



Formation of assimilable organic carbon (AOC) during drinking water disinfection: A microbiological prospect of disinfection byproducts

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ABSTRACT

Disinfection processes might alter the chemical structure of biological recalcitrant natural organic matter (NOM) in source water to form assimilable organic carbon (AOC), which can be readily utilized by microbes for growth. However, AOC has not been classified as disinfection byproducts (DBPs) before and little is known about the chemical and structural nature of AOC. This study, for the first time, considers the disinfection-induced AOC as DBPs from a microbiological perspective. The AOC formation by three types of disinfection processes, i.e., chlorination, UVC irradiation (254 nm) and photocatalysis represented by TiO₂-UVA in drinking water containing two reference NOM materials of Suwannee River and Nordic Reservoir (SRNOM and NRNOM, respectively) were comparatively benchmarked using *Pseudomonas aeruginosa* as inoculum. Results showed that chlorination caused a substantial increase in AOC content, whereas TiO₂-UVA led to a moderate increase in AOC content and UVC rendered the AOC content unchanged, independent of the types of NOM. Molecular weight indicated by spectral slope ratio and fluorescence fingerprint were found to not provide critical information about the AOC formation potential. FTIR and FT-ICR-MS results indicated that the AOC formation by chlorination was attributed to the oxidation and chlorine substitution on aromatic molecules to form molecules with carboxylic- and alcohol- functionalities, as well as chlorinated aromatics. These molecules could be metabolized and assimilated by *Pseudomonas* species by a catechol pathway. The results obtained in this study can provide valuable insight regarding the selection of proper technologies for disinfection to prevent microbial growth/regrowth in the distributing system and is intended to encourage more thinking and research on AOC as a new prospect of DBPs during disinfection of drinking water.

1. Introduction

Disinfection is an essential step at the end of drinking treatment plant for the final abatement of opportunistic pathogens in drinking water. However, microbial growth/regrowth could occur in the drinking water distribution systems (DWDS). This phenomenon poses a threat to the safety of the consumers (LeChevallier et al., 1996; Falkinham et al., 2001; Berry et al., 2006). The occurrence of assimilable organic carbon (AOC) in water is the controlling factor responsible for microbial growth and related water quality deterioration in the DWDS (Vanderkooij, 1992). AOC refers to the fraction of the total organic carbon (TOC) pool that can be readily taken up by heterotrophic microbes for proliferation, resulting in an increase in cell population

(Escobar and Randall, 2001). Therefore, the AOC level could be used as an important indicator to access microbial growth potential/biological stability of water in the DWDS (Hem and Efraimsson, 1999; Escobar and Randall, 2001).

The organic matter in surface water is generally referred as natural organic matter (NOM). NOM can be described as a complex mixture originated from a vast variety of living matters (i.e., animals, plants, microorganisms, etc.) that have been chemically or microbiologically degraded in diverse environment (Hudson et al., 2007; Matilainen et al., 2011; Sleighter and Hatcher, 2011). Some are derived from surrounding terrestrial landscapes and transported to water bodies (i.e. allochthonous), whereas some are created autochthonously through microbial activity (Hudson et al., 2007). Given the allochthonous- and

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autochthonous-derived NOM in surface water (e.g. main stream, reservoir) is likely to expose to microbes in the wild environment for some amount of time, AOC could have been selectively consumed by heterotrophic metabolism. Therefore, NOM that enters the drinking water sources is generally supposed to be relatively microbial refractory fraction such as humic substances. In drinking water source, the AOC may be originated from dissolved organic matter (DOM) released from microorganisms such as algal (Chen and Wangersky, 1996). For the source water with high AOC content, engineering bioreactors such as biofiltration (Halle et al., 2009) and biological aerated filters (BAF) (Huang et al., 2011) can be acted as pretreatment to specifically remove the AOC constituent. However, it is noteworthy that the chemical- and structural- properties of NOM pool would be reshaped by the disinfection process. This has initiated enormous researches on the formation of conventional disinfection byproducts (DBP) such as trihalo-methanes (THMs) and haloacetic acids (HAAs) during disinfection (Gjessing et al., 1999; Hua and Reckhow, 2007). These compounds are toxicological contaminants with mutagenicity, teratogenicity and carcinogenicity. Another concern is that the disinfected NOM may become AOC and pose uncertainty to the biological stability of the finished drinking water. The presence of AOC can support bacterial (re) growth and thus a certain level disinfectant residues is often applied in the pipeline system. Although the parameter of AOC was introduced several decades ago (Vanderkooij, 1992) and the formation of AOC was previously observed after a variety of disinfection methods (Ramseier et al., 2011; Liu et al., 2015; Li et al., 2018), AOC has not been classified as DBPs before. By definition, any new compounds formed after water disinfection should be considered as DBPs. Considering the microbiological nature of AOC as compared with the conventional toxicological DBPs, we propose that the disinfection-induced AOC should be regarded as DBPs from a microbial perspective. To the best of the author's knowledge, it is the first time that AOC is regarded as a new group of DBPs. More crucially, AOC is not yet monitored as a routine parameter in practical drinking water facilities. There is a general lack of law to regulate the AOC concentrations in the treated drinking water before entering the DWDS. The AOC, especially its formation after disinfection, is often overlooked by the operators of drinking water treatment plant (Wang et al., 2007; WHO, 2011; Grellier et al., 2015). The main reason could be attributed to the complex nature of AOC occurrence as a mixture of chemicals and the existing AOC assays are time and labour consuming. Therefore, it is desirable to understand the chemical- and structural- transformation of NOM during disinfection and link the corresponding AOC formation with instrumental surrogates.

The biological stability of the treated drinking water depends on the disinfection methods since each disinfection method reacts with organic matter through its own mechanism (Swietlik et al., 2009; Ramseier et al., 2011; Pigeot-Remy et al., 2012). In general, for a typical drinking water source, only a small fraction of the NOM is biodegradable because most of the organic matter is the recalcitrant fraction remaining from natural decomposition (Hudson et al., 2008; Henderson et al., 2009). At present, chlorine-based method is the most widely used disinfection method due to its relatively low cost and high effectiveness. However, there are increasing environmental concerns regarding formation of potentially carcinogenic and mutagenic DBP through chlorine reacted with NOM (Sharma et al., 2014). As such, the use of alternative disinfection technologies for drinking water disinfection has been extensively explored. One other treatment option is to use germicidal lamps which emits short-wavelength UVC (200–280 nm) light to inactivate the microorganisms by disrupting their DNA, leaving them unable to reproduce (Santos et al., 2013). Despite its high effectiveness, UVC might cause harmful effect to operators and requires expensive set up for lighting equipment. Another attractive technology is photocatalysis, which is regarded as treatment of future in disinfection due to its potential to utilize a cost-effective and renewable source of energy, namely solar energy, to drive disinfection process (Shannon et al.,

2008; Huang et al., 2017). Currently, the most widely used photocatalyst is titanium dioxide (TiO_2). Although the TiO_2 -based photocatalytic disinfection only makes use of small portion of the sunlight (< 5%), the majority of which is UVA (315–380 nm), the practicality of the technology still receives much research interest (Dalrymple et al., 2010). Previous literature has paid much efforts to elucidate and compare the mechanisms by which different disinfection technologies inactivate bacteria, and different depth of knowledge regarding the advantages, limitation and usefulness of different disinfection have been obtained (Pigeot-Remy et al., 2012; Rubio et al., 2013). However, little attempt has been made to understand how and to what extent AOC are formed from NOM treated by various disinfection technologies and their relationships with the NOM transformation.

In the present study, a most commonly found bacterial species in pipeline of DWDS, *Pseudomonas aeruginosa*, was selected to assess the extent of AOC formation from NOM upon chlorination, UVC irradiation and photocatalysis (TiO_2 -UVA). Moreover, various techniques including UV–VIS spectrum, fluorescence excitation-emission matrix (EEM), Fourier Transform infrared spectroscopy (FTIR), and Fourier Transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) were employed in an attempt to link the changes in structure- and chemical-transformations of the NOM to the corresponding AOC formation. The obtained results herein have significant implication for the drinking treatment design, operation as well as the disinfected downstream water quality assessment in the DWDS.

