



# Characterization of dissolved organic matter in wastewater during aerobic, anaerobic, and anoxic treatment processes by molecular size and fluorescence analyses

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## ABSTRACT

Changes in the characteristics of dissolved organic matter (DOM: the dissolved fraction of natural organic matter) during a series of wastewater treatment plant (WWTP) processes were investigated by using a combination of molecular size analysis and excitation emission matrix (EEM) spectroscopy coupled with parallel factor analysis. The characteristics of DOM were compared following aerobic, anoxic, and anaerobic treatments. Three peaks at about 100,000 Da (high-molecular-size DOM, Peak 1) and about 900–1,100 Da (intermediate-molecular-size DOM, Peak 2; low-molecular-size DOM, Peak 3 as the shoulder of Peak 2) were observed in the distribution of total organic carbon molecular sizes in the influent of the WWTPs. In this study, five fluorescent components (C1 to C5) were identified in the EEM spectra. Molecular size analysis and molecular size fractionation revealed that the C3 (humic-like) and C5 (specific to sewage) fluorophores had intermediate or low molecular sizes. Comparison of the changes of the concentrations of dissolved organic carbon in each reaction tank and investigation of the removal selectivity of each treatment (aerobic, anaerobic, and anoxic) suggested that the heterogeneous compounds present in DOM of the influent were homogenized into intermediate-molecular-size DOM with high hydrophobicity and aromaticity, or into C4 fluorophores (DOM-X), during anaerobic or anoxic treatment. DOM-X was able to be transformed or removed by aerobic treatment. The results suggested that introduction of aerobic treatment at the appropriate stage of wastewater treatment or inclusion of physical or chemical treatment should be an effective way to optimize DOM removal.

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## 1. Introduction

As the world's population and economic activity continue to grow, scarcity of fresh water has become an urgent global issue to be resolved. "Ensure access to water and sanitation for all," is one of the Sustainable Development Goals of the United Nations General Assembly. Wastewater reclamation, in which secondarily treated wastewater is purified by additional tertiary treatment such as coagulation/flocculation, ozonation, and membrane filtration, is one of the successful strategies developed to meet this goal (Bixio et al., 2006; Intriago et al., 2018). This strategy has been implemented especially in urban areas to provide recycled water for purposes such as toilet flushing, landscape improvement, and road cleaning (Meneses et al., 2010; Pintillie et al., 2016; Xu et al., 2016;

Jeong et al., 2018). To ensure the quality of the reclaimed water, concerns associated with dissolved organic matter (DOM) must be addressed, because DOM causes a variety of problems, including coloration and odor of the water and membrane fouling during reclamation treatment (Chon and Cho, 2016; Wang et al., 2017). Furthermore, DOM can be a major precursor of carcinogenic disinfection by-products (e.g. trihalomethanes and haloacetic acids) produced during the chlorination of water. Those by-products are a serious problem if the reclaimed water is destined for reuse for drinking (Zhang et al., 2013).

Because the influent to a wastewater reclamation plant (WWRP) is typically the effluent from a wastewater treatment plant (WWTP), it is essential to have a good understanding of the characteristics of DOM in WWTP effluent. Knowledge of which treatment process in a WWTP efficiently removes problematic DOM compounds such as humic substances can facilitate optimization of WWRP treatment processes.

DOM is a heterogeneous mixture of organic compounds that

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include proteins, sugars, carboxylic acids, nucleic acids, and aquatic humic substances (Imai et al., 2001); its characteristics have been studied by using a variety of analytical approaches. In this study, we focused on two approaches to elucidate changes of DOM characteristics during the biological processes in WWTPs. The first approach was analysis of the molecular size distributions of DOM. Traditionally, an ultrafiltration technique has been used to fractionate DOM on the basis of molecular size cut-off (Xu et al., 2006; Wei et al., 2008). Recently, a tangential-flow system has been most frequently used (Ma et al., 2013; Han et al., 2015; Broek et al., 2017). Our method of analyzing the molecular size distribution in DOM was high-performance liquid chromatography – size exclusion chromatography (HPLC-SEC). Shon et al. (2004) analyzed the molecular size distribution of DOM of biologically treated sewage effluent and found that its range was 300–400,000 Da. Tang et al. (2014) analyzed the molecular size distribution of resin-fractionated DOM in WWTP effluents by using HPLC-SEC. They estimated molecular size with a total organic carbon (TOC) detector. In previous studies, the molecular size distribution of DOM has been determined by using several different methods. For example, Liu et al. (2019) used a fluorescence detector to characterize the DOM in textile wastewater and to examine the molecular size distribution of the protein-like fluorophores. The molecular size distribution of DOM has typically been discussed on the basis of the results of a single detection method. Few studies have compared the molecular size distributions of DOM on the basis of measurements of TOC concentrations, ultraviolet (UV) absorbance, and fluorescence emission (Her et al., 2003). In this study, a homemade HPLC-SEC system with TOC, UV and fluorescence detectors was used to quantitatively evaluate the molecular size distribution of DOM in terms of TOC, UV and fluorescence. The system was able to detect DOM with molecular sizes as high as 100,000 Da. Although such DOM plays an important role in biological processes, it has relatively low UV absorbance and has been hardly detected by UV absorbance detectors in previous studies (Rosario-Ortiz et al., 2007). Furthermore, we discuss the differences between the molecular size distributions detected by TOC, UV, and fluorescence.

The second approach for characterizing DOM was fluorescence analysis. It has been suggested that 40%–60% of Natural Organic Matter is fluorescent organic matter (Baker, 2001). A fluorescence technique such as excitation-emission matrix spectroscopy coupled with parallel factor analysis (EEM-PARAFAC) has proven to be a powerful monitoring tool for assessing DOM in wastewater (Carstea et al., 2016). It is a simple, rapid, and sensitive method for monitoring DOM and requires only a small volume of sample. Cohen

et al. (2014) have used EEM-PARAFAC to monitor the effects of various treatment processes on DOM in a WWTP in Israel and have found that the fluorescence of a tryptophan-like component can be used as an indicator of biological treatment efficacy. Yu et al. (2013) have compared the removal efficiency of each treatment process in a WWTP; they have found that the protein-like components are more easily removed than fulvic-like components during the anaerobic-anoxic-aerobic process. The EEM-PARAFAC method has recently been used for both the online and real-time monitoring. For example, Murphy et al. (2011) have used EEM-PARAFAC to characterize a large and diverse data-set of WWTP effluents ( $n = 1479$ ). The EEMs calculated by the PARAFAC model and the original EEM were compared. The comparison suggested that monitoring with fluorimeters and selection of appropriate wavelengths should capture the same information as a full EEM. Goffin et al. (2018) have used the concentration scores of multiple components for online monitoring of biological oxygen demand (BOD) and have produced a multiple linear predictive model of BOD.

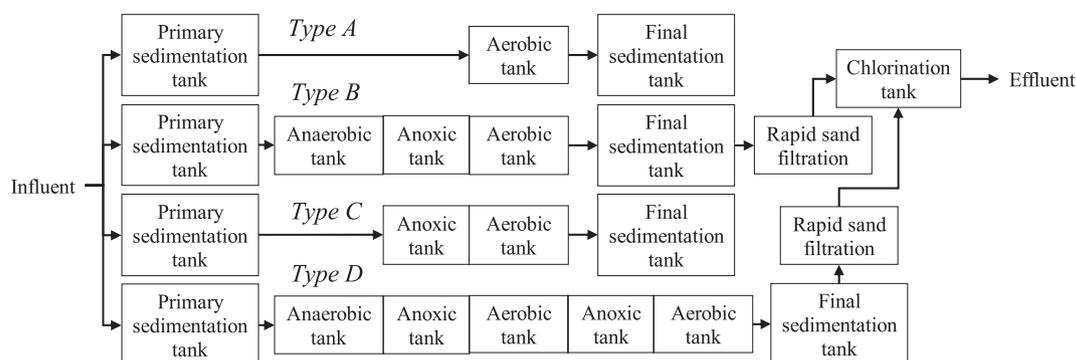
Various previous studies have investigated the characteristics of DOM in the effluent of WWTPs. The changes of DOM characteristics in biological treatments such as the activated sludge process or in chemical treatments such as ozonation have also been investigated (Tran et al., 2015; Jin et al., 2016). However, few studies have compared the characteristics of DOM in the influent and effluent of WWTPs (Park et al., 2010; Hur et al., 2011). Information on the characteristics of DOM in influent is especially scarce.

The objectives of this study were to characterize the changes in the characteristics of DOM during a series of WWTP processes and to compare these changes during DOM removal under aerobic, anoxic, and anaerobic conditions. We characterized DOM by using a combination of EEM-PARAFAC and HPLC-SEC systems. In addition, we used an ultrafiltration technique to separate DOM into high- and low-molecular size fractions.

