

A study on the reactivity characteristics of dissolved effluent organic matter (EfOM) from municipal wastewater treatment plant during ozonation



Pengkang Jin ^{a,*}, Xin Jin ^{a,**}, Viggo A. Bjerkelund ^b, Stein W. Østerhus ^b, Xiaochang C. Wang ^a, Lei Yang ^c

^a School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi Province, 710055, China

^b Department of Hydraulic and Environmental Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, N-7491, Norway

^c Department of Chemistry-Ångström Laboratory, Uppsala University, Uppsala, 75120, Sweden

ARTICLE INFO

Article history:

Received 17 August 2015
Received in revised form
29 October 2015
Accepted 30 October 2015
Available online 4 November 2015

Keywords:

Ozonation
Dissolved effluent organic matter (EfOM)
Fluorescence characteristics
XPS analysis

ABSTRACT

The reactivity of dissolved effluent organic matter (EfOM) in the process of ozonation was examined. Under different ozone dosages (0.42 ± 0.09 , 0.98 ± 0.11 and 2.24 ± 0.17 mgO₃/mg DOC), the EfOM before and after ozonation could be classified into four fractions according to their hydrophobicities. By ozonation, the hydrophobic fractions, especially hydrophobic acid (HOA) and hydrophobic neutral (HON), were found to undergo a process of transformation into hydrophilic fractions (HI), of which the HOA were first transformed into HON, and then the majority of the HON fraction was later converted to HI by further ozonation. It was noticeable that after ozonation, the fluorescence intensity in the humic-like and protein-like regions decreased as indicated by the excitation and emission matrix (EEM) spectra for the hydrophobic fractions. By coupling the EEM spectra with the molecular size analysis using high performance size exclusion chromatography (HPSEC), the difference between the characteristic distributions of the humic-like and protein-like fluorophores were further revealed. It could thus be extrapolated that ozone might have preferentially reacted with the protein-like hydrophobic fraction with molecular weight (MW) less than 100 kDa. Moreover, by X-ray photoelectron spectroscopy (XPS) analysis, it was identified that with increasing ozone dosage (from 0 to 2.24 ± 0.17 mgO₃/mg DOC), the aromaticity of HON decreased dramatically, while aliphatics and ketones increased especially at the low ozone dose (0.42 ± 0.09 mgO₃/mg DOC). Of the EfOM fractions, the HON fraction would have a higher content of electron enriched aromatics which could preferentially react with ozone rather than the HOA fraction.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Dissolved effluent organic matter (EfOM) after conventional biological treatment is a mixture of soluble microbial products (SMP) (Barker and Stuckey, 1999), natural organic matter (NOM) conveyed from drinking water sources (Filloux et al., 2012) and xenobiotic micropollutants (Audenaert et al., 2013). In cases where the effluent is disinfected by chlorine, EfOM can also contain chlorinated disinfection byproducts (Krasner et al., 2009). The

majority of residual organic compounds present in EfOM are slowly biodegradable or recalcitrant.

Reclaimed water has become a very important water source in water deficient areas and one of the top issues concerning the water reuse is the safety of reclaimed water. However, conventional water reclamation process (coagulation-sedimentation-filtration) is not considered sufficient to secure the reuse safety. In this case, oxidation processes are often applied to minimize the risk of negative environmental and health effects due to exposure to micropollutants and disinfection byproducts (Galapate et al., 2001; Jin et al., 2013). Among the different oxidation processes, ozone is often applied because of its high oxidation potential (Sadrnourmohamadi and Gorczyca, 2015; Rodríguez et al., 2012; Owen et al., 1995; Reckhow and Singer, 1984) and compared to

* Corresponding author.

** Corresponding author.

E-mail addresses: pkjin@hotmail.com (P. Jin), jinxinjasen2006@163.com (X. Jin).

advanced oxidation processes (like e.g. UV/H₂O₂), ozonation is an energy efficient solution for transformation of most recalcitrant organic micropollutants (Sonntag and Von Gunten, 2012). Therefore, knowledge about changes in the chemical properties of EfOM during ozonation is important because it is closely related to their reaction pathways, the trihalomethanes formation potential (THMFP), and downstream treatment process efficiency.

Ozonation directly influences both the composition and chemical properties of EfOM. Especially important chemical features such as hydrophobicity/hydrophilicity and acid/base behaviours, which are important for EfOM distribution in the water phase and its interactions with other dissolved compounds and colloids/particles (Gong et al., 2008), are altered. Therefore, knowledge about changes in these chemical properties of EfOM during ozonation are important, when wastewater effluents are discharged to the environment or being reused (Chiang et al., 2002). Fractionation through XAD macroporous resins, which are commonly used for the fractionation of dissolved organic matter (Matilainen et al., 2011), is a favourable way to obtain information about changes in the hydrophobicity/hydrophilicity and acid/base behaviours of EfOM during ozonation. Fractionation by XAD resin has been frequently applied to study the reactivity between NOM and oxidants, such as ozone and chlorine dioxide (Galapate et al., 2001; Chiang et al., 2002; Marhaba et al., 2000; Świetlik et al., 2004; Yan et al., 2007; Zhang et al., 2008a; Bose and Reckhow, 2007). Nonetheless, the detailed transformation pathways remained poorly understood in these studies. As for the fractionation of EfOM in general, Imai et al. (2002) analysed the fraction distribution of EfOM from wastewater treatment plants (WWTPs), while Zhang et al. (2009) studied the THMFP and haloacetic acids formation potential (HAAFP) of isolated EfOM fractions. However, there is relatively little data available. Furthermore, there are few studies describing the effects of ozonation on EfOM (Gong et al., 2008).

Fluorescence spectroscopy can provide important information about the chemical nature of the EfOM because the position, shift and intensity of fluorescence peaks can be correlated with molecular structural information (Chen et al., 2003). To characterize the fluorescent dissolved organic matter (DOM) in wastewater effluents, this technique has been extensively used (Quaranta et al., 2012; Wang et al., 2009). Further, high-performance size exclusion chromatography (HPSEC) can provide information about the molecular size of DOM (Sarathy and Mohseni, 2007; Bazri et al., 2012). The molecular size of fluorescent DOM can be determined when HPSEC is combined with fluorescence detection, by using selected pairs of excitation/emission (Ex/Em) wavelengths in the EEM spectra. This was previously done by Nagao et al. (2003), Bourven et al. (2012) and Bhatia et al. (2013). However, they focused on NOM and extracellular polymeric substance (EPS), instead of EfOM. HPSEC coupled with fluorescence detection is well-suited to resolve different fluorophores within different fractions in EfOM. Furthermore, the variation of functional groups of different fractions in EfOM is important reactivity characteristics during ozonation as well. However, there are very few works focussing on the functional groups transformation in different fractions during ozonation. Zheng et al. (2014) only obtained the isolated fractions from EfOM using ¹³C nuclear magnetic resonance (NMR) without further information about molecular structure variation during ozonation. X-ray photoelectron spectroscopy (XPS) can be used to determine the functional groups in organic matters, and it has been extensively used in the analyses of functional groups in EPS in recent years (Yin et al., 2015; Hou et al., 2015; Badireddy et al., 2010).

Thus, the main objective of this study was to investigate the reactivity of dissolved EfOM during ozonation at different ozone dosages to provide useful information for evaluation of reaction

pathways, which subsequently can be used for ozone dosage selection during water reclamation, improved risk control for reclaimed water, and possible downstream treatment process optimization. In order to obtain a better understanding of the fingerprints variation of humic-like and protein-like substances in different fractions during ozonation, HPSEC with fluorescence detection was applied. XPS was used to analyse the effect of ozonation on functional groups for the different fractions of EfOM to facilitate determining the detailed transformation pathways between different fractions.

