

**Effect of oxidation ditch and anaerobic-anoxic-oxic processes on CX3R-type disinfection by-product formation during wastewater treatment**

Peng, Liqi; Wang, Feifei; Zhang, Di; Fang, Chao; Hoek, Jan Peter van der; Chu, Wenhai

**DOI**

[10.1016/j.scitotenv.2021.145344](https://doi.org/10.1016/j.scitotenv.2021.145344)

**Publication date**

2021

**Document Version**

Final published version

**Published in**

Science of the Total Environment

**Citation (APA)**

Peng, L., Wang, F., Zhang, D., Fang, C., Hoek, J. P. V. D., & Chu, W. (2021). Effect of oxidation ditch and anaerobic-anoxic-oxic processes on CX3R-type disinfection by-product formation during wastewater treatment. *Science of the Total Environment*, 770, 1-9. Article 145344.  
<https://doi.org/10.1016/j.scitotenv.2021.145344>

**Important note**

To cite this publication, please use the final published version (if applicable).  
Please check the document version above.

**Copyright**

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

**Takedown policy**

Please contact us and provide details if you believe this document breaches copyrights.  
We will remove access to the work immediately and investigate your claim.

***Green Open Access added to TU Delft Institutional Repository***

***'You share, we take care!' - Taverne project***

**<https://www.openaccess.nl/en/you-share-we-take-care>**

Otherwise as indicated in the copyright section: the publisher is the copyright holder of this work and the author uses the Dutch legislation to make this work public.



# Effect of oxidation ditch and anaerobic-anoxic-oxic processes on CX<sub>3</sub>R-type disinfection by-product formation during wastewater treatment

Liqi Peng<sup>a,b</sup>, Feifei Wang<sup>c</sup>, Di Zhang<sup>a,b</sup>, Chao Fang<sup>a,b</sup>, Jan Peter van der Hoek<sup>d,e</sup>, Wenhai Chu<sup>a,b,\*</sup>

<sup>a</sup> State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

<sup>b</sup> Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China

<sup>c</sup> School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China

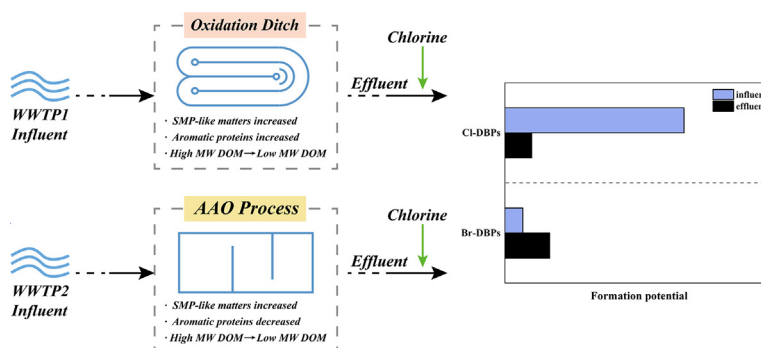
<sup>d</sup> Department of Water Management, Delft University of Technology, P.O. Box 5048, 2600 GA Delft, the Netherlands

<sup>e</sup> Research & Innovation, Waternet, Korte Ouderkerkerdijk 7, 1096 AC Amsterdam, the Netherlands

## HIGHLIGHTS

- Biological treatment enhanced the formation of a series of DBPs, especially Br-DBPs.
- Biological treatment reduced the DOC and UV<sub>254</sub> but increased the DON and SUVA.
- Biological treatment increased the DON/DOC ratio and bromide/DOC ratio.
- SMP- and humic acid-like matters increased significantly after OD and AAO treatment.
- High MW fractions were transformed into low MW fractions after OD and AAO processes.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 10 November 2020

Received in revised form 27 December 2020

Accepted 17 January 2021

Available online 22 January 2021

## ABSTRACT

The high chlorine dosages in wastewater treatment plants during the COVID-19 pandemic may result in increased formation of disinfection by-products (DBPs), posing great threat to the aquatic ecosystem of the receiving water body and the public health in the downstream area. However, limited information is available on the effect of biological wastewater treatment processes on the formation of CX<sub>3</sub>R-type DBPs. This study investigated the effect of oxidation ditch (OD) and anaerobic-anoxic-oxic (AAO), two widely used biological wastewater treatment processes, on the formation of five classes of CX<sub>3</sub>R-type DBPs, including trihalomethanes (THMs),

**Abbreviations:** AAO, Anaerobic-anoxic-oxic; BCAA, Bromochloroacetic acid; BCAL, Bromochloroacetaldehyde; BCAN, Bromochloroacetonitrile; BDCAA, Bromodichloroacetic acid; BDCAL, Bromodichloroacetaldehyde; BDCAN, Bromodichloroacetonitrile; BDCM, Bromodichloromethane; BIFs, Bromine incorporation factors; Br-DBPs, Brominated disinfection by-products; C-DBPs, Carbonaceous disinfection by-products; Cl-DBPs, Chlorinated disinfection by-products; COVID-19, Coronavirus Disease 2019; DBAA, Dibromoacetic acid; DBAL, Dibromoacetaldehyde; DBAN, Dibromoacetonitrile; DBCAA, Dibromochloroacetic acid; DBCAL, Dibromochloroacetaldehyde; DBCAN, Dibromochloroacetonitrile; BDCM, Dibromochloromethane; DBPs, Disinfection by-products; DCAA, Dichloroacetic acid; DCAL, Dichloroacetaldehyde; DCAN, Dichloroacetonitrile; DCNM, Dichloronitromethane; DHANs, Dihaloacetonitriles; DIN, Dissolved inorganic nitrogen; DOC, Dissolved organic carbon; DOM, Dissolved organic matter; DON, Dissolved organic nitrogen; EEM, Excitation-emission matrix; FP, Formation potential; FRI, Fluorescence region integration; GC/ECD, Gas chromatography/Electron capture detection; GC/MS, Gas chromatography/Mass spectrometer; HAAs, Haloacetic acids; HALs, Haloacetaldehydes; HANs, Haloacetonitriles; HBrO, Hypobromous acid; HClO, Hypochlorous acid; HNMs, Halonitromethanes; MBAA, Monobromoacetic acid; MCAA, Monochloroacetic acid; MW, Molecular weight; N-DBPs, Nitrogenous disinfection by-products; OD, Oxidation ditch; SEC, Size exclusion chromatography; SMPs, Soluble microbial products; SUVA, Specific ultraviolet absorption; TBAA, Tribromoacetic acid; TBAN, Tribromoacetonitrile; TBM, Tribromomethane; TBNM, Tribromonitromethane; TCAA, Trichloroacetic acid; TCAL, Trichloroacetaldehyde; TCAN, Trichloroacetonitrile; TCM, Trichloromethane; TCNM, Trichloronitromethane; TDN, Total dissolved nitrogen; THANs, Trihaloacetonitriles; THMs, Trihalomethanes; WWTPs, Wastewater treatment plants.

\* Corresponding author at: Room 407A Engineering Center, College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Yangpu District, Shanghai 200092, China. E-mail address: [1world1water@tongji.edu.cn](mailto:1world1water@tongji.edu.cn) (W. Chu).

Editor: Paola Verlicchi

**Keywords:**

Disinfection by-products  
Wastewater disinfection  
Oxidation ditch  
Anaerobic-anoxic-oxic  
Formation reactivity

haloacetic acids (HAAs), haloacetaldehydes (HALs), haloacetonitriles (HANs) and halonitromethanes (HNMs), during chlorination. Experimental results showed that biological treatment effectively reduced the dissolved organic carbon (DOC) and UV<sub>254</sub>, while it increased the dissolved organic nitrogen (DON), and therefore the ratio of DON/DOC. In addition, increases in the contents of soluble microbial product- and humic acid-like matters, and the transformation of high molecular weight (MW) fractions in the dissolved organic matter into low MW fractions were observed after OD and AAO processes. Although biological treatment effectively decreased the formation of Cl-THMs, Cl-HAAs, Cl-HANs and Cl-HNMs, the formation of DBCM, DBAA, BDCAA, DBCAA, DCAL, TCAL and DBAN (where C = chloro, B = bromo, D = di, T = tri) all increased significantly, due to the increased formation reactivity. Moreover, biological treatment increased the ratio of bromide/DOC and bromine incorporation into THMs, HAAs and DHANs except for HALs and THANs. Different from previous studies, this study revealed that biological treatment increased the formation of some DBPs, especially brominated DBPs, despite the efficient removal of organic matters. It provides insights into the DBP risk control in wastewater treatment, particularly during the COVID-19 pandemic.

© 2021 Elsevier B.V. All rights reserved.