## 2. Materials and methods

### 2.1. Collection of water samples

Water samples were collected in October 2016 and March, June, and August 2017 from a WWTP operating four types of advanced treatment processes: Type A (activated sludge process); Type B (anaerobic–anoxic–aerobic process [A<sub>2</sub>O]); Type C (recirculating denitrification process); and Type D (modified Bardenpho process) (Fig. 1). Influent sewage was branched into the four processes and treated in parallel. The confluent of the finished waters was then



**Fig. 1.** Four types of advanced treatment processes operated by the WWTP in this study.

Type A: Activated sludge process

Type B: Anaerobic–anoxic–aerobic process (A<sub>2</sub>O)

Type C: Recirculating denitrification process

Type D: Modified Bardenpho process.

treated further by rapid sand filtration and chlorination and discharged to a river. For comparison, influent and effluent water samples were collected from a human excreta treatment plant. Samples of water from the surface and bottom layer of the river a short distance downstream from the outlet of the WWTP were also collected. All samples were immediately cooled in an ice cooler and brought back to the laboratory. The water was filtered through a precombusted Whatman GF/F filter (nominal pore size: 0.7  $\mu\text{m}$ ).

## 2.2. Measurement of basic characteristics of DOM

DOM concentrations were quantified in terms of dissolved organic carbon (DOC) concentrations and UV absorbance (UVA) at a wavelength of 254 nm (UVA<sub>254</sub>). DOC concentrations were measured as non-purgeable organic carbon with a Shimadzu TOC-V TOC analyzer equipped with a Pt catalyst on quartz wool (Shimadzu Corp., Kyoto). Potassium hydrogen phthalate (Kanto Chemical Co., Tokyo) was used as a standard. At least three measurements were made for each sample, and the analytical precision was typically less than  $\pm 2\%$ . UVA<sub>254</sub> was measured with a Shimadzu UV-4500 UV-Vis spectrometer; samples were placed in a quartz cell with a path length of 1 cm. Milli-Q water was used as a blank.

## 2.3. Measurement of molecular size distributions

Molecular size distributions were determined with a home-made prototype HPLC-SEC system with a UV absorbance (UVA) detector (SPD-6A Shimadzu), a fluorescence detector (RF-10A Shimadzu), and a nondispersive infrared total organic carbon (TOC) detector. The wavelength of the UVA detector was set at 254 nm. A mixture of disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) and monobasic dihydrate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) was adjusted to pH 6.8 and used as a mobile phase. The concentration of phosphate and ionic strength of the mobile phase were 0.02 mol/L and 0.0335 M, respectively. Sodium polystyrene sulfonate (PSS) was used as a standard for molecular size analysis. The SEC column was a TSKgel G3000SW<sub>XL</sub> (Tosoh Bioscience LLC, King of Prussia); the flow rate of the mobile phase was set at 0.5 mL/min and the sample volume was set at 100  $\mu\text{L}$ . The molecular size range of the PSS was 1,920–220,000 Da. The apparent molecular size of DOM in the samples was calculated from the elution time by using a calibration curve based on the PSS standards. Kawasaki et al. (2011) have provided further details about the measurement procedure.

## 2.4. Molecular size fractionation

To fractionate water samples into a high-molecular-size fraction (HMF) and low-molecular-size fraction (LMF), we used a 10,000-Da cut-off ultrafiltration membrane set in a centrifuge tube (Macrosep Advance Centrifugal Device, Pall Corp., New York). Before use, each membrane was cleaned by being rinsed with Milli-Q water with centrifuging for 5 min (2000g; 3300 rpm in our device) at least three times. It was then soaked for more than 24 h, and then further cleaned three times with Milli-Q water because the membranes were coated with glycerin, which would have contaminated the samples with DOC. We found that this cleaning procedure reduced the contamination level of the filtrate to less than 0.3 mg C/L. A water sample of about 15 mL was filtered by centrifuging the sample at 2000g for 15 min. The filtrate from the first filtration was considered to be the LMF of DOM. DOM remaining on the filter was rinsed with Milli-Q water, and then centrifuged at 2000g for 15 min, three times and was considered to be the HMF of DOM. The ability of this rinsing procedure to remove the LMF of DOM remaining on the membrane was verified with the HPLC-SEC system described in Section 3.3.

## 2.5. EEM spectra measurement and PARAFAC analysis

Fluorescence spectra were measured with a multiwavelength fluorescence spectrophotometer (F-7000, Hitachi, Tokyo) equipped with a 150-W xenon lamp and a quartz flow-cell in an autosampler (AS-3000, Hitachi, Tokyo). The operational conditions were identical to those described by Komatsu et al. (2019). The spectra were corrected for instrument biases via an excitation correction spectrum derived from a solution of Rhodamine B (lot no. MNF3287, Wako, Osaka) and an emission correction spectrum derived by using a ground quartz diffuser, as recommended by the manufacturer of the spectrophotometer. To allow for the comparison of our results with those of previous studies, we standardized our EEM spectral data by using integrated Raman scattering data for Milli-Q water after subtraction of the EEM spectrum of Milli-Q water, as suggested by Determann et al. (1994), Matthews et al. (1996), and Mostafa et al. (2005). PARAFAC uses an alternating least-squares algorithm to minimize the sum of squared residuals across an EEM dataset, and the EEM data are decomposed into a set of trilinear terms and a residual array, as follows:

$$x_{ijk} = \sum_{f=1}^F a_{if} b_{jf} c_{kf} + \varepsilon_{ijk}$$

where  $x_{ijk}$  is the intensity of the  $i$ th sample at the  $j$ th emission variable and  $k$ th excitation variable;  $\varepsilon_{ijk}$  is the unexplained signal (residuals);  $a_{if}$  is directly proportional to the concentration of the  $f$ th analyte of the  $i$ th sample;  $b_{jf}$  and  $c_{kf}$  are estimates of the emission and excitation spectra, respectively, for the  $f$ th fluorophore; and  $F$  is the number of components of fluorescent DOM.

The analyses were performed for measured EEMs of DOM samples by using Matlab 7.10.0 (R2016b) with the DOMFluor toolbox according to the methodology described by Stedmon and Bro (2008). Targeted data were excitation wavelengths (260–550 nm) and emission wavelengths (260–550 nm). The data in the region influenced by Rayleigh and Raman peaks and the region where the emission wavelength was less than the excitation wavelength were omitted and replaced with missing values (“NaN” [Not A Number] in Matlab). The appropriate number of components was validated by split-half analysis and random initialization, as suggested by Murphy et al. (2013).

## 2.6. Comparison of changes of DOM characteristics among treatment types

The WWTP treatment processes that we studied were implemented by using various types of reaction tanks with aerobic, anaerobic, and anoxic treatments (see Fig. 1). In a series of wastewater processes involving completely mixed tanks, the DOM concentration in the previous tank means the DOM concentration in the effluent from the previous tank as well as the DOM concentration in the influent to the present reaction tank. Thus, the removal selectivity of each tank could be determined by comparing the characteristics of DOM in a reaction tank with those of DOM in the previous tank. For example, the first treatments in the processes were aerobic for Type A, anaerobic for Type B, anoxic for Type C, and anaerobic for Type D. The treatment efficiencies of the aerobic, anaerobic, and anoxic processes could therefore be examined by comparing the water quality between the first reaction tank and the primary sedimentation tank.

To determine the removal selectivity of each type of treatment, the difference between DOM concentration ([DOM]) in the reaction tank and that in the previous tank was calculated as follows. Comparisons were made between the  $\Delta\text{DOC}$  values and various

parameters of  $\Delta$ DOM.

$$\Delta[\text{DOM}] = [\text{DOM}] \text{ previous tank} - [\text{DOM}] \text{ present reaction tank}$$

[DOM] could be replaced with DOM parameters such as DOC or  $\text{UVA}_{254}$ .

### 3. Results and discussion

#### 3.1. Total DOM concentrations of WWTP influent and effluent

On the one hand, the influents were characterized by a wide range of DOC concentrations, 14.3–27.2 mg C/L [coefficient of variation (CV), 0.28] and a wide range of  $\text{UVA}_{254}$ , 282–457 mAbs/cm (CV, 0.21) (Table 1). On the other hand, the effluents were characterized by relatively narrow ranges of DOC concentrations, 4.1–5.9 mg C/L (CV, 0.18) and  $\text{UVA}_{254}$ , 79.5–98.2 mAbs/cm (CV, 0.10). The narrow ranges in the effluent imply that the concentrations of recalcitrant DOM were relatively constant in the effluent, presumably because wastewater treatment is a biological process intended to stabilize organic matter. The efficiency of  $\text{UVA}_{254}$  reduction by the WWTP was 72%–79%, similar to the percent removal of DOC (70%–78%). In both the influent and effluent samples obtained in October 2016, the specific ultraviolet absorbance (SUVA), calculated as  $\text{UVA}_{254}/\text{DOC}$ , was substantially lower than the values obtained in the other seasons. The SUVA indicates the hydrophobicity and aromaticity of DOM (Yang et al., 2013); the substantially lower values in October 2016 thus indicate lower concentrations of hydrophobic compounds in the influent.

