Fate of dissolved organic nitrogen during biological nutrient removal wastewater treatment processes

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Abstract
Due to its potential to form toxic nitrogenous disinfection byproducts (N-DBPs), dissolved organic nitrogen (DON) is considered as one of the most important parameters in wastewater treatment plants (WWTP). This study describes a comprehensive investigation of variations in DON levels in orbal oxidation ditches. The results showed that DON increased gradually from 0.71 to 1.14 mg l⁻¹ along anaerobic zone, anoxic zone, aerobic zone 1 and aerobic 2. Molecular weight fractionation of DON in one anaerobic zone and one aerobic zone (aerobic zone 2) was performed. We found that the proportion of small molecular weight (<6 kDa) decreased and large molecular weight (>20 kDa) showed opposite trend. This variation may have been caused due to the release of different types of soluble microbial products (SMPs) during biological processes. These SMPs contained both tryptophan protein-like and aromatic protein-like substances, which were confirmed by three-dimensional excitation-emission matrix (EEM) analysis.

Key words
Wastewater treatment plant, Dissolved organic nitrogen, Soluble microbial products, Orbal oxidation ditch

Introduction
During wastewater treatment processes, chlorine is frequently used for final disinfection prior to discharge. However, undesirable disinfection byproducts (DBPs) can be produced during chlorination. Trihalomethanes (THMs) and haloacetic (HAA) are typical halogen-DBPs, and are considered as carcinogenic or suspected carcinogens (Gopal et al., 2007). Recent research suggests that nitrogenous disinfection byproducts (N-DBPs) with higher toxicity may exist (Gopal et al., 2007). The N-DBPs including a variety of -NO₂ and -CN contain chemical compounds like nitrosoamines, halonitroalkanes and nitriles (Choi and Valentine, 2002; Plewa et al., 2004; Chen and Valentine, 2006; Krasner et al., 2006; Lee et al., 2007). These compound are far more genotoxic to mammalian cells than halogen- DBPs (Cowman and Singer, 1996; Brosillon et al., 2009). Increased contributions from wastewater discharge can led to elevated levels of N-DBPs in drinking water sources. This is one of the reasons why, DON is attracting so much attention in wastewater treatment field. DON is composed of series of compounds containing a variety of nitrogen-containing functional groups. These mainly include NH classes, amino category, nitriles, purine, pyrimidine and nitro compounds (Westerhoff and Mash, 2002). It has been reported that DON concentration can range from 0.5 to 3 mg l⁻¹ in the final effluent. This figure holds true for many different treatment levels and strategies (Chakkr et al., 2009). With total nitrogen and inorganic nitrogen in effluent reduced to low levels, DON became an important component in final effluent (Chakkr et al., 2009). Nowadays, the studies on DON are focusing on its analytical measurement, structural composition, occurrence and potential in N-DBPs formation (Chu et al., 2009), and few profiles of typical wastewater biological treatment processes have been reported.
Generally, soluble microbial products (SMPs) are generated during biological treatment processes by bacteria as a result of substrate metabolism and biomass decay (Barker and Stuckey, 1999). They are considered a main source of DON (Westerhoff and Mash, 2002), so the biological treatment processes requires attention. In wastewater treatment plants (WWTP), conventional biological wastewater treatment systems including oxidation ditch, trickling filter and activated sludge, are designed and operated to meet the BOD and ammonia effluent standards. Orbital oxidation ditch, as one of the typical such processes, has presented effective removals of NH$_4^+$-N and DOC. It has been reported that SMPs will be released in both anaerobic and aerobic system (Barker and Stuckey, 1999). Therefore, the transformation of DON in orbital oxidation ditches is of significant importance and interest.

In the present study, the fate or occurrence of DON in orbital oxidation ditches involving anaerobic zone, anoxic zone, aerobic zone 1 and aerobic zone 2 were investigated. Correspondingly, the distribution of DON of different molecular weights were also studied. Furthermore, EEM was applied to determine the mechanism of the DON variation during orbital oxidation ditch process.