2. Materials and methods

2.1. Materials

Two well-characterized and purified IHSS NOM materials of the Suwannee River and Nordic Reservoir (SRNOM and NRNOM, respectively), which represent dominantly terrestrial and relatively more autochthonous sources of NOM in aquatic ecosystems, respectively. A detailed description of the reference samples is available online (<http://www.humicsubstances.org/>). TiO_2 (Degussa, P-25) was purchased from Evonik (Germany). All reagents used were of analytical grades. All glassware used in the experiments was AOC-free and was washed with ultrapure water and then autoclaved at 121 °C for 20 min to ensure sterility. Ten liters of real tap water was collected at the Chinese University of Hong Kong and used as the background water in all the disinfection experiments. Chlorination was applied as disinfection for tap water in Hong Kong. The collected tap water was filtered through a 0.22 μm sterile nitrocellulose membrane (Millipore, USA) to remove any existing microbes and stored in 4 °C prior to use (maximum 14 days). Real tap water was used because it could provide essential elements such as N, P, K and Na etc. for bacterial growth in a level similar to that of the pipeline of DWDS. Detailed information on the tap water quality including the $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ content were determined using the standard examination methods (Federation and Association, 2005) and can be found in the Table S1 in the Supporting Information (SI).

2.2. Disinfection experiments

The two NOM samples were dissolved in 50 mL of the collected tap water and adjust to a final concentration of 10 mg NOM/L, respectively. Then the prepared water samples were treated with various disinfection methods, including chlorination, UVC irradiation and photocatalysis, for different reaction time.

For chlorination, a free chlorine (Cl_2) stock solution (3000 mg Cl_2 /L) was prepared by diluting $\geq 4\%$ sodium hypochlorite (NaClO) (Aldrich, USA) and buffered to pH 8.0 ± 0.2 . The chlorine stock solution was then added into the NOM solution to a working concentration of 5 mg Cl_2 per mg carbon of NOM and stirred for 30, 60 and 120 min. After treatment, a certain volume of Na_2SO_3 (2 g/L) was

added into the solution, which would be capable of quenching all the Cl_2 added at the initial (Kristiana et al., 2014). The concentration of chlorine was determined at the initial and the end of the experiments with a HACH chlorine pocket colorimeter (Hach Co., Loveland, CO) in accordance with Standard Method 4500-Cl B (APHA, 2005). No chlorine was detected at the end of each chlorination experiment. For UVC irradiation, the NOM solution was irradiated under a UVC tube (peak at 254 nm) (Cole-Parmer, USA) at intensity 2 mW cm^{-2} for 60, 120 and 240 min. For photocatalysis, 100 mg/L of TiO_2 was added into the solution and then irradiated under a UVA-LED (LAMPLIC, China) at an intensity of 0.3 mW cm^{-2} for 15, 30 and 60 min. After each photocatalytic treatment, the TiO_2 particles were filtered by $0.22 \mu\text{m}$ sterile AOC-free nitrocellulose membrane. The disinfected water were then inoculated with bacterial cells and sterilely sealed with tinfoil for AOC quantification. A schematic illustration of the experimental procedures can be found in the SI (Fig. S1). All treatments were conducted in triplicate. The degree of NOM degradation was expressed by the change of UV absorbance at 254 nm (UV_{254}), which is attributable to the π - π^* transitions of aromatic ring (Matilainen et al., 2011). Different reaction time for various treatments was selected due to their power to react with NOM is different as shown by the decrease of UV_{254} (Fig. 1a). However, the comparison of AOC formation by different disinfection methods was still reasonable because all disinfection treatments can achieve disinfection purpose within the selected time. All treatment inactivated 5 log *Escherichia coli*, in the designed reaction times (data not shown). Moreover, the selected time was within the range of typical hydraulic retention time (HRT) for disinfection in water industry or previous literature (Paul et al., 2012; Pigeot-Remy et al., 2012; Wang et al., 2013).

2.3. Analysis of assimilable organic carbon (AOC)

To evaluate the AOC contents in water, *Pseudomonas aeruginosa* (*P. aeruginosa*) was chosen as inoculum because it is a commonly dominant species in tap water pipeline systems (Wang et al., 2013). *P. aeruginosa* was obtained from ATCC (10145). *P. aeruginosa* cells were maintained on Nutrient Agar (Lab M, Lancashire, United Kingdom) plate and stored at 4°C . Prior to AOC measurement, cells from single colony of the agar plate were inoculated into 50 mL liquid Nutrient Broth “E” medium and then incubated at 30°C with constant shaking until stationary growth phase (18 h). Then, the *P. aeruginosa* culture were harvested by centrifugation at 5000 rpm for 5 min and washed twice with sterile ultrapure water. Finally, they were re-suspended in ultrapure water. The cell density corresponded to approximately 2×10^9 colony-forming unit per milliliter (CFU/ml).

For each treated, untreated water sample and control experiment with solely Na_2SO_3 , inoculum of the as-prepared *P. aeruginosa* cells was added into the water and adjusted to an initial cell density of 2×10^4 CFU/ml. Then the water samples were incubated at 30°C with agitation. One milliliter aliquot was sampled every 24 h to monitor the cell concentration of *P. aeruginosa* using heterotrophic plate count methods (Huang et al., 2015) until stationary phase was reached (4 days). Then the final cell density was converted to carbon equivalents using the following equation:

$$\text{AOC}(\mu\text{gCL}^{-1}) = \frac{\text{net grown cell density (CFU L}^{-1})}{\text{conversion factor (CFU } \mu\text{gC}^{-1})} = \frac{S_{\text{final}} - S_{\text{initial}}}{k} \quad (1)$$

where S_{initial} and S_{final} represent the initial and final cell density of *P. aeruginosa* of the water, respectively; k was the conversion factor determined by the total cell numbers of 50 mL of *P. aeruginosa* cells at stationary phase divided by the corresponding TOC of freeze-dried cells. The k for *P. aeruginosa* was calculated as 5.63×10^6 CFU/ $\mu\text{g C}$, that is, 5.65×10^6 cells giving 1 $\mu\text{g AOC}$. Details of the calculation procedure of the conversion factor are described in the SI.

2.4. Characterization of NOM transformation

To better understand the observed effects of different disinfection methods on AOC formation, possible modification of NOM characteristics was investigated by using various array of instrumental tools including UV-VIS spectrometer, fluorescence EEM, FTIR and FT-ICR-MS, with an attempt to probe the potential characteristics of these complex compounds corresponding to the AOC formation. Detailed description of these methods can be found in the SI.

2.5. Statistical analyses

In this study, the cluster analysis was applied to the EEM and FT-ICR-MS datasets Matlab 12.0. Cluster analysis has been previously applied to NOM research to ascertain similarities/dissimilarities between NOM samples (Kujawinski et al., 2009; Meng et al., 2013). A shorter distance between clusters indicates a greater similarity. The FTIR datasets were analyzed with principal component analysis (PCA). PCA is a technique that is used to reduce the number of variables in a complex system to obtain significant principal components. The dataset of each FTIR spectrum was standardized by the sum of its data point between the region between 1800 and 450 cm^{-1} to increase the influence of variables with little variance, as well as to render the data dimensionless. PCA was carried out using Matlab software. In addition, the obtained AOC data of the three disinfection methods was analyzed by one-way ANOVA followed by Tukey test to determine whether the significant differences occur between the control (untreated NOM) and within individual treatment using SPSS software.

3. Results and discussion

3.1. Biological stability of treated NOM

The tap water mixing with 10 mg/L of SRNOM or NRNOM was firstly treated by chlorination, UVC or TiO_2 -UVA for different reaction time. The UV_{254} , a rough indicator of NOM concentration, dropped to different extents (Fig. 1a) but no significant variation ($p > 0.05$) in TOC (Fig. 1b) were observed for both NOMs treated by these three disinfection methods, implying that other than mineralization, the structural- or chemical- transformation of NOM mainly occurred on the chromophores of NOM.

