered to 4.85 mg/L. For the secondary BAC, NO₂⁻-N levels showed completely complementary trends relative to corresponding DON levels, i.e. DON concentration increased when NO₂⁻-N decreased. The mass balance relationship of NO₂⁻-N and DON was further investigated, and it was found that the absolute value of increased DON was not strictly equal to that of disappeared NO₂⁻-N from any two adjacent sampling points. This was because bacterial decay and soluble microbial products (SMPs) release in the biofilters could bring nitrogen-containing matters related to DON, which were effected by nutrients, the backwash cycle and so on.

Microbial biomass and microbial activity (Fig. 5) were studied because microorganisms are critical to DON increase through N-containing SMPs release. Generally, both filters had the same trends for biomass levels. Biomass increased sharply from 33.1 nmolP/cm² AC on the top media surface, to about 49.1 nmolP/cm² AC at a 0.10 m depth for the first BAC. They then dropped close to the initial level of about 33.0 nmolP/cm² AC at a depth of 0.20 m. This was maintained for a significant depth until 1.90 m, and then showed a clear drop to 26.6 nmolP/cm² AC. For the secondary BAC filter, the biomasses were often less than those of the primary filter, except those from the first three sampling ports. In these, the biomass sharply increased from 31.8 to 52.6 nmolP/cm² AC, and then dropped to 28.5 nmolP/cm² AC. Specific oxygen uptake rate (SOUR) was used to indicate microbial activity. In terms of SOUR per biomass for average activity, variations were also similar for both filters. In the first 0.1 cm depth, SOUR decreased significantly when biomass increased sharply. The SOUR dropped from 0.55 to 0.47 mgO₂/nm10⁶P/h×10⁻³ for the primary filter, and from 0.40 to 0.29 mgO₂/nm10⁶P/h×10⁻³ for the secondary filter. SOUR then kept increasing slowly for both filters from 0.10 m to 1.50 m depth until reaching maximum values of 0.74 and 0.52 for the primary and secondary biofilters. Finally, the SOUR values dropped to 0.65 and 0.19 at the bottom points. SOUR values were always higher in the primary biofilter than in the secondary biofilter. In terms of SOUR per volume of AC for general activity, the variations were same as the biomass trends. General microbial activities in the first BAC were all above 1.5 mgO₂/cm² AC/h×10⁻², which was demonstrably higher than in the second BAC, which had a maximum value of about 1.5 mgO₂/cm² AC/h×10⁻².

The fractions DON by EEM, DOC, and SUVA₂₅⁴₄ varying along the media depth are showed in Fig. 6 and Table 3. The effluents and four water samples along the media depth (0, 0.30, 1.00, 1.50 and 1.75/1.70 m) were selected for EEM analysis according to the peaks and/or vales of DON concentration for both biofilters. There were two fluorophore regions with Ex/Em=220-240/280-360 nm and Ex/Em=220-290/280-360 nm for all samples. They represent tyrosine/tryptophan amino acid and tyrosine/tryptophan protein, respectively. For the primary BAC, the strongest fluorophores appeared at Ex/Em=235.0/340.0 and Ex/Em=275.0/320.0. Table 2 shows that the fluorescent intensities of each sample generally correlated well with corresponding DON concentrations. However, for the secondary filter, a reverse correlation was observed at Ex/Em=230.0/340.0 nm and Ex/Em=275.0/320.0 nm. Otherwise, amino acid and protein fluorescent intensities became stronger or weaker simultaneously for both biofilters.
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Fig. 4. NO₃⁻N variations along the depth of each stage of BAC