2. Materials and methods

2.1. WWTP and water samples

The water used in this study was effluent wastewater with pH of 7.33 ± 0.23 collected before the disinfection step at the effluent of the sedimentation tank in a municipal wastewater treatment plant (WWTP) in Xi'an, China. The WWTP consists of a biological Anaerobic-Anoxic-Oxic treatment process that treats mainly domestic wastewater. The capacity of the WWTP is 500,000 m³/d with hydraulic retention time (HRT) 20 h and sludge retention time (SRT) 19 d. The effluent of the WWTP typically has the following characteristics: 42.2 ± 4.6 mg/L COD, 12.8 ± 1.7 mg/L total nitrogen and 0.24 ± 0.13 mg/L total phosphorus. The water samples were filtered using a 0.45 μm filter (Shanghai Xinya, China) for the removal of particles prior to ozonation. The filtered raw water quality is shown in Table 1.

2.2. Experimental setup

The experimental ozonation setup is shown in Fig. 1. The effective volume of the reactor was 2.0 L. The ozonated water sample was collected to meet the demand of concentration and fractionation. The feed gas flow from the ozone generator (WH-S-B2, Nanjing Wohuan, China) was maintained at 40 L/h. Unreacted ozone in the off gas from the reactor was destructed in a 20% KI trap. The reactor temperature was kept constant at 15 °C by a water bath. Nitrogen gas was fed to the reactor for 5 min to strip the remaining ozone from the water in the reactor. The ozone dosage was determined according to Kasprzyk-Hordern et al. (2006) and Galapate et al. (2001). The reacted ozone dosage in this study was determined by the difference between pre-determined in-gas amount of ozone and off-gas ozone trapped by 20% KI solution. The reacted dosage of the ozone was adjusted by the reaction time. The ozonation experiments were performed in triplicate and the results are presented as the mean values with standard deviations. In this study, three different ozone dosages were applied respectively, 0.42 ± 0.09 , 0.98 ± 0.11 and 2.24 ± 0.17 mgO₃/mg DOC.

2.3. Fractionation procedure

All of the filtered samples, raw water and ozonated samples, were concentrated by a reverse osmosis membrane system (Mosutech, China). The concentration degree was 20, namely each 1-L water sample was concentrated to 50 ml. The characteristics of the concentrated samples before and after ozonation are shown in Table 2. The fractionation was performed with the XAD resin method, which was modified from Imai et al. (2002), Leenheer (1981), Pernet-coudrier et al. (2008) and Zhang et al. (2009). XAD-8 resin from Supelco was used in this study. The resin was first purified by Soxhlet extraction with methanol before being used in fractionation. During the fractionation, 150 mL of a concentrated water sample (raw or ozonated) was pumped through the resin. The first adsorbent from XAD-8, hydrophobic base (HOB), was then

Table 1
The water quality variation before and after ozonation.

| Parameter | Raw water | Dosage (mgO ₃ /mg DOC) | | |
|---|---------------|-----------------------------------|---------------|---------------|
| | | 0.42 (5 min) | 0.98 (30 min) | 2.24 (60 min) |
| DOC (mg/L) | 17.08 ± 1.81 | 17.56 ± 1.95 | 16.65 ± 1.39 | 16.18 ± 1.57 |
| Colour (c.u.) | 2.86 ± 0.03 | 1.83 ± 0.03 | 0.73 ± 0.01 | 0.57 ± 0.01 |
| UV ₂₅₄ (cm ⁻¹) | 0.135 ± 0.005 | 0.105 ± 0.003 | 0.077 ± 0.002 | 0.061 ± 0.003 |
| UV ₂₈₀ (cm ⁻¹) | 0.111 ± 0.003 | 0.078 ± 0.003 | 0.055 ± 0.001 | 0.042 ± 0.001 |
| SUVA (L.mg ⁻¹ .m ⁻¹) | 0.790 ± 0.047 | 0.598 ± 0.038 | 0.462 ± 0.020 | 0.377 ± 0.026 |

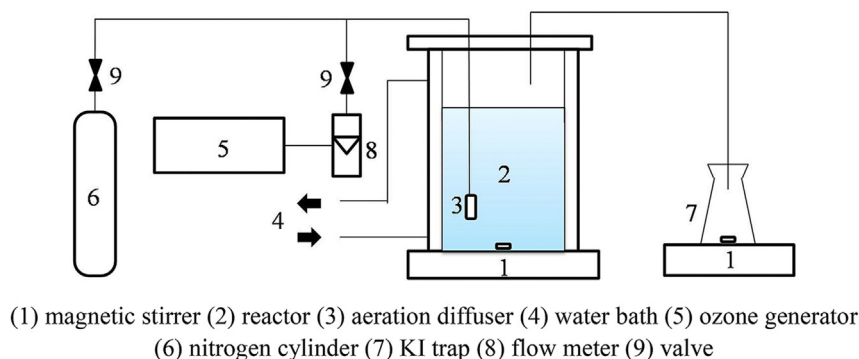


Fig. 1. Experimental setup for ozonation.

Table 2
The concentrated water quality variation before and after ozonation.

| Parameter | Raw water | Dosage (mgO ₃ /mg DOC) | | |
|---|---------------|-----------------------------------|---------------|---------------|
| | | 0.42 (5 min) | 0.98 (30 min) | 2.24 (60 min) |
| DOC (mg/L) | 139.99 ± 1.81 | 123.95 ± 1.95 | 122.00 ± 1.39 | 122.38 ± 1.57 |
| UV ₂₅₄ (cm ⁻¹) | 1.697 ± 0.004 | 1.108 ± 0.003 | 0.972 ± 0.003 | 0.534 ± 0.003 |
| UV ₂₈₀ (cm ⁻¹) | 1.411 ± 0.002 | 0.828 ± 0.003 | 0.704 ± 0.002 | 0.390 ± 0.001 |
| SUVA (L.mg ⁻¹ .m ⁻¹) | 1.212 ± 0.054 | 0.894 ± 0.029 | 0.797 ± 0.047 | 0.436 ± 0.038 |

eluted by a sequential flow of 0.1 M HCl. The XAD-8 effluent was acidified by HCl to pH 2 and recycled through the column. Then, the hydrophobic acid (HOA) was adsorbed onto the resin and the hydrophilic fraction (HI) was the effluent. The HOA was then eluted by 0.1 M NaOH. The XAD-8 resin was then Soxhlet-extracted with methanol after air drying in order to obtain hydrophobic neutral (HON). All of the elutions in this procedure were performed in a forward direction or by gravity flow.

2.4. Analytical method

2.4.1. DOC and UV absorbance measurement

DOC was measured by a Shimadzu TOCV_{CPH} analyser with infrared detection. The DOC analyser was calibrated with potassium hydrogen phthalate standard solutions before each run. All of the samples were filtered with a 0.45 μm filter, acidified with H₂SO₄ and purged with nitrogen to remove inorganic carbon before measurement. UV₂₅₄ was measured at 254 nm by UV-VIS spectrophotometer (UV-2102C UNIC™, China) using 1-cm path length quartz cells. All analyses were performed in triplicate for all of the measurements of DOC and UV₂₅₄.

2.4.2. Fluorescence excitation-emission matrix analysis

Fluorescence measurements were conducted using a spectrofluorometer (FP-6500, Jasco, Japan) with a 150 W xenon lamp. The analyses were performed at ambient temperature. A 1-cm quartz cuvette with four optical windows was used for the analyses.

Emission scans were performed from 280 to 550 nm with 5 nm steps, while excitation wavelengths were measured from 220 to 480 nm with 2 nm intervals. The slit widths for excitation and emission were 5 nm. The detector was set to high sensitivity and the scanning speed was kept at 2000 nm/min. The fluorescence spectra for distilled water was measured with the same method, and subtracted from all of the sample spectra to eliminate water Raman scattering and reduce other background noises throughout the experimental period. The EEM spectra were plotted as contours. The X-axis represents the emission spectra, while the Y-axis represents the excitation wavelength and the third dimension, i.e., the contour line, is given to express the fluorescence intensity.