Comparison of SUVA between the influent and effluent did not show a clear pattern. In both October 2016 and August 2017, SUVA

was higher in the effluent than in the influent. This pattern is consistent with the results of Park et al. (2010) and Musikavong and Wattanachira (2007). However, SUVA was lower in the effluent than in the influent in the other seasons. The percentage removal of DOC during wastewater treatment was relatively low when the influent SUVA was relatively high (Table 1); this pattern is consistent with a finding reported by Hur et al. (2011).

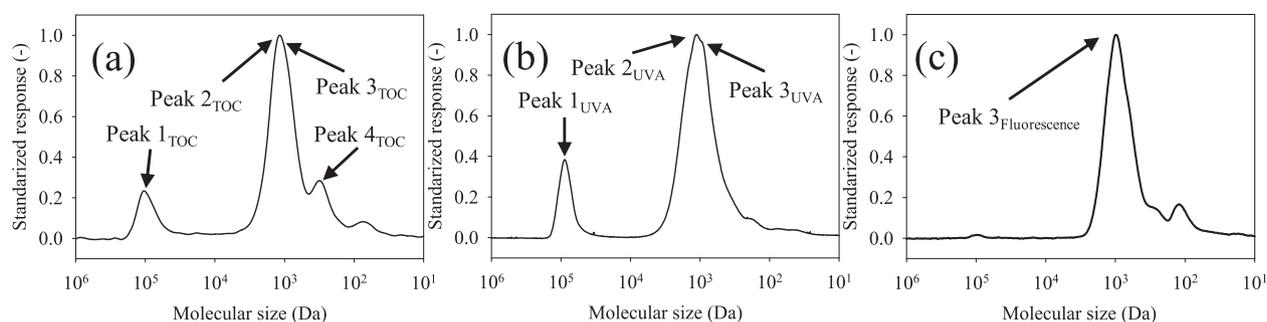
#### 3.2. Changes in molecular size distributions during WWTP processes

Molecular size distributions were measured in terms of TOC (corresponding to DOC in this study) and UVA (Fig. 2a and b). In the TOC detection, three peaks, at about 100,000 Da (high-molecular-size DOM, Peak 1) and about 900–1,100 Da (intermediate-molecular-size DOM, Peak 2; low-molecular-size DOM, Peak 3 as the shoulder on Peak 2) were observed in the influent. It was difficult to distinguish between Peaks 2 and 3 on the basis of TOC detection (Fig. 2a); because of the overlap of Peaks 2 and 3, the top of the merged peak was a little wider than expected for a single peak. Furthermore, in the cases of the treated water samples, a shoulder was clearly apparent on both Peaks 2 and 3 because Peak 3 was selectively reduced (Fig. S1 in the Supplemental material). In addition, Peak 4 at around 300 Da was observed only in the sample obtained in June 2017.

Peaks 1–3 were also observed in the SEC chromatograms as UVA (Fig. 2b). The Peak 1 areas expressed as percentages of the total peak area in the influent were 0.5%, 13%, and 20% in October 2016, June 2017, and August 2017, respectively. Evaluation of the UVA detection results showed that the area of Peak 1 in the influent was much smaller in October 2016 than in the other seasons.

**Table 1**  
Data on influent, effluent, and removal/reduction percentages during WWTP.

		Basic parameters			EEM-PARAFAC				
		DOC	$\text{UV}_{254}$	SUVA	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5
		(mgC/L)	(mAbs/cm)	(mAbs·L)/(mgC·cm)	(Score)	(Score)	(Score)	(Score)	(Score)
October 2016	Influent	27.2	282	10.4	1.22	0.51	1.66	0.66	3.00
	Effluent	5.9	79.5	13.4	0.14	0.40	0.83	0.64	1.50
	Removal/reduction %	78%	72%	–	88%	22%	50%	3%	50%
March 2017	Influent	19.1	455	23.9	1.80	0.57	1.48	0.74	2.92
	Effluent	4.5	98.2	21.9	0.16	0.39	0.71	0.64	1.75
	Removal/reduction %	76%	78%	–	91%	31%	52%	14%	40%
June 2017	Influent	14.3	457	31.9	1.28	0.65	1.60	1.12	3.39
	Effluent	4.3	97.6	22.7	0.19	0.45	0.84	0.84	1.60
	Removal/reduction %	70%	79%	–	85%	31%	47%	25%	53%
August 2017	Influent	17.9	370	20.6	1.27	0.54	1.47	0.88	1.75
	Effluent	4.1	89.3	22.0	0.17	0.44	0.77	0.77	0.97
	Removal/reduction %	77%	76%	–	87%	20%	48%	13%	44%
		HPLC-SEC							
		TOC detection			UVA detection				
		Peak 1	Peak 2	Peak 3 (and 4)	Peak 1	Peak 2	Peak 3		
		(Area)	(Area)	(Area)	(Area)	(Area)	(Area)		
October 2016	Influent	1283004	7586154	20809968	49662	5013606	4894044		
	Effluent	220724	2686482	2496860	28804	3463460	1735356		
	Removal/reduction %	83%	65%	88%	42%	31%	65%		
March 2017	Influent	–	–	–	–	–	–		
	Effluent	–	–	–	–	–	–		
	Removal/reduction %	–	–	–	–	–	–		
June 2017	Influent	2567490	7327640	10655250	1486370	5508675	4433625		
	Effluent	275133	3093937	2439484	65697	3048592	2335802		
	Removal/reduction %	89%	58%	77%	96%	45%	47%		
Aug. 2017	Influent	3077034	6629784	13187802	2962674	4662972	6954942		
	Effluent	207181	3225615	3108931	36568	2537282	3619719		
	Removal/reduction %	93%	51%	76%	99%	46%	48%		



**Fig. 2.** Size exclusion chromatograms of the WWTP influent (June 2017), detected by (a) TOC, (b) UVA (254 nm), and (c) fluorescence (excitation/emission of 340/430 nm).

Nevertheless, the ratios were higher in the influent than in aquatic environments such as rivers and lakes. For example, [Kawasaki et al. \(2011\)](#) reported that the area of the UVA in Peak 1 accounted for only about 1% of the total area of UVA in lake waters. In other words, the WWTP influent includes unique high-molecular-size DOMs having abundant UV-absorbing, which was not detected in aquatic environments.

The removal percentage of each peak in the WWTP was calculated by using the peak areas in the influent and effluent samples ([Table 1](#)). The results on the basis of TOC detection revealed that the removal percentages were 83%–93% (Peak 1), 51%–65% (Peak 2), and 76%–88% (Peaks 3 + 4). The implication is that the DOM associated with Peak 1 was easily degraded or transformed to the DOM with a lower molecular size by biological treatment in the WWTP and that the DOM associated with Peaks 3 + 4 was also efficiently biodegraded. In previous studies, low-molecular-size or intermediate-molecular-size DOMs in the range of 100–3,000 Da ([Audenaert et al., 2013](#)) or smaller than 1,000 Da ([Gong et al., 2008](#)) remained in the effluent discharged from WWTPs. In contrast to the case for Peak 1, the DOM associated with Peak 2 (around 1,100 Da) appeared to be relatively resistant to biological treatment. The refractory nature of the DOM associated with Peak 2 might have been due to the presence of decomposition products of Peak 1. The DOM associated with Peak 1 might not have been completely degraded to carbon dioxide and thus might have accumulated as DOM that was detected as Peak 2. This suggestion is consistent with the results of previous studies. [Wang and Wu \(2009\)](#) studied the molecular size distribution of DOM in a municipal wastewater treatment plant in Shanghai. They suggested that the large-molecular-size DOM in the influent was metabolized and degraded by microorganisms into relatively low-molecular-size DOM.

In terms of UVA detection, the removal percentages of Peak 1, Peak 2, and Peak 3 were 96%–99% (with the exception of a very low removal percentage of 42% in October 2016), 31%–46%, and 47%–65%, respectively ([Table 1](#)). A comparison of the removal percentages of each peak on the basis of UVA and TOC detections revealed that UVA > TOC for Peak 1 (except in October 2016), UVA < TOC for

Peak 2, and UVA < TOC for Peak 3. In summary, the biological treatments in the WWTPs were able to selectively remove “Peak 1 DOM with high UVA,” “Peak 2 DOM with low UVA,” and “Peak 3 DOM with low UVA.” Many studies have reported that high-molecular-size DOM with a peak location similar to that of Peak 1 is likely to be biopolymers or polysaccharide materials (e.g. [Her et al., 2002](#); [Myat et al., 2012](#); [Quaranta et al., 2012](#); [Kimura et al., 2014](#)); these high-molecular-size biopolymers or polysaccharide materials have been associated with low UVA, and their associated DOM was therefore likely to differ from “Peak 1 DOM with high UVA” in our study. Peak 3 DOM with low UVA seemed to correspond to low-molecular-size acids, as reported by [Huber et al. \(2011\)](#).