Fig. 5. The biomass and SOUR of each stage of BAC
A most widely used advanced drinking water treatment process, O$_3$-BAC is designed to further remove hazardous substances and improve the quality of drinking water following the conventional treatment processes. With respect to DON elimination, ozone elimination proved to be an effective technology. Unfortunately, the DON concentration rebounded after biological activated carbon filtration. This result was consistent with our previous study (Gu et al., 2010). This finding is thus widespread in biological processes, and must receive more attention because DON is a very important precursor to N-DBPs as mentioned above. In our previously study, in an individual biofilter without advanced oxidation pretreatment, DON concentration presented a rapid decrease from 0.73 to 0.44 mg/L in the top media (0-10 cm), and a slow increase from 0.44 to 1.08 mg/L in the bottom media (10-100 cm) (Liu et al., 2011). However, DON variation was different in biofilter with ozonation pretreatment as shown above; DON increased along the first 0.30 m depth, then decreased in the middle media, and rebounded from a 1.00 m to about a 2.00 m depth finally. Comparing the results of these two kinds of biofilters suggests that the residual ozone in the influent had a demonstrably significant effect on DON generation. Due to strong oxidation ability, ozone could decompose DOM to low molecular weight compounds. More importantly, ozonation could inhibit microbial activity and accelerate bacterial decay (Valde et al., 2002). When microbial activity is strong, more SMPs are released by bacteria into water, and the N-containing part causes an increase in DON. Similarly, bacteria decay releases SMPs into water, thus increasing DON. Microbial biomass and microbial activities in biofilters are shown in Fig. 5. Microorganisms in shallow media could obtain the most available nutrients. This is especially true for carbon sources. Ozone can oxidize DOM with high molecular weights to DOM with smaller molecular weights that are more likely to be utilized by microorganisms. Although assimilable organic carbon (AOC)

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**Table 2. Fluorescent intensities of the water samples along the media depth**

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Ex/Em (nm)</th>
<th>0</th>
<th>0.30</th>
<th>1.00</th>
<th>1.75/1.70</th>
<th>2.00/1.90</th>
</tr>
</thead>
<tbody>
<tr>
<td>The primary BAC</td>
<td>235.0/340.0</td>
<td>4540</td>
<td>4846</td>
<td>4717</td>
<td>4960</td>
<td>4985</td>
</tr>
<tr>
<td>The secondary BAC</td>
<td>275.0/320.0</td>
<td>6303</td>
<td>6344</td>
<td>6467</td>
<td>6287</td>
<td>6089</td>
</tr>
</tbody>
</table>

**Table 3. DOC (mg/L) and SUVA$_{254}$ (L/mg/m) of the water samples along the media depth**

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>1.75/1.7</th>
<th>2.0/1.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>The primary BAC</td>
<td>DOC</td>
<td>4.49</td>
<td>4.19</td>
<td>3.92</td>
<td>5.24</td>
<td>3.86</td>
<td>4.40</td>
<td>4.48</td>
<td>4.09</td>
</tr>
<tr>
<td>The secondary BAC</td>
<td>SUVA$_{254}$</td>
<td>1.43</td>
<td>1.52</td>
<td>1.72</td>
<td>1.28</td>
<td>1.73</td>
<td>1.67</td>
<td>1.54</td>
<td>1.69</td>
</tr>
<tr>
<td>The primary BAC</td>
<td>SUVA$_{254}$</td>
<td>4.23</td>
<td>4.65</td>
<td>4.22</td>
<td>5.12</td>
<td>3.70</td>
<td>4.26</td>
<td>7.47</td>
<td>4.51</td>
</tr>
<tr>
<td>The secondary BAC</td>
<td>DOC</td>
<td>1.14</td>
<td>1.08</td>
<td>1.31</td>
<td>1.01</td>
<td>1.49</td>
<td>1.20</td>
<td>0.67</td>
<td>1.11</td>
</tr>
</tbody>
</table>
Dissolved Organic Nitrogen