The disinfected water was subsequently inoculated with *P. aeruginosa* to examine the biological stability of NOM after treatments by the three disinfection methods (Fig. S2). Firstly, *P. aeruginosa* were subjected to different growth kinetics and reached different final cell density in water treated by various disinfection methods. Secondly, identical treatment had similar growth curve independent of the type of NOM. Thirdly, prolonged reaction time did not have significant impact on cell density in all the treatments. Specifically, as shown in the representative growth curves (Fig. 1c and 1d), the cell density in chlorination and TiO_2 -UVA treated waters both first increased and then leveled off for SRNOM and NRNOM, respectively. Chlorination resulted in the highest growth in cell density (~ 7 log) regardless of the NOM types and chlorination reaction time, whereas TiO_2 -UVA treated NOM only led to slightly increase in cell densities (1–2 log). Conversely, a drop in cell density for both the UVC and control were observed after 1-day incubation, then the cell density gradually increased to the level of the initial concentrations after 4-day incubation for both SRNOM and NRNOM. This drop in cell density could be attributed to the cells underwent viable but nonculturable (VBNC) state in response to environmental stress such as starvation at the initial inoculation stage (Heim et al., 2002). These combined results indicated a trend of growing potential of NOM treated by the various disinfection methods follow an order from high to low as: chlorination > TiO_2 -UVA > UVC $\approx$ control (untreated), independent of the types of the NOM used in this study.

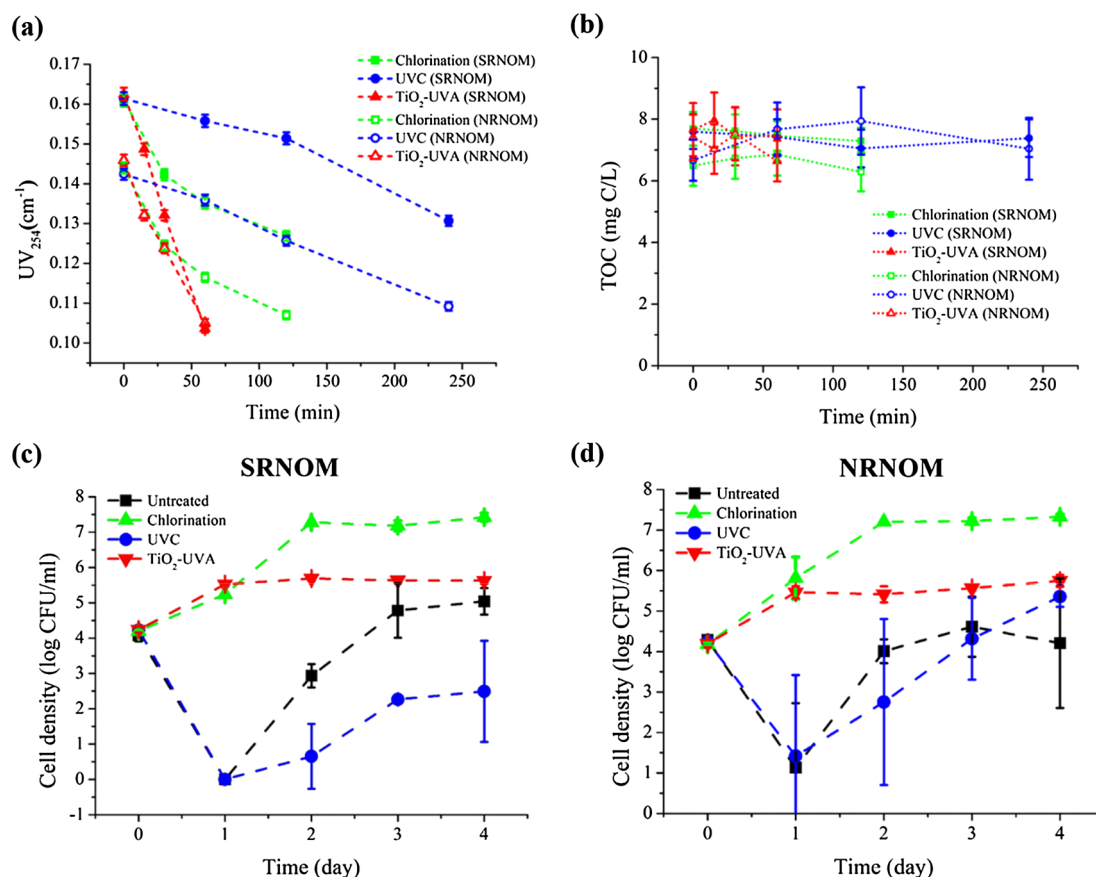


Fig. 1. Evolution of (a) UV absorbance at 254 nm (UV_{254}) and (b) TOC of SRNOM and NRNOM treated by various disinfection treatments. Representative growth curves of *Pseudomonas aeruginosa* cultivated in (c) SRNOM and (d) NRNOM after chlorination (60 min), UVC (240 min) and TiO₂-UVA (30 min). Bars represent standard deviation of three replicates.

To further quantify the biological stability of NOM by different disinfection methods, the assimilable organic carbon (AOC) were calculated using the final colony counts and expressed as acetate-carbon equivalents and shown in Fig. 2. The control experiment with solely Na₂SO₃ did not increase or decrease the cell density (data not shown), suggesting that the addition of Na₂SO₃ did not have an impact on AOC measurement of the chlorinated samples. In parallel with the cell density, chlorination yielded the highest ($p < 0.05$) AOC contents for both SRNOM and NRNOM (ranging from 373 to 455 $\mu\text{g C/L}$ for SRNOM and from 339 to 458 $\mu\text{g C/L}$ for NRNOM, respectively) among the three treatments. Interestingly, no significant difference of AOC for each treatment was observed among different reaction time for the both NOM as indicated by the high p values (> 0.05).

As for UVC, the formation of AOC was negligible compared with the control for both SRNOM and NRNOM ($p > 0.05$). As a consequence, AOC production can be avoided when UVC is applied for disinfection. For TiO₂-UVA, the AOC at reaction time of 15 and 30 min were slightly higher than the control, while at 60 min the AOC content was decreased to level similar to the control ($p > 0.05$) for both NOM. The major disinfectant in TiO₂-UVA is reported to be the highly reactive hydroxyl radical ($\cdot\text{OH}$) with an oxidation potential of 2.8 eV (Dalrymple et al., 2010), which means that it can virtually oxidize any forms of organics. Moreover, unlike chlorination, the disinfectant $\cdot\text{OH}$ would not decay but keep being produced in a TiO₂-UVA system if irradiation is continuously provided. It is reasonable to deduce that generated AOC can be further degraded with prolonged reaction time. Ideally, AOC can be completely migrated if sufficient reaction time is provided in a TiO₂-UVA system. It is worth noted that some organics may adsorb on the TiO₂ surface and could possibly contribute to AOC (Phong and Hur, 2016). However, after TiO₂-UV disinfection, TiO₂ particles must be

removed from the water and should not enter the drinking water distributing system. As such, the TiO₂ particles together with the adsorbed organics were filtered after each photocatalytic treatment and the adsorbed organics were not considered. To sum up, we can infer that different disinfection methods lead to different extent of AOC formation with the order from high to low as: chlorination $>$ TiO₂-UVA $>$ UVC $\approx$ control (untreated); and the AOC formation could be attributable to the physical-chemical transformation of NOM.

3.2. Transformation of NOM by various disinfection methods

To explore the possible transformation of the NOM and link the AOC formation with the corresponding transformation, various techniques including spectral slope ratio of UV-VIS spectrum, EEM, FITR and FT-ICR-MS, were applied to probe the characteristics of NOM induced by disinfection treatments. Since the most reaction time points did not have significant effect on the AOC formation in the individual disinfection treatment (Fig. S2), only one reaction time point was selected for each treatment for comparison. The rationale behind the selection of reaction time for each treatment was based on their ability to decrease the UV_{254} to the similar extent ($\sim 0.2 \text{ cm}^{-1}$ decrease) (Fig. 1a). Therefore, the NOM samples treated for reaction time of 60 min, 240 min and 30 min for chlorination, UVC and TiO₂-UVA, respectively, were selected for comparison.

