



# Effect of ozone pretreatment on characteristics of dissolved organic matter formed in aerobic and anaerobic digestion of waste-activated sludge

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## Abstract

The characteristics of dissolved organic matter (DOM) formed in aerobic and anaerobic digestion of waste-activated sludge (WAS) after ozone pretreatment were investigated with three ozone dosages (4.72, 10.96, and 13.8 mg O<sub>3</sub>/min) and four ozonation times (0, 10, 20, and 30 min) using six aerobic and six anaerobic digestion reactors. High decreasing rate of volatile suspended solid/total suspended solid indicated enhanced destruction of volatile solids and efficient sludge reduction. The results of TOC and UV absorbance indicated that increasing ozone dosage and time significantly enhanced hydrolysis and degradation of DOM. Data analysis with a first-order sequential reaction model revealed that, for aerobic digestion,  $k_h$  increased in the range of 0.00049–0.00154 day<sup>-1</sup>; and for anaerobic digestion of WAS,  $k_h$  increased in the range of 0.00302–0.00796 day<sup>-1</sup> and  $k_d$  increased in the range of 0.24910–0.54548 day<sup>-1</sup>. Detailed analysis of the composition of DOM showed that ozone pretreatment increased irreversible membrane resistance (IMR) and enhanced the breakdown of large DOM molecules, the formation of aromatic proteins, and tryptophan- and tyrosine-aromatic amino acids, as well as the accumulation of humic acid- and fulvic acid-like substances.

**Keywords** Aerobic/anaerobic digestion · Dissolved organic matter · Humic substances · Irreversible membrane resistance · Ozonation · Waste-activated sludge

## Introduction

Huge amount of waste-activated sludge (WAS) is generated in municipal wastewater treatment plants (WWTPs), and its quantity is increasing because of population growth and rapid urbanization (Li and Yu 2011). The treatment and disposal of WAS are challenging tasks in WWTPs and represent up to 60% of their operating cost (Ramdani et al. 2010). Reducing WAS production in WWTPs thus presents an obvious economic interest. WAS is also undesirable for the following reasons: (1) its high content of biodegradable constituents

causes viruses, bacteria, and other pathogenic microorganisms; and (2) its high water content (containing more than 99% of water) requires dewatering prior to disposal (Ramdani et al. 2012).

Aerobic and anaerobic digestions have been regarded as efficient methods for reduction and stabilization of WAS and energy recovery (Zhang et al. 2017; Chiavola et al. 2013). But, in the liquid digestate of the WAS, some intermediates and final products are still remaining as dissolved organic matter (DOM) that includes some non-biodegradable organic constituents (Du and Li 2016). Most biodegradable DOM constituents can be removed if the liquid digestate is recycled for treatment in the water treatment line of WWTPs, but some may remain together with non-biodegradable constituents, thus worsening the quality of the natural water systems, and can contribute to the formation of more hazardous disinfection byproducts in drinking water treatment systems if they are merged in the source water for drinking water production (Du and Li 2016; Cheng et al. 2009). Clarifying the characteristics of DOM is of great importance for water environment conservation and safe drinking water production.

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WAS is rich in organic matter and easy to decay under normal environmental condition (Grosser et al. 2017). The first step of its aerobic and anaerobic digestion (hydrolysis) is considered rate-limiting. Pretreatment of WAS prior to aerobic and anaerobic digestion, aiming to enhance sludge hydrolysis, degradability, and final dewaterability, has been tested for many methods, including alkalization, ozonation, and thermal, ultrasound, or microwave decomposition (Li et al. 2016; Zawieja et al. 2015; Appels et al. 2008). Among these methods, ozonation has demonstrated its effect in reducing WAS (Chiavola et al. 2013; Chiavola et al. 2019). Ozone can cause cell lysis, breakdown of extracellular polymeric substances (EPS), release of intracellular and extracellular organic matter, and the subsequent oxidation of some organic substances into carbon dioxide (mineralization) (Jaime et al. 2017). This improves volatile solids (VS) destruction and alters the floc structure of sludge into a more compact sludge aggregate (Saktaywin et al. 2005; Lee et al. 2005). Chacana et al. (2017) investigated the ozonation effect of anaerobic-digested sludge on methane production with the ozone doses ranging from 0 to 192 mg O<sub>3</sub>/g COD. An optimal methane yield was obtained at 86 mg O<sub>3</sub>/g COD, which was 52% higher than those without ozonation. Chiavola et al. (2019) investigated the effects of ozonation on the anaerobic digestion of activated sludge alone (3.5 to 53.6 mg O<sub>3</sub>/g TS) and a mixture of activated sludge and primary sludge (4.8 to 73.2 mg O<sub>3</sub>/g TS). The best result was obtained at the lowest dosages of 3.5 and 4.8 mg O<sub>3</sub>/g TS for activated sludge and the mixed sludge, respectively, which corresponded to additional methane production of about 30% and 6% and the maximum production rate increase by about 33% and 21%, respectively. Fall and Silvahernandez (2017) evaluated bacterial inactivation and regrowth in ozonated activated sludge and found that ozonation promoted the selection of fast-growing bacteria in the activated sludge after transitory inactivation and long lag times. Chiavola et al. (2013) conducted techno-economic evaluation of the application of ozone oxidation in a full-scale aerobic digestion plant with three ozone flow rates of 60, 500, and 670 g O<sub>3</sub>/h, and their effects were evaluated in terms of total and soluble COD, N, P, TS, VS, and sludge volume index (SVI). The results determined an average reduction of about 60% in the biological sludge. Jin et al. (2015) investigated the effect of ultrasonic and ozone pretreatments on pharmaceutical WAS's digestion efficiency and found that the sludge solubilization reached 30% under the ultrasonic treatment condition of 150,000 kJ/kg TS and 28% under the ozone treatment condition of 0.1 g O<sub>3</sub>/g TS. These pioneering studies have provided very useful information relating to the effect of ozone pretreatments on WAS's digestion as well as characteristic change of DOM formed in the digestion of WAS.

However, since the WAS remains as the residue of wastewater treatment with unpredictable composition and fluctuating concentration and DOM is a complex mixture of polydispersed components, characterization of the

composition of DOM requires more indexes that are well used in water quality evaluations. Ozone dosage and ozonation time greatly affect DOM formation and release from WAS as well as its hydrolysis and degradation rate in following aerobic and anaerobic digestion. Increasing the ozone dosage and ozonation time leads to more destroyed bacterial cells and released intracellular constituents (Hashimoto et al. 2020). This probably changes the properties of the formed DOM, a point that remains unclear in available literatures.