Materials and Methods

Wastewater treatment processes: The present study was performed at the Jimei wastewater treatment plant (WWTP), which has a capacity of approximately 3.50×10$^4$ m$^3$ d$^{-1}$. The treatment process included grid, primary sedimentation tank, orbital oxidation ditch and secondary sedimentation tank (Fig. 1). There are two orbital oxidation ditches in parallel, providing a total working volume of 20,000 m$^3$. The orbital oxidation ditch consist of 4 ditches including anaerobic zone, anoxic zone, aerobic zone 1 and aerobic zone 2. The concentration of solids suspended in mixed liquor (MLSS) in the orbital oxidation ditch was 2500–3000 mg l$^{-1}$ and the returned activated sludge (RAS) was roughly equal to the influent flow. The operating sludge retention time (SRT) was about 20 days.

Sampling: Water samples were collected from anaerobic zone, anoxic zone, aerobic zone 1 and aerobic zone 2. Before analysis, all water samples were filtered through 0.45 µm pore-sized membranes. In four water samples, the DO concentration was 0.14, 0.32, 2.75, 4.71 mg l$^{-1}$, and the pH value was 7.25, 7.11, 6.79 and 6.57, respectively.

Analytical methods: DOC was determined using a Shimadzu TOC-VCHS analyzer. NH$_4^+$-N was measured using the salicylate-hypochlorite method. NO$_3^-$-N was measured using the N-(1-naphthyl)-ethylenediamine photometric method. NO$_2^-$-N was determined using UV spectro photometry method. Total dissolved nitrogen (TDN), sum of NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N and DON was measured using the alkaline potassium persulfate digestion-UV spectrophotometric method. All the determinations were performed as described in Chinese National Standard Methods (SEPA, 2002). DON was quantified as the difference between TDN and three dissolved inorganic nitrogen species (DIN, including NH$_4^+$-N, NO$_3^-$-N and NO$_2^-$-N).

Molecular weight fractionation: The DON was fractionated using molecular sieves. Two different kinds of regenerated cellulose membranes (Millipore Corp) were used: 20,000 and 6,000 nominal molecular weight limit (NWML). Water samples (200 ml) were filtered through the first and second membranes in series. 50 ml of raw water and each filtrate was retained for further analysis. The percentage of DON in each size range was calculated as follows:

\[ \% < 6 \text{ kDa} = \frac{C_{\text{6K Permeate}}}{C_{\text{raw}}} \times 100\% \]

(1)

\[ \% < 6 \text{ kDa} - 20 \text{ kDa} = \frac{C_{20K Permeate} - C_{6K Permeate}}{C_{\text{raw}}} \times 100\% \]

(2)

\[ \% > 20 \text{ kDa} = \frac{C_{\text{raw}} - C_{20K Permeate}}{C_{\text{raw}}} \times 100\% \]

(3)

Here, $C_i$ is the measured parameter of fraction i and the percentage difference between the measured total mass and the sum of the masses for each mass fraction was calculated.

EEM fluorescence spectroscopy: Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy (F-4600 FL Spectrophotometer, Hitachi, Japan) was used to identify different types of organic materials dissolved in the sampled 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 emission slits were set at 5 nm and the scanning speed was set at 1200 nm min$^{-1}$. Double-distilled water provided a blank spectrum. Data were processed using Software Origin 7.5 (OriginLab Inc, USA). The EEM spectra were plotted as the elliptical shape of contours. The X-axis here represents the emission spectra from 280 to 500 nm, and the Y-axis here represents the excitation from 200-500 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 and Discussion

Data regarding nitrogen species and other related parameters, such as the concentrations of DOC, C/N and DON/TDN in anaerobic zone, aerobic zone 1 and aerobic zone.
Fig. 1: Wastewater treatment processes