3.2.1. Spectral slope ratio

Molecular weight is reported to be an important parameter that affects NOM bioavailability (Hammes et al., 2006). In general, low molecular weight NOM are smaller and expected to be easily taken-up by microbes. The spectral slope ratio (S_R), which is an indicator

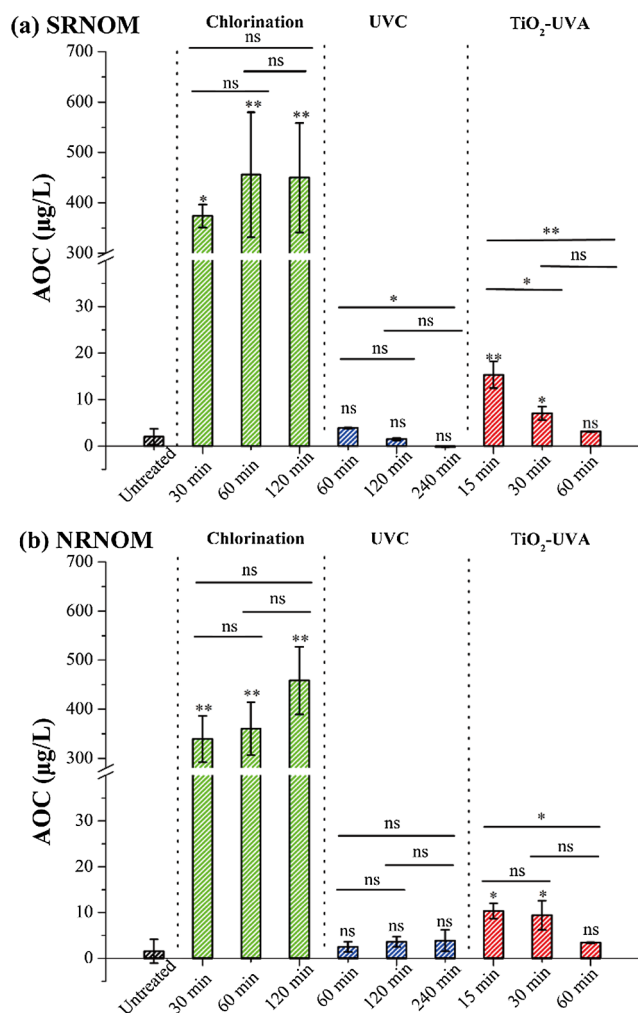


Fig. 2. Comparison of assimilable organic carbon (AOC) formation of NOM treated by various disinfection methods. (a) SRNOM and (b) NRNOM. Bars represent the standard deviation of three replicates. * ($p < 0.05$), ** ($p < 0.01$), and ns (not significant) indicate the significance of the difference between control (above the columns) and different reaction time point of individual treatment (above the transverse), obtained by one-way ANOVA.

reversely correlated with average molecular weight (MW) (Helms et al., 2008), was determined for the two NOM before and after different treatments (Table S2). Generally, S_R showed similar variation trend for the same treatment, whereas independent of the types of NOM. Specifically, an increase for SRNOM and NRNOM (from 0.80 to 0.95 and from 0.75 to 0.87, respectively) by TiO₂-UVA was observed, suggesting a decrease in MW. It is consistent with the previous findings that the photo-generated $\cdot\text{OH}$ from photocatalysis can break the high MW NOM into low MW NOM. Conversely, S_R values of the NOM by chlorination and UVC treatment exhibited a decline with chlorination to a greater extent, implying that chlorination and UVC preferentially reacted with the low MW NOM and hence resulting in an increase in the percentage of the high MW NOM. This result is somehow inconsistent with some previous studies, which emphasized a positive relation between the amount of low MW NOM and their bioavailability. In this study, chlorination treated NOM led to the highest AOC formation, whereas the high MW NOM was the dominant portion of the treated NOM. It should be noted that MW is not the only determinant factor that affects the NOM bioavailability, the structural- and chemical- characteristics of the NOM should also have significant impact. Therefore, MW is not a good surrogate to indicate AOC formation potential by different disinfection methods.

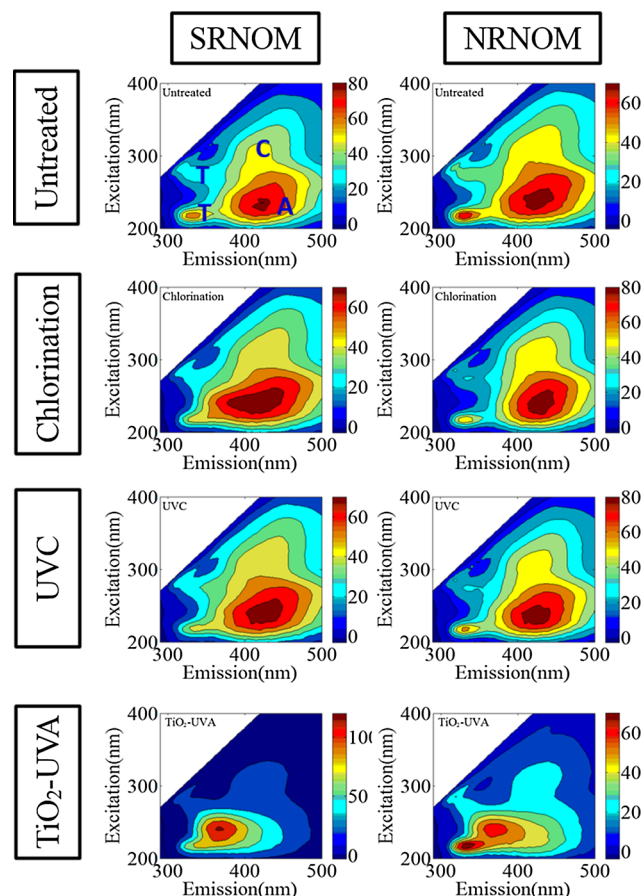


Fig. 3. Comparison of fluorescence excitation-emission-matrix (EEM) spectra of SRNOM (left panel) and NRNOM (right panel) treated by various disinfection methods, as well as the pristine NOM.

3.2.2. Fluorescence EEM

Numerous studies have applied fluorescence signature as a tool to track the source, characteristics, transformation as well as biodegradability of NOM (Hudson et al., 2007; Valencia et al., 2014). Fig. 3 displays the EEM contour plots of the two untreated NOM samples as well as their products treated by the three disinfection methods. Two distinct peaks at Ex/Em of 235/420 nm (peak A) and 305/420 nm (peak C) were both observed for the untreated SRNOM and NRNOM. Peak A and C were widely determined in previous literature and ascribed to humic-like substances that predominate in natural water (Hudson et al., 2007). Two minor peaks located at Ex/Em of 220/335 nm and 280/340 nm together were indicative of tryptophan-like compounds (peak T) (Hudson et al., 2007), which were originally present in the tap water (data not shown). Peak T generally predominates in wastewater and associated with biodegradable fraction therein. Similar to the spectral slope ratio, the EEM contour plots were generally found to be independent of the types of NOM in this study. Specifically, EEM peak intensities and contour shapes of water treated by chlorination and UVC were less impacted by visual inspection, whereas TiO₂-UVA led to substantially decrease in peak A and C intensities and occurrence of a new peak at Ex/Em of 240/370 nm. This observation was further evidenced by the cluster analysis of the EEM spectra (Fig. S3), in which the NOM samples treated by chlorination and UVC were grouped with the untreated samples, while the TiO₂-UVA treated samples were grouped into another cluster. These results suggest that TiO₂-UVA photocatalysis has strong ability to degrade the humic-like fractions of NOM. Similar observation was reported by Valencia and his coworkers (Valencia et al., 2014) who concluded the reduction in the NOM fluorescence intensities (peak A and C) could be