## 1. Introduction

Reclamation and reuse of treated wastewater are important for sustainable water resource management (Tian et al., 2017). In order to minimize the potential public health risks, disinfection is of vital importance to inactivate pathogens and prevent the transmission of waterborne diseases (Costán-Longares et al., 2008). For instance, it was reported that prolonged virus shedding was observed in the feces of patients infected with 2019 novel coronavirus (2019-nCoV) (Jiehao et al., 2020), and it is known that the toilet water enters the wastewater treatment plants (WWTPs) via the sewer. Therefore, the wastewater could contain the 2019-nCoV. The chlorine disinfection is able to inactivate the 2019-nCoV in wastewater, blocking the virus transmission, and therefore has played an important role in effectively controlling the spread of Coronavirus Disease 2019 (COVID-19) in China during the past few months. However, a variety of disinfection by-products (DBPs) will be unintentionally formed when chlorine disinfection is practiced (Richardson et al., 2007), which were shown to be associated with adverse health effects, such as cancer induction (Plewa and Wagner, 2015). Moreover, the DBP formation potentials (FPs) were found to be higher in bio-treated wastewater than drinking water due to the higher disinfectant dosages and the abundant organic compositions, which are the main precursors of DBPs (Sirivedhin and Gray, 2005). Du et al. (2017) reported that carbonaceous DBPs (C-DBPs) such as trihalomethanes (THMs), haloacetic acids (HAAs) and chloral hydrate, and nitrogenous DBPs (N-DBPs) like *N*-nitrosodimethylamine in reclaimed water were present at higher levels than those in drinking water. In addition, a recent study conducted by the same group also found that chlorinated effluent organic matter formed more N-DBPs and total organic halogen than natural organic matter, accompanied by higher toxicity (Du et al., 2020). Additionally, during the COVID-19 pandemic, most WWTPs have increased their chlorine dosages to ensure the disinfection effectiveness. A study conducted during the COVID-19 pandemic reported that among the 56 WWTPs surveyed, the chlorine dosages applied in 14% of them were too high (>6 mg/L), with the highest being 11.3 mg/L (Li et al., 2020). This may result in enhanced formation of DBPs during chlorination and hence pose great threat to the aquatic ecosystem of the receiving water body (Liu and Zhang, 2014; Luan et al., 2020). Moreover, when the effluents discharged from WWTPs is utilized downstream as a potable water supply, the DBPs as well as their precursors in the effluents will be simultaneously introduced into the downstream drinking water treatment plants (Pehlivanoglu-Mantas and Sedlak, 2006), increasing potential health risks. Therefore, improving the quality of wastewater effluents is of great significance not only for reducing the ecological risk, but also for controlling the potential DBP risks in the downstream area.

Oxidation ditch (OD) process, a modification of conventional activated sludge process, is widely used due to easy management, low

energy demand and stable effluent quality (Zhang et al., 2016). Owing to its longer sludge retention time, the sludge yield of OD process is relatively lower than that of conventional activated sludge process. Anaerobic-anoxic-oxic (AAO), another widely applicable biological wastewater treatment process, has good removal performance of nitrogen and phosphorous with low operation cost (Lim et al., 2009).

Since biological wastewater treatment processes are mainly designed to remove organic matters and nutrients such as chemical oxygen demand, nitrogen and phosphorous to meet the wastewater discharge standards, most studies focused on these water quality indicators, whereas the impact of biological wastewater treatment processes on the formation of DBPs during subsequent disinfection has remained largely unknown. During biological treatment, microbial activities may alter the concentrations, compositions and properties of the organic materials in the influents, thus affecting the DBP formation during subsequent disinfection. On the other hand, soluble microbial products (SMPs), which mainly consist of polysaccharides, proteins, humic acids, fulvic acids, amino acids and nucleic acids (Parkin and McCarty, 1981; Urbain et al., 1998), are released into the water through substrate metabolism and microbial cell decay (Barker and Stuckey, 1999), contributing to various DBP formation during disinfection (Wu et al., 2019). Taken together, the characteristics of dissolved organic matter (DOM) may be different after biological treatment, which may lead to different DBP formation and speciation during chlorination. However, up to now, only a few studies have evaluated the effect of biological wastewater treatment processes on DBP formation (Han et al., 2018; Xue et al., 2017), and the DBP species involved in these studies were quite limited (e.g., THMs and HAAs). Moreover, the effect on the formation of brominated DBPs (Br-DBPs), which are more toxic than their chlorinated analogues (i.e., Cl-DBPs) (Wagner and Plewa, 2017), was neglected. Hence, a systematic study on the effect of biological wastewater treatment processes on DBP formation (C- and N-DBPs, Cl- and Br-DBPs) is quite necessary to further understand DBP risk control in wastewater treatment.

Though emerging DBPs with higher toxic potency have been identified recently (Zhang et al., 2018), the focus of most studies remain on several classes of DBPs such as THMs, HAAs, haloacetaldehydes (HALs), haloacetonitriles (HANs) and halonitromethanes (HNMs) owing to their higher concentration levels and/or greater toxicity (Bond et al., 2011; Shah and Mitch, 2012), which can also be termed as CX<sub>3</sub>R-type DBPs (X = H, Cl, Br or I) due to their similar molecular structure (Ding et al., 2019; Hou et al., 2018).

Therefore, the objectives of this study were to 1) examine the variation of DOM characteristics after OD and AAO treatment, including spectroscopic characteristics and molecular weight (MW) distribution; 2) investigate the effect of OD and AAO processes on CX<sub>3</sub>R-type DBP formation, especially to assess the formation reactivity and speciation of CX<sub>3</sub>R-type DBPs, during subsequent disinfection. This study would

be helpful to better understand the DBP risk control in wastewater treatment, particularly during the COVID-19 pandemic.

## 2. Materials and methods

### 2.1. Materials and samples

Detailed information on the DBP standards involved in this study is listed in Table S1. Free chlorine stock solution was prepared by diluting a sodium hypochlorite solution (active chlorine >5%). The ultrapure water was produced with a Millipore Milli-Q Gradient water purification system (Billerica, USA). All other materials were of at least analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) unless otherwise noted.

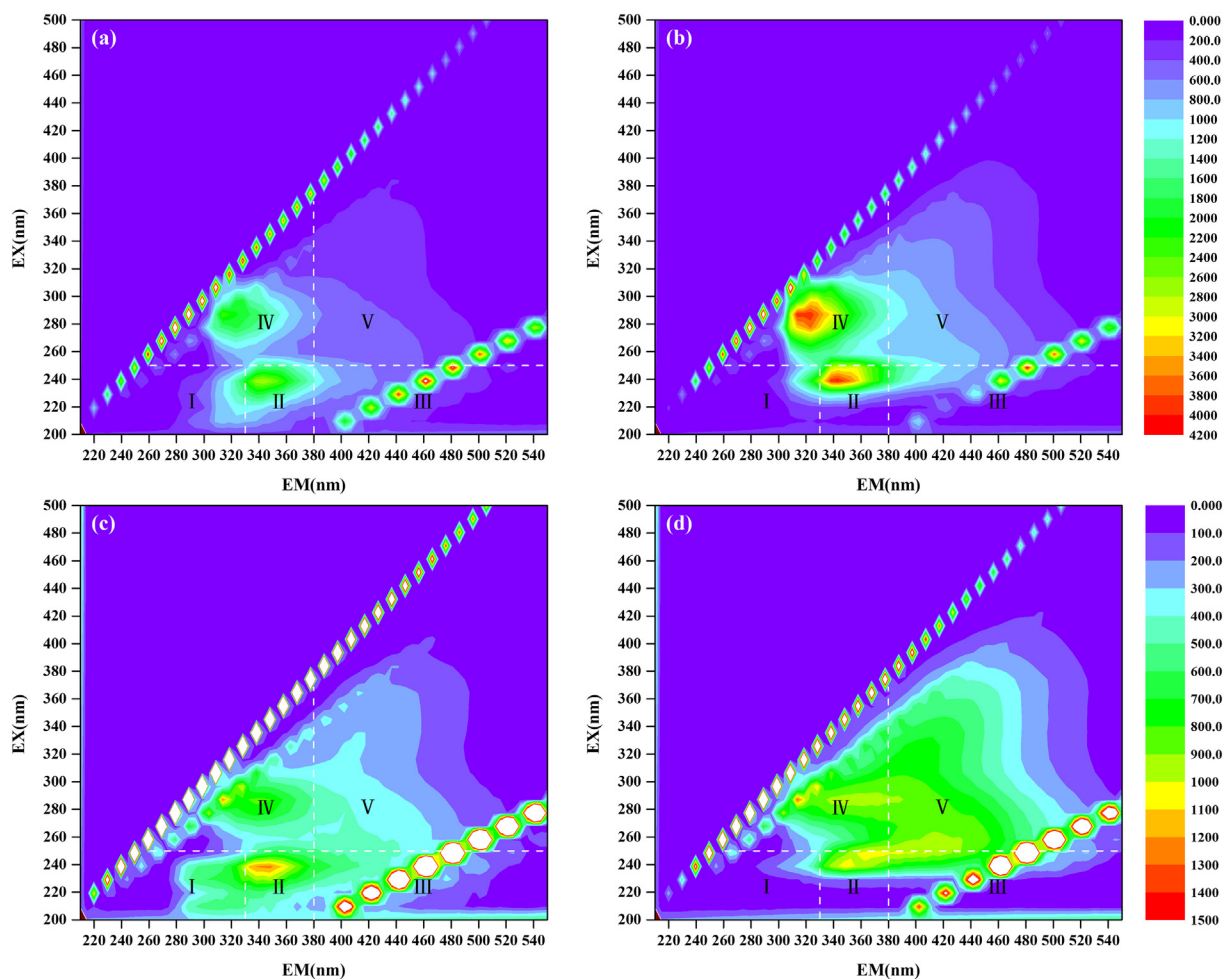
Water samples were collected more than three times from October 2019 to December 2019, from two different WWTPs in Shanghai, including the influents and effluents of biological wastewater treatment processes. WWTP A adopts OD process, while WWTP B uses AAO process, both of which fed with domestic wastewater. The operating parameters of OD and AAO processes including capacity, food to mass ratio (F/M), mixed liquor suspended solids (MLSS), sludge retention time (SRT) and hydraulic retention time (HRT) are listed in Table S2. Once collected, the water samples were filtered through a 0.45  $\mu\text{m}$  glass fiber filter (Millipore, USA) and stored at 4  $^{\circ}\text{C}$  for less than 2 weeks until use. The water quality characteristics of the studied water samples are listed in Table S3.