Accordingly, in order to generate more information for better understanding the effect of ozone pretreatment on the characteristics of DOM formed and remaining in the digestion of WAS under aerobic and anaerobic conditions, in this study, six aerobic and six anaerobic digestion reactors were operated at 35 °C in parallel after ozone pretreatment with three different ozone dosages (4.72, 10.96, and 13.8 mg O<sub>3</sub>/min) and four different ozonation times (0, 10, 20, and 30 min). Previous reports suggested that the ozone-activated sludge process would be economically viable with doses between 25 and 45 mg O<sub>3</sub>/g COD approximately (Chu et al. 2009). Yan et al. (2009) reported that at a high dosage, the oxidant can gradually lose its ability to attack the solids matrix efficiently, due to the release of radical scavengers such as lactic acid and SO<sub>4</sub><sup>2-</sup> from the decayed bacteria. DOM was evaluated using the overall concentration indexes of total organic carbon (TOC) and ultraviolet absorbance at the wavelength of 220 nm (UV<sub>220</sub>), 260 nm (UV<sub>260</sub>), and 390 nm (UV<sub>390</sub>) and the composition-related properties, including the fluorescence EEM, Z-potential, and particle size distribution. Moreover, irreversible membrane resistance (IMR) caused by DOM was measured. The effect of different ozone dosages and ozonation times on WAS stabilization was also evaluated with pH, oxidation-reduction potential (ORP), electrical conductivity (EC), total solids (TS), volatile solids/total solids (VS/TS), and volatile suspended solid/total suspended solid (VSS/TSS). Principal component analysis (PCA) was performed for better understanding the interrelations among all measured quality indexes and the changes of DOM properties during the digestion process.

## Materials and methods

### WAS preparation

Fresh returned activated sludge was obtained from a representative municipal WWTP operated in the sequential anaerobic/aerobic mode in Gifu, Japan. The measurement results for the concentrated sludge were as follows: temperature 25 °C, pH 6.47, DO 1.74 mg/L, ORP -41 mV, EC 40.5 mS/m, VS/TS 75.5% and VSS/TSS 78.8% for the sludge, and TOC 9.86 mg/L, UV<sub>220</sub> 0.396 cm<sup>-1</sup>, UV<sub>260</sub> 0.158 cm<sup>-1</sup>, and UV<sub>390</sub> 0.014 cm<sup>-1</sup> for water after filtration.

## Ozone pretreatment of WAS

Ozonation was performed with an ozone gas generator (Pressure Swing Adsorption, Fujii PO-20). Air was used as feed gas and converted to ozone with a high-voltage converter. The applied ozone was quantified using 0.1 M of  $\text{Na}_2\text{S}_2\text{O}_3$  and KI in terms of ozone dosage ( $\text{mg O}_3/\text{min}$ ) (Morali et al. 2016). One liter of the concentrated sludge (TS: 1.23%) was respectively added into six aerobic and six anaerobic digestion reactors (fluorinated polyethylene bottle, 2 L) and ozonated with three ozone dosages (4.72, 10.96, and 13.8  $\text{mg O}_3/\text{min}$ ) and four ozonation times (0, 10, 20, and 30 min). A stone diffuser was installed to produce fine ozone bubbles and to enhance ozone mass transfer. Residue ozone from the outlet gas was monitored by 0.1 M of  $\text{Na}_2\text{S}_2\text{O}_3$  and KI to determine the effective ozone consumption in the reactor.

## Digestion experiments

Aerobic digestion was ensured by consistent air bubbling with an air pump (power: 2.5 W, air flow rate: 2 L/min; DO: 6.25–6.33  $\text{mg/L}$ ). Anaerobic digestion was ensured by tightly sealing the opening of the reactors with rubber stoppers lined with Teflon sheet immediately after the addition of the WAS samples (DO: below 1.0  $\text{mg/L}$ ). The converted TOC concentrations of the WAS for ozonation time of 0, 10, 20, and 30 min with ozone dosage of 10.96  $\text{mg O}_3/\text{min}$  as well as ozone dosage of 4.72 and 13.8  $\text{mg O}_3/\text{min}$  with ozonation time of 20 min were 4711, 4688, 4638, 4590, 4542, and 4529  $\text{mg/L}$ , respectively. The reactors were placed on a water bath shaker adjusted to 35 °C and shaken gently to ensure uniform reactions within each reactor. All twelve reactors were operated without inoculation and additional feed for 30 days.

During the digestion experiments, from each reactor, samples were collected at designated time points. After filtration through 0.2- $\mu\text{m}$  membrane filters (Toyo Roshi Co., Ltd., Japan), the filtrates were subjected to quality analysis for DOM and IMR caused by DOM. The quality analysis for DOM mainly focused on TOC,  $\text{UV}_{220}$ ,  $\text{UV}_{260}$ ,  $\text{UV}_{390}$ , fluorescence EEM, Z-potential and size distribution, and IMR, the details of which will be described below. In addition to these measurements, pH, ORP, EC, TS, VS/TS, and VSS/TSS inside all reactors were monitored, and PCA was performed using the SPSS Statistics 17.0 software.

## Quality analysis

### Overall quality indexes of DOM and irreversible membrane resistance

pH, ORP, and EC were analyzed by a pH meter (HM-21P, Toaok), ORP meter (RM-20P, Toaok), and EC meter (CM-14P, TOA Electronics Ltd.), respectively. TS and VS were measured using an oven at 105 °C and 600 °C, respectively. TSS and

VSS were measured by filtering the sludge sample through 1  $\mu\text{m}$  of a glass microfiber filter and drying using an oven at 105 °C and 600 °C, respectively. TOC was analyzed by a TOC analyzer (TOC-V DS, Shimadzu, Japan).  $\text{UV}_{220}$ ,  $\text{UV}_{260}$ , and  $\text{UV}_{390}$  were analyzed by a spectrophotometer (UV-1600 GLP, Shimadzu, Japan). Z-potential and particle size distribution were analyzed by a nanoparticle analyzer (Zetasizer Nano Series Nano-ZS, Malvern, England). IMR caused by DOM was measured according to the time needed for filtering DOM solution (digested solution filtered by 1  $\mu\text{m}$  of a glass microfiber filter) through 0.2  $\mu\text{m}$  of a cellulose acetate filter (Ebie and Azuma 2002).

### Fluorescence EEM of DOM

Fluorescence EEM was analyzed using a spectrofluorometer (RF-5300, Shimadzu, Japan). The excitation and emission scans were performed between 220 and 550 nm with 5-nm increments. The bandwidth for both excitation and emission was 1 nm. The obtained EEM was normalized into the quinine sulfate unit (QSU) by dividing the fluorescence intensity values of all samples with the fluorescence intensity value of 10 ppb quinine sulfate (in 0.05 M  $\text{H}_2\text{SO}_4$  solution) at the designated wavelengths of  $E_x/E_m = 350/450$  nm (Lee et al. 2015). Comparison of the composition of DOM was further made according to the fluorescence peaks emerged on the obtained EEM images. To minimize the inner filter effect, all samples subjected to the analysis were diluted using pure water to TOC below 2  $\text{mg/L}$  (Lee et al. 2015).