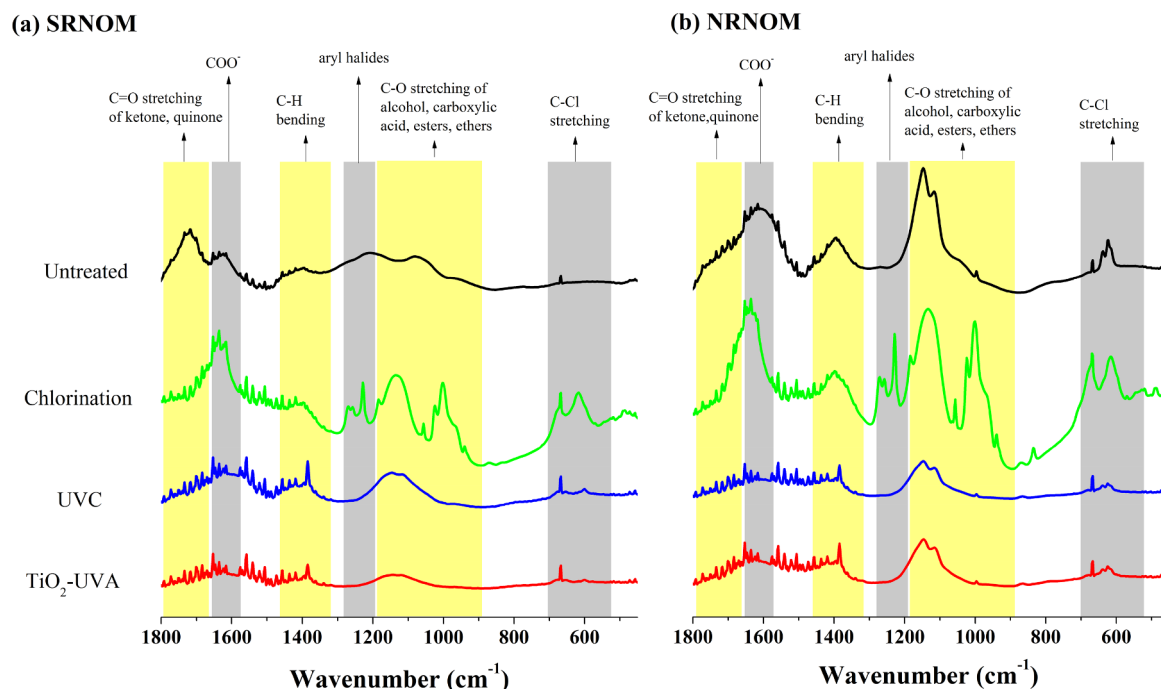


Fig. 4. FTIR spectra of NOM treated by chlorination, UVC and TiO_2 -UVA. (a) SRNOM and (b) NRRNOM. Shown are bands in the spectral region between 1800 and 450 cm^{-1} .

explained by the preferential break-up of high MW and with high UV_{254} molecules by TiO_2 photocatalysis, as a result leading to the reduction of aromatic compounds and formation of low MW molecules. Nevertheless, TiO_2 -UVA treatment only resulted in a moderate amount of AOC formation compared with chlorination, treated by which, the fluorescent signature of NOM remained almost unchanged. Therefore, fluorescence fingerprint cannot be used as a good indicator for AOC formation. It was also noted that the peak T in all the treatments were not obviously affected, suggesting that the difference in the AOC formation by the three disinfection treatments were not attributable to peak T.

3.2.3. FTIR analysis

Fig. 4 shows the FTIR spectral analysis of the two untreated NOM samples together with their responses to the three disinfection treatments. FTIR spectroscopy is a well-established, simple and non-destructive technique used to probe the functional aspects of NOM. The wavenumber region of $500\text{--}1800 \text{ cm}^{-1}$ is considered and assignment of major peaks are listed in Table S3. Similar to the result from EEM, the variation in FTIR spectrum generally depends on the type of disinfection method regardless of the NOM types. It was observed that TiO_2 -UVA and UVC treatments resulted in considerably decreased in the major peaks in the regions of $1800\text{--}1670$, $1470\text{--}1340$ and $1218\text{--}1030 \text{ cm}^{-1}$, which were attributed to C=O stretching of ketone, C-H bend of alkanes and C-O stretching (Matilainen et al., 2011), together with some jagged-like peaks in the region from 1400 to 1800 cm^{-1} , suggested a decrease in functionalities of NOM treated by TiO_2 -UVA and UVC and as a result noises appeared. Conversely, a common and prominent feature of chlorination was that chlorination resulted in enhancement of some functionalities compared with the pristine NOM. Peaks in the region of $710\text{--}505 \text{ cm}^{-1}$, which is indicative of C-Cl stretching vibration ($\nu_{\text{C-Cl}}$) were enhanced (Sundaraganesan et al., 2009), suggesting aromatic or aliphatic substitution of chlorine. Meanwhile, the peaks in region of $1650\text{--}1590 \text{ cm}^{-1}$, corresponding to asymmetric stretching of carboxylic functional group (COO^-) (Hay and Myneni, 2007), was also found to increase, suggesting oxidation of NOM. More interestingly, it was observed that chlorination was creating a group of new peaks in the regions of $1300\text{--}1210$ and

$1200\text{--}950 \text{ cm}^{-1}$, which could be attributable to aryl halides (Pein et al., 2010) and C-O (alcohols, carboxylic acids, esters, or ethers) (Matilainen et al., 2011).

More detail information on the variation of FTIR spectra was obtained by the principal component analysis (PCA) and the loadings and scores are shown in Fig. 5. Two principal component (PC) were found to explain the major variation (70%) of the FTIR spectra with PC1 and PC2 made up 40% and 30%, respectively, of the total variation. The loading of PC1 had a strong negative correlation at regions of $1800\text{--}1686$, $1360\text{--}1186$, 1027 and 940 cm^{-1} , which together indicative of oxygenated functionalities (Fig. 5a). The loading of PC2 showed a strong negative correlation at regions of $1691\text{--}1598$, $1293\text{--}1215$, $1028\text{--}945$ and $710\text{--}600 \text{ cm}^{-1}$, suggesting a component reversely correlated with COO^- , C-O containing, halogenated molecules (Fig. 5b). The scores of PC1 separated the untreated samples of SRNOM and NRRNOM (Fig. 5c), indicating difference in oxygenated functionalities possibly due to different sources. UVC and TiO_2 -UVA led to increase in the PC1, suggesting a decrease of overall oxygenated functionalities by these two treatments. On the other hand, the scores of PC2 separated the profiles of chlorinated NOM from those of other samples. These observations indicated that chlorination of NOM lead to formation of carboxylic acid (COO^-) and alcohol-like (C-O), and chlorinated aromatic compounds, which is well in line with the most proposed reaction pathway for chlorination of NOM molecules: (i) oxidation and (ii) electrophilic substitution, where a hydrogen is replaced by Cl (Deborde and von Gunten, 2008).