2.4.3. Molecular weight distribution analysis

The characterization of the apparent molecular weight distribution (MWD) of the EfOM was performed using size exclusion chromatography (SEC) coupled with a fluorescence detector. The detection wavelengths were Ex/Em 355/430 nm and Ex/Em 230/340 nm for humic-like substances and protein-like substances, respectively. The high performance liquid chromatography (HPLC) system was a Shimadzu LC-2010AHF with a Zenix SEC-100 column (Sepax Technologies, USA). The injection volume was 10 μL and the column temperature was 30 °C. The mobile phase was 150 mM phosphate buffer solution (PBS) with a pH of 7.0 ± 0.1, and the flow rate was 0.8 mL/min. Polyethylene glycol (PEG) and Bull serum albumin (BSA) were used for humic-like and protein-like apparent molecular weight (AMW) calibration, respectively.

2.4.4. X-ray photoelectron spectroscopy analysis

X-ray photoelectron spectroscopy (XPS) was used to determine the ratio of different carbon species (aromatic carbon, aliphatic carbon, ketonic carbon and carboxylic carbon) in the EfOM fractions. The different fractions were dried at 50 °C to complete dryness before XPS analyses. The XPS analyses were performed with an X-ray photoelectron spectrometer (K-Alpha, Thermo Fisher Scientific, UK). Each analysis commenced with a survey scan in the binding energy range from 100 to 1000 eV, with a step size of 1 eV. A high energy resolution scan was applied with a step size of 0.1 eV. The binding energies of the photoelectrons were calibrated using the aliphatic adventitious hydrocarbon C1s peak at 284.6 eV.

3. Results and discussions

3.1. Characterization of EfOM

3.1.1. DOC and UV₂₅₄ distribution in EfOM fractions

Fig. 2 shows the DOC and UV₂₅₄ distribution in the EfOM fractions. The DOC values in Fig. 2 show that HOA and HON were the dominant fractions in EfOM before ozonation, and accounted for approximately 41% and 30%, respectively. Furthermore, HOB represented approximately 9% of the DOC in EfOM, while the HI represented approximately 20% of the DOC. This fraction distribution is different from the research of Yan et al. (2007), who studied typical micro-polluted surface water from north China. According to their results, HOA was the overwhelmingly dominant fraction, while HON was the smallest fraction. Zhang et al. (2008a) found that after sand filtration of river water, HOA and HON were the dominant fractions, which highly agreed with the results in this study. However, according to Marhaba et al. (2000) and Korshin et al. (1997), the dominant NOM fraction in surface water was hydrophilic. Furthermore, Gong et al. (2008) found that HOA and HI fractions were relatively similar in EfOM, and Zheng et al. (2014) revealed that the HOA and HI represented the majority fractions of EfOM. There are relatively large variations between different studies in the fraction distributions of DOM from both NOM and EfOM. This indicates that the DOM fraction distributions are highly site specific. It is known that UV₂₅₄ represents the content of aromatic structures and other unsaturated carbon bonds of organic matters in EfOM. It can be seen from Fig. 2b that HOA had highest UV₂₅₄ value, which is consistent with the results of Gong et al. (2008). In addition, HON also had very high UV₂₅₄ value comparing with HOB and HI.

3.1.2. Fluorescence characteristics of EfOM fractions

EEM spectra obtained by fluorescence spectroscopy provides important information on the chemical nature of the DOM from EfOM, such as the position, shift and intensity of the fluorescence peaks, which can be correlated to structural information, such as functional groups (electron-donating/withdrawing groups), polycondensation, aromaticity, heterogeneity and dynamic properties related to their intramolecular and intermolecular interactions (Chen et al., 2003). Fig. 3 shows the EEM spectra of the four EfOM fractions before ozonation in this study. Three apparent EEM peaks, exhibiting peak maxima at Ex/Em 220–250/320–370 nm, 260–300/320–370 nm and 300–380/400–450 nm, were observed for all raw DOM fractions from the EfOM. The peak at Ex/Em 300–380/400–450 nm corresponds to humic-like structures, which are classified as Type C according to Coble (1996) and Leenheer (2009). Additionally, the peaks at Ex/Em 220–250/320–370 nm and 260–300/320–370 nm are assigned as tyrosine-like (Type B) and tryptophan-like (Type T) constituents, respectively, which represent protein-like substances in general. As shown in Fig. 3, HOA and HON were rich in humic-like organic matters. Whereas, HOB and HI mainly contained protein-like organics. The results of this study are consistent with the early studies from Leenheer (1981), Marhaba et al. (2000) and Barber et al. (2001). Nonetheless, Świetlik and Sikorska (2004) and Zhang et al. (2008b), who used river water, revealed that the HOA, HON and HI fractions only had two well-resolved peaks, corresponding to humic and/or fulvic materials. The differences observed between our study and others, are most likely caused by the different raw waters used. Effluents from biological processes contain SMP which is from substrate metabolism and biomass decay (Barker and Stuckey, 1999; Kunacheva and Stuckey, 2014). Therefore, the HOA, HON and HI fractions in this study contain protein-like substances according to EEM spectra while these fractions from NOM only comprised humic-like materials.

3.1.3. Molecular weight distribution of EfOM fractions

Two fixed excitation and emission wavelengths were selected for the fluorescence detector during the SEC analyses. These corresponded to the peaks of humic-like and protein-like substances in Fig. 3 with specific wavelengths of Ex/Em 355/430 nm and Ex/Em 230/340 nm shown in Fig. 4a and b, respectively. Fig. 4a shows the MWD chromatograms of humic-like substances for the four EfOM fractions before ozonation. The MWD for the hydrophobic fractions show that the majority of the fluorescent organics are approximately 1000 Da. This results is consistent with the results from

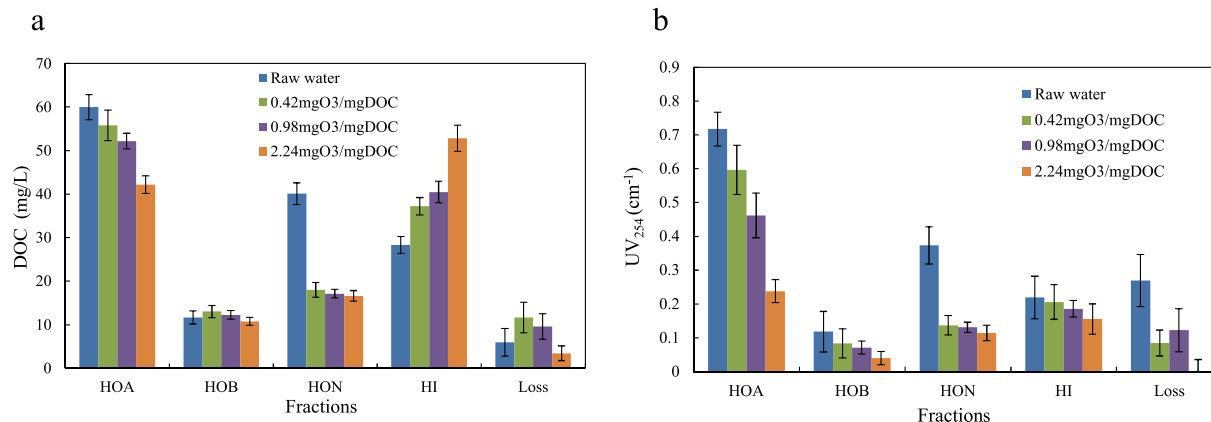


Fig. 2. (a) DOC and (b) UV₂₅₄ distribution of the four fractions before and after ozonation.