### Principal component analysis

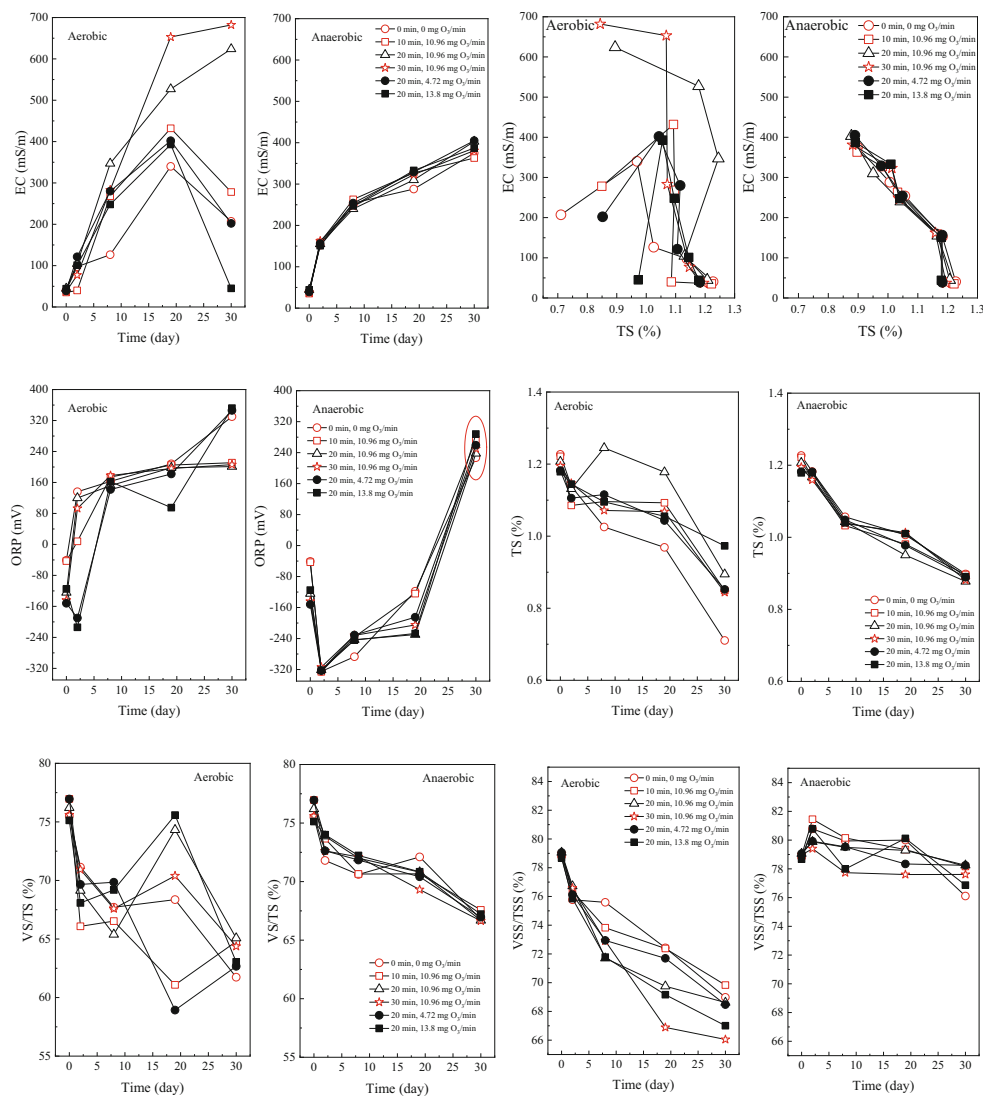
The PCA was performed using the SPSS software (IBM SPSS Statistic 21.0, IBM Corp., Armonk, NY) for better understanding the interrelations of all measured quality indexes (including pH, EC, ORP, TOC,  $\text{UV}_{220}$ ,  $\text{UV}_{260}$ ,  $\text{UV}_{390}$ , Z-potential, VS/TS, VSS/TSS, IMR, and the major fluorescence peaks emerged in the fluorescence EEM measured for all samples) and the change of DOM properties during the process of the aerobic and anaerobic digestion after ozone pretreatment. For this, all the measurement results in the series of aerobic and the series of anaerobic digestion were used separately as the pooled data for analysis after standardization treatment for each index in order to obtain general information on the differences of the interrelations among all quality indexes between aerobic and anaerobic digestions.

## Results and discussion

### Effect of ozone on sludge reduction performance

The changing trends of EC, ORP, and TS, and the relations of EC and TS, VS/TS, and VSS/TSS over the total digestion time for 30 days are shown in Fig. 1. Under aerobic conditions, the

**Fig. 1** Effect of ozone on change of EC, the relation between EC and TS, ORP, TS, VS/TS, VSS/TSS during 30 days' digestion



EC significantly increased with ozonation time of 20 and 30 min, reaching the highest value of 682 mS/m. A trend of a rapid EC increase (until day 19) followed by a rapid decrease was observed with short ozonation time of 0 and 10 min, displaying no significant changes with the ozone dosage. While under anaerobic conditions, the EC significantly increased with all the ozonation conditions, reaching the highest value of 405 mS/m. The ORP decreased at the initial time (day 2) and then increased, with higher increasing rate under aerobic conditions. The initial decrease of the ORP was probably due to the release of intracellular substances from cell lysis induced by ozone pretreatment (Cheng et al. 2018). At the end of the digestion experiment, ORP for the aerobic reactors reached to levels in the range about 200–360 mV. The accumulation of oxidative substances, such as  $\text{SO}_4^{2-}$  (standard redox potential of 0.240 V) and the nitrification product of  $\text{NO}_3^-$  (standard redox potential of 0.835 V), was the most possible reason contributing to the observed increase. For

the anaerobic reactors, in reflection of anaerobic biological reactions, negative ORP values were detected for samples before 20 days of digestion. However, for the samples at the end of digestion, contrary to expectation, ORP turned to positive levels even if the controlling condition for DO was kept below 1.0 mg/L. The reason for this will be investigated. ORP is an important index for sludge reduction because sludge in the particulate form was transformed to DOM by hydrolysis and acidification (Saby et al. 2003). The TS showed a remarkable decrease, with slightly higher effect by ozonation under aerobic conditions. The relation of EC and TS under aerobic conditions was significantly affected by the ozonation and that under anaerobic conditions displayed nearly linear trend, with almost no effect of ozonation. VS/TS and VSS/TSS of all the reactors significantly decreased. The VS/TS was used as an index to determine the organic stability of the treated sludge (Bresters et al. 1997). Improved VS/TS reduction in all the reactors assured that the ozone pretreatment resulted in

solubilization of sludge solids and the formation of more stable final digestion product (Tian et al. 2015). VSS destruction is frequently regarded as an index to evaluate the performance of digestion systems (Elsayed et al. 2016). The decrease of VSS/TSS reflects the effect of sludge reduction (Ge et al. 2013). The VSS/TSS under aerobic conditions displayed much higher decreasing rate than that under anaerobic conditions, indicating enhanced destruction of volatile solids and more efficient sludge reduction.