concentration increased after ozonation (Lou et al., 2009), the microbial biomass and the general activity were at low levels on the shallow media layer. This was because of the oxidation effect of the remaining ozone in the influent on microorganisms. In comparison, the microbial biomass and activity were more influenced by the toxicity of ozone than by the availability of nutrients on the top media. The biomass and the general activity reached peak values at a 0.10 m depth for both biofilters. There, the ozonation disappeared and microorganisms were under a carbon-rich situation. At this stage, a lot of available carbon source was consumed, which led to a carbon lack for microorganisms in lower places. SMPs include utilization associated products (UAP) and biomass associated products (BAP). UAP was used by microorganisms, while the biodegradation rate of BAP was far slower than that of UAP (Rittmann et al., 2004). At the depths of 0.30-1.00 m, quite low biomass and general activity were observed and so lower levels of SMPs were produced. Due to the lack of easily available nutrients, SMPs, especially BAP, were utilized by microorganisms to maintain growth, and so DON levels declined. At the places near the bottom, sand intercepted microbial shedding, resulting in a DON rebound again because of sands smaller size, relative to activated carbon. Overall, although DON variations were complicated during the medial depth of biofilter pretreated by ozonation, the variation was still reasonable. Several factors, like ozonation and the amount of available nutrients, had great influences on DON generation and degradation in the O3-BAC treatment. Microorganisms synthesized proteins and nucleotides, utilizing NH$_4^+$ as the primary nitrogen source. As mentioned above, NH$_4^+$ was not detectable in the O$_3$-BAC influent, so another nitrogen had to be utilized. Table 4 lists the reactions of cell synthesis using different forms of nitrogen (Rittmann et al., 2004). NO$_3^-$ was a more easily substitutable nitrogen source by comparison (Rittmann et al., 2004; Shen et al., 2006), and it did show an ebb and flow pattern in accordance with DON, particularly for the secondary biofilter (Figs 3 and 4). However, the mass balance was not observed between increased DON and disappeared NO$_3^-$ (Figs 3 and 4) from the same two adjacent sampling points. Due to the discrepancy of the amounts of net growth and net death of microorganisms at different stages of a backwashing cycle, the biomass and activity were also causing SMPs amounts to differ. DON concentration decreased at the very beginning of a backwashing cycle, which indicated that the new accumulated SMPs were less than those consumed. This data was not listed here. There should be a balance between DON and NO$_3^-$ in a whole backwashing cycle involving the nitrogen in the influent, effluent, and backwashing water, and the nitrogen synthesized by microorganisms. This will be further studied in the laboratory. EEM of both biofilters showed that DON fractions were mainly tyrosine/tryptophan amino acids and tyrosine/tryptophan proteins. This indicated that microorganisms could use these two kinds of amino acids in a distribution system without disinfection. Unfortunately, both amino acids were dichloroacetamide (emerging N-DBPs) precursors according to Chu et al. (Chu et al., 2010). Bougeard indicated that high SUVA$_{254}$ values indicated that the DON was hydrophobic, and low values indicated that DON was hydrophilic (Bougeard et al., 2010). In this study, the SUVA$_{254}$ values were situated in the middle (Table 3). While tyrosine and tryptophan amino acids are hard soluble and slightly soluble in water, respectively, many hydrophilic substances existed in the effluent. The processes developed based on this characteristic will help to control DON in drinking water treatment plants.

Table 4. Cell synthesis utilizing different forms of nitrogen sources

<table>
<thead>
<tr>
<th>Number of reaction</th>
<th>Reaction Equation</th>
<th>Product</th>
<th>Reaction Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Ammonia</td>
<td>$5/2CO_2+1/2NO_2^-+1/46NH_4^++H^++e^- = 1/46C_3H_7O_2N+8/46H_2O$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2) Nitrate</td>
<td>$1/16CO_2+1/46NO_2^-+1/46NH_4^++29/46H^++e^- = 1/46C_3H_7O_2N+11/46H_2O$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) Nitrite</td>
<td>$5/26CO_2+1/26NO_2^-+27/26H^++e^- = 1/26C_5H_7O_2N+10/26H_2O$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) Nitrogen gas</td>
<td>$5/23CO_2+1/46NO_2^-+H^++e^- = 1/23C_3H_7O_2N+8/23H_2O$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSION

This full-scale study investigated the DON in a two-stage O₃-BAC process. DON concentrations increased from 0.12 to 0.56 mg/L in the primary biofilter and from 0.20 to 0.31 mg/L in the secondary biofilter. Tracing DON along the media depth found that DON increased in the first 0.30 m depth, decreased in the depths of 0.30 to about 1.00 m, and then rebounded in the depths of 1.00 m to about 2.00 m finally. Although there were fluctuations with the increase of the media depth, the DON concentrations generally increased. There was an ebb and flow pattern between NO₃⁻N and DON along the media depth in the absence of NH₄⁺-N and NO₂⁻-N. The ozonation and nutrients significantly impacted the microbial biomass and microbial activities along the media depth, which was very important to DON formation through their effects on SMPs release. EEM results demonstrated that DON fractions were mainly tyrosine/tryptophan amino acids and tyrosine/tryptophan proteins for both BAC filters, and the fluorescent intensities of each sample generally correlated well with the corresponding DON.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support of the Hundred Talents Program, Chinese Academy of Sciences; Financial support from Xiamen Municipal Bureau of Science and Technology (3502ZZ0116007 and 3502ZZ0101010) and Urban Environmental Quality and Health Effects, Innovative Team of Overseas Chinese Academy of Sciences Partner Program (KZCX2-YW-T08).

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