3.2.4. FT-ICR-MS analysis

The broadband spectra of the two NOM samples treated by the three disinfection methods are presented in Fig. S4. Several thousands of peaks were observed for the untreated NOM and NOM treated by chlorination and UVC, whereas no obvious peaks were detected after TiO_2 -UVA treatment. The resolving power was found to significantly improve when the samples were further analyzed in a narrow scan mode, namely sequential selective ion accumulation (SSIA) mode (Fig. S5). Therefore, the following discussion will be based on the SSIA results of the FT-ICR-MS analysis. Noted that the mass spectra were collected in segment in the SSIA mode, thus the scale of intensity was not

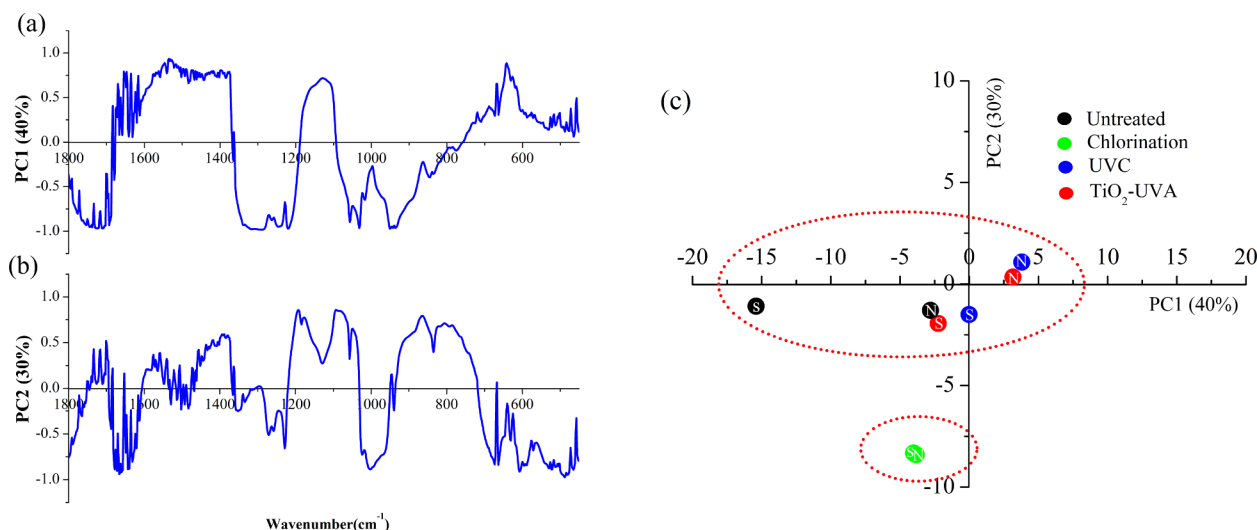


Fig. 5. Principal component analysis (PCA) of the FTIR spectra. Loadings of (a) principal component 1 (PC1), and (b) principal component 2 (PC2); and (c) scores of the NOM samples. S and N denotes the SRNOM and NRNOM, respectively. Shown are bands in the spectral region between 1800 and 450 cm^{-1} .

uniform in each slice, and the results therefore are only being used in presence/absence basis. The number of peaks observed for the samples were listed in Table S4. Thousands of peaks were found in all the samples. Insight into the reactivity of NOM was obtained by examining the mass spectra in greater detail. Fig. 6 compares the counts of new and raw (originally presented in the untreated NOM) peaks in every 50 m/z region. It was found that all the treatments led to the formation of enormous new peaks, which was indicative of the formation of new compounds by all the three methods. Therefore, the observed AOC formation by chlorination and TiO_2 -UVA could be attributed to the new peaks in the mass spectrum while those raw peaks were indicative of recalcitrant compounds to corresponding disinfection method. Cluster analysis of the data indicated that the transformation of NOM were mainly dependent on the disinfection methods, while independent of the types of NOM samples (Fig. S6). Particularly, the UVC treatment were clustered away from these treatments. This observation somehow paralleled with the formation of AOC by the three disinfection treatments, where AOC content were changed after chlorination and TiO_2 -UVA but not UVC treatments.

The KMD mass defect analysis shown that the untreated NOM samples consist of numerous homologous series molecules, which differs only by one/multiple CH_2 group (Figs. S7 and S8). The exact masses (in amu) of ^{12}C , ^1H , and ^{16}O are 12.000000, 1.007825, and 15.994915, respectively. Hydrogen has a positive mass defect, while oxygen has a negative mass defect. Thus, compounds that are oxygen-rich and/or hydrogen-poor will occur as peaks at a lower KMD mass defect (~ 0.0 – 0.2), while compounds that are hydrogen-rich and/or oxygen-poor give peaks with a higher mass defect (Kim et al., 2003). We found that new mass features with lower KMD appeared in the Kendrick mass defect plots of the NOM samples treated by TiO_2 -UVA, which were indicative of oxidation (Fig. S9). Interestingly, prominent clusters appeared in the bottom-left and top-right regions of the NOM samples treated by chlorination (Fig. 7 and S10). These clusters were responsible for chlorine substitution of hydrogen (H/Cl) on the NOM molecules as revealed by the H/Cl-mass defect analysis, in which the clusters had exact mass interval equivalent to the mass difference between ^{35}Cl and ^{37}Cl (1.997 Da). The FT-ICR-MS results herein provide a qualitative method for tracking the AOC content in similar matrices. While we acknowledge that the FT-ICR-MS analysis in this study has its own limitations and may not be able to give a full reflection of the complex AOC content in water. Firstly, the result is limited that only positive mode for electrospray ionization (ESI) was tested since the negative ionization mode may give very different mass spectra

(Sleighter and Hatcher, 2007). Secondly, the FT-ICR-MS have a relatively high mass cutoff (290 m/z in this study), suggesting that molecules with low MW are neglected, while these fractions were proposed to contribute to AOC. Thirdly, the electrospray ionization is biased to easily ionized compounds and hence compounds with low ionization efficiency would be suppressed, especially when different treatment processes were applied to the NOM samples. Finally, solid-phase extraction (SPE) was not performed to enrich the samples as three distinct disinfection methods were employed in this study. It is reasonable to probe differences in assigned molecular formulas and their relative abundance in similar samples from a treatment process, but not comparable between different treatments because sample-type dependent lost in the organic composition may occur during SPE process (Chen et al., 2016). SPE is generally an indispensable pretreatment for the enrichment of organic contents in surface water samples to ensure sufficient resolution for FT-ICR-MS analysis (Zhang et al., 2012). Therefore, formula assignments that require high resolution were not valid in this study owing to the low concentrations of organics in the samples. Researches using FT-ICR-MS instruments with ultrahigh resolution (e.g. 15 T) should thus be warranted in the future.

Nevertheless, it was still possible to qualitatively conclude that the FT-ICR-MS analyses together with the FTIR results, suggested the formation of oxidation products as well as chlorinated NOM after chlorination. These were also consistent with the experimental results in earlier studies. For example, Ramseier et al. (2011) reported oxalate (highly oxidized form organic compounds) were detected during the chlorination of NOM. Lavonen et al. (2013) found that substitution of chlorine preferentially occurred on the aromatic NOM with oxygen-containing functional groups. Therefore, the significant increase of AOC formation by chlorination of NOM could be attributable to the formation of carboxylic acid, alcohol-like substances, as well as the chlorinated aromatic. Besides, the comparison of the four analytical techniques is summarized in Table S5.

3.3. Utilization of AOC

The bioavailability of carboxylic acid- and alcohol-like organic matters were well established and previous studies on biodegradability of NOM and their oxidation products have mainly focused on carbohydrates and organic acids (Hammes et al., 2006; Hammes et al., 2007; Swietlik et al., 2009). However, the bacterial utilization of aromatic, especially the halogenated aromatic molecules, has seldom been taken into consideration though aromatic compounds are relatively rich in