### 2.2. Experimental procedures

To avoid detector saturation, chlorination experiments were conducted in 500 mL headspace-free brown glass bottles in the dark at  $25 \pm 0.5$   $^{\circ}\text{C}$  with dissolved organic carbon (DOC) diluted to 2.0 mg/L and buffered at  $7.0 \pm 0.2$  with 10 mM phosphate buffer. The chlorine dosage for each water sample was determined according to a preliminary demand test such that a final free chlorine residue of at least 1–2 mg/L remained after 72 h (Ma et al., 2013; Meng et al., 2016). After 72 h incubation, stoichiometric amounts of sodium sulfite were added to quench the disinfectant residuals, and the water samples were analyzed as soon as possible. Error bars in the figures represent the relative standard deviation of two replicates.

### 2.3. Analytical methods

DOC and total dissolved nitrogen (TDN) were measured using a TOC analyzer (Shimadzu TOC-VCPH, Japan). Concentrations of different dissolved inorganic nitrogen (DIN) species (i.e. ammonia, nitrite and nitrate) were measured using their respective HACH test kits with a UV–vis spectrophotometer (HACH DR6000, USA). Dissolved organic nitrogen (DON) was determined by subtracting DIN from TDN.  $\text{UV}_{254}$  was measured using the HACH DR6000 spectrophotometer (Loveland, USA) as well. Specific ultraviolet absorption (SUVA) was calculated as the ratio of  $\text{UV}_{254}$  to DOC multiplied by 100. Free chlorine was measured with a HACH Pocket Colorimeter™ II spectrophotometer (Loveland, USA). Bromide concentration was determined by ion chromatography (Dionex 2000; USA).



**Fig. 1.** Fluorescence EEM spectra of the DOM in (a) OD influent; (b) OD effluent; (c) AAO influent; (d) AAO effluent. Solution DOC was adjusted to 1 mg/L. EEM spectra were divided into five regions (I: aromatic protein I; II: aromatic protein II; III: fulvic acid-like; IV: SMP-like; V: humic acid-like).

Three-dimensional spectrofluorometry (F-7100 Fluorescence, HITACHI, Japan) was used for DOM characterization. Fluorescence scans ( $\lambda_{ex}$ : 200–500 nm;  $\lambda_{em}$ : 210–550 nm) were performed with 5 nm slits for excitation and emission. Fluorescence excitation-emission matrix (EEM) spectra was divided into five regions (I: aromatic protein I; II: aromatic protein II; III: fulvic acid-like; IV: SMP-like; V: humic acid-like) (Chen et al., 2003). Fluorescence region integration (FRI) method was utilized for the quantitative analysis of EEM spectra (Chen et al., 2003):

$$\Phi_i = \int_{\lambda_{ex}} \int_{\lambda_{em}} I(\lambda_{ex}\lambda_{em}) d\lambda_{ex} d\lambda_{em} \quad (1)$$

where  $\Phi_i$  is the EEM volume at region  $i$ , which represents the cumulative fluorescence response of DOM with similar properties at each region;  $I(\lambda_{ex}\lambda_{em})$  is the fluorescence intensity at each excitation-emission wavelength pair. Given that the fluorescence intensities of organics in different regions were inconsistent, the  $\Phi_i$  of different regions might not be comparable. Hence, the variation of fluorescence response in the same region was evaluated to investigate the effect of OD and AAO processes on DOM characteristics.

Size exclusion chromatography (SEC) was utilized to evaluate the MW distribution of DOM. SEC was carried out using a polymethacrylates-packed column (250 mm  $\times$  20 mm, 3  $\mu$ m), which was installed on a Waters Alliance high performance liquid chromatography (e2695, MA, USA) equipped with a Sievers M9 SEC DOC detector. Polyethylene glycol standards were used for MW calibration. A mobile phase solution of 5.6 mM disodium hydrogen phosphate and 14.4 mM sodium dihydrogen phosphate was pumped at 1.0 mL min<sup>-1</sup>, and the injection volume was 250  $\mu$ L.

THMs, HALs, HANs and HNMs were detected using gas chromatography/electron capture detection (GC/ECD, QP2010plus, Shimadzu Corporation, Japan) while HAAs were measured with a gas chromatography/mass spectrometer (GC/MS-QP2020, Shimadzu Corporation, Japan). Detailed information on the analytical methods of these DBPs was presented in previous studies (Fang et al., 2019a; He et al., 2020b) and is summarized in Table S4.

To examine the variation of bromination extent after biological treatment, bromine incorporation factors (BIFs) for CX<sub>3</sub>R-type DBPs were calculated, based on Eq. (2).

$$BIF = \frac{\sum_{m=1}^n m \times CH_{3-n}Cl_{n-m}Br_mR}{n \times \sum_{m=0}^n CH_{3-n}Cl_{n-m}Br_mR}, n = 1, 2 \text{ or } 3 \quad (2)$$

where  $m$  and  $n$  represent the number of bromines and halogens, respectively.

### 3. Results and discussion

#### 3.1. Variation of water quality characteristics after OD and AAO processes

The water quality characteristics of the influents and effluents of OD and AAO processes are presented in Table S3. Considering the variation trends presented by the water samples collected from October 2019 to December 2019 were the same, here we only show the results of one of them. As expected, DOC was effectively removed after biological treatment, decreasing by 66.8% and 75.3% for OD and AAO processes, respectively. However, the DON levels were significantly higher in the effluents than the influents, even though the concentrations of TDN and ammonia declined substantially. Accordingly, a substantial increase in the DON/DOC ratio was observed after biological treatment. Although biological treatment reduced the UV<sub>254</sub>, it led to an increase in the aromaticity of DOM (e.g., phenolic structures), as indicated by the increased SUVA. This is in agreement with previous studies showing that low UV-absorbing fraction was preferentially removed during biological treatment (Han et al., 2018; Krasner et al., 2009b; Xue et al., 2017). Besides, the relatively low SUVA values (<2) of the studied

water samples indicated that the DOM were mostly non-humics and featuring low hydrophobicity and low MW (Edzwald and Tobiason, 1999). This is in agreement with the results of DOM characterization which will be discussed in Section 3.2. After OD and AAO processes, the bromide concentrations only decreased by 10.2% and 22.8%, respectively, suggesting that biological treatment had little effect on bromide removal, which was consistent with previous studies (Han et al., 2018; Krasner et al., 2009a). As a result, a significant increase in the bromide/DOC ratio was also observed.