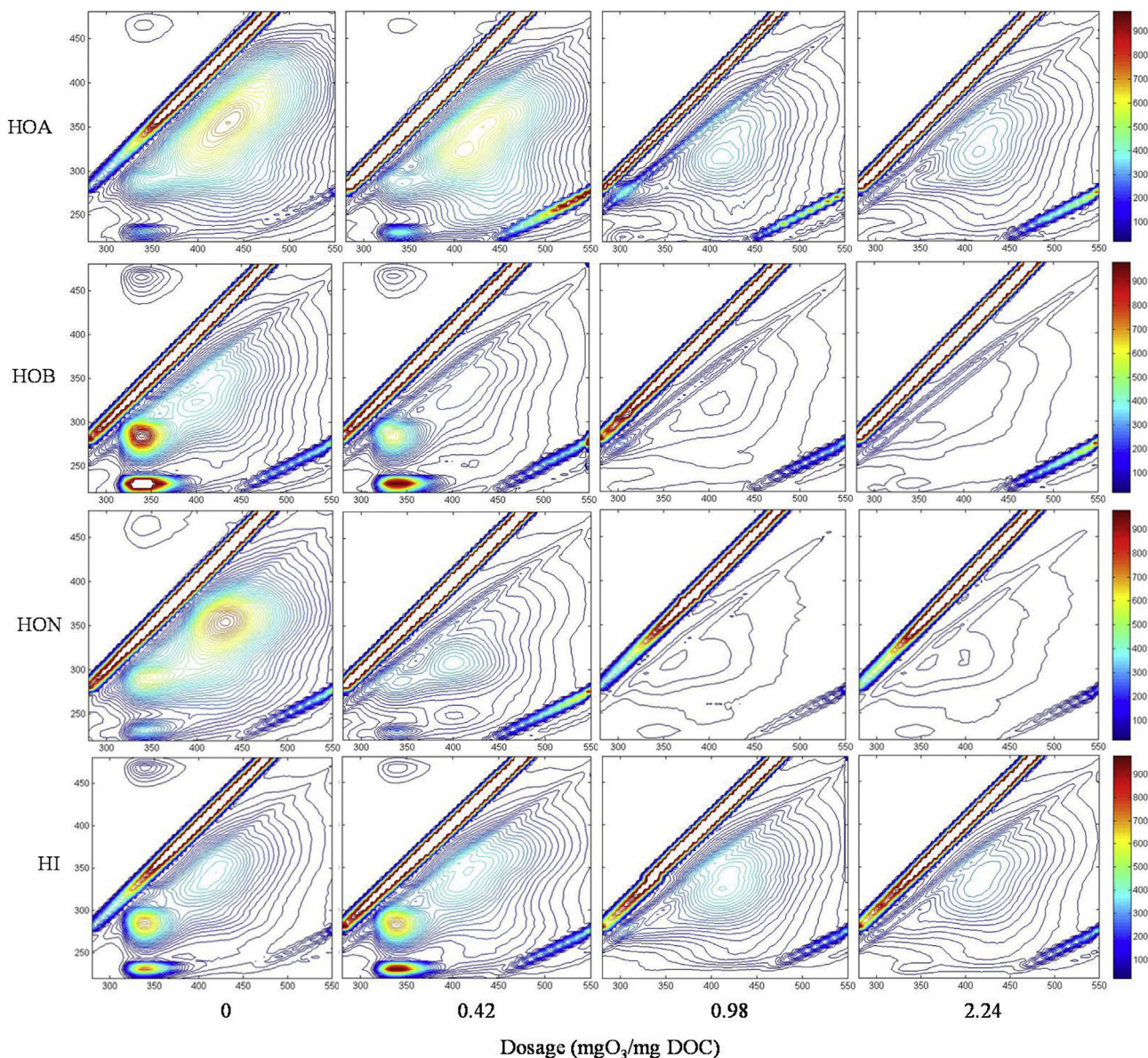


Fig. 3. EEM spectra of the four EfOM fractions before and after ozonation. Fractionation was done after ozonation and no single fractions are oxidized.

Audenaert et al. (2013) who also analysed the molecular weight of EfOM and revealed that the EfOM had molecular weight from 100 Da to 3000 Da. Gong et al. (2008) indicated that the majority of EfOM had molecular weight smaller than 1000 Da. In this case, ozone oxidation process resulted in a decrease of intensity values over the entire MWD range as reported by Zhang et al. (2008a), Ratpukdi et al. (2010) and Gonzales et al. (2012). For the protein-like substances shown in Fig. 4b, the size distribution of the HOA, HOB and HON fractions from raw water were broader than that of the humic-like substances. Protein-like HI fraction had lower molecular weight comparing with protein-like hydrophobic fractions.

3.1.4. Chemical structures of EfOM fractions

In order to gain an increased understanding of the characteristics of EfOM, the C1s high resolution spectra for each of the four fractions were obtained by XPS analyses. Subsequently, the results were analysed by XPSpeak software for Gaussian fitting to obtain the ratio of total carbon, as illustrated in Fig. 5. The C1s high resolution spectra were shown in the Supplementary data (Fig. S1).

Four types of chemical binding signals appeared in Fig. S1, which were assigned to aromatic carbon (C=C), aliphatic carbon (C–C), ketonic carbon (C=O) and carboxylic carbon (O–C=O), according to Monteil-Rivera et al. (2000) and Lin et al. (2014). As shown in Fig. 5, the four fractions in raw EfOM exhibited a different carbon species composition. Raw HOA and HON showed considerably high ratios of aromatic carbon, which is in accordance with the high UV₂₅₄ value in raw HOA and HON, as shown in Fig. 2. However, HON had higher ratio of aliphatic carbon and carboxylic carbon than HOA. Raw HOB and HI had low contents of aromatic carbon and the majority of the carbon species were assigned to ketonic carbon and aliphatic carbon. This explains why they showed a low fluorescent intensity in humic-like region compared with raw HOA and HON.

3.2. Effect of ozonation on EfOM fractions distribution

Fig. 2 shows the changes in the EfOM fractions due to ozonation. For an ozone dose of 0.42 mgO₃/mg DOC, the DOC of HOA and HOB barely changed. However, the content of HON decreased