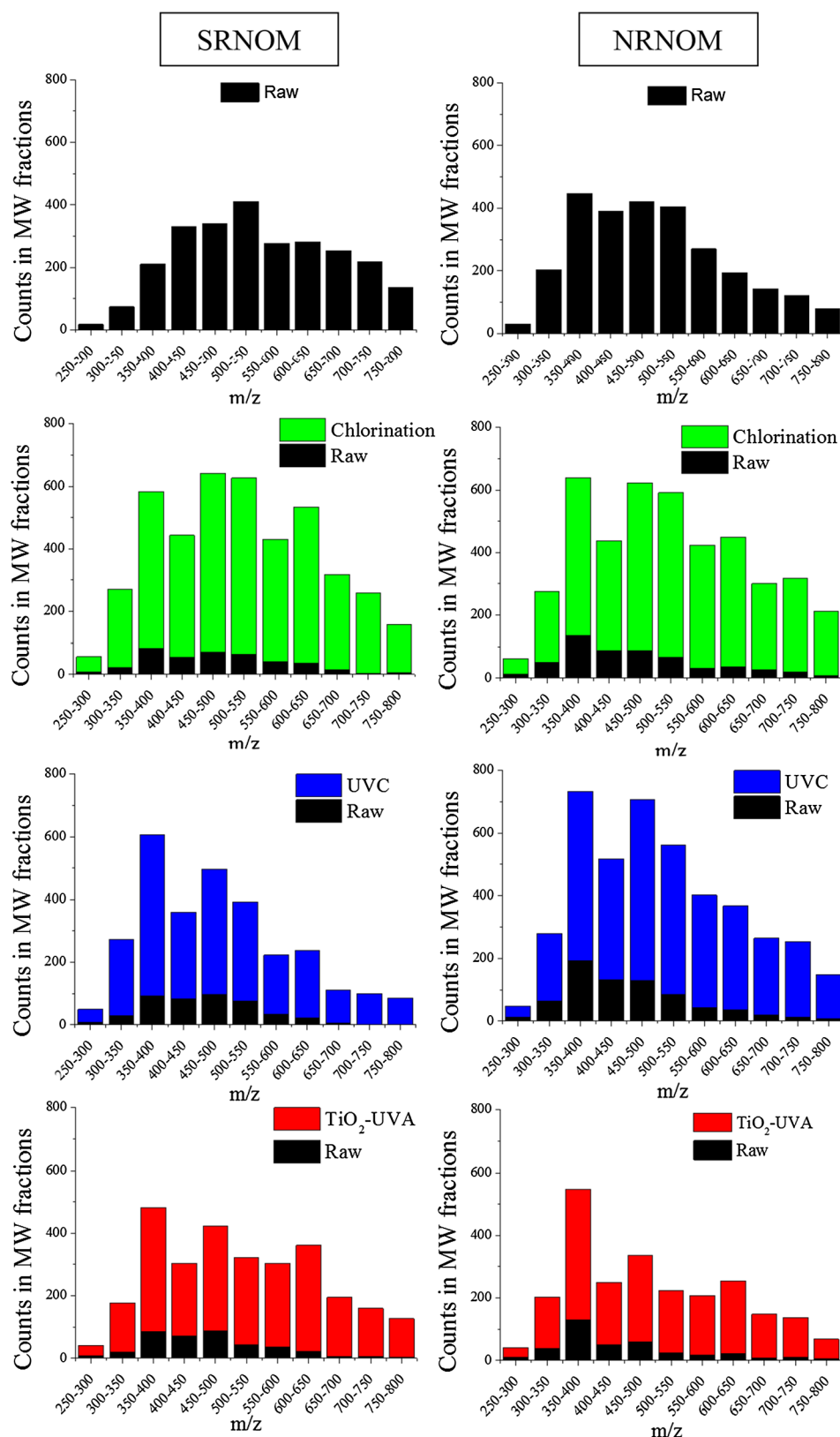


Fig. 6. Counts of new and raw (originally presented in the untreated NOM) peaks in every 50 m/z molecular weight (MW) segment.

the NOM. Aromatic compounds is often implicated as a reason for microbial persistence due to the exceptional stability associated with the benzene rings. Furthermore, chlorination of aromatic molecules typically renders them become the notoriously toxic compounds. Yet bacteria such as *Pseudomonas* and *Rhodococcus* species have evolved to

metabolize oxidized and chlorinated benzene derivatives. An example is the benzoate and 2-chlorobenzoate metabolism through a catechol (a central intermediate) pathway (Fig. 8), ultimately directing its carbon into the tricarboxylic acid (TCA) cycles (Słonczewski and Foster, 2013). Therefore, the metabolites in the TCA cycles could provide substrates as

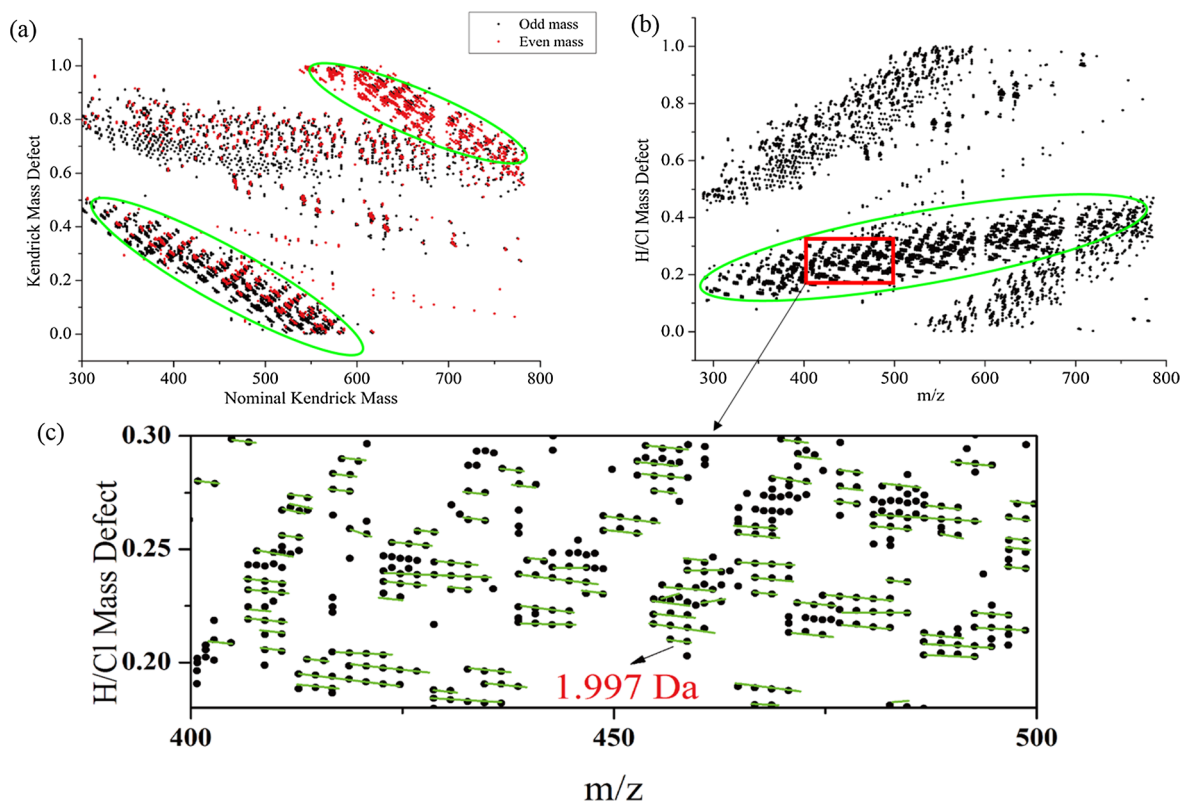


Fig. 7. Kendrick mass defect (using a CH_2 group) vs nominal Kendrick mass plot (a) and H/Cl (substitution of a hydrogen atom by a chlorine atom) mass defect vs measured m/z plot (b) for SRNOM treated by chlorination. Even and odd mass were plotted separately in the nominal Kendrick mass for simpler visual interpretation of homologous series differed by CH_2 . The red box (c) is expanded from (b) to highlight the clusters (green line) that consists of isotopic peaks separated by 1.997 Da, which is the exact mass difference between ^{35}Cl and ^{37}Cl , indicating that they are chlorinated NOM molecules. The green ellipses are indicative of chlorinated NOM clusters.

well as energy for biosynthesis and thus contribute to significant source of AOC from NOM treated by chlorination. In this regard, it is of great importance to note that the obtained AOC data in this study is bacterial-species-dependent and may be different if other bacterial species were used as the inoculum. Indeed, the concept of AOC in drinking water was originally developed by Van Der Kooij et al. (1982). The typical inoculum for the AOC bioassay can be laboratory pure cultures such as *Pseudomonas fluorescens* P-17 and *Spirillum NOX* (Escobar and Randall, 2001). Later, other species such as *Escherichia coli*, *P. aeruginosa* (Vital et al., 2010) or mixtures of environmental bacteria (Hammes et al., 2006; Ramseier et al., 2011) were selected as inoculum to obtain a more realistic interpretation of AOC under relevant environmental conditions. Despite the relevance of AOC data to the water industry, the formation of AOC by disinfection has often been neglected in practice. Currently, there is no consensus on using which bacterial species to measure the AOC concentration. In the present study, we found that significant amount of AOC were generated from NOM upon chlorination using *P. aeruginosa*, and the AOC can be ascribed to the formation of carboxylic acid- and alcohol-like organic matters as well as the oxidized or chlorinated aromatic molecules. These phenomena could imply that the disinfection method may have a selection effect on the bacterial species in the DWDS and presumably a reason to the query that why *P. aeruginosa* is a commonly dominant species in the DWDS, given the fact that chlorination is the most widely applied disinfection method in practice. Future work using natural microbial consortium as the test organisms, together with molecular biological approaches to examine the microbial diversity and activities upon NOM treated by various disinfection methods would be of great merit to provide more relevant results (Douterelo et al., 2014; Ng et al., 2015).