#### 3.2. Variation of DOM characteristics after OD and AAO processes

##### 3.2.1. Fluorescence EEM spectra

Fluorescence EEM spectra of the DOM in the influents and effluents of the two investigated biological wastewater treatment processes is shown in Fig. 1. As is shown in Fig. 1, the DOM in the influents of OD and AAO processes were both dominated by aromatic proteins and SMP-like substances. Fig. S1 (Supplementary material) shows the variation of fluorescence intensity of different EEM regions of DOM before and after biological treatment. The fluorescence intensity of SMP-like peak after OD and AAO processes increased by 78.8% and 38.0%, respectively, indicating the formation of SMP-like substances during biological treatment. In addition, significant increases in the fluorescence intensity of humic acid-like peak were also observed after biological treatment

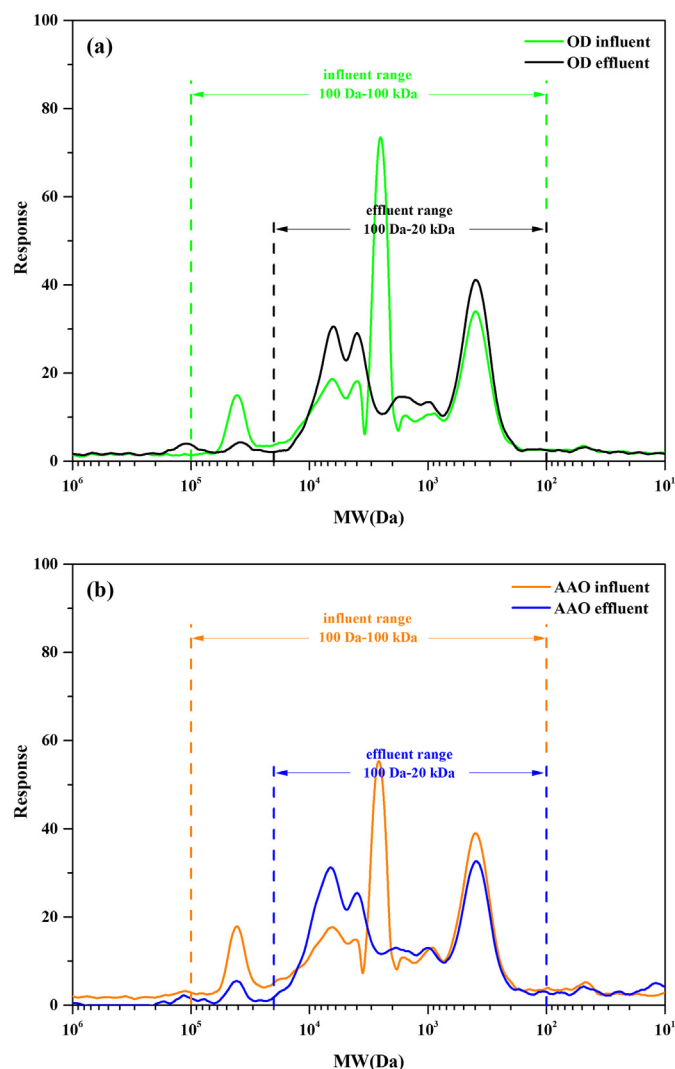


Fig. 2. MW distribution of the DOM in the influents and effluents of (a) OD and (b) AAO processes determined with SEC.

(by 78.9% and 134.4% for OD and AAO processes, respectively), which was probably due to the ineffective removal of humic acid-like substances during biological treatment as reported in previous studies (Han et al., 2015; Liu and Li, 2010). Although the fluorescence intensity of aromatic protein I both decreased (by 13.5% and 58.7% for OD and AAO processes, respectively), the fluorescence intensity of aromatic protein II increased by 18.9% after OD process while decreased by 19.4% after AAO process. This could be explained by the fact that the content of aromatic proteins depends on their formation and degradation. On the one hand, the aromatic proteins in the influents may undergo degradation during biological treatment, while on the other hand, microbial cell decay can also release aromatic proteins. As is shown in Table S2, the SRT of OD process is longer than that of AAO process, suggesting that higher proportion of microorganism may decay and release more aromatic proteins during OD process, thus may lead to the different variation trends of the aromatic protein contents for OD and AAO processes.

### 3.2.2. Molecular weight distribution

The MW distribution of the DOM in the influents and effluents of the two investigated biological wastewater treatment processes is presented in Fig. 2. OD and AAO processes exhibited similar trends in the variation of MW distribution. After biological treatment, compounds with MW of 20–100 kDa and 2–3 kDa decreased dramatically, while those with MW of 3–10 kDa increased. A minor increase in the compounds with MW of 1–2 kDa was also observed. In addition, the MW of the DOM in the influents was mainly in the range of 100 Da–100 kDa, while the MW of the DOM in the effluent was mainly in the

range of 100 Da–20 kDa. These results indicated that the higher MW changed to lower MW after biological treatment, which was in agreement with a previous study (Han et al., 2018). This is probably because the breakdown of high MW substances played a major role, even though the polymerization of microorganism metabolites and DOM may produce high MW substances. However, the compounds with MW of 100–1000 Da increased after OD process while decreased after AAO process. This could be explained by the differences in F/M and SRT between OD and AAO processes. As is shown in Table S2, the F/M of OD process is slightly higher than that of AAO process, suggesting that the degree of degradation of compounds with MW of 100–1000 Da may be relatively lower for OD process. On the other hand, the SRT of OD process is also longer, implying that higher proportion of microorganism may undergo decay during OD process and release more compounds with MW of 100–1000 Da.

### 3.3. Effect of OD and AAO processes on Cl-DBP formation

Fig. 3 shows the FP of Cl-DBPs during chlorination of the influents and effluents from OD and AAO processes. It is noteworthy that to avoid detector saturation, the chlorination experiments were conducted with a diluted DOC concentration of 2 mg/L, and certain amounts of chlorine were dosed to reach a final free chlorine residual of at least 1.0–2.0 mg/L after 72 h reaction. Afterwards, the DBP FP corresponding to the original DOC concentrations in the influents and effluents of OD and AAO processes were calculated. As is shown in Fig. 3, biological treatment effectively reduced the formation of Cl-THMs, Cl-HAAs, Cl-