### Effect of ozone on content of DOM assessed by TOC and UV absorbance

Over the total digestion time for 30 days, a trend of a rapid TOC increase followed by either rapid increases or gradual decreases was observed, as could be seen from Fig. 2. The increase reflected the rate of solubilization being higher than the rate of degradation and mineralization of DOM. In contrary, the observed decrease reflected that the rate of degradation exceeded the rate of solubilization or solubilization had terminated. After ozone pretreatment, the TOC under aerobic conditions was obviously higher than that under anaerobic conditions, with a significant increase by increasing either the ozone dosage or ozonation time, while the changing trend of TOC in the reactor without ozone pretreatment was very similar to those in the reactors under anaerobic conditions.

This probably indicated that ozone pretreatment leads to much higher degradation and mineralization rate of DOM under anaerobic conditions, which will be discussed later in Table 1. UV-absorbing organic constituents can be resistant to biodegradation (Pathak et al. 2018). The UV<sub>260</sub> under anaerobic conditions displayed an increasing trend followed by a gradual decrease, while that under aerobic conditions increased constantly with much small extent. This indicated that the release/hydrolysis of WAS was more significantly enhanced by ozone pretreatment under anaerobic conditions. The UV<sub>390</sub>/UV<sub>260</sub> under anaerobic conditions increased dramatically after ozone pretreatment and showed much higher values than those under aerobic conditions, indicating the presence of more color-causing constituents in the humic-like substances under anaerobic conditions. The UV<sub>220</sub> showed a very similar changing trend with the TOC. While for the reactor without ozone pretreatment, the values of UV<sub>220</sub> under aerobic condition were still much higher than those under anaerobic conditions. This phenomenon suggested that more inorganic ions that can be detected by UV<sub>220</sub> (such as NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>) were formed and accumulated under aerobic conditions. The TOC and UV absorbance results showed that increasing ozone dosage and/or ozonation time significantly enhanced hydrolysis of sludge and degradation of DOM (Tian et al. 2015; Jaime et al. 2017; Chiavola et al. 2019; Hashimoto et al. 2020).

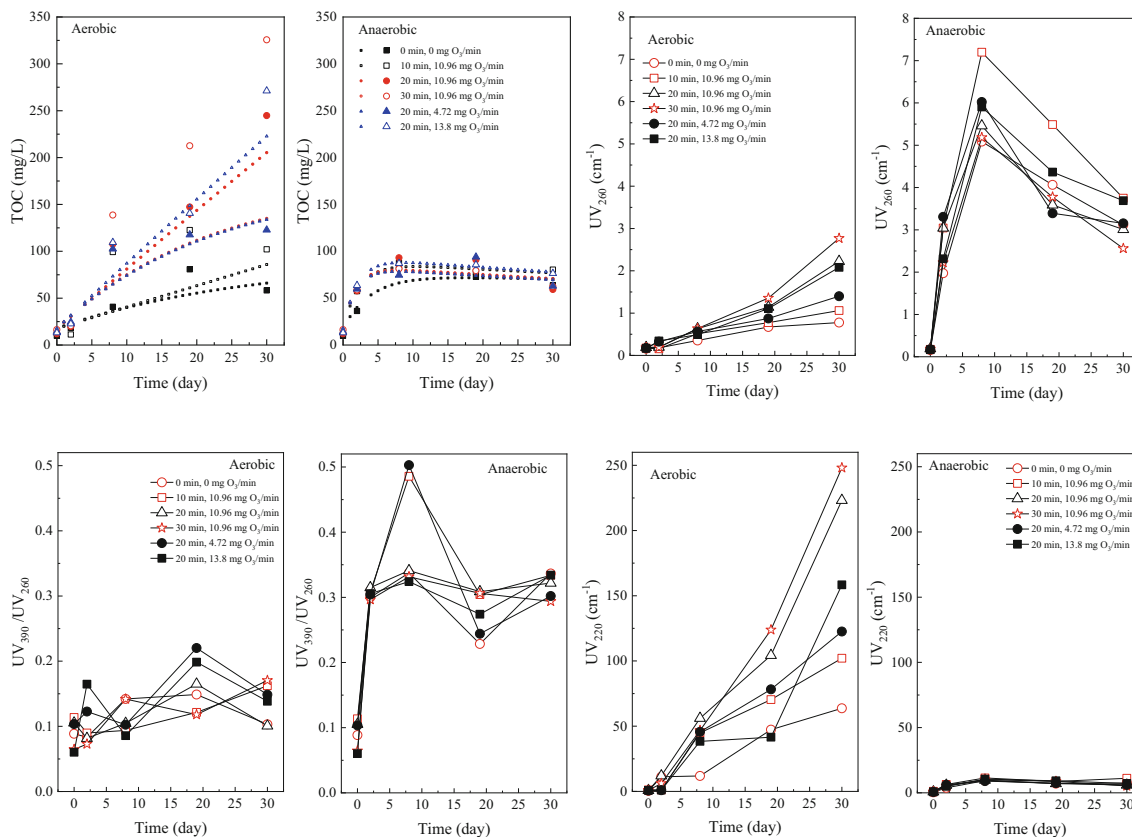


Fig. 2 Effect of ozone on change of the TOC, UV<sub>220</sub>, UV<sub>260</sub>, and UV<sub>390</sub> during 30 days’ digestion



**Table 1** The rate parameters of hydrolysis and degradation of DOM in aerobic and anaerobic digestion of excess activated sludge estimated using a sequential reaction model

	$k_h$	$k_d$	Error
<b>Aerobic</b>			
0 min, 0 mg O <sub>3</sub> /min	0.00049	0.03579	0.173
10 min, 10.96 mg O <sub>3</sub> /min	0.00056	0	0.356
20 min, 10.96 mg O <sub>3</sub> /min	0.00153	0	0.134
30 min, 10.96 mg O <sub>3</sub> /min	-	-	-
20 min, 4.72 mg O <sub>3</sub> /min	0.00138	0.04155	0.058
20 min, 13.8 mg O <sub>3</sub> /min	0.00154	0	0.085
<b>Anaerobic</b>			
0 min, 0 mg O <sub>3</sub> /min	0.00302	0.24910	0.244
10 min, 10.96 mg O <sub>3</sub> /min	0.00624	0.41591	0.027
20 min, 10.96 mg O <sub>3</sub> /min	0.00778	0.54548	0.062
30 min, 10.96 mg O <sub>3</sub> /min	-	-	-
20 min, 4.72 mg O <sub>3</sub> /min	0.00772	0.54396	0.025
20 min, 13.8 mg O <sub>3</sub> /min	0.00796	0.47951	0.024

$k_h$ , first-order hydrolysis rate parameter (day<sup>-1</sup>);  $k_d$ , first-order degradation rate parameter (day<sup>-1</sup>); *Error*, the difference between calculated and observed TOC concentrations defined by equation (5) in our previous study (Du and Li 2016)