### 3.3. Variations in PARAFAC components during wastewater treatment

Five fluorescent components (C1 to C5) were identified in the EEM spectra ([Table 2](#)). From previous studies, C1 is likely to have originated from tryptophan-like compounds, and C2–C4 from humic-like or fulvic-like compounds ([Yamashita and Tanoue, 2003](#); [Stedmon and Markager, 2005](#)). The origin of C5, however, is unknown, but it may be related to the characteristics of WWTPs ([Galapate et al., 1998](#)). The PARAFAC scores were in the order of C5 > C3 ≈ C1 > C4 > C2 in the influent ([Table 1](#)), and it was apparent that protein-like DOM was abundant in the influent. In the case of the effluent, the order of the PARAFAC scores was C5 > C3 ≥ C4 > C2 > C1, and humic/fulvic-like DOM was abundant.

Removal percentages of the PARAFAC components were calculated by comparing the PARAFAC scores of each component between the influent and effluent; they were in the range of 85%–91% for C1, 20%–31% for C2, 47%–52% for C3, 3%–25% for C4, and 40%–53% for C5 ([Table 1](#)). The greater removal percentage of C1 than that of DOC (70%–78%) suggested that the fluorophore associated with the protein-like C1 was more easily and selectively removed than the other types of PARAFAC components. The higher removal percentage of C1 was consistent with the findings of [Riopel et al. \(2014\)](#), who reported that a WWTP removed an average of 60% of

**Table 2**  
PARAFAC components identified in this study.

Component	Excitation wavelength (nm)	Emission wavelength (nm)	Description
C1	280	350	Protein-like, derived from tryptophan
C2	330	385	Microbial, fulvic-like, and observed in wastewater samples Decomposed by photo-irradiation
C3	360	440	Humic-like with terrestrial origin; present in all freshwater samples
C4	420	480	Humic-like, observed in wetland samples
C5	490	520	Decomposes biologically after photodegradation Might be specific to sewage samples Information on this component is scarce

protein-like material. The C1 fluorophore is likely to be labile. For example, Lønborg et al. (2010) have studied the characteristics of the DOM in the Ria de Vigo coastal area of Spain and have found a highly linear relationship between protein-like fluorescence and the concentration of bioavailable DOC. Moreover, C1 is a good indicator of fecal coliforms (Sorensen et al., 2016). The high percentage removal of C1 in the biological treatment at the WWTP seemed to be due to the high bioavailability of C1 fluorophores.

Molecular size distributions of C3 fluorophores, the scores of which were the highest among the “humic/fulvic-like” components in the influent, were measured by using the fluorescence detector in the HPLC-SEC system at excitation/emission wavelengths of 340/430 nm. There was a peak in the molecular size distribution of the C3 fluorophores at about 900 Da—a peak that seemed to correspond to Peak 3 of the TOC and UVA distributions—with an additional small peak at about 100 Da (Fig. 2c). These results suggested that the humic-like fluorophore probably had a low molecular size of around 900 Da. Her et al. (2003) have also shown that humic-like DOM has low-molecular-size peaks on a molecular size distribution chromatogram; their findings are consistent with ours.

The removal percentages of the humic-like components C2, C3, and C4 were lower than those of DOC; the humic-like fluorophores were barely removed, especially in the case of C4. The percent removal of C4 was the lowest during the wastewater treatment processes. C4 has been described as a soil fulvic-like compound associated with anomeric acetal and ketal carbon (Chen et al., 2003; Williams et al., 2010).

C5 fluorophores, which may be specific to sewage (Galapate et al., 1998), were present in substantial amounts in the WWTP influents during this study. It has been suggested that C5 is not detected in natural bodies of water such as rivers, lakes, and coastal seas (Matthews et al., 1996; Galapate et al., 1998; Komatsu et al., 2005). In this study, a local thermocline in the river formed because of the WWTP effluent, which was warmer than the river water. The spread of effluent across the surface layer of the river was clearly apparent in the C5 analyses. In the river water near the outlet of the WWTP, the C5 PARAFAC score in the surface layer was 3.1 times the score in the bottom layer. This result implies that C5 was a component specific to the WWTP effluent. C5 may therefore be useful for detecting or tracing WWTP effluents in environmental waters. The absence of the peak on the EEM corresponding to C5 in both the influent and effluent of the human excreta treatment plant

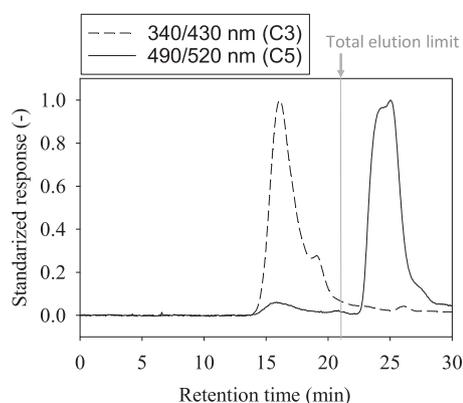


Fig. 4. Comparison in size exclusion chromatogram between C3 and C5, measured on fluorescence detection.

(Fig. 3) suggested that its source was not human waste but domestic, non-fecal wastewater that contained a detergent or fluorescent whitening agent.

C5 fluorophores have a peak at an excitation wavelength of 490 nm and emission wavelength of 520 nm (Table 2); no reported DOM studies have included an investigation of the source of C5 fluorophores. However, it is known that the fluorescence spectrum of fluorescein (one of the green fluorescence dyes) has a peak at the same location as C5 (Smart and Laidlaw, 1977). Fluorescein has a very high quantum efficiency and has been used as a tracer or marker of specific proteins especially in microscopic studies. In Japan, fluorescein has been used for coloring household bath salts. The source of C5 might therefore be wastewater from bathtubs in households or industrial wastewater, because wastewater from bathtubs accounts for a substantial part of household wastewater.

To compare the characteristics of C5 and fluorescein, the molecular size distribution of C5 was measured with the fluorescence detector in the HPLC-SEC system at excitation/emission wavelengths of 490/520 nm; however, the C5 fluorophores were eluted so slowly that the peak extended past the total elution time. The retention time of the peak could therefore not be converted to a molecular size (Fig. 4). Because C5 fluorophores may be adsorbed onto the column carrier by hydrophobic interactions, the C5

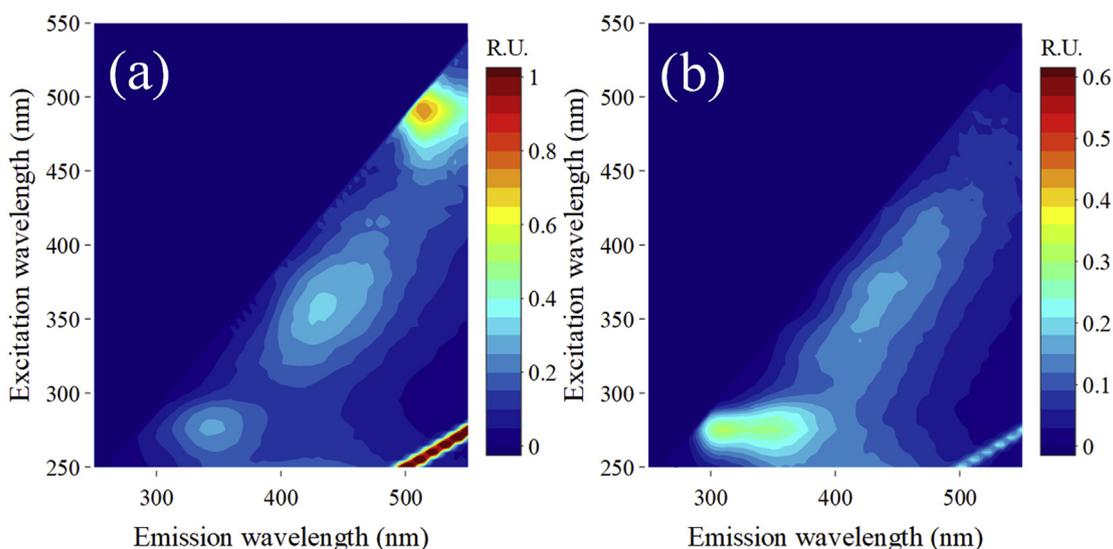


Fig. 3. EEM of (a) the influent of WWTP (June 2017) and (b) influent of human excreta treatment plant.

fluorophores may be very hydrophobic. Huber et al. (2011) have also suggested that hydrophobic interactions cause strong retention on a column medium composed of hydrophobic organic carbon. The implication is that the C5 fluorophores are likely to be very high in hydrophobicity. This line of reasoning seems to be in accord with the high density of benzene rings in fluorescein.