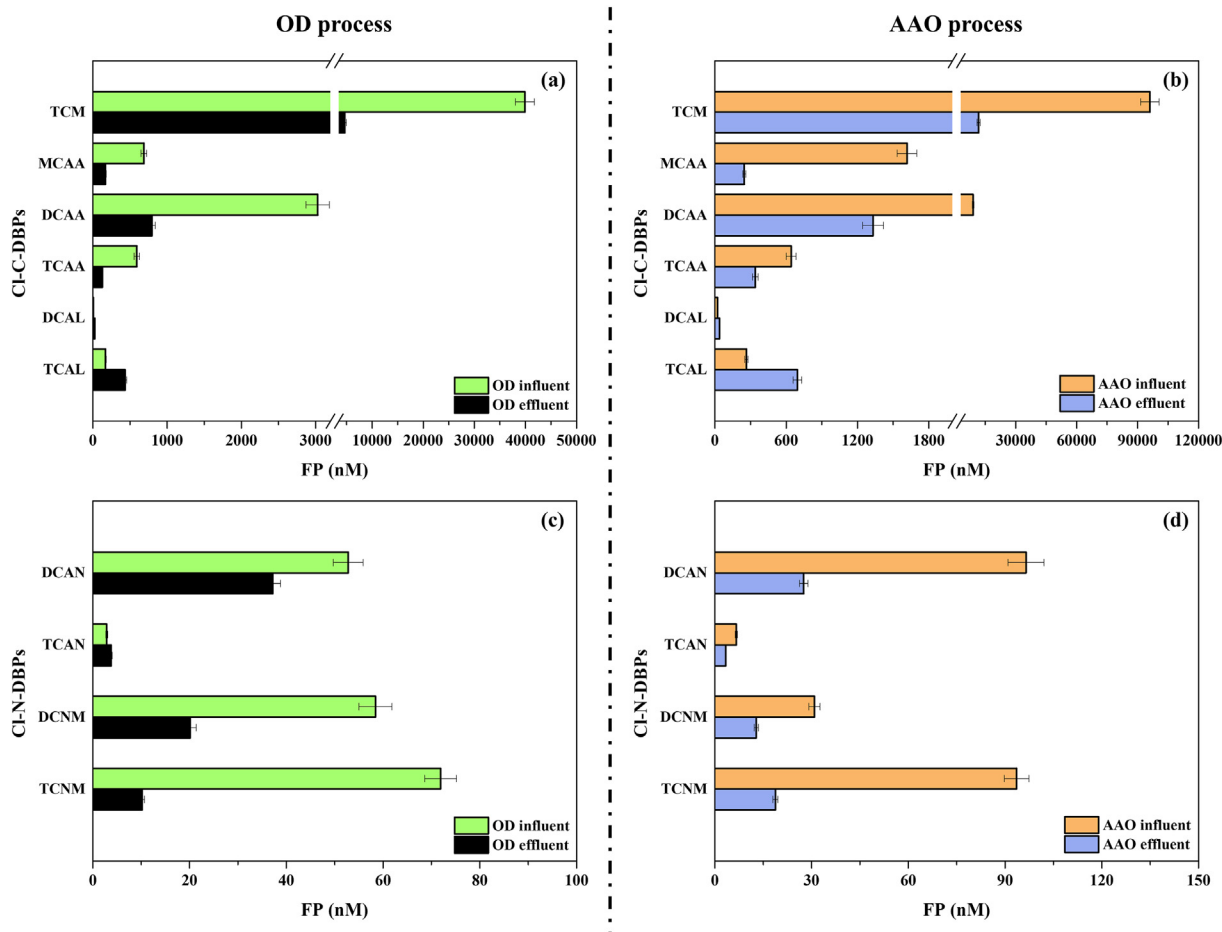
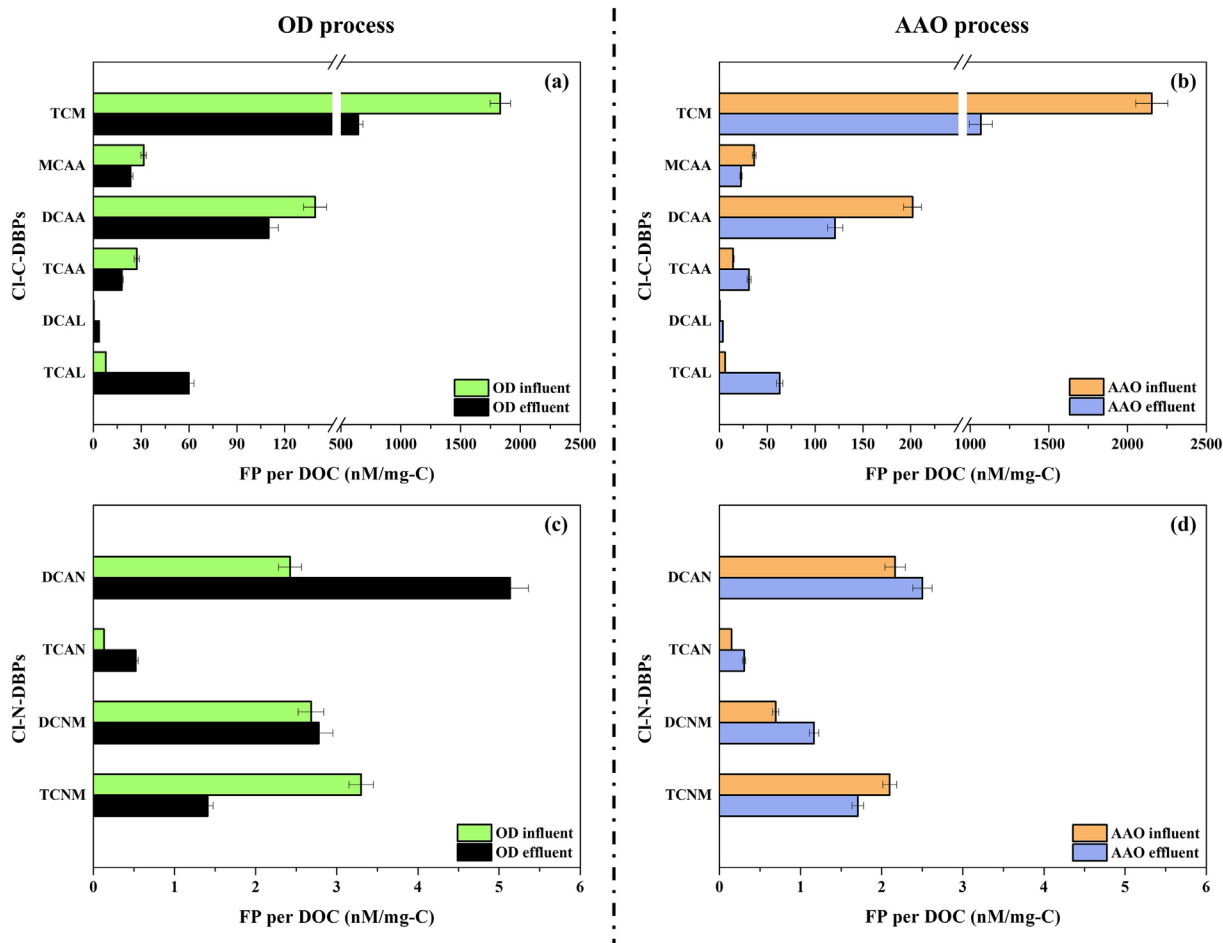


Fig. 3. Effect of OD and AAO processes on Cl-DBP FP. Experimental conditions: DOC = 2 mg/L, chlorine dose = 1.0–2.0 mg/L residual after 72 h reaction, T = 25 ± 0.5 °C, pH = 7 ± 0.2.



**Fig. 4.** Effect of OD and AAO processes on Cl-DBP formation reactivity. Experimental conditions: DOC = 2 mg/L, chlorine dose = 1.0–2.0 mg/L residual after 72 h reaction, T = 25 ± 0.5 °C, pH = 7 ± 0.2.

HANs and Cl-HNMs. However, an evident increase in Cl-HAL formation was observed, despite the effective reduction of DOC. This is likely due to the increase in the DBP formation reactivity of DOM. Therefore, a systematic evaluation of the DBP formation reactivity of DOM may foster our understanding of the Cl-DBP FP variation.

According to previous studies (He et al., 2020a; Meng et al., 2017; Xue et al., 2017), DBP FP per unit of DOC can be used to assess the reactivity of DOM in DBP formation. As is shown in Fig. 4, the formation reactivity of Cl-THM and Cl-HAA decreased after OD and AAO treatment, despite the increased aromaticity of DOM (i.e., increased SUVA) may lead to increased THM and HAA formation. This is probably because the impact of SUVA increase was offset by the production of less reactive precursors of THMs and HAAs during biological treatment. For instance, polysaccharides, one of the major components of SMPs, were reported to have low THM and HAA yields upon chlorination due to the low reactivity of aliphatic and alcohol groups toward chlorine (Bond et al., 2009a; Bond et al., 2009b; Hong et al., 2008). A recent study reported that increased production of polysaccharides led to a higher proportion of hydrophilic organics in DOM and ultimately contributed to the substantial decrease (>80%) in THM formation reactivity (Xia et al., 2016). Moreover, Hong et al. (2009) found that the THM yields of 20 selected amino acids were rather low except for tyrosine and tryptophan. This could be another reason for the decrease of Cl-THM formation reactivity, since amino acids is another major component of SMPs, which were found to increase after OD and AAO processes (Figs. 1 and S1). Decreased formation reactivity of Cl-THMs and Cl-HAAs, together with the efficient removal of DOC, led to the substantial reduction in the formation of Cl-THMs and Cl-HAAs. In addition, the trichloromethane (TCM)

formation reactivity for AAO effluent was much higher than that for OD effluent. It could be explained by that AAO effluent contained higher content of humic substances (Figs. 1 and S1), which were reported to be the main THM precursors (Adin et al., 1991).

Unlike Cl-THMs and Cl-HAAs, the formation reactivity of Cl-HALs increased significantly after OD and AAO processes. For instance, the formation reactivity of trichloroacetaldehyde (TCAL) after OD and AAO treatment increased by 665.0% and 956.5%, from 7.8 nM/mg-C and 6.0 nM/mg-C to 59.9 nM/mg-C and 63.1 nM/mg-C, respectively. This may be ascribed to the increase of phenolic structures, which were reported to be the predominant HAL precursors (Chuang et al., 2015). Significant concentrations of phenols were identified in SMPs by solid phase extraction plus GC/MS in a previous study (Trzcinski and Stuckey, 2009). In addition, a relatively strong correlation between SUVA and HAL yields was recently observed (Jian et al., 2016), suggesting that the increase in SUVA may result in the increase in HAL formation. Because the effect of formation reactivity increase outweighed the effect of DOC reduction, the formation of Cl-HALs increased abundantly after biological treatment.