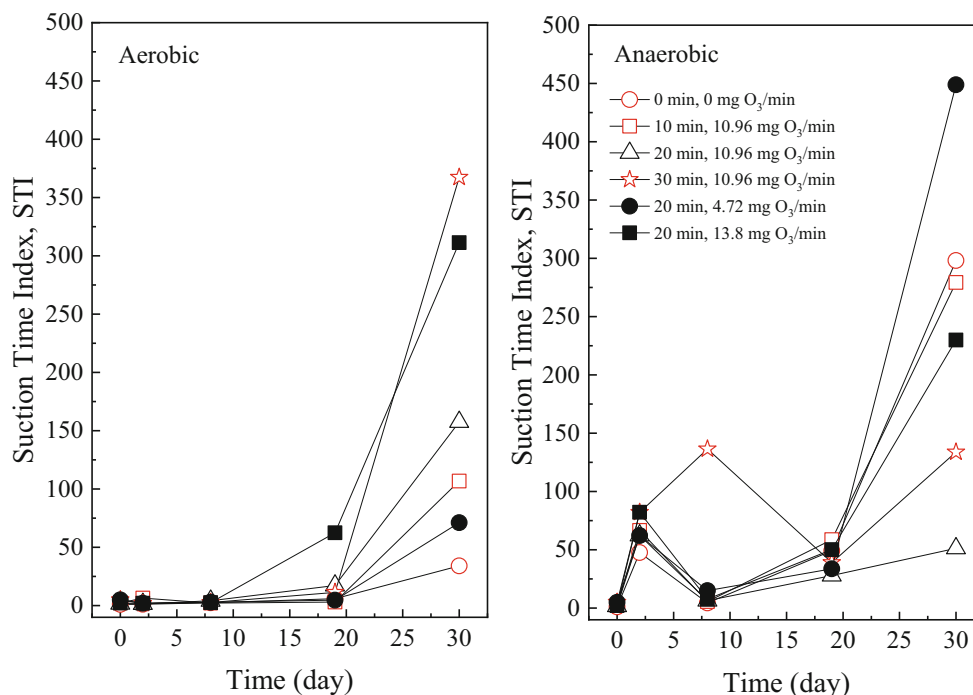
### Effect of ozone on rate parameters of hydrolysis and degradation in WAS digestion

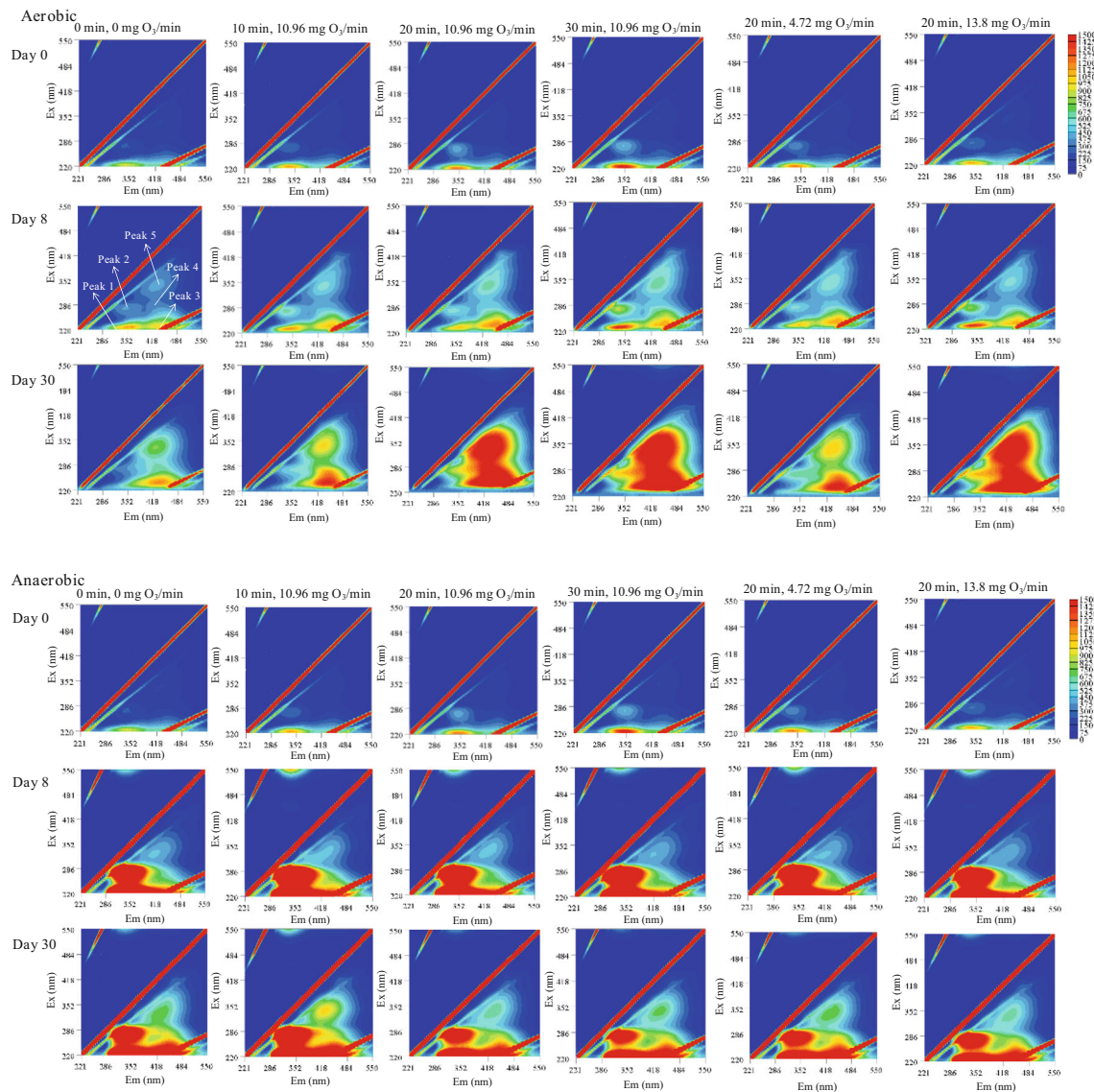
The digestion of WAS is a sequential process of hydrolysis and degradation (Mani et al. 2016). The DOM detected during

digestion is the combined results of formation by hydrolysis involving both cell disruption and the release of intracellular materials (such as proteins, carbohydrates, and lipids), and degradation of the products of the hydrolysis process (such as sugars, amino acids, and fatty acids). First-order reaction model is well used for simple simulation of the hydrolysis and degradation process of WAS (Du and Li 2016; Mani et al. 2016; Adak et al. 2011). In this study, using the first-order sequential reaction model used in our previous study (Du and Li 2016), the observed concentration profiles of DOM were described and involved rate parameters were estimated. The initial TOC values required in this model were converted from the concentration of TS based on the conversion ratio (38.4% of TOC in TS of the WAS used in this study).