To investigate the molecular size of the C5 fluorophores, the influent during March 2017 was fractionated into two fractions, HMF and LMF, via a 10,000-Da cut-off ultrafiltration membrane. Successful separation of the HMF and LMF was verified by measuring the distribution of the molecular sizes of each fraction (Fig. 5). Interestingly, the presence of the EEM peak corresponding to C5 in only the LMF (Fig. 6) indicated that the C5 fluorophores were associated with DOM that had a molecular size below 10,000 Da on the basis of the HPLC-SEC analysis (intermediate- or low-molecular-size DOM).

Furthermore, the protein-like peak on the EEM (at excitation/emission wavelengths of 280/340 nm) corresponding to C1 was observed for both the HMF and LMF (Fig. 6); however, the peak intensity of the protein-like compounds in the HMF accounted for only 3% of the total (HMF + LMF). This result is a new discovery. No previous studies have quantified the partitioning of protein-like fluorophores between the HMF and LMF.

#### 3.4. Comparison of changes of DOM characteristics among treatment types

To determine the removal selectivity of each type of treatment processes, we compared various characteristics of  $\Delta$ DOM and  $\Delta$ DOC (Fig. 7). The points plotted for the samples collected in October 2016 were apparent as outliers (circled by dotted lines in Fig. 7). These data were outliers because the influent sample in October of 2016 had water quality characteristics that were very different from those of the samples collected during the other seasons (The SUVA was substantially lower than the SUVA during the other seasons as shown in Section 3.1.).

Plots of  $\Delta$ DOC against  $\Delta$ Peak 1 were linear, and no differences in this relationship were found among the treatment types (aerobic, anaerobic, and anoxic) (Fig. 7a and 7b). The concentration of high-molecular-size DOM (Peak 1 of the TOC detection) therefore decreased along with the total concentration of DOM (DOC concentration) in all treatment tanks. The implication is that the high-molecular-size DOM was removed efficiently, regardless of the treatment types.

Plotting  $\Delta$ DOC against  $\Delta$ Peak 2 (TOC detection) revealed a positive correlation, regardless of the treatment types (Fig. 7c). In the aerobic treatment,  $\Delta$ Peak 2 (TOC detection) was positive even when  $\Delta$ DOC was nearly zero. The concentration of intermediate-molecular-size DOM therefore decreased even when the total

concentration of DOM was not changed in the aerobic treatment. These results suggested that the intermediate-molecular-size DOM (Peak 2) was not so much biodegraded but instead was bio-transformed into low-molecular-size DOM during the aerobic treatment.

In the case of UVA detection, the relationship between  $\Delta$ DOC and  $\Delta$ Peak 2 (UVA detection) differed among the treatment types (Fig. 7d). In the aerobic treatment, the  $\Delta$ Peak 2 (UVA detection) values were positive, even when  $\Delta$ DOC was nearly zero. This pattern was very similar to the analogous pattern determined by TOC detection. However, in both the anaerobic and anoxic treatments,  $\Delta$ Peak 2 (UVA detection) was negative, even when  $\Delta$ DOC exceeded 10 mgC/L. In other words, in both anaerobic and anoxic treatments, the concentration of intermediate-molecular-size DOM with UVA seemed to increase, even when the DOM concentration (as DOC) decreased (i.e. was removed). Therefore, the high- or intermediate-molecular-size DOM with substantially low UVA seemed to be transformed into intermediate-molecular-size DOM with high UVA (with high hydrophobicity and aromaticity). This transformation appeared to result in the negative values of  $\Delta$ Peak 2 (UVA detection) in the anaerobic or anoxic treatment.

A strong linear relationship between  $\Delta$ DOC and  $\Delta$ Peak 3 (TOC detection) was observed, regardless of the treatment types (Fig. 7e). A plot of  $\Delta$ DOC versus  $\Delta$ Peak 3 (UVA detection) was weakly linear, and differences among the treatment types were not clearly apparent (Fig. 7f). This low-molecular-size DOM seemed to have been completely and easily biodegraded without transformation.

Plotting of  $\Delta$ DOC against  $\Delta$ C1 (Fig. 8a) revealed no differences among the treatment types (aerobic, anaerobic, and anoxic). The linearity between  $\Delta$ DOC and  $\Delta$ C1 suggested that protein-like DOM should be removed efficiently, regardless of the treatment types.

Plotting  $\Delta$ DOC against  $\Delta$ C2,  $\Delta$ C3, or  $\Delta$ C4 (humic-like) (Fig. 8b to d) revealed that these fluorophores could be removed more efficiently and selectively by aerobic treatment than by anaerobic/anoxic treatment. Furthermore, in aerobic treatment,  $\Delta$ C2,  $\Delta$ C3, and  $\Delta$ C4 were all positive, whereas  $\Delta$ DOC was nearly zero. In other words, in aerobic treatment, the concentrations of C2, C3, and C4 fluorophores decreased even when the total concentration of DOM was not changed. When  $\Delta$ DOC was low (typically in the latter stages of wastewater processing), humic-like DOM might have been transformed into DOM that lacked the fluorescence signatures of C2 to C4 and was not completely removed. The anaerobic and anoxic processes resulted in negative values of  $\Delta$ C4, despite a positive  $\Delta$ DOC. Other DOM, without the fluorescence of C4, might have been degraded or transformed into DOM with a C4 signature during these processes and thus might have contributed to the increase of C4 after the treatment.

In the targeted WWTP, treatment types A, B, C and D were prepared for the purpose of removing nutrients (not DOM).

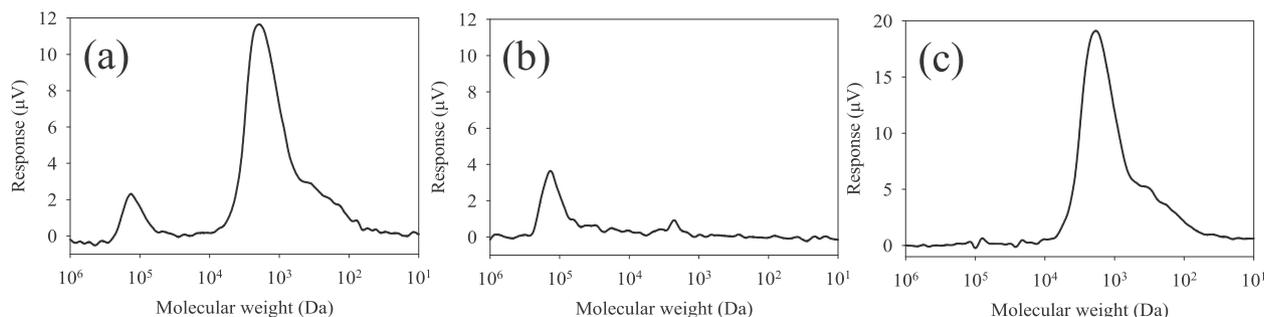


Fig. 5. Size exclusion chromatograms on TOC detection of (a) influent (b) high-molecular-size fraction of the influent, and (c) low-molecular-size fraction of the influent. Influent samples were collected in Mar. 2017.

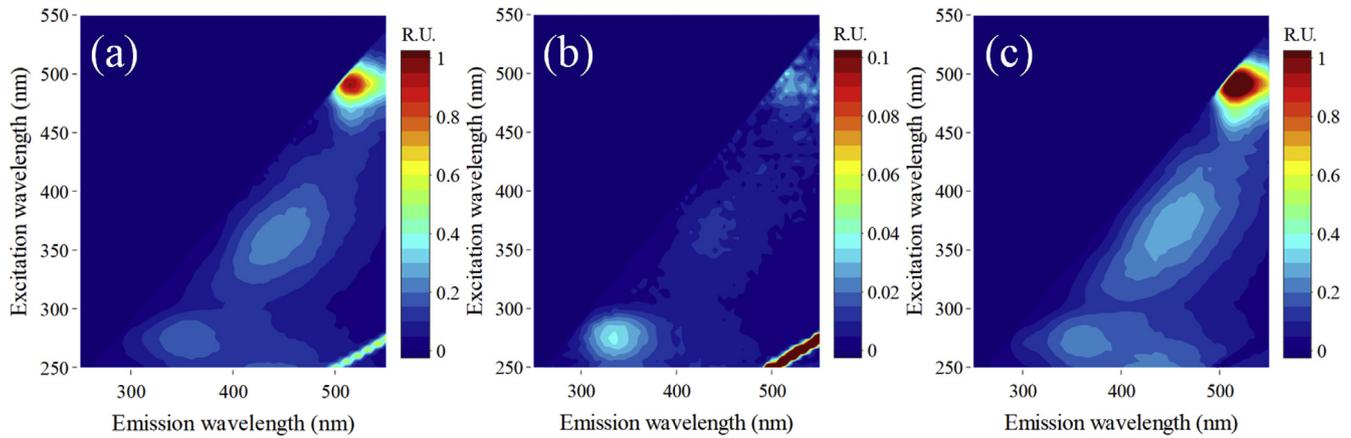


Fig. 6. EEM of (a) influent (b) high-molecular-size fraction of the influent, and (c) low-molecular-size fraction of the influent. Influent samples were collected in Mar. 2017.