Unexpectedly, the formation reactivity of Cl-HANs was observed to increase after OD and AAO processes. This was mainly due to the increase of the DON/DOC ratio (Table S3). More specifically, the increase in the formation reactivity of Cl-HANs was probably induced by the production of proteins and/or amino acids during OD and AAO processes, both of which were main components of SMPs and reported to have relatively high HAN yields (Chu et al., 2016; Fang et al., 2019b). Nevertheless, the concentrations of Cl-HANs in effluents were still lower than in



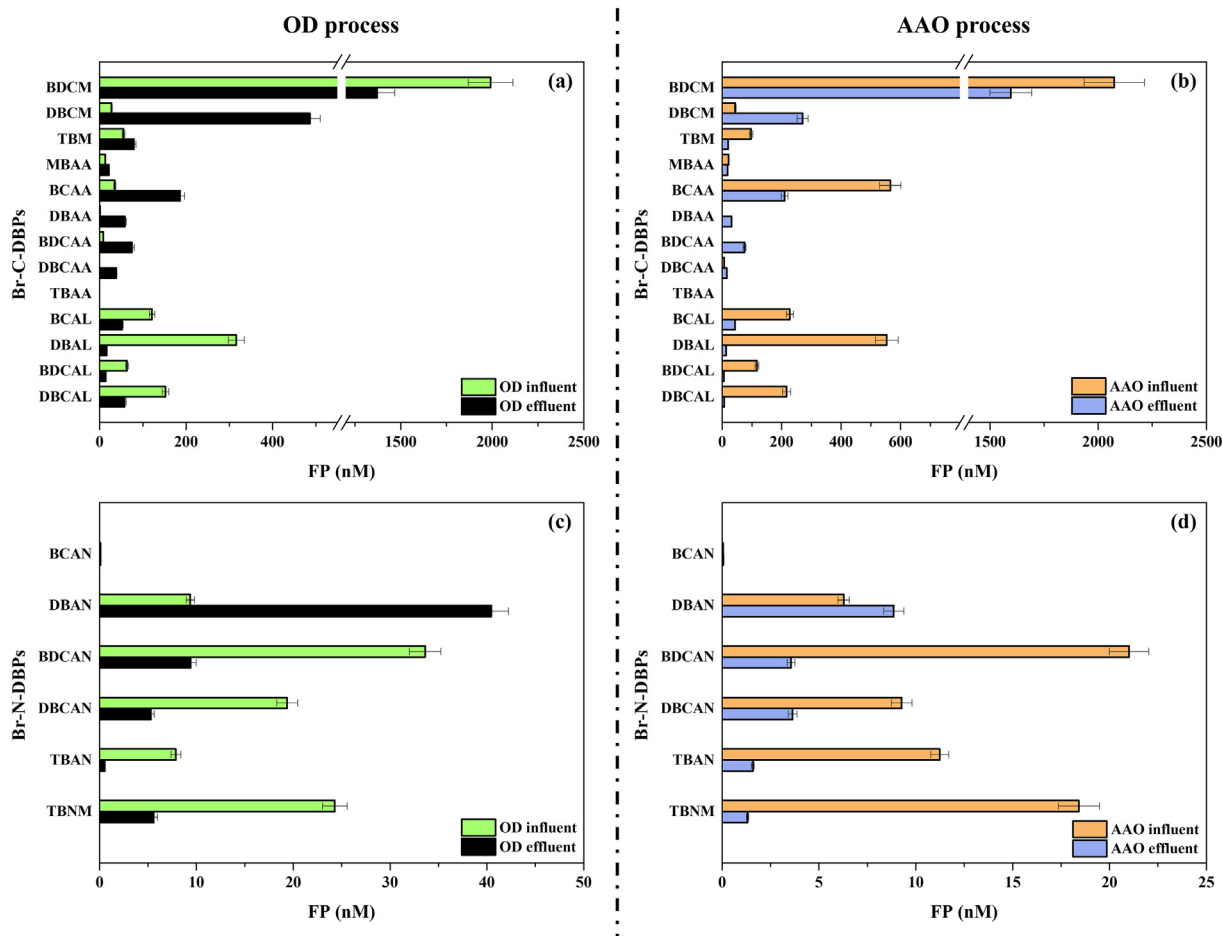


Fig. 5. Effect of OD and AAO processes on Br-DBP FP. Experimental conditions: DOC = 2 mg/L, chlorine dose = 1.0–2.0 mg/L residual after 72 h reaction, T = 25 ± 0.5 °C, pH = 7 ± 0.2.

influent, due to the efficient removal of DOC. Besides, the formation reactivity of dichloroacetonitrile (DCAN) for OD effluent is much higher than that for AAO effluent. It could be explained by that OD effluent contained higher contents of aromatic proteins and SMP-like substances (Figs. 1 and S1), which were reported to have high HAN yields (Fang et al., 2019b; Wu et al., 2019). After biological treatment, the formation reactivity of dichloronitromethane (DCNM) increased while that of trichloronitromethane (TCNM) decreased, which was probably due to the different properties of precursors. But after OD and AAO treatment, the formation of DCNM and TCNM both decreased.

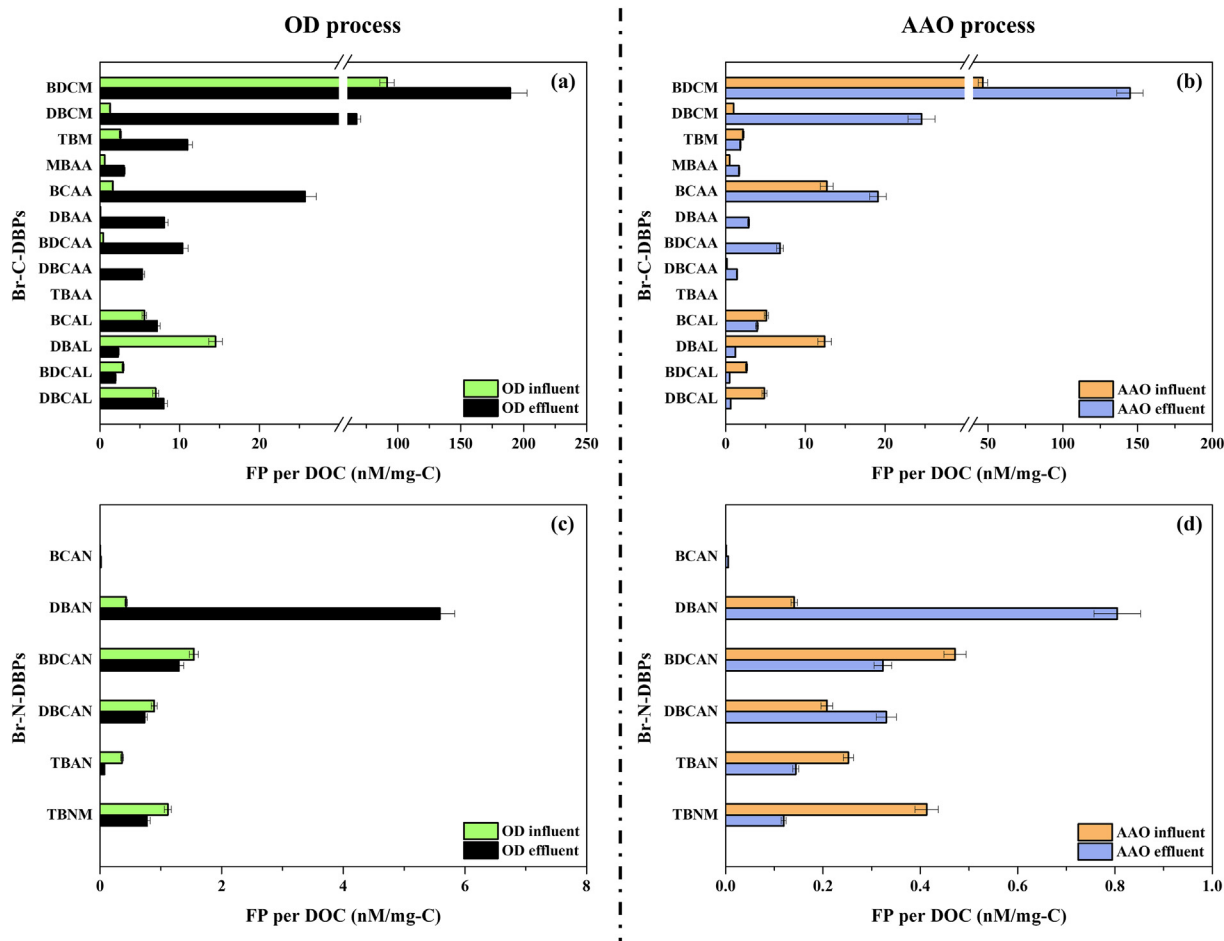
### 3.4. Effect of OD and AAO processes on Br-DBP formation

In the presence of hypochlorous acid (HClO), the bromide in the water is rapidly oxidized to hypobromous acid (HBrO), a stronger halogenating agent than HClO, which can then react with DOM to form Br-DBPs. Therefore, apart from the DBP formation reactivity of DOM, bromide also plays an important role in the formation of Br-DBPs. Fig. 5 presents the FP of Br-DBPs during chlorination of the influents and effluents from OD and AAO processes. Given that only one Br-HNM, tribromonitromethane was investigated in this study, the results related to Br-HNMs were not comprehensive enough and thus were not discussed here. As shown in Fig. 5, after biological treatment, the formation of dibromochloromethane (DBCM), dibromoacetic acid (DBAA), bromodichloroacetic acid (BDCAA), dibromochloroacetic acid (DBCAA) and dibromoacetonitrile (DBAN) all increased, while the formation of Br-HALs all decreased, which was quite different from

the variation of Cl-DBPs. Surprisingly, from the perspective of formation reactivity, it can be observed that the formation reactivity of Br-THMs and Br-HAAs all increased while the formation reactivity of Br-HALS decreased (Fig. 6). The variation of BIF values also indicated increased bromine incorporation into THMs, monohaloacetic acids (MHAAs), dihaloacetic acids (DHAAs) and trihaloacetic acids (THAAs) (Fig. S2). This was likely due to the increase in the bromide/DOC ratio (Table S3), enhancing the generation of HBrO during chlorination. Moreover, as discussed in Section 3.2.2, high MW fractions in the DOM were transformed into low MW fractions after biological treatment (Fig. 2), which were reported to be more prone to form Br-DBPs (Farré et al., 2013).