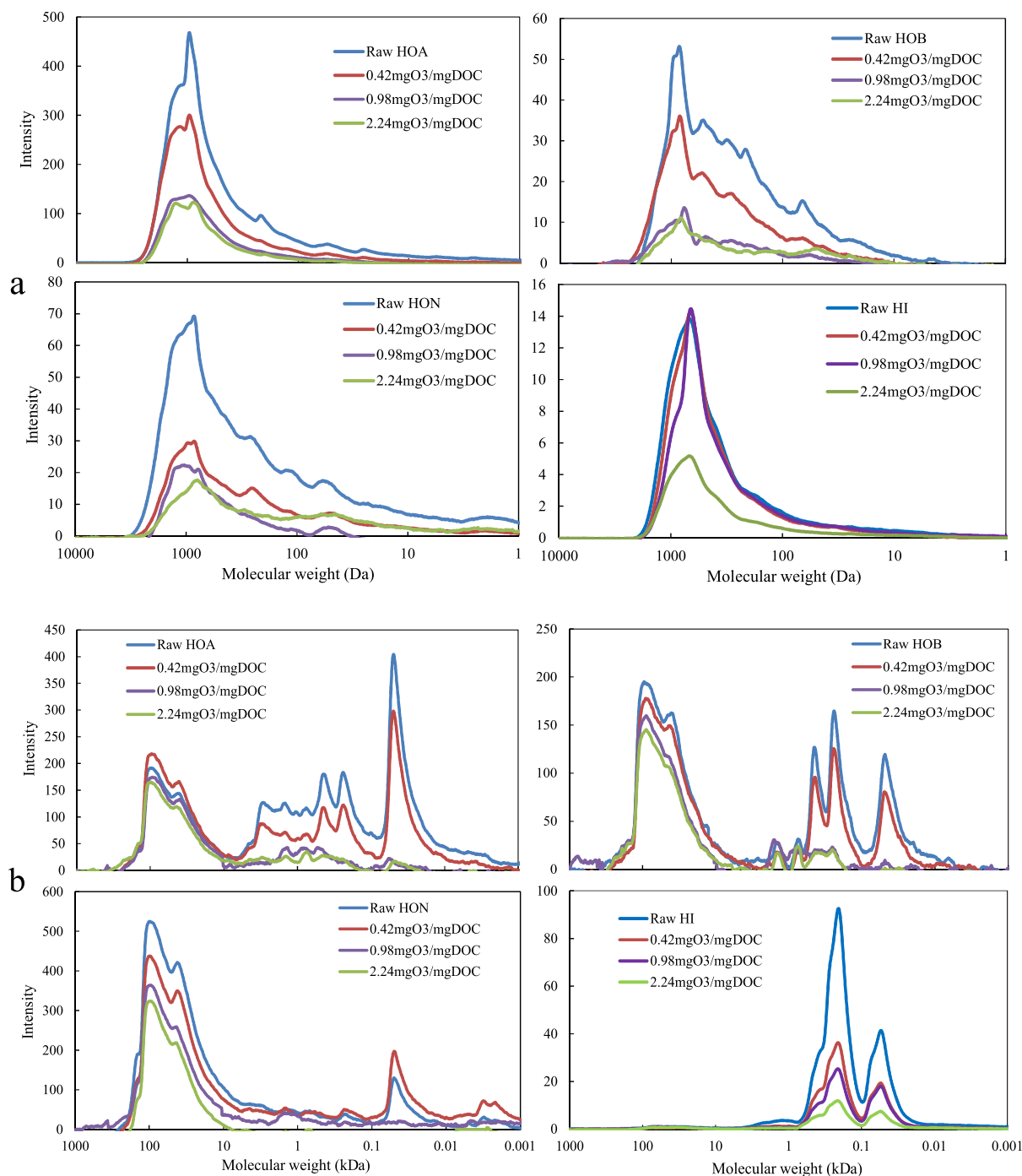


Fig. 4. Chromatograms of MWD of (a) humic-like and (b) protein-like substances in the four EDOM fractions. Fractionation was done after ozonation and no single fractions are oxidized.

significantly and the HI fraction increased. Furthermore, as observed in Fig. 2, the content of HOA decreased gradually, while an analogous increase in HI was observed, with a gradually increasing ozone dose. On the other hand, the content of HOB and HON remained relatively constant, regardless of the ozone dose. According to Fig. 2, it can be inferred that the HOA and HON fractions were transformed into the HI fraction after ozonation, leading to the DOC increase of the HI fraction, which also have been observed by elsewhere (Marhaba et al., 2000; Świetlik et al., 2004; Molnar et al., 2013). According to Chiang et al. (2009), ozonation leads to

oxidative cleavage of large molecules and increases polar and acidic functional groups, which interprets the increasing HI fraction during ozonation. During the fractionation of raw water and ozonated water, some loss of DOC in the desorption step was observed. Most likely this can be explained due to changes in the physical and chemical properties of some NOM molecules, which results in irreversible bindings with the resin (Świetlik et al., 2004).

In general, the organic matter composition in terms of UV₂₅₄ became less UV-sensitive after ozonation. The effective destruction of these unsaturated structures during ozonation is caused by the

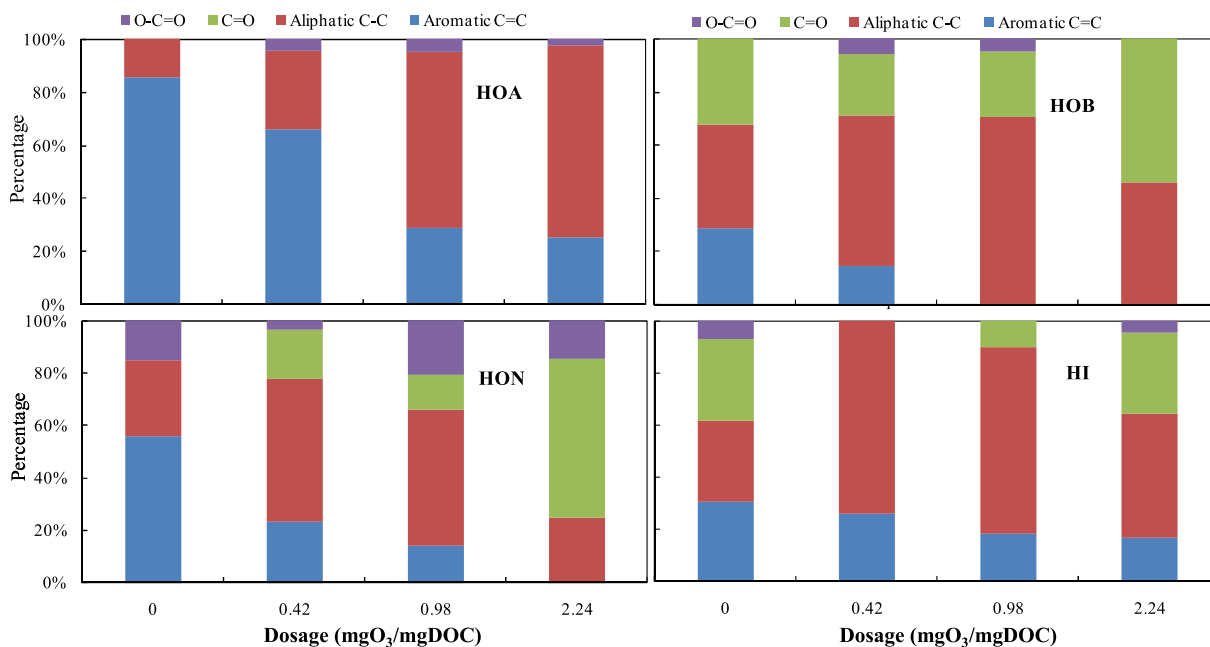


Fig. 5. Carbon species of the four fractions before and after ozonation. Fractionation was done after ozonation and no single fractions are oxidized.

electrophilic attack of ozone (Zhang et al., 2008a). Similar to DOC, the UV₂₅₄ absorption of the HON fraction decreased dramatically at an ozone dose of 0.42 mgO₃/mg DOC. However, with further increase in the ozone dose, the UV₂₅₄ absorption of the HON fraction remained relatively constant. For the other three fractions, the UV₂₅₄ absorption gradually decreased with the increasing ozone dosages. The HOA fraction had the highest decrease rate, while the HOB and HI fractions exhibited a more moderate decline. The DOC and UV₂₅₄ absorption in Fig. 2 demonstrate that it was primarily the HOA and HON fractions that were reduced at low ozone dosage (0.42 mgO₃/mg DOC). However, their behaviours (reaction properties) were different at higher ozone dosage than 0.42 mgO₃/mg DOC. This is probably due to the chemical composition of the HOA and HON fractions from EfOM, which will be further explained below.

According to literature, hydrophobic fractions were found to have higher potential to form THM than hydrophilic fraction (Galapate et al., 2001; Kitis et al., 2001). However, the hydrophilic fractions had great affinity for bromine to form brominated THMs during chlorination, such as dichlorobromomethane (DCBM), dibromomonochloromethane (DBCM) (Chiang et al., 2009). In this study, with the increasing ozone dosages, the amount of hydrophobic fractions decreased and hydrophilic fraction increased. To ensure the water reuse safety, very high ozone dosage was not suitable for water reclamation due to the risk of high potential to form carcinogenic brominated THMs (Molnar et al., 2013). Thus, low ozone dosage (0.42 mgO₃/mg DOC) was recommended for water reclamation as the HON fractions decreased significantly at low ozone dosage while HOA and HOB barely changed. In addition, the HI fraction just increased slightly according to Fig. 2. Therefore, the total THMFP could be controlled at low ozone dosage without predominant increase of brominated THMFP.