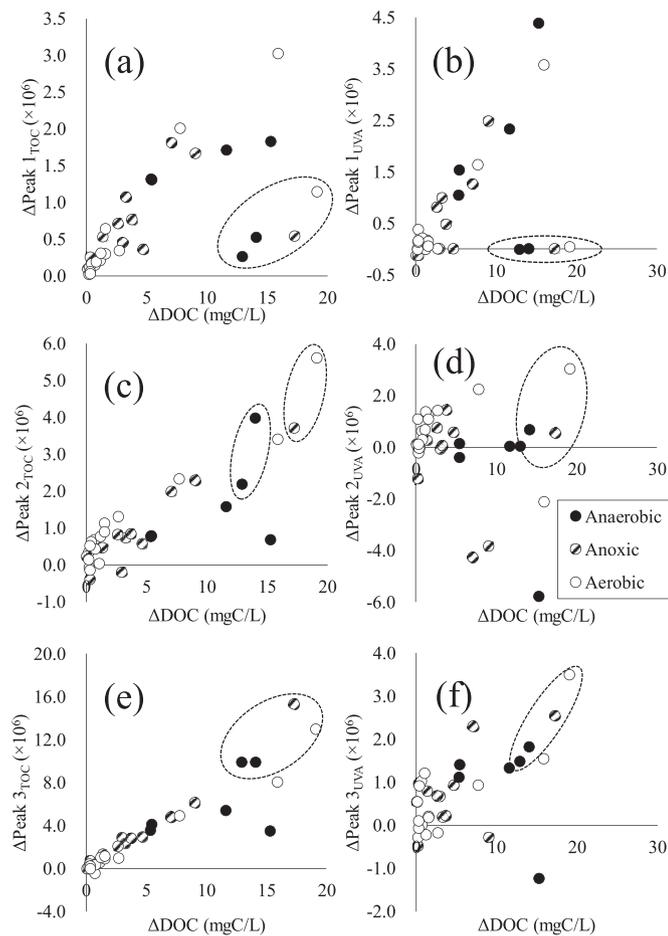


Fig. 7. Plots of  $\Delta$ DOC versus (a)  $\Delta$ Peak 1 (TOC), (b)  $\Delta$ Peak 1 (UVA), (c)  $\Delta$ Peak 2 (TOC), (d)  $\Delta$ Peak 2 (UVA), (e)  $\Delta$ Peak 3 (TOC), and (f)  $\Delta$ Peak 3 (UVA). The exceptional plotted points of the samples collected in October 2016 were circled by dotted line.

Therefore, the DOM removal feature did not differ much among treatment types A to D as a whole. However, detailed comparisons of the DOM removal performances among the treatment types revealed that the removal behavior was controlled by the aeration conditions or dissolved oxygen concentrations regardless of the preceding treatments. From the results of this study, the appropriate order of aerobic/anoxic/anaerobic treatment might be

proposed for the purpose of DOM removal.

### 3.5. Comparisons with previous studies, and a proposal for optimizing DOM removal in WWTPs

As described in Section 3.2., the high-molecular-size DOM with high UV absorbance (Peak 1) in this study seemed to differ from the “biopolymer” category to which high-molecular-size DOM is generally assigned (Huber et al., 2011). However, the removal efficiency of Peak 1 was similar to that of biopolymer—that is, it was relatively high, regardless of aerobic/anoxic/anaerobic treatment.

Intermediate-molecular-size DOM (Peak 2), the molecular size of which was around 1,000Da, is often classified as “humic substances” (Huber et al., 2011). In the field of aquatic chemistry, humic substances are operationally and strictly defined as the hydrophobic compounds in the fractionation by using XAD-8 resin (Leenheer, 1981; Imai et al., 2001; Komatsu et al., 2019) and DOM of around 1,000Da is not completely equivalent to humic substances. However, the single peak at the same retention time as Peak 2 was observed when the standard solution of humic substances provided by the International Humic Substances Society was analyzed by using the HPLC-SEC used in this study (data not shown). In previous studies, humic substances have been reported to be resistant to biological treatment and Peak 2 has been clearly observed in the effluent of WWTPs (Imai et al., 2002). In the PAR-AFAC components, C2, C3, and C4 are probably related to humic substances. This study revealed that the intermediate-molecular-size DOM with high UV absorbance and the fluorophores of C4 (named DOM-X) were increased in abundance or synthesized by both anoxic and anaerobic treatments. DOM-X was part of the humic substances and might contribute to the lower removal ratio of the total humic substances in biological treatments. Many studies (e.g. Goldman et al., 2012; Yu et al., 2013) have reported humic-like materials to be less decomposed than other DOMs by biological treatment; however, our study revealed for the first time that humic substances such as DOM-X may be produced by biological treatment in WWTPs.

In this study, low-molecular-size DOM (Peak 3) had low UV absorbance and was removed efficiently in aerobic, anoxic or anaerobic treatment. This result is consistent with previous studies, which have suggested that DOM under 1,000Da is easily biodegraded. For example, Guo et al. (2011) have reported that the peak of low-molecular-size organic materials (<500 Da) on the chromatogram of the influent vanishes after biological treatment. Furthermore, Gonzalez et al. (2013) have revealed that low molecular-size acid is easily removed by biological treatment.

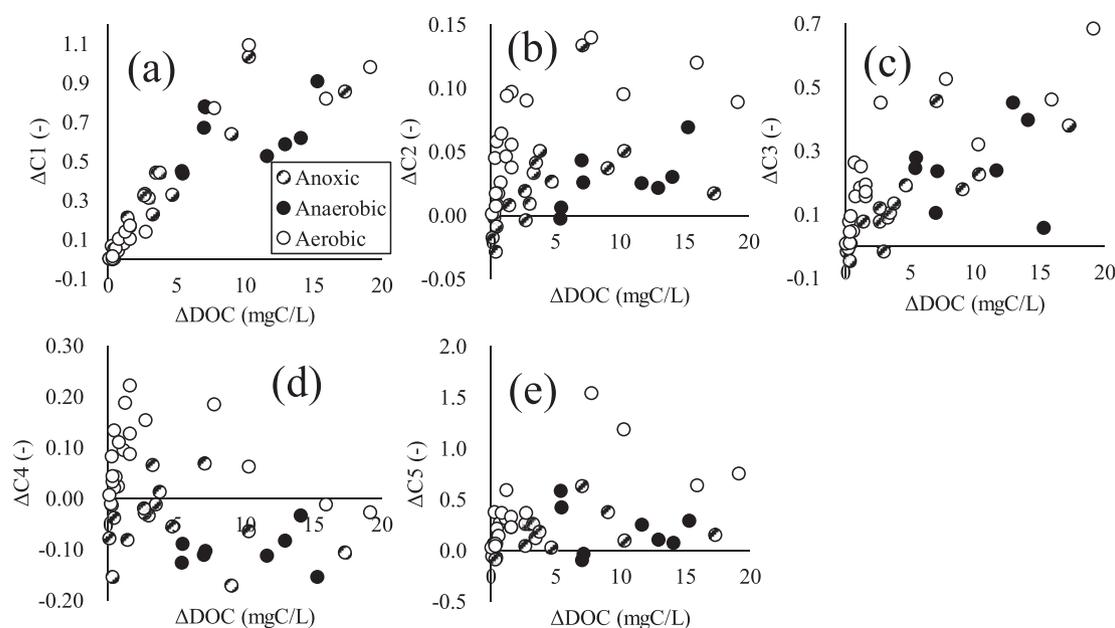


Fig. 8. Plots of  $\Delta$ DOC versus (a)  $\Delta$ C1, (b)  $\Delta$ C2, (c)  $\Delta$ C3, (d)  $\Delta$ C4, and (e)  $\Delta$ C5.