Ozonation was shown to increase the solubilization of sludge mainly through partial solubilization of the sludge matrix and damage to the cell membrane integrity, and to increase the biodegradability of organic products (Jaime et al. 2017). The calculated TOC profiles that best fitted the observed data are depicted in Fig. 2 as dot lines, and the searched hydrolysis rate constant  $k_h$  and degradation rate constant  $k_d$  are summarized in Table 1. Compared with aerobic digestion, the rates of hydrolysis and degradation in anaerobic digestion were faster. As shown in Table 2, for aerobic digestion,  $k_h$  increased from 0.00049<sup>1</sup> to 0.00153 day<sup>-1</sup> as ozonation time increased from 0 to 20 min with an ozone dosage of 10.96 mg O<sub>3</sub>/min, and from 0.00138 to 0.00154 day<sup>-1</sup> as ozone dosage increased from 4.72 to 13.8 mg O<sub>3</sub>/min with an ozonation time of 20 min. For anaerobic digestion, the corresponding increase of  $k_h$  with the increase of ozonation time was from 0.00302 to 0.00778 day<sup>-1</sup> and that with

**Fig. 3** Effect of ozone on change of the irreversible membrane resistance during 30 days' digestion





**Fig. 4** The fluorescence EEM of DOM during aerobic and anaerobic digestion after ozone pretreatment

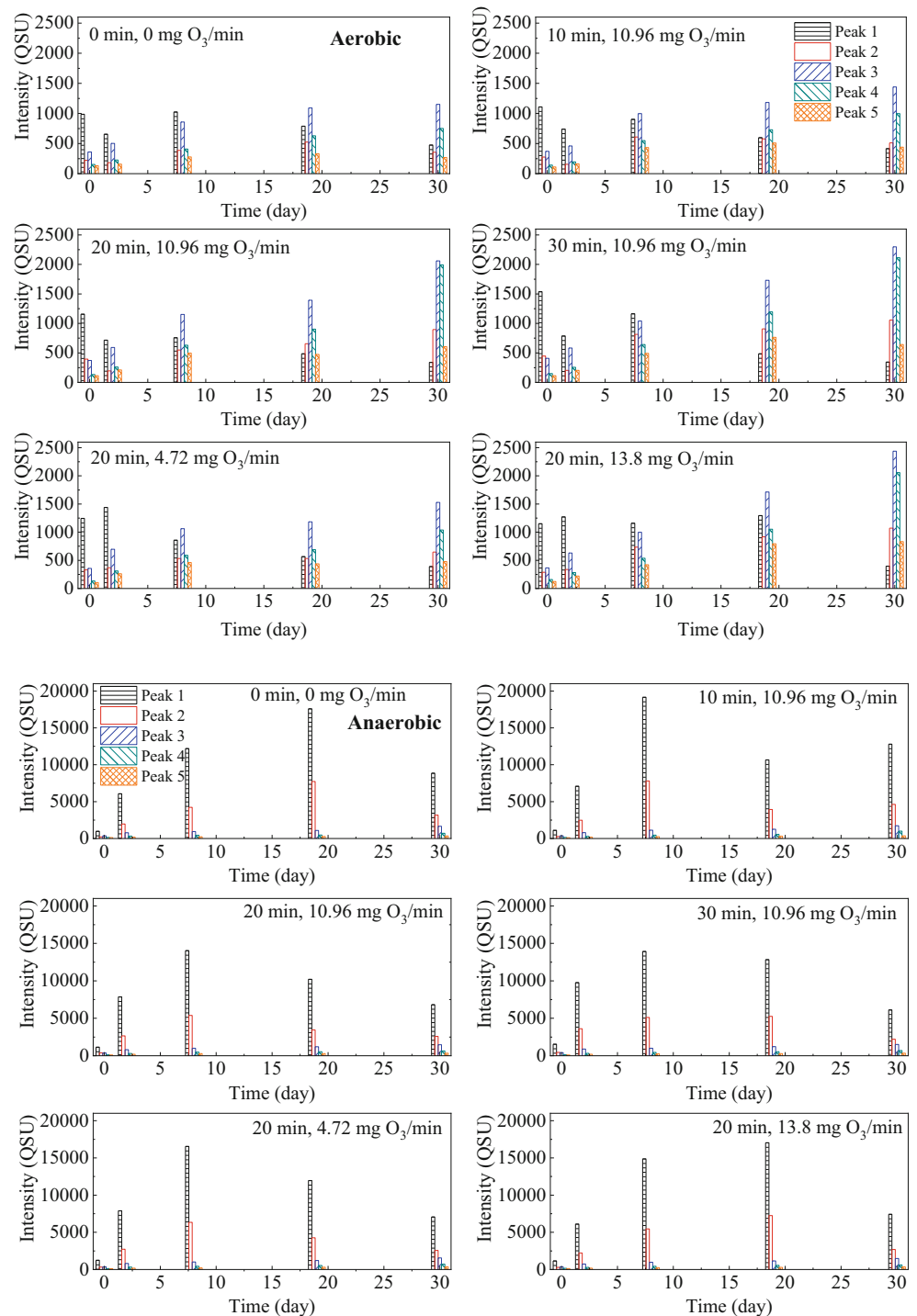
the increase of ozone dosage was from 0.00772 to 0.00796 day<sup>-1</sup>, respectively, with the values being generally higher than those estimated for the aerobic digestion. The reactors with 10.96 mg O<sub>3</sub>/min for 30 min under both aerobic and anaerobic conditions did not followed the first-order reaction model, which was probably because of much higher releasing rate of intracellular materials when ozonation time increased.

In regard of the degradation rate, the estimated *k<sub>d</sub>* values for the aerobic digestion varied greatly with the ozonation conditions, showing not a trend that could be satisfactorily explained. The estimated *k<sub>d</sub>* for the anaerobic digestion increased from 0.249 to 0.545 day<sup>-1</sup> as ozonation time increased from 0 to 20 min with an ozone dosage of 10.96 mg O<sub>3</sub>/min, and the highest value of 0.545 day<sup>-1</sup> was obtained with 10.96 mg O<sub>3</sub>/min for 20 min, with its values being obviously higher than those of the aerobic digestion. Compared with aerobic digestion, the hydrolysis and degradation rates of anaerobic digestion were higher.

