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Migration and transformation of nitrogen in algae organic matter (AOM) during the growth of Microcystis aeruginous

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ABSTRACT

Microcystis aeruginous were cultured to study on the algae organic matter (AOM) migration, transformation and potential to produce nitrogenous disinfection by-products (N-DBPs) during algae growth, dissolved organic nitrogen (DON) concentration shows an upward tendency. The migration of nitrogen in different inorganic ions were studied. Large molecular weight substances in EOM gradually transform into small molecules with the growth of algae, while the molecular weight distribution of IOM stay stable. EOM is mainly composed of humic acid-like materials and soluble microbial products, while IOM is mainly composed of aromatic proteins and soluble microbial products. The strongly hydrophobic organic matter (HPO) possesses the highest proportion. In IOM, but the hydrophilic fraction of IOM presents the highest N-DBPs formation potential. The hydrophilic organic matter (HPI) is the major content of EOM, but the HPO presents the highest N-DBPs formation potential.

Keywords: *Microcystis aeruginous*; Transformation of nitrogen forms; Dissolved organic nitrogen (DON); Nitrogenous disinfection by-products (N-DBPs)

1. Introduction

Frequent occurrence of algal blooms in lakes, reservoirs and slow-moving streams poses great challenges to drinking water safety and potable water production processes. During algal blooming, the concentration of algae increases abruptly, and the algae cells release undesirable organic matters via metabolites excretion. Algal organic matter (AOM) consists of extracellular organic matter (EOM) and intracellular organic matter (IOM). Usually, the EOM derives from metabolites excretion of algal cells, while the IOM is released when cells are dying or algal-laden water goes through water treatment processes, such as pre-oxidation before coagulation. AOM appears to contain an appreciable amount of dissolved organic nitrogen (DON) and hydrophilic substances, but a low content of aromatic carbon [1-4]. Both EOM and IOM contain biopolymers, such as proteins, peptides and amino acids, contributing to the high concentration of DON in algae-containing eutrophication water. The portion of protein in IOM is reported larger than that in EOM [5].

DON is an important fraction of dissolved organic matter (DOM) and a key element in the formation of nitrogenous disinfection byproducts (N-DBPs). DON contains amino acids, proteins, amino sugars, amides, nitriles, pyrroles, purines and pyrimidines, which can be widespread found in surface water. Typically, DON commonly derives from soluble microbial products (SMPs), extracellular organic matter of algae, forest litter and agricultural fertilizers [6]. Although DON comprises a small portion of natural organic matter (NOM) by weight (0.5–10%), compositions of the DON. It can react with chlorine or chloramine during disinfection process and form halogenated nitrogenous dis-

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infection by-products (N-DBPs), such as haloacetamides (HAcAms), halonitromethanes (HNMs), haloacetonitriles (HANs) [1,2,7,8]. W. Lee