Fig. 2: Variations in (A) DON concentration; (B) DIN and TDN concentration; (C) DOC concentration and (D) Ratios of C/N and DON/TDN of different process.
2 are shown in Fig. 2. As shown in Fig. 2A and B, along anoxic zone, aerobic zone 1 and aerobic zone 2, the DON concentration increased gradually from 0.71 to 1.14 mg l\(^{-1}\) and about 61% of DON increased. The concentrations of NH\(_4\)^+-N and TDN decreased gradually, which was 10.08, 5.58, 1.28, 0.11 mg l\(^{-1}\) and 12.42, 10.41, 10.39, 10.42 mg l\(^{-1}\), respectively. The sum of NO\(_3\)^-N and NO\(_2\)^-N concentration increased gradually from 1.68 to 9.17 mg l\(^{-1}\).

Also, related parameters including DOC concentration and the ratios of C/N, DON/TDN were evaluated as shown in figures 2C and D. The concentration of DOC decreased gradually from 16.32 to 7.36 mg l\(^{-1}\). The C/N ratio decreased significantly from 22.98 to 6.45 and of DON/TDN ratio increased gradually from 5.69 to 10.94.

It is generally thought that SMPs are produced in biological process and contain organic matters such as polysaccharides, proteins, nucleic acids, amino acids and steroids, all of which are nitrogen enriched compounds (Rittmann et al., 1987). It was reported that these SMPs presented a lower biodegradability and C/N ratio (Ciner and San, 2001). The normalized production of SMP in aerobic system appears to be higher than that in anaerobic system (Barker and Stuckey, 1999). So, in aerobic zone 1 and aerobic zone 2, the rate of SMPs generation tended to be higher than that of substrate utilization, and SMPs were accumulated gradually along the flow, which increased the DON concentration.

Due to nitrification, the concentration of NH\(_4\)^+-N decreased gradually, while the sum of NO\(_3\)^-N and NO\(_2\)^-N concentration increased gradually. The TDN and DOC concentration decreased in anoxic zone because of denitrification. In aerobic zone, DOC was removed owing to hydrolysis. The gradual decrease of DOC and TDN concentration and gradual increase of DON concentration caused the ratio of DOC/DON to decrease gradually and DON/TDN ratio to increase gradually.

The distribution of DON of different molecular weights in the anaerobic zone and in aerobic zone 2 is shown in Fig. 3. It could be seen that in the anaerobic zone small molecular weight (<6 kDa) accounted for a high proportion of 85%, and large molecular weight (>20 kDa) and middle molecular weight (6 kDa–20 kDa) accounted for a small proportion of 15%. However, from anaerobic zone to aerobic zone 2, a distinct increase from 8 to 31% in >20 kDa molecular weight fraction, while the <6 kDa molecular weight fraction was greatly reduced from 85 to 64%. Middle molecular weight fraction (6 kDa–20 kDa) decreased slowly from 7 to 4%, but was not apparent.

Previous studies have reported that SMPs can vary widely in molecular weight (Ni et al., 2010). Furthermore, SMPs are composed of both growth related utilization associated products (UAPs) and non-growth related biomass products (BAPs) (Carlson and 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 (Carlson and Amy, 2000). UAPs in SMPs was found to be carbonaceous compounds derived from the substrate utilization with smaller molecular weight, BAPs, however, were found to result mainly from the cellular decay of larger macromolecules (Ni et al., 2010). In the anaerobic zone, microbes were in the microbial growth and substrate utilization phases, which resulted in production of UAP with lower molecular weight. As shown in Fig. 3, this might cause that the DON of small molecular weight (<6 kDa) was absolutely predominant. Owing to decrease of the substrate, the behavior of the microbe was gradually transitioned from substrate utilization to endogenous respiration in aerobic zone 2. The BAPs was gradually accumulated and UAPs was gradually decreased, which significantly increased the organic matter with large molecular weight. Therefore, compared with the molecular weight distribution of DON in the anaerobic zone, the DON of large molecular weight (>20 kDa) in aerobic zone 2 was