### Irreversible membrane resistance caused by DOM

Aerobic and anaerobic digestions of WAS result in different intermediate and final organic compounds (Martinez-Garcia et al. 2014; Fall et al. 2014). As shown in Fig. 3, for aerobic conditions, there was almost no IMR in the initial 8 days and an obvious increase of IMR was observed from day 8, with the IMR being significantly increased as the ozone dosage and/or ozonation time increased. For the anaerobic condition, a significant increase of IMR was observed from day 0 and the increasing rate of IMR decreased significantly as the ozone dosage and/or ozonation time increased. The observed differences in IMR also indicated clearly that, judging from the presence of organic constituents that could cause irreversible membrane fouling, the composition of DOM formed in aerobic and anaerobic digestion

**Fig. 5** Fluorescence intensity of all peaks of DOM during 30 days' aerobic and anaerobic digestion after ozone pretreatment. \*Peaks 1 and 2, aromatic proteins and tryptophan-/tyrosine-like aromatic amino acids; peaks 3, 4, and 5, fulvic acid- and humic acid-like substances



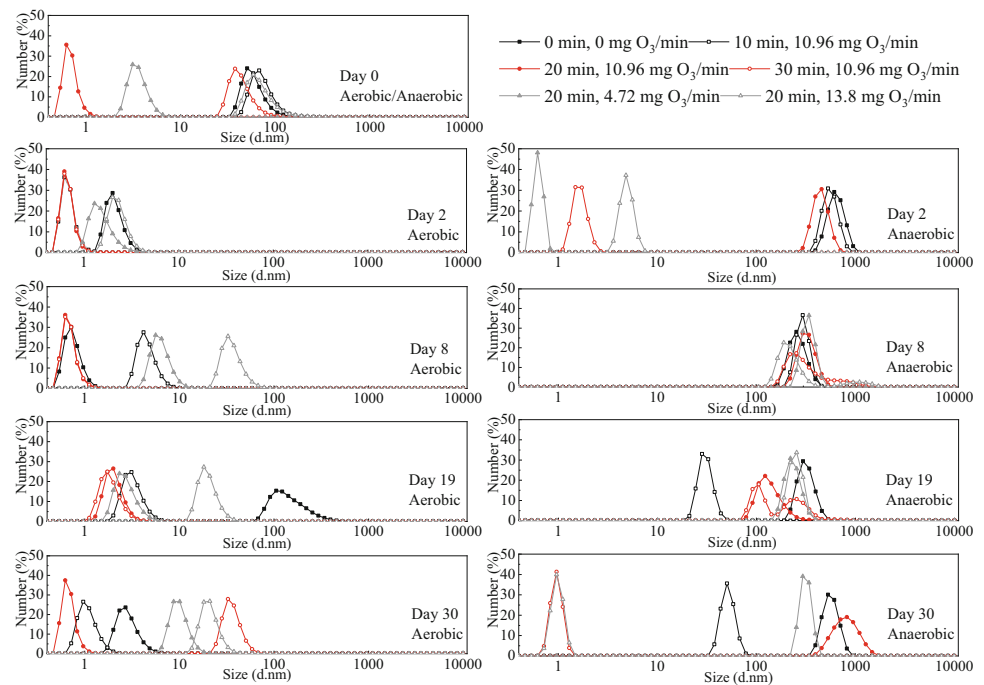
of activated sludge was different, and the extent of difference also differs with and without the ozone pretreatment. Yu et al. (2015) studied the effect of sludge retention time on membrane fouling and explained that the accumulation of EPS induced membrane fouling and protein-like substances and some of the humic-like substances (or polysaccharide) are major components of the organic material causing membrane fouling.

### Effect of ozone on composition of DOM assessed by fluorescence EEM

As shown in Fig. 4, peaks 1 and 2 at the excitation/emission ( $E_x/E_m$ ) of 225/340 and 280/344, which are reported to reflect aromatic proteins and tryptophan-/tyrosine-like aromatic amino acids (Hudson et al. 2008), were two specific peaks emerged mainly during anaerobic digestion; peaks 3, 4, and



**Fig. 6** Effect of ozone on change of the particle size distribution during 30 days’ digestion



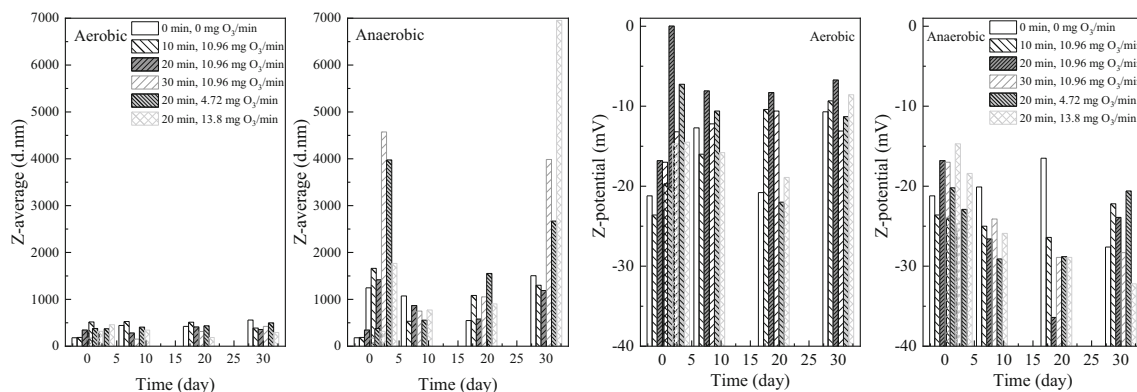
5 at the  $E_x/E_m$  of 240/436, 340/428, and 405/468 reported to reflect fulvic acid- and humic acid-like substances, respectively (Chen et al. 2003), emerged mainly during aerobic digestion. This phenomenon was consistent with our previous study (Du and Li 2016).

The fluorescence intensity values of all peaks during the whole operation are plotted in Fig. 5. The total fluorescence intensity was higher for the anaerobic digestion, which was consistent with our previous study (Du and Li 2016). Higher percentages of peaks 3, 4, and 5 under aerobic conditions and peaks 1 and 2 under anaerobic conditions during the whole operation obviously indicated that DOM formed and remaining after aerobic digestion contained higher content of humic acid- and fulvic acid-like substances. After anaerobic digestion, higher contents of aromatic proteins and tryptophan- and tyrosine-aromatic amino acids were found existent (Du and Li 2016; Shao et al. 2013; Ramdani et al. 2012). For the aerobic

conditions, increasing ozone dosage and/or ozonation time enhanced formation of aromatic proteins in the initial period of the operation and accumulation of tryptophan- and tyrosine-aromatic amino acids and humic acid- and fulvic acid-like substances during the whole operation. For the anaerobic conditions, there was a significant increase, followed by a decrease of aromatic proteins and tryptophan- and tyrosine-aromatic amino acids. Increasing ozone dosage and/or ozonation time shortened the time for aromatic proteins and tryptophan- and tyrosine-aromatic amino acids reaching their maximum values.