3.4. Engineered implications for control of microbial growth/regrowth in DWDS

Water is an environmental resource that is essential to ensure good physical health and wellbeing of human. The water supply systems must be dimensioned to send acceptable and safe water to the end users. Although various effective disinfection methods have been developed and applied, microbial growth/regrowth is frequently found in the DWDS (Muyima and Ngcakani, 1998). Therefore, attention has recently been transferred from the water quality in the plant effluent to the water quality in the end users. The AOC is a key parameter that determining the degree of microbial growth/regrowth in the system (Escobar and Randall, 2001). As disinfection may have a potential to affect the AOC profiles, this study attempt to estimate the AOC formation potential from NOM upon chlorination, UVC and TiO_2 -UVA disinfection. The results from this study suggest that, under tested conditions, chlorination led to the highest increase in the AOC formation followed by TiO_2 -UVA, whereas UVC showed no effect on the AOC formation, regardless types of the NOM and reaction time. This is expected to provide more in-depth information on the microbial growth/regrowth phenomenon in the DWDS and its causes and therefore proper technology may be selected on a case by case basis in the design of drinking water treatment plants. For instance, considering the selection of proper disinfection technology for surface water containing NOM, UVC is preferred to minimize the AOC formation to prevent microbial growth in the DWDS. Nevertheless, the UVC disinfection suffers from a serious disadvantage, for example, the reactivation and regrowth of bacteria (Hu et al., 2005). Bacteria have evolved mechanism to repair the damage induced by UVC irradiation (Sancar, 1996), thus the disinfection efficiency is reduced. If chlorination is applied, secondary treatment such as adding low levels of disinfectants into DWDS is

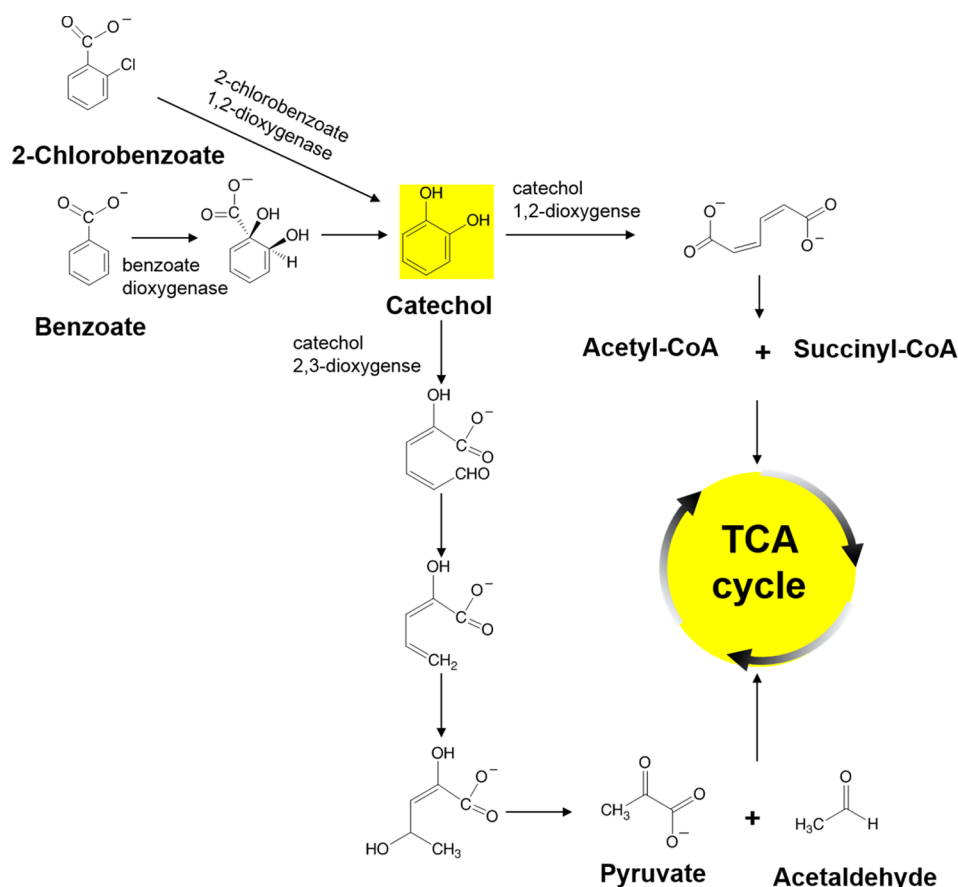


Fig. 8. Schematic representation of the pathways of benzoate and chlorinated benzoate (2-chlorobenzoate) metabolisms. Benzoate and chlorinated derivatives are each converted to catechol by a specific dioxygenase. The intermediate catechol then undergoes another key oxidation by a catechol dioxygenase, either oxidized the 1, 2 position, or the 2, 3 position. The carbon skeleton in each case is further oxidized and directed into the TCA cycle.

essential to prevent potential microbial growth. Photocatalysis has emerged as a “green” technology for disinfection, however, there exists a concern that photocatalytic disinfection no residual effect in the DWDS. In the present study, only moderate amount of AOC was produced by the TiO_2 -UVA (from 3 to 15 $\mu\text{g C/L}$), which is below the limit of 50 $\mu\text{g C/L}$ to avoid microbial growth as suggested in the literature (Lechevallier et al., 1991), and more crucially, it is expected to further decrease with prolonged reaction time. Therefore, the concern regarding the residual effect of photocatalytic disinfection might be overestimated and the prevention of potential microbial growth in the DWDS could be achieved by employing a sufficient enough reaction time of the photocatalytic treatment. We acknowledge that this study is limited to only three disinfection technology, namely chlorination, photocatalysis and UVC. It would be of great value to examine the AOC formation potential by other practical disinfection methods such as chloramination or ozonation in the future.

4. Conclusions

This study evaluated one potential downstream effect of chlorination, UVC irradiation and TiO_2 -UVA disinfections on the formation of a new prospect of disinfection byproducts – AOC. Instead of carcinogenic properties like other DBPs such as THM when one consumes, this new DBP will promote microbial growth (i.e. biological safety) in finished drinking water. This DBP is totally against the original objectives of water disinfection. The major conclusions can be drawn as follows:

- Bench-scale experiments demonstrated that: AOC formation potential follows the order from high to low as: Chlorination > TiO_2 -UVA > UVC, regardless of the types of NOM used in this study. This has significant implications for the disinfected drinking water since AOC has been correlated with increased bacterial populations

in DWDS, especially in the absence of secondary disinfection.

- Molecular weight indicated by spectral slope ratio and fluorescence fingerprint can be used as a fast monitoring tool, but they did not provide critical information about the AOC formation potential. FTIR and FT-ICR-MS can be easily obtained and used to characterize NOM in the disinfected water and to quickly predict AOC formation. A major disadvantage of FT-ICR-MS is the relatively high mass cutoff (290 m/z in this study), which means that the low molecular AOC (< 290 Da) was not evaluated.
- FTIR and FT-ICR-MS results revealed the significant increase in AOC formation of NOM treated by chlorination was attributed to the oxidation and chlorine substitution on aromatic molecules, and these molecules could be metabolized and assimilated by *Pseudomonas* species by a catechol pathway.

Declaration of Competing Interest

We declare there is no conflict of interest.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105389>.

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