![Fig. 3: Profiles of molecular weight fractionation of DON in anaerobic zone and aerobic zone 2](image)

**Table 2**: Fluorescence spectral parameters of different water sample

<table>
<thead>
<tr>
<th>Samples</th>
<th>Peak A Ex/Em</th>
<th>Peak B Ex/Em</th>
<th>Peak C Ex/Em</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic zone</td>
<td>275/335</td>
<td>230/310</td>
<td>230/345</td>
</tr>
<tr>
<td>Aerobic zone</td>
<td>275/330</td>
<td>230/310</td>
<td>235/345</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>Peak A Int.</th>
<th>Peak B Int.</th>
<th>Peak C Int.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic zone</td>
<td>1410</td>
<td>569</td>
<td>2107</td>
</tr>
<tr>
<td>Aerobic zone</td>
<td>1737</td>
<td>591</td>
<td>2364</td>
</tr>
</tbody>
</table>
distinctly increased and the DON of small molecular weight was evidently decreased.

The variation in DON concentration suggests that nitrogenous organic compounds might be eliminated or regenerated in orbal oxidation ditch. In order to find an evidence of variation in DON concentration, EEM was used to identify the dissolved organic substances in water samples. EEM fluorescence spectra of water samples in anaerobic zone and aerobic zone 2 are illustrated in Fig. 4, and their spectral characteristics are listed in Table 1.

From the EEM spectra, three main peaks (A, B and C) could be identified. It is worth noting that there were still many peaks with relatively low intensity which could not be clearly evident. Peak A was found at the excitation/emission (Ex/Em) wavelengths of 275/330-340 nm, while Peak B was found at the excitation/emission (Ex/Em) wavelengths of 225-230/295-310 nm and Peak C was found at the Ex/Em wavelengths of 225-230/345 nm. Peaks A, B and C have been shown to be protein-like compounds, associated with tryptophan protein-like and aromatic protein-like substances, respectively (Chen et al., 2003; Yamashita and Tanoue, 2003; Aryal et al., 2009). The fluorescence parameters of the spectra including peak locations and fluorescence intensity were summarized in Table 1. Compared to anaerobic zone, the intensity of peak A, B and C were increased in aerobic zone 2. SMPs contain a series of organic matters, such as proteins, nucleic acids, amino acids, humic acids and fulvic acids, which are all nitrogenous organic compounds (Barker and Stuckey, 1999).

It has been proved that three characteristic peaks (Fig. 4) were typical compounds of SMPs, which were associated with the microbial activity (Esparza-Soto and Westerhoff, 2001; Westerhoff et al., 2001; Her et al., 2003; Lee and Ahn, 2004; Sheng and Yu, 2006). EEM results (Fig. 4 and Table 1) revealed that the relative concentrations of the three characteristic peaks in aerobic zone 2 were higher than these in anaerobic zone. As shown in Fig. 4 and Table 1, the EEM parameters indicate a good relationship between the variations in DON and the intensity of three characteristic peaks. This suggests that the variations in DON during the process are closely depended on the number of SMPs. Therefore, the results of EEM (Fig. 4 and Table 1) also confirmed that SMPs should count for the changes of DON in orbal oxidation ditch process.

SMPs generated during orbal oxidation ditch could be essential to DON production during biological processes. With the decrease of substrate concentration and increasing proportion of aged biomass, part of microbe went into the endogenous respiration, and BAPs became gradually predominant in SMPs, causing DON concentration to increase gradually. EEM results also confirmed that the changes in the concentration of SMPs could contribute to the variations in the concentration of DON in orbal oxidation ditch process.

Based on the above conclusions, the security of biological wastewater treatment processes should be re-considered for its potential of the increased DON in forming N-DPBs.
Acknowledgments

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References