To obtain more detailed information about transformation reactions of the HOA and HON fractions at a low ozone dosage (0.42 mgO₃/mg DOC), both fractions were first isolated with the XAD-8 resin before ozonation started. Both fractions were then ozonated separately for 1, 2, 3, 4 and 5 min, respectively. The ozonated samples were then fractionated again by XAD-8 resin to

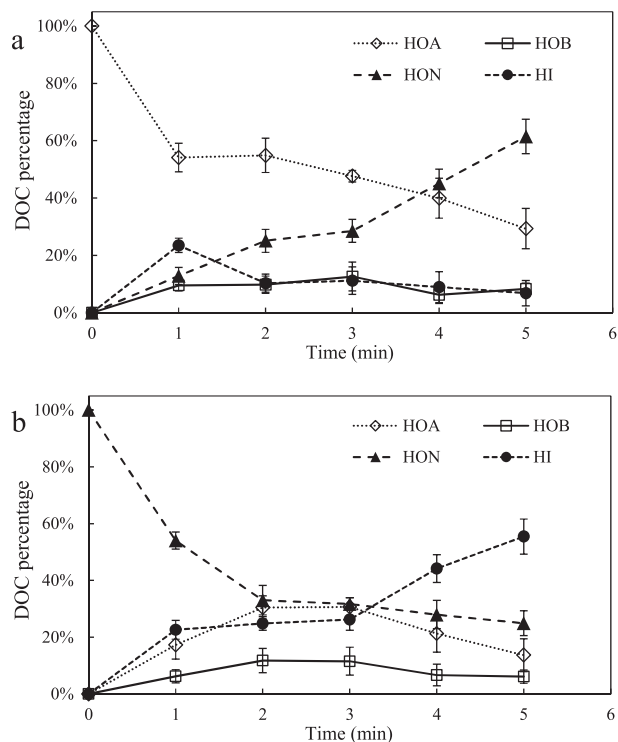


Fig. 6. DOC transformation of (a) HOA and (b) HON isolated fractions during the first 5 min of ozonation.

determine the changes in the HOA, HOB, HON and HI fractions, as shown in Fig. 6. As observed in Fig. 6a, during the 5 min ozonation, the HOA fraction was gradually transformed into HON. On the other hand, ozonation transformed the HON fraction into HI (Fig. 6b). Therefore, a feasible reaction pathway is proposed that HOA is first transformed into HON, subsequently, the HON is further converted into HI. As a result, the HI will increase in general at a low dosage,

due to the rapid transformation of HON into HI. Furthermore, with increasing ozone dosages, the HI fraction will gradually increase, as the HOA fraction is slowly converted into HON, which are then rapidly transformed into HI.

3.3. Effect of ozonation on fluorescence characteristics of EfOM fractions

After ozonation, the fluorescence intensity generally decreased with increasing ozone dosages, except for the region representing protein-like substances in the HI fraction at 0.42 ± 0.09 mgO₃/mg DOC (Fig. 3). Ozone reacts preferentially with the aromatic constituents, and changes the structure of aromatic structures (Westerhoff et al., 1999). According to Uyguner and Bekbolet (2005) and Świetlik and Sikorska (2004), the intensity decrease indicates the depletion or variation of aromatic structures and the increase of electron withdrawing groups in aromatic compounds such as –COOH. For all four fractions, the intensity of protein-like substances dropped significantly at the ozone dose of 0.98 ± 0.11 mgO₃/mg DOC. Moreover, small changes in the fluorescence intensity of HOA were observed at an ozone dose of 0.42 ± 0.09 mgO₃/mg DOC. However, an obvious fluorescence decrease was observed in the regions for both humic- and protein-like substances at a dose of 0.98 ± 0.11 mgO₃/mg DOC. There was a significant fluorescence intensity decline for HON at the lowest ozone dosage. This is consistent with the relatively large drop in DOC for the HON fraction at an ozone dose of 0.42 ± 0.09 mgO₃/mg DOC shown in Fig. 2. With regard to the HI fraction, the variation of fluorescence intensity was different from that of the hydrophobic fractions. The intensity of protein-like materials increased at the lowest ozone dosage. According to Zhang et al. (2008b), this may indicate that ozonation of hydrophobic fractions led to the emergence of hydrophilic aromatic amino acid functionalities. Świetlik and Sikorska (2004) revealed that the appearance of aromatic amino acids functional groups was attributed to the breakdown of macromolecular proteinaceous substances by ozonation. In this case, the newly formed aromatic amino acid substances had a hydrophilic character and increased the intensity of the protein-like fluorophores at low ozone dosages. With increasing ozone dosages, the intensity of humic-like substances varied slightly for the HI fraction. A possible explanation for this might be that equilibrium between the oxidation of humic-like materials from the raw HI fraction itself and the generation of humic-like materials from oxidation of HOA and HON fractions.

In addition to the intensity reduction of the fluorophores, ozonation also caused peak-shifts for some EfOM fractions, as shown in Fig. 3. Ozonation shifted both the maxima excitation and emission wavelengths of humic-like substances to shorter wavelengths (blue shift) for both the HOA and HON fractions as the ozone dosages increased from 0 to 0.42 ± 0.09 mgO₃/mg DOC. A blue shift is related to the decomposition of condensed aromatic moieties into smaller molecules or a reduction in the degree of π -electron systems, such as decreasing the number of aromatic rings or conjugated bonds in a chain structure (Coble, 1996; Liu et al., 2011). On the other hand, the protein-like substances in EEM spectra had neither red nor blue shifts after ozonation in this study.

3.4. Molecular weight analysis of the four EfOM fractions

According to Fig. 4a, the MWD of the HOA, HOB and HON fractions became slightly less divergent after ozonation shown by the vanishing peaks in these three fractions, especially for a molecular weight lower than 1000 Da. However, there was no emergence or disappearance of any peaks in the HI fraction during the ozonation process, instead only a general decrease in intensity was observed

at the highest ozone dose. For the protein-like substances shown in Fig. 4b, ozonation showed no effect on the size distribution of the HOA, HOB and HON fractions at low ozone dosage except for the intensity decrease. The MWD diversity of the HOA, HOB and HON fractions decreased at higher ozone doses than 0.98 mgO₃/mg DOC, especially for the small molecular weight protein-like substances. This indicated that ozone preferentially reacts with fluorophores in small molecular weight protein-like substances, i.e. less than 10 kDa, which can be attributed to the thorough depletion of conjugated bonds or aromatic rings in small molecular weight protein-like substances. Typically, organic matter in EfOM with molecular weight higher than 10 kDa refers to biopolymers generated from substrate metabolism and biomass decay (Audenaert et al., 2013) and (Huber et al., 2011). Furthermore, Gonzales et al. (2012) and Siembida-Lösch et al. (2015) indicated that biopolymers had lower reactivity to ozone. Similar effects were not seen at higher ozone doses for the protein-like substances in the HI fraction as this fraction was already less divergent with only small molecular weight protein-like substances.

3.5. Effect of ozonation on EfOM chemical structure

As shown in Fig. 5, with the increasing ozone dosage, the aromaticity of HON had a pronounced decrease and the aliphatic and ketonic carbon obviously increased even at the lowest ozone dosage (0.42 mgO₃/mg DOC). This demonstrates the evident blue shift of the humic-like peak for HON at this dosage, as shown in Fig. 3. However, the HOA, HOB and HI fractions had lower aromaticity decrease rates at low ozone dose. The ozone will react sequentially with aromatic hydrophobics and then hydrophilics (Gong et al., 2008). Westerhoff et al. (1999) demonstrated that ozone reacts preferentially with the aromatic constituents, specifically electron enriched aromatics substituted by oxygen and nitrogen including phenol, ether and amine groups. Moreover, raw HON had more oxygen-containing functional groups than HOA according to Fig. 5. It can be speculated that the raw HON fraction had more electron enriched aromatics than raw HOA fraction, and that ozone will therefore react with HON prior to HOA. Hence, compared with Fig. 6, HON tends to be first ozonated into HI, subsequently HOA is ozonated into HON followed by further ozonation into HI. The aromatic carbon further decreased with increasing ozone dosage for the hydrophobic fractions, which was in accordance with the results shown in Fig. 3. The HI fraction exhibited a remarkably low aromatic carbon decrease rate, which may be attributed to the formation of aromatic carbon in the HI fraction from the hydrophobic fractions. This can account for the low UV₂₅₄ decrease rate presented in Fig. 2.