As for Br-HALS, however, although the elevated bromide/DOC ratio may increase the formation of Br-HALS, the Br-HALS are less stable in water and more prone to degrade into corresponding THMs than their chlorinated analogues (Koudjonou and LeBel, 2006), thus resulting in the decreased formation reactivity of Br-HALS in this study. As is shown in Fig. S2, the BIF values of dihaloacetaldehydes (DHALS) and trihaloacetaldehydes (THALS) both decreased, which was consistent with the variation of the Br-HAL formation reactivity. Decreased Br-HAL formation reactivity, accompanied by DOC reduction, led to the substantial decrease in Br-HAL formation (Fig. 5).

HANs can also undergo degradation in water, but contrary to HALs, brominated dihaloacetonitriles (Br-DHANs) are more stable (Yu and Reckhow, 2015), together with the increased bromide/DOC ratio, leading to the substantial increase in the formation reactivity of Br-DHANs. For instance, the formation reactivity of DBAN after OD and AAO



**Fig. 6.** Effect of OD and AAO processes on Br-DBP formation reactivity. Experimental conditions: DOC = 2 mg/L, chlorine dose = 1.0–2.0 mg/L residual after 72 h reaction, T = 25 ± 0.5 °C, pH = 7 ± 0.2.

treatment increased by 1199.4% and 471.4%, respectively, due to which the DBAN concentrations in effluents were much higher than those in influents (Fig. 5). However, after biological treatment, the formation reactivity of brominated trihaloacetone nitriles (Br-THANs) decreased. In addition, the variation of BIF values also revealed decreased bromine incorporation into THANs (Fig. S2). This distinction might lie in the nature of precursors.

#### 4. Conclusions

Biological treatment effectively reduced the DOC and UV<sub>254</sub> but increased the DON, SUVA, DON/DOC ratio and bromide/DOC ratio. In addition, increases in the contents of SMP- and humic acid-like substances, and transformation of high MW fractions in the DOM into low MW fractions were observed after OD and AAO processes. Although biological treatment effectively decreased the formation of Cl-THMs, Cl-HAAs, Cl-HANs and Cl-HNMs, the formation of DBCM, DBAA, BDCAA, DBCAA, DCAL, TCAL and DBAN all significantly increased, due to the increased formation reactivity. Moreover, biological treatment increased bromine incorporation into THMs, HAAs and DHANs except for HALs and THANs. As is known, biological wastewater treatment process is able to effectively control the formation of DBPs. However, for the first time this study revealed that biological treatment increased the formation of some DBPs, especially Br-DBPs (e.g., DBAN), despite the efficient removal of organic materials. Furthermore, during the COVID-19 pandemic, the high chlorine dosages in WWTPs may lead to increased formation of DBPs, posing great threat to the aquatic ecosystem of the

receiving water body and the public health in the downstream area. Therefore, apart from the main water quality indicators regulated by wastewater discharge standards, enough attention should also be paid to the reduction of DON and bromide in WWTPs to achieve the integrated risk control.

#### CRediT authorship contribution statement

**Liqi Peng:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Feifei Wang:** Formal analysis, Writing – review & editing. **Di Zhang:** Writing – review & editing. **Chao Fang:** Writing – review & editing. **Jan Peter van der Hoek:** Writing – review & editing. **Wenhai Chu:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

This work was supported by the Shanghai Science and Technology Project (No. 20230714100), National Natural Science Foundation of China (Nos. 51822808; 52091542), Tongji University Youth 100 program and Fundamental Research Funds for the Central Universities.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.145344>.