The protein-like component observed in this study was only C1. The removal characteristics of the protein-like component during biological treatments have been reported by many studies. Yu et al. (2014) have investigated DOM characteristics in the A<sub>2</sub>O process; they have suggested that tryptophan-like material is decomposed by both anaerobic and aerobic bacteria. Bridgeman et al. (2013) have investigated the change of two tryptophan-like peaks on EEM and have found that the fluorescence intensities of both peaks were reduced drastically in biological treatment with activated sludge. These results are consistent with our findings. However, Cheng et al. (2018) have characterized DOM during textile wastewater treatment and have found that, although the protein-like component was removed efficiently by aerobic process, the protein-like component was removed only slightly by anaerobic or anoxic processes. The removal efficiency of the protein-like DOM might be affected by the type of influent.

In summary, the heterogenous compounds in the DOM of the influent of WWTPs seemed to be homogenized into intermediate-molecular-size DOM with high hydrophobicity/aromaticity or into C4 fluorophores (DOM-X) by microorganisms under anoxic or anaerobic conditions. Furthermore, we discovered that the DOM-X could be transformed or removed by aerobic treatment. In addition, DOM-X seemed to have properties similar to those of humic substances, and it has been reported that humic substances could be selectively removed by physical or chemical treatments such as coagulation or adsorption by activated carbon (Komatsu et al., 2005; Patricia et al., 2005). The implication is, therefore, that introduction of aerobic treatment, or insertion of physical/chemical treatments at an appropriate stage in WWTP treatment processes should be an effective way to optimize DOM removal in WWTP.

#### 4. Conclusions

In this study, we investigated changes of DOM characteristics during WWTP processes. Three peaks (Peak 1 to Peak 3) were found on the HPLC-SEC chromatogram based on TOC detection. In those peaks, the DOM at Peak 2 (intermediate-molecular-size DOM) was relatively resistant to biological treatment. In the EEM-PARAFAC analysis, five fluorescent components (C1 to C5) were

identified. HPLC-SEC analysis and molecular size fractionation techniques revealed that C3 (humic-like) and C5 fluorophores (specific to WWTP effluent) were low-molecular-size DOM. Furthermore, the C1 fluorophores (protein-like) were observed in both the high-molecular-size fraction and the low-molecular-size fraction. Comparison of the changes of the DOC concentrations in each reaction tank and investigation of the removal selectivity of each treatment (aerobic, anaerobic, and anoxic) suggested that heterogenous compounds of DOM in the influent were homogenized into intermediate-molecular-size DOM with high hydrophobicity and aromaticity, or into C4 fluorophores (DOM-X), in anaerobic or anoxic treatment. We also discovered that DOM-X could be transformed or removed by aerobic treatment.

#### 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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watres.2019.115459>.

#### References

Audenaert, W.T.M., Vandierendonck, D., Van Hulle, S.W.H., Nopens, I., 2013. Comparison of ozone and HO induced conversion of effluent organic matter (EfOM)

- using ozonation and UV/H<sub>2</sub>O<sub>2</sub> treatment. *Water Res.* 47, 2387–2398.
- Baker, A., 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *ES T (Environ. Sci. Technol.)* 35, 948–953.
- Bixio, D., Thoeue, C., Koning, J.D., Joksimovic, D., Savic, D., Wintgens, T., Melin, T., 2006. Wastewater reuse in europe. *Desalination* 187, 89–101.
- Bridgeman, J., Baker, A., Carliell-Marquet, C., Carstea, E., 2013. Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. *Environ. Technol.* 34 (23), 3069–3077.
- Broek, T.A.B., Walker, B.D., Guilderson, T.P., McCarthy, M.D., 2017. Coupled ultrafiltration and solid phase extraction approach for the targeted study of semilabile high molecular weight and refractory low molecular weight dissolved organic matter. *Mar. Chem.* 194, 146–157.
- Carstea, E.M., Bridgeman, J., Baker, A., Reynolds, D.M., 2016. Fluorescence spectroscopy for wastewater monitoring: a review. *Water Res.* 95, 205–219.
- 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.
- Cheng, C., Wu, J., You, L., Tang, J., Chai, Y., Liu, B., Khan, M.F.S., 2018. Novel insights into variation of dissolved organic matter during textile wastewater treatment by fluorescence excitation emission matrix. *Chem. Eng. J.* 335, 13–21.
- Chon, K., Cho, J., 2016. Fouling behavior of dissolved organic matter in nanofiltration membranes from a pilot-scale drinking water treatment plant: an autopsy study. *Chem. Eng. J.* 295, 268–277.
- Cohen, E., Levy, G.J., Borisover, M., 2014. Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment. *Water Res.* 55, 323–334.
- Determann, S., Reuter, R., Wagner, P., 1994. Fluorescent matter in the eastern Atlantic Ocean. Part 1: method of measurement and near-surface distribution. *Deep Sea Res.* 1 41 (4), 659–675.
- Galapate, R.P., Baes, A.U., Ito, K., Mukai, T., Shoto, E., Okada, M., 1998. Detection of domestic wastes in Kurose River using synchronous fluorescence spectroscopy. *Water Res.* 32 (7), 2232–2239.
- Goffin, A., Guerin, S., Rocher, V., Varrault, G., 2018. Towards a better control of the wastewater treatment process: excitation-emission matrix fluorescence spectroscopy of dissolved organic matter as a predictive tool of soluble BOD<sub>5</sub> in influents of six Parisian wastewater treatment plants. *Environ. Sci. Pollut. Control Ser.* 25, 8765–8776.
- Goldman, J.H., Rounds, S.A., Needoba, J.A., 2012. Applications of fluorescence spectroscopy for predicting percent wastewater in an urban stream. *Environ. Sci. Technol.* 46 (8), 4374–4381.
- Gong, J., Liu, Y., Sun, X., 2008. O<sub>3</sub> and UV/O<sub>3</sub> oxidation of organic constituents of biotreated municipal wastewater. *Water Res.* 42, 1238–1244.
- González, O., Justo, A., Bacardit, J., Ferrero, E., Malfeito, J.J., Sans, C., 2013. Characterization and fate of effluent organic matter treated with UV/H<sub>2</sub>O<sub>2</sub> and ozonation. *Chem. Eng. J.* 226, 402–408.
- Guo, J., Peng, Y., Guo, J., Ma, J., Wang, W., Wang, B., 2011. Dissolved organic matter in biologically treated sewage effluent (BTSE): characteristics and comparison. *Desalination* 278 (1–3), 365–372.
- Han, Q., Yan, H., Zhang, F., Xue, N., Wang, Y., Chu, Y., Gao, B., 2015. Trihalomethanes (THMs) precursor fractions removal by coagulation and adsorption for biotreated municipal wastewater: molecular weight, hydrophobicity/hydrophilicity and fluorescence. *J. Hazard Mater.* 297, 119–126.
- Her, N., Amy, G., Foss, D., Cho, J., 2002. Variations of molecular weight estimation by HP-size exclusion chromatography with UVA versus online DOC detection. *Environ. Sci. Technol.* 36, 3393–3399.
- Her, N., Amy, G., McKnight, D., Sohn, J., Yoon, Y., 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. *Water Res.* 37 (17), 4295–4303.
- 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.
- Hur, J., Lee, T.-H., Lee, B.-M., 2011. Estimating the removal efficiency of refractory dissolved organic matter in wastewater treatment plants using a fluorescence technique. *Environ. Technol.* 32 (16), 1843–1850.
- Imai, A., Fukushima, T., Matsushige, K., Kim, Y.H., 2001. Fractionation and characterization of dissolved organic matter in a shallow eutrophic lake, its inflowing rivers, and other organic matter sources. *Water Res.* 35 (17), 4019–4028.
- 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.
- Intriago, J.C., Lopez-Galvez, F., Allende, A., Vivaldi, G.A., Camposo, S., Nicolas Nicolas, E., Alarcon, J.J., Pedrero Salcedo, F., 2018. Agricultural reuse of municipal wastewater through an integral water reclamation management. *J. Environ. Manag.* 213, 135–141.
- Jeong, H., Broesicke, O.A., Drew, B., Crittenden, J.C., 2018. Life cycle assessment of small-scale greywater reclamation systems combined with conventional centralized water systems for the City of Atlanta, Georgia. *J. Clean. Prod.* 174, 333–342.
- Jin, P., Jin, X., Bjerkelund, V.A., Østerhus, S.W., Wang, X.C., Yang, L., 2016. A study on the reactivity characteristics of dissolved effluent organic matter (EfOM) from municipal wastewater treatment plant during ozonation. *Water Res.* 88, 643–652.
- Kawasaki, N., Matsushige, K., Komatsu, K., Kohzu, A., Nara, F.W., Ogishi, F., Yahata, M., Mikami, H., Goto, T., Imai, A., 2011. Fast and precise method for HPLC-size exclusion chromatography with UV and TOC (NDIR) detection: importance of multiple detectors to evaluate the characteristics of dissolved organic matter. *Water Res.* 45 (18), 6240–6248.
- Kimura, K., Tanaka, K., Watanabe, Y., 2014. Microfiltration of different surface waters with/without coagulation: clear correlations between membrane fouling and hydrophilic biopolymers. *Water Res.* 49, 434–443.
- Komatsu, K., Nakajima, F., Furumai, H., Miki, O., 2005. Characterization of dissolved organic matter (DOM) removed by iron coagulation using spectrofluorimetry and pyrolysis GC/MS analysis. *Water Supply* 54 (3), 157–163.
- Komatsu, K., Imai, A., Kawasaki, N., 2019. Comparison between humic-like peaks in excitation-emission matrix spectra and resin-fractionated humic substances in aquatic environments. *Limnology* 20 (1), 109–120.
- 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.
- Liu, B., Wu, J., Cheng, C., Tang, J., Khan, M.F.S., Shen, J., 2019. Identification of textile wastewater in water bodies by fluorescence excitation emission matrix-parallel factor analysis and high-performance size exclusion chromatography. *Chemosphere* 216, 617–623.
- Lønborg, C., Álvarez-Salgado, X.A., Davidson, K., Martínez-García, S., Teira, E., 2010. Assessing the microbial bioavailability and degradation rate constants of dissolved organic matter by fluorescence spectroscopy in the coastal upwelling system of the Ría de Vigo. *Mar. Chem.* 119, 121–129.
- 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.
- Matthews, B.J.H., Jones, A.C., Theodorou, N.K., Tudhope, A.W., 1996. Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs. *Mar. Chem.* 55, 317–332.
- Meneses, M., Pasqualino, J.C., Castells, F., 2010. Environmental assessment of urban wastewater reuse: treatment alternatives and applications. *Chemosphere* 81, 266–272.
- Mostafa, K.M.G., Yoshioka, T., Konohira, E., Tanoue, E., Hayakawa, K., Takahashi, M., 2005. Three-dimensional fluorescence as a tool for investigating the dynamics of dissolved organic matter in the Lake Biwa watershed. *Limnology* 6, 101–115.
- Murphy, K.R., Hambly, A., Singh, S., Henderson, R.K., Baker, A., Stuetz, R., Khan, S.J., 2011. Organic matter fluorescence in municipal water recycling schemes: toward a unified PARAFAC model. *Environ. Sci. Technol.* 45 (7), 2909–2916.
- Murphy, K.R., Stedmon, C.A., Graeber, D., Bro, R., 2013. Fluorescence spectroscopy and multi-way techniques. *PARAFAC. Analytical Methods* 5, 6557–6566.
- Musikavong, C., Wattanachira, S., 2007. Reduction of dissolved organic matter in terms of DOC, UV-254, SUVA and THMFP in industrial estate wastewater treated by stabilization ponds. *Environ. Monit. Assess.* 134 (1–3), 489–497.
- Myat, D.T., Mergen, M., Zhao, O., Stewart, M.B., Orbell, J.D., Gray, S., 2012. Characterisation of organic matter in IX and PACI treated wastewater in relation to the fouling of a hydrophobic polypropylene membrane. *Water Res.* 46 (16), 5151–5164.
- Park, M.H., Lee, T.H., Lee, B.M., Hur, J., Park, D.H., 2010. Spectroscopic and chromatographic characterization of wastewater organic matter from a biological treatment plant. *Sensors (Basel)* 10 (1), 254–265.
- Patricia, A., Quinlivan, L., Detlef, R.U.K., 2005. Effects of activated carbon characteristics on the simultaneous adsorption of aqueous organic micropollutants and natural organic matter. *Water Res.* 39, 1663–1673.
- Pintillie, L., Torres, C.M., Teodosiu, C., Castells, F., 2016. Urban wastewater reclamation for industrial reuse: an LCA case study. *J. Clean. Prod.* 139, 1–14.
- 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.
- Riopel, R., Caron, F., Siemann, S., 2014. Fluorescence characterization of natural organic matter at a northern ontario wastewater treatment plant. *Water, Air, Soil Pollut.* 225, 2126.
- Rosario-Ortiz, F.L., Snyder, S.A., Suffet, I.H., 2007. Characterization of dissolved organic matter in drinking water sources impacted by multiple tributaries. *Water Res.* 41, 4115–4128.
- Shon, H.K., Vigneswaran, S., Kim, I.S., Cho, J., Ngo, H.H., 2004. The effect of pre-treatment to ultrafiltration of biologically treated sewage effluent: a detailed effluent organic matter (EfOM) characterization. *Water Res.* 38 (7), 1933–1939.
- Smart, P.L., Laidlaw, I.M.S., 1977. An evaluation of some fluorescent dyes for water tracing. *Water Resour. Res.* 13 (1), 15–33.
- Sorensen, J.P.R., Sadhu, A., Sampath, G., Sugden, S., Dutta Gupta, S., Lapworth, D.J., Marchant, B.P., Pedley, S., 2016. Are sanitation interventions a threat to drinking water supplies in rural India? An application of tryptophan-like fluorescence. *Water Res.* 88, 923–932.
- Stedmon, C.A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol Oceanogr. Methods* 6, 572–579.
- Stedmon, C.A., Markager, S., 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnol. Oceanogr.* 50 (2), 686–697.
- Tang, X., Wu, Q.Y., Zhao, X., Huang, H., Shi, X.J., Hu, H.Y., 2014. A fingerprint analysis method for characterization of dissolved organic matter in secondary effluents of municipal wastewater treatment plant. *Environ. Sci. Pollut. Res. Int.* 21 (24), 14211–14218.
- Tran, N.H., Ngo, H.H., Uruse, T., Gin, K.Y., 2015. A critical review on characterization strategies of organic matter for wastewater and water treatment processes. *Bioresour. Technol.* 193, 523–533.