In addition, it has been reported that when pre-ozonation is used, it could assist the coagulation performance (Sadrnourmohamadi and Gorczyca, 2015). However, very high ozone dosages is not recommended before coagulation. It has been found that hydrophobic fractions are more easily removed by coagulation than hydrophilic fractions (Yan et al., 2007; Wang et al., 2013), and that organic matter with aromatic structure is easy to be removed by coagulation (Bose and Reckhow, 2007). Moreover, hydrophilic fractions increased significantly and hydrophobic fractions declined (Fig. 2), and the content of aromatic structure in the hydrophobic fractions of EfOM would decrease at high ozone dosages according to Fig. 5. Therefore, low ozone dosage (0.42 mgO₃/mg DOC) is preferred before coagulation for water reclamation.

4. Conclusions

The EfOM herein was isolated into four fractions (HOA, HOB,

HON, and HI) by XAD resin method before and after ozonation at different ozone dosages. The results indicated that HOA and HON constituted the major part of the EfOM while HOB contributed the least part of EfOM. During ozonation, hydrophobic fractions, especially HOA and HON, were transformed into HI. In addition, the HOA was transformed into HON at a low ozone dosage and then further converted into the HI fraction.

Ozonation decreased the fluorescence intensity in humic-like and protein-like regions in EEM spectra for the hydrophobic fractions. The protein-like peak intensity declined sharply at 0.98 mgO₃/mg DOC. Based on the EEM spectra, MWD analyses indicated that humic-like fluorophores in HOA, HON and HOB fractions were mainly distributed at approximately 1000 Da, and that the intensity decreased with increasing ozone dosages. Furthermore, ozone tends to react with the fluorescent protein-like hydrophobic fraction with MW less than approximately 10 kDa.

Raw HOA and HON contained very high ratios of aromatic carbon although HON had higher ratios of aliphatic carbon and carboxylic carbon than HOA. During ozonation, the aromaticity of HON decreased sharply at the lowest ozone dosage (0.42 mgO₃/mg DOC) and the aliphatic and ketonic carbon increased. It can be concluded that raw HON contains more electron enriched aromatics than raw HOA and that ozone tends to react with HON prior to HOA.

Acknowledgement

This study was financially supported by National Natural Science Foundation of China (Grant No. 51378414, 51178376), National Key Technology Support Program (Grant No. 2014BAC13B06), the Program for Innovative Research Team in Shaanxi (Grant No. 2013KCT-13) and the Program for New Century Excellent Talents in University of Ministry of Education of China (NCET-12-1043).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2015.10.060>.

References

- Audenaert, W.T., Vandierendonck, D., Van Hulle, S.W., Nopens, I., 2013. Comparison of ozone and HO. Induced conversion of effluent organic matter (EfOM) using ozonation and UV/H₂O₂ treatment. *Water Res.* 47 (7), 2387–2398.
- Badireddy, A.R., Chellam, S., Gassman, P.L., Engelhard, M.H., Lea, A.S., Rosso, K.M., 2010. Role of extracellular polymeric substances in bioflocculation of activated sludge microorganisms under glucose-controlled conditions. *Water Res.* 44 (15), 4505–4516.
- Barber, L.B., Leenheer, J.A., Noyes, T.I., Stiles, E.A., 2001. Nature and transformation of dissolved organic matter in treatment wetlands. *Environ. Sci. Technol.* 35 (24), 4805–4816.
- Barker, D.J., Stuckey, D.C., 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Res.* 33 (14), 3063–3082.
- Bazri, M.M., Barbeau, B., Mohseni, M., 2012. Impact of UV/H₂O₂ advanced oxidation treatment on molecular weight distribution of NOM and biostability of water. *Water Res.* 46 (16), 5297–5304.
- Bhatia, D., Bourven, I., Simon, S., Bordas, F., van Hullebusch, E.D., Rossano, S., Lens, P.N., Guibaud, G., 2013. Fluorescence detection to determine proteins and humic-like substances fingerprints of exopolymeric substances (EPS) from biological sludges performed by size exclusion chromatography (SEC). *Bioresour. Technol.* 131, 159–165.
- Bourven, I., Costa, G., Guibaud, G., 2012. Qualitative characterization of the protein fraction of exopolymeric substances (EPS) extracted with EDTA from sludge. *Bioresour. Technol.* 104, 486–496.
- Bose, P., Reckhow, D., 2007. The effect of ozonation on natural organic matter removal by alum coagulation. *Water Res.* 41 (7), 1516–1524.
- Chen, J., LeBoeuf, E.J., Dai, S., Gu, B., 2003. Fluorescence spectroscopic studies of natural organic matter fractions. *Chemosphere* 50 (5), 639–647.
- Chiang, P.C., Chang, E.E., Liang, C.H., 2002. NOM characteristics and treatabilities of ozonation processes. *Chemosphere* 46 (6), 929–936.
- Chiang, P.C., Chang, E.E., Chang, P.C., Huang, C.P., 2009. Effects of pre-ozonation on the removal of THM precursors by coagulation. *Sci. Total Environ.* 407 (21), 5735–5742.
- Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Mar. Chem.* 51 (4), 325–346.
- Filloux, E., Labanowski, J., Croue, J.P., 2012. Understanding the fouling of UF/MF hollow fibres of biologically treated wastewaters using advanced EfOM characterization and statistical tools. *Bioresour. Technol.* 118 (0), 460–468.
- Galapate, R.P., Baes, A.U., Okada, M., 2001. Transformation of dissolved organic matter during ozonation: effects on trihalomethane formation potential. *Water Res.* 35 (9), 2201–2206.
- Gong, J., Liu, Y., Sun, X., 2008. O₃ and UV/O₃ oxidation of organic constituents of biotreated municipal wastewater. *Water Res.* 42 (4–5), 1238–1244.
- Gonzales, S., Peña, A., Rosario-Ortiz, F.L., 2012. Examining the role of effluent organic matter components on the decomposition of ozone and formation of hydroxyl radicals in wastewater. *Ozone Sci. Eng.* 34 (1), 42–48.
- Hou, X.L., Liu, S.T., Zhang, Z.T., 2015. Role of extracellular polymeric substance in determining the high aggregation ability of anammox sludge. *Water Res.* 75 (0), 51–62.
- Huber, S.A., Balz, A., Abert, M., Pronk, W., 2011. Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography–organic carbon detection–organic nitrogen detection (LC-OCD-OND). *Water Res.* 45 (2), 879–885.
- Imai, A., Fukushima, T., Matsushige, K., Kim, Y.H., Choi, K., 2002. Characterization of dissolved organic matter in effluents from wastewater treatment plants. *Water Res.* 36 (4), 859–870.
- Jin, P.K., Jin, X., Wang, X.C., Shi, X., 2013. An analysis of the chemical safety of secondary effluent for reuse purposes and the requirement for advanced treatment. *Chemosphere* 91 (4), 558–562.
- Kasprzyk-Hordern, B., Raczek-Stanislawski, U., Świetlik, J., Nawrocki, J., 2006. Catalytic ozonation of natural organic matter on alumina. *Appl. Catal. B Environ.* 62 (3–4), 345–358.
- Kitis, M., Kilduff, J.E., Karanfil, T., 2001. Isolation of dissolved organic matter (dom) from surface waters using reverse osmosis and its impact on the reactivity of dom to formation and speciation of disinfection by-products. *Water Res.* 35 (9), 2225–2234.
- Korshin, G.V., Benjamin, M.M., Sletten, R.S., 1997. Adsorption of natural organic matter (NOM) on iron oxide: effects on NOM composition and formation of organo-halide compounds during chlorination. *Water Res.* 31 (7), 1643–1650.
- Krasner, S.W., Westerhoff, P., Chen, B., Rittmann, B.E., Amy, G., 2009. Occurrence of disinfection byproducts in United States wastewater treatment plant effluents. *Environ. Sci. Technol.* 43 (21), 8320–8325.
- Kunacheva, C., Stuckey, D.C., 2014. Analytical methods for soluble microbial products (SMP) and extracellular polymers (ECP) in wastewater treatment systems: a review. *Water Res.* 61, 1–18.
- Leenheer, J.A., 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. Technol.* 15 (5), 578–587.
- Leenheer, J.A., 2009. Systematic approaches to comprehensive analyses of natural organic matter. *Ann. Environ. Sci.* 3 (1), 1–130.
- Lin, J.L., Huang, C., Dempsey, B., Hu, J.Y., 2014. Fate of hydrolyzed Al species in humic acid coagulation. *Water Res.* 56, 314–324.
- Liu, T., Chen, Z.L., Yu, W.Z., You, S.J., 2011. Characterization of organic membrane foulants in a submerged membrane bioreactor with pre-ozonation using three-dimensional excitation-emission matrix fluorescence spectroscopy. *Water Res.* 45 (5), 2111–2121.
- Marhaba, T.F., Van, D., Lippincott, R.L., 2000. Changes in NOM fractionation through treatment: a comparison of ozonation and chlorination. *Ozone Sci. Eng.* 22 (3), 249–266.
- Matilainen, A., Gjessing, E.T., Lahtinen, T., Hed, L., Bhatnagar, A., Sillanpaa, M., 2011. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. *Chemosphere* 83 (11), 1431–1442.
- Molnar, J., Agbaba, J., Dalmacija, B., Tubić, A., Krčmar, D., Maletić, S., Tomašević, D., 2013. The effects of matrices and ozone dose on changes in the characteristics of natural organic matter. *Chem. Eng. J.* 222 (0), 435–443.
- Monteil-Rivera, F., Brouwer, E.B., Masset, S., Deslandes, Y., Dumonceau, J., 2000. Combination of X-ray photoelectron and solid-state ¹³C nuclear magnetic resonance spectroscopy in the structural characterisation of humic acids. *Anal. Chim. Acta* 424 (2), 243–255.
- Nagao, S., Matsunaga, T., Suzuki, Y., Ueno, T., Amano, H., 2003. Characteristics of humic substances in the Kuji river waters as determined by high-performance size exclusion chromatography with fluorescence detection. *Water Res.* 37 (17), 4159–4170.
- Owen, D.M., Amy, G.L., Chowdhury, Z.K., Paode, R., McCoy, G., Viscosil, K., 1995. NOM characterization and treatability. *J. Am. Water Works Assoc.* 81 (1), 46–63.
- Pernet-coudrier, B., Clouzot, L., Varrault, G., Tusseau-vuillemin, M.H., Verger, A., Mouchel, J.M., 2008. Dissolved organic matter from treated effluent of a major wastewater treatment plant: characterization and influence on copper toxicity. *Chemosphere* 73 (4), 593–599.
- Quaranta, M.L., Mendes, M.D., MacKay, A.A., 2012. Similarities in effluent organic matter characteristics from Connecticut wastewater treatment plants. *Water Res.* 46 (2), 284–294.
- Ratpukdi, T., Siripattanakul, S., Khan, E., 2010. Mineralization and biodegradability enhancement of natural organic matter by ozone-VUV in comparison with ozone, VUV, ozone-VUV, and UV: effects of pH and ozone dose. *Water Res* 44 (11), 3531–3543.
- Reckhow, D.A., Singer, P.C., 1984. Removal of organic halide precursors by pre-