## References

- Adin, A., Katzhendler, J., Alkaslassy, D., Rav-Acha, C., 1991. Trihalomethane formation in chlorinated drinking water: a kinetic model. *Water Res.* 25, 797–805.
- Barker, D.J., Stuckey, D.C., 1999. A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Res.* 33, 3063–3082.
- Bond, T., Goslan, E.H., Jefferson, B., Roddick, F., Fan, L., Parsons, S.A., 2009a. Chemical and biological oxidation of NOM surrogates and effect on HAA formation. *Water Res.* 43, 2615–2622.
- Bond, T., Henriot, O., Goslan, E.H., Parsons, S.A., Jefferson, B., 2009b. Disinfection byproduct formation and fractionation behavior of natural organic matter surrogates. *Environ. Sci. Technol.* 43, 5982–5989.
- Bond, T., Huang, J., Templeton, M.R., Graham, N., 2011. Occurrence and control of nitrogenous disinfection by-products in drinking water – a review. *Water Res.* 45, 4341–4354.
- Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation–emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* 37, 5701–5710.
- Chu, W., Li, D., Deng, Y., Gao, N., Zhang, Y., Zhu, Y., 2016. Effects of UV/PS and UV/H<sub>2</sub>O<sub>2</sub> pre-oxidations on the formation of trihalomethanes and haloacetonitriles during chlorination and chloramination of free amino acids and short oligopeptides. *Chem. Eng. J.* 301, 65–72.
- Chuang, Y.-H., McCurry, D.L., Tung, H.-h., Mitch, W.A., 2015. Formation pathways and trade-offs between haloacetamides and haloacetaldehydes during combined chlorination and chloramination of lignin phenols and natural waters. *Environ. Sci. Technol.* 49, 14432–14440.
- Costán-Longares, A., Montemayor, M., Payán, A., Méndez, J., Jofre, J., Mujeriego, R., Lucena, F., 2008. Microbial indicators and pathogens: removal, relationships and predictive capabilities in water reclamation facilities. *Water Res.* 42, 4439–4448.
- Ding, S., Wang, F., Chu, W., Fang, C., Pan, Y., Lu, S., Gao, N., 2019. Using UV/H<sub>2</sub>O<sub>2</sub> pre-oxidation combined with an optimised disinfection scenario to control CX<sub>3</sub>R-type disinfection by-product formation. *Water Res.* 167, 115096.
- Du, Y., Lv, X.-T., Wu, Q.-Y., Zhang, D.-Y., Zhou, Y.-T., Peng, L., Hu, H.-Y., 2017. Formation and control of disinfection byproducts and toxicity during reclaimed water chlorination: a review. *J. Environ. Sci.* 58, 51–63.
- Du, Y., Wang, W.-L., He, T., Sun, Y.-X., Lv, X.-T., Wu, Q.-Y., Hu, H.-Y., 2020. Chlorinated effluent organic matter causes higher toxicity than chlorinated natural organic matter by inducing more intracellular reactive oxygen species. *Sci. Total Environ.* 701, 134881.
- Edzwald, J.K., Tobiasson, J.E., 1999. Enhanced coagulation: US requirements and a broader view. *Water Sci. Technol.* 40, 63–70.
- Fang, C., Ding, S., Gai, S., Xiao, R., Wu, Y., Geng, B., Chu, W., 2019a. Effect of oxoanions on oxidant decay, bromate and brominated disinfection by-product formation during chlorination in the presence of copper corrosion products. *Water Res.* 166, 115087.
- Fang, C., Hu, J., Chu, W., Ding, S., Zhao, T., Lu, X., Zhao, H., Yin, D., Gao, N., 2019b. Formation of CX<sub>3</sub>R-type disinfection by-products during the chlorination of protein: the effect of enzymolysis. *Chem. Eng. J.* 363, 309–317.
- Farré, M.J., Day, S., Neale, P.A., Stalter, D., Tang, J.Y.M., Escher, B.I., 2013. Bioanalytical and chemical assessment of the disinfection by-product formation potential: role of organic matter. *Water Res.* 47, 5409–5421.
- Han, X., Wang, Z., Ma, J., Zheng, J., Wang, P., Wu, Z., 2015. Formation and removal of dissolved organic nitrogen (DON) in membrane bioreactor and conventional activated sludge processes. *Environ. Sci. Pollut. Res.* 22, 12633–12643.
- Han, H., Miao, H., Zhang, Y., Lu, M., Huang, Z., Ruan, W., 2018. Carbonaceous and nitrogenous disinfection byproduct precursor variation during the reversed anaerobic–oxic process of a sewage treatment plant. *J. Environ. Sci.* 65, 335–346.
- He, J., Shi, M., Wang, F., Duan, Y., Zhao, T., Shu, S., Chu, W., 2020a. Removal of CX<sub>3</sub>R-type disinfection by-product precursors from rainwater with conventional drinking water treatment processes. *Water Res.* 185, 116099.
- He, J., Wang, F., Zhao, T., Liu, S., Chu, W., 2020b. Characterization of dissolved organic matter derived from atmospheric dry deposition and its DBP formation. *Water Res.* 171, 115368.
- Hong, H.C., Mazumder, A., Wong, M.H., Liang, Y., 2008. Yield of trihalomethanes and haloacetic acids upon chlorinating algal cells, and its prediction via algal cellular biochemical composition. *Water Res.* 42, 4941–4948.
- Hong, H.C., Wong, M.H., Liang, Y., 2009. Amino acids as precursors of trihalomethane and haloacetic acid formation during chlorination. *Arch. Environ. Contam. Toxicol.* 56, 638–645.
- Hou, M., Chu, W., Wang, F., Deng, Y., Gao, N., Zhang, D., 2018. The contribution of atmospheric particulate matter to the formation of CX<sub>3</sub>R-type disinfection by-products in rainwater during chlorination. *Water Res.* 145, 531–540.
- Jian, Q., Boyer, T.H., Yang, X., Xia, B., Yang, X., 2016. Characteristics and DBP formation of dissolved organic matter from leachates of fresh and aged leaf litter. *Chemosphere.* 152, 335–344.
- Jiehaio, C., Jin, X., Daojiong, L., Zhi, Y., Lei, X., Zhenghai, Q., Yuehua, Z., Hua, Z., Ran, J., Pengcheng, L., Xiangshi, W., Yanling, G., Aimei, X., He, T., Hailing, C., Chuning, W., Jingjing, L., Jianshe, W., Mei, Z., 2020. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clin. Infect. Dis.* 71, 1547–1551.
- Koudjonou, B.K., LeBel, G.L., 2006. Halogenated acetaldehydes: analysis, stability and fate in drinking water. *Chemosphere.* 64, 795–802.
- Krasner, S.W., Westerhoff, P., Chen, B., Rittmann, B.E., Amy, G., 2009a. Occurrence of disinfection byproducts in United States wastewater treatment plant effluents. *Environ. Sci. Technol.* 43, 8320–8325.
- Krasner, S.W., Westerhoff, P., Chen, B., Rittmann, B.E., Nam, S.-N., Amy, G., 2009b. Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effluent organic matter. *Environ. Sci. Technol.* 43, 2911–2918.
- Li, J., Wang, Y., Xiong, H., Tan, Z., Lv, Z., Zheng, K., Zou, L., Luo, G., Ye, L., Zhang, Z., Wang, M., 2020. Investigation and optimization strategies on the operation of disinfection facilities in municipal WWTPs. *China Water & Wastewater.* 36, 7–19.
- Lim, E.-T., Jeong, G.-T., Bhang, S.-H., Park, S.-H., Park, D.-H., 2009. Evaluation of pilot-scale modified A<sub>2</sub>O processes for the removal of nitrogen compounds from sewage. *Bioresour. Technol.* 100, 6149–6154.
- Liu, J.-L., Li, X.-y., 2010. Biodegradation and biotransformation of wastewater organics as precursors of disinfection byproducts in water. *Chemosphere.* 81, 1075–1083.
- Liu, J., Zhang, X., 2014. Comparative toxicity of new halophenolic DBPs in chlorinated saline wastewater effluents against a marine alga: Halophenolic DBPs are generally more toxic than haloaliphatic ones. *Water Res.* 65, 64–72.
- Luan, X., Liu, X., Fang, C., Chu, W., Xu, Z., 2020. Ecotoxicological effects of disinfected wastewater effluents: a short review of in vivo toxicity bioassays on aquatic organisms. *Environ. Sci.-Wat. Res. Technol.* 6, 2275–2286.
- Ma, D., Gao, B., Sun, S., Wang, Y., Yue, Q., Li, Q., 2013. Effects of dissolved organic matter size fractions on trihalomethanes formation in MBR effluents during chlorine disinfection. *Bioresour. Technol.* 136, 535–541.
- Meng, Y., Wang, Y., Han, Q., Xue, N., Sun, Y., Gao, B., Li, Q., 2016. Trihalomethane (THM) formation from synergic disinfection of biologically treated municipal wastewater: effect of ultraviolet (UV) irradiation and titanium dioxide photocatalysis on dissolve organic matter fractions. *Chem. Eng. J.* 303, 252–260.
- Meng, Y., Wang, M., Guo, B., Zhu, F., Wang, Y., Lu, J., Ma, D., Sun, Y., Gao, B., 2017. Characterization and C-, N-disinfection byproduct formation of dissolved organic matter in MBR and anaerobic-anoxic-oxic (AAO) processes. *Chem. Eng. J.* 315, 243–250.
- Parkin, G.F., McCarty, P.L., 1981. A comparison of the characteristics of soluble organic nitrogen in untreated and activated sludge treated wastewaters. *Water Res.* 15, 139–149.
- Pehlivanoglu-Mantas, E., Sedlak, D.L., 2006. The fate of wastewater-derived NDMA precursors in the aquatic environment. *Water Res.* 40, 1287–1293.
- Plewa, M.J., Wagner, E.D., 2015. Charting a new path to resolve the adverse health effects of DBPs. In: Karanfil, T., Mitch, B., Westerhoff, P., Xie, Y. (Eds.), *Recent Advances in Disinfection By-products*, pp. 3–23.
- Richardson, S.D., Plewa, M.J., Wagner, E.D., Schoeny, R., DeMarini, D.M., 2007. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat. Res.-Rev. Mutat. Res.* 636, 178–242.
- Shah, A.D., Mitch, W.A., 2012. Halonitroalkanes, halonitriles, haloamides, and N-nitrosamines: a critical review of nitrogenous disinfection byproduct formation pathways. *Environ. Sci. Technol.* 46, 119–131.
- Sirivedhin, T., Gray, K.A., 2005. 2. Comparison of the disinfection by-product formation potentials between a wastewater effluent and surface waters. *Water Res.* 39, 1025–1036.
- Tian, Y., Hu, H., Zhang, J., 2017. Solution to water resource scarcity: water reclamation and reuse. *Environ. Sci. Pollut. Res.* 24, 5095–5097.
- Trzcinski, A.P., Stuckey, D.C., 2009. Continuous treatment of the organic fraction of municipal solid waste in an anaerobic two-stage membrane process with liquid recycle. *Water Res.* 43, 2449–2462.
- Urbain, V., Mobarry, B., de Silva, V., Stahl, D.A., Rittmann, B.E., Manem, J., 1998. Integration of performance, molecular biology and modeling to describe the activated sludge process. *Water Sci. Technol.* 37, 223–229.
- Wagner, E.D., Plewa, M.J., 2017. CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review. *J. Environ. Sci.* 58, 64–76.
- Wu, M., Liang, Y., Zhang, Y., Xu, H., Liu, W., 2019. The effects of biodegradation on the characteristics and disinfection by-products formation of soluble microbial products chemical fractions. *Environ. Pollut.* 253, 1047–1055.
- Xia, C., Ma, D., Gao, B., Hu, X., Yue, Q., Meng, Y., Kang, S., Zhang, B., Qi, Y., 2016. Characteristics and trihalomethane formation reactivity of dissolved organic matter in effluents from membrane bioreactors with and without filamentous bulking. *Bioresour. Technol.* 211, 183–189.
- Xue, S., Jin, W., Zhang, Z., Liu, H., 2017. Reductions of dissolved organic matter and disinfection by-product precursors in full-scale wastewater treatment plants in winter. *Chemosphere.* 179, 395–404.
- Yu, Y., Reckhow, D.A., 2015. Kinetic analysis of haloacetonitrile stability in drinking waters. *Environ. Sci. Technol.* 49, 11028–11036.
- Zhang, Q.H., Yang, W.N., Ngo, H.H., Guo, W.S., Jin, P.K., Dzakpasu, M., Yang, S.J., Wang, Q., Wang, X.C., Ao, D., 2016. Current status of urban wastewater treatment plants in China. *Environ. Int.* 92–93, 11–22.
- Zhang, D., Chu, W., Yu, Y., Krasner, S.W., Pan, Y., Shi, J., Yin, D., Gao, N., 2018. Occurrence and stability of chlorophenylacetamides: a new class of nitrogenous aromatic DBPs in chlorinated and chloraminated drinking waters. *Environ. Sci. Technol. Lett.* 5, 394–399.