- Wang, Z., Wu, Z., 2009. Distribution and transformation of molecular weight of organic matters in membrane bioreactor and conventional activated sludge process. *Chem. Eng. J.* 150 (2–3), 396–402.
- Wang, L., Li, Y.J., Xiong, Y., Tan, W.B., Zhang, L.Y., Li, X., Wang, X.S., Xu, J.F., Li, T.T., Wang, J.S., Cai, M.X., Xi, B.D., Wang, D.H., 2017. Spectroscopic characterization of DOM and the nitrogen removal mechanism during wastewater reclamation plant. *PLoS One* 12 (11), e0187355.
- Wei, Q., Wang, D., Wei, Q., Qiao, C., Shi, B., Tang, H., 2008. Size and resin fractionations of dissolved organic matter and trihalomethane precursors from four typical source waters in China. *Environ. Monit. Assess.* 141 (1–3), 347–357.
- Williams, C.J., Yamashita, Y., Wilson, H.F., Jaffé, R., Xenopoulos, M.A., 2010. Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. *Limnol. Oceanogr.* 55 (3), 1159–1171.
- Xu, Y.D., Yue, D.B., Zhu, Y., Nie, Y.F., 2006. Fractionation of dissolved organic matter in mature landfill leachate and its recycling by ultrafiltration and evaporation combined processes. *Chemosphere* 64 (6), 903–911.
- Xu, M., Bai, X., Pei, L., Pan, H., 2016. A research on application of water treatment technology for reclaimed water irrigation. *Int. J. Hydrogen Energy* 41 (35), 15930–15937.
- Yamashita, Y., Tanoue, E., 2003. Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. *Mar. Chem.* 82, 255–271.
- Yang, W., Li, X., Pan, B., Lv, L., Zhang, W., 2013. Effective removal of effluent organic matter (EfOM) from bio-treated coking wastewater by a recyclable aminated hyper-cross-linked polymer. *Water Res.* 47 (13), 4730–4738.
- Yu, H., Song, Y., Tu, X., Du, E., Liu, R., Peng, J., 2013. Assessing removal efficiency of dissolved organic matter in wastewater treatment using fluorescence excitation emission matrices with parallel factor analysis and second derivative synchronous fluorescence. *Bioresour. Technol.* 144, 595–601.
- Yu, H., Song, Y., Liu, R., Pan, H., Xiang, L., Qian, F., 2014. Identifying changes in dissolved organic matter content and characteristics by fluorescence spectroscopy coupled with self-organizing map and classification and regression tree analysis during wastewater treatment. *Chemosphere* 113, 79–86.
- Zhang, F., Wang, Y., Chu, Y., Gao, B., Yue, Q., Yang, Z., Li, Q., 2013. Reduction of organic matter and trihalomethane formation potential in reclaimed water from treated municipal wastewater by coagulation and adsorption. *Chem. Eng. J.* 223, 696–703.