- ozonation and alum coagulation. *J. Am. Water Works Assoc.* 76 (4), 151–157.
- Rodríguez, F.J., Marcos, L.A., Núñez, L.A., 2012. Influence of preozonation on the formation of chlorination disinfection by-products – a case study: the Úzquiza reservoir water. *Ozone Sci. Eng.* 34 (3), 213–224.
- Sadrnourmohamadi, M., Gorczyca, B., 2015. Effects of ozone as a stand-alone and coagulation-aid treatment on the reduction of trihalomethanes precursors from high DOC and hardness water. *Water Res.* 73, 171–180.
- Sarathy, S.R., Mohseni, M., 2007. The impact of UV/H₂O₂ advanced oxidation on molecular size distribution of chromophoric natural organic matter. *Environ. Sci. Technol.* 41 (24), 8315–8320.
- Sonntag, C., Von Gunten, U., 2012. *Chemistry of Ozone in Water and Wastewater Treatment: from Basic Principles to Applications*. IWA publishing, UK, ISBN 9781843393139.
- Siembida-Lösch, B., Anderson, W.B., Wang, Y., Bonsteel, J., Huck, P.M., 2015. Effect of ozone on biopolymers in biofiltration and ultrafiltration processes. *Water Res.* 70, 224–234.
- Świetlik, J., Dąbrowska, A., Raczyk-Stanisławiak, U., Nawrocki, J., 2004. Reactivity of natural organic matter fractions with chlorine dioxide and ozone. *Water Res.* 38 (3), 547–558.
- Świetlik, J., Sikorska, E., 2004. Application of fluorescence spectroscopy in the studies of natural organic matter fractions reactivity with chlorine dioxide and ozone. *Water Res.* 38 (17), 3791–3799.
- Uyguner, C.S., Bekbolet, M., 2005. Evaluation of humic acid photocatalytic degradation by UV–vis and fluorescence spectroscopy. *Catal. Today* 101 (3–4), 267–274.
- Wang, D., Zhao, Y., Xie, J., Chow, C.W.K., van Leeuwen, J., 2013. Characterizing DOM and removal by enhanced coagulation: a survey with typical Chinese source waters. *Sep. Purif. Technol.* 110, 188–195.
- Wang, Z., Wu, Z., Tang, S., 2009. Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy. *Water Res.* 43 (6), 1533–1540.
- Westerhoff, P., Aiken, G., Amy, G., Debroux, J., 1999. Relationships between the structure of natural organic matter and its reactivity towards molecular ozone and hydroxyl radicals. *Water Res.* 33 (10), 2265–2276.
- Yan, M., Wang, D., Shi, B., Wang, M., Yan, Y., 2007. Effect of pre-ozonation on optimized coagulation of a typical North-China source water. *Chemosphere* 69 (11), 1695–1702.
- Yin, C.Q., Meng, F.G., Chen, G.H., 2015. Spectroscopic characterization of extracellular polymeric substances from a mixed culture dominated by ammonia-oxidizing bacteria. *Water Res.* 68 (0), 740–749.
- Zhang, H., Qu, J., Liu, H., Zhao, X., 2009. Characterization of isolated fractions of dissolved organic matter from sewage treatment plant and the related disinfection by-products formation potential. *J. Hazard. Mater.* 164 (2–3), 1433–1438.
- Zhang, T., Lu, J., Ma, J., Qiang, Z., 2008a. Comparative study of ozonation and synthetic goethite-catalyzed ozonation of individual NOM fractions isolated and fractionated from a filtered river water. *Water Res.* 42 (6–7), 1563–1570.
- Zhang, T., Lu, J., Ma, J., Qiang, Z., 2008b. Fluorescence spectroscopic characterization of DOM fractions isolated from a filtered river water after ozonation and catalytic ozonation. *Chemosphere* 71 (5), 911–921.
- Zheng, X., Khan, M.T., Croué, J.P., 2014. Contribution of effluent organic matter (EfOM) to ultrafiltration (UF) membrane fouling: isolation, characterization, and fouling effect of EfOM fractions. *Water Res.* 65 (0), 414–424.