**Effect of ozone on composition of DOM assessed by particle size distribution, Z-average, and Z-potential**

As shown in Fig. 6, the particle size distribution under aerobic conditions has an obvious peak value corresponding to the smaller particle size, and the distribution produced by



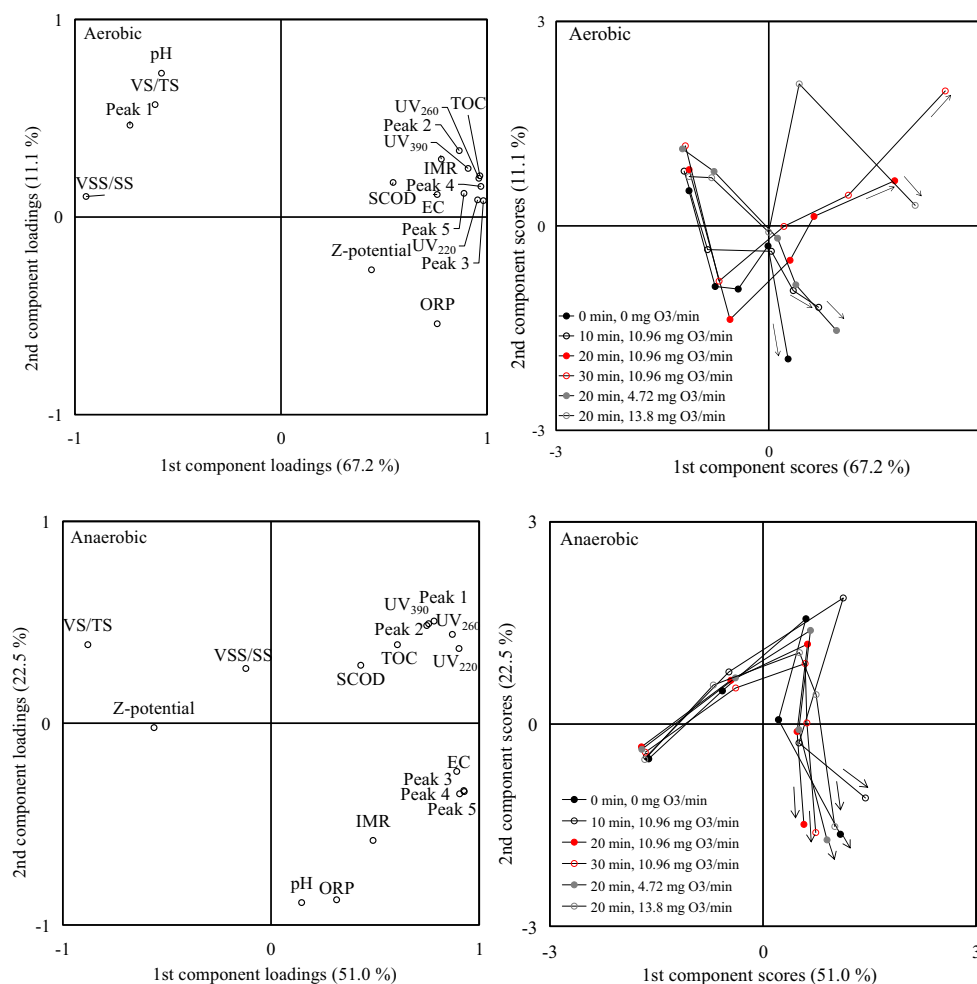
**Fig. 7** Effect of ozone on change of the Z-average and Z-potential during 30 days’ digestion

anaerobic digestion is located on the right side, reflecting the relatively large particle size. The values of the Z-average and Z-potential during the whole operation are plotted in Fig. 7. The Z-average of the aerobic digestion was significantly lower than that of the anaerobic digestion, and the Z-average of anaerobic digestion very similar to that of IMR discussed above and significantly increased in the initial period of operation. This was a good explanation for the enhanced dissolution of large DOM molecules during anaerobic digestion after ozone pretreatment, which led to the formation of IMR. The Z-potential of aerobic digestion increased significantly, while, for anaerobic digestion, the Z-potential decreased during the whole operation. Negatively charged protein was probably the main reason for the lower Z-potential (Li et al. 2017).

### Principal component analysis

The component loading and score plots of aerobic and anaerobic digestion after ozone pretreatment are shown in Fig. 8. For aerobic digestion, the loadings of TOC, UV<sub>220</sub>, UV<sub>260</sub>,

UV<sub>390</sub>, peaks 2–5, EC, ORP, IMR, SCOD, and Z-potential as PC1 were very high; the loadings of pH, VS/TS, and peak 1 as PC2 were relatively high. Compared with that for the reactor (0 min, 0 mg O<sub>3</sub>/min) without ozone pretreatment, the scores of PC1 obviously increased, indicating significantly enhanced formation of aromatic proteins and tryptophan/tyrosine-like aromatic amino acids after ozone pretreatment and corresponding increase of IMR (Yu et al. 2015). Increasing the ozonation time and/or ozone dosage dramatically increased the scores of PC1. The scores of PC2 decreased for the reactors with lower ozonation time (0 and 10 min) and/or ozone dosage (4.72 mg O<sub>3</sub>/min) while they decreased in the initial period (2–8 days) and then adversely increased in a rapid trend for the reactors with longer ozonation time (20 and 30 min) and/or ozone dosage (13.8 mg O<sub>3</sub>/min). This indicated that ozone pretreatment enhanced degradation of aromatic proteins and tryptophan/tyrosine-like aromatic amino acids while higher ozonation time and/or ozone dosage greatly enhanced formation of fulvic acid- and humic acid-like substances. For anaerobic digestion, compared with the result for aerobic



**Fig. 8** The results of the principal component analysis (component loadings and component scores) using all observed data during the whole process of aerobic and anaerobic digestions

digestion, the indexes reflecting the concentration and composition of DOM (TOC, UV<sub>220</sub>, UV<sub>260</sub>, UV<sub>390</sub>, peaks 1–5) also appeared as PC1 with higher loadings. The index of ORP that showed a higher loading as PC1 for aerobic digestion appeared as PC2, and the index of VSS/SS as PC 1 with a higher loading in the aerobic digestion turned to an index with lower loading either as PC1 or as PC2, reflecting the significant difference in the changing trends of organic matter content in SS of the anaerobic reactors with that of the aerobic reactors. The scores of PC1 and PC2 for all the reactors were very similar. Combination of the loading results with the corresponding score plots indicated that, for all reactors, the aromatic proteins and tryptophan-/tyrosine-like aromatic amino acids increased from day 0 to day 8 and then gradually decreased; the fulvic acid- and humic acid-like substances slightly increased, which caused an increase of IMR.

## Conclusions

The effect of ozone pretreatment on the characteristics of DOM formed in the liquid of the aerobic and anaerobic digestion of activated sludge was investigated. Higher destruction effect for volatile solids by ozone pretreatment was found in aerobic digestion than in anaerobic digestion. The results of TOC and UV absorbance at 220, 260, and 390 nm showed that ozonation enhanced sludge hydrolysis and DOM degradation. Model simulation indicated that, compared with aerobic digestion, anaerobic digestion had higher hydrolysis and degradation rate. The composition analysis of DOM based on the fluorescence EEM indicated that increasing ozone dosage and ozonation time enhanced aromatic protein-like substances in the initial time and accumulation of tryptophan- and tyrosine-aromatic amino acids and humic acid- and fulvic acid-like substances in the whole time of the aerobic digestion, while for the anaerobic digestion, ozone pretreatment promoted the formation of aromatic protein-like and tryptophan- and tyrosine-aromatic amino acid-like substances. The evaluation based on IMR further indicated that more membrane fouling substances were contained in the DOM of the anaerobic digestion of the sludge with ozone pretreatment. The PC analysis results indicated that ozone pretreatment enhanced degradation of aromatic proteins and tryptophan-/tyrosine-like aromatic amino acids while increasing ozone dosage and ozonation time enhanced the formation of humic and fulvic acid-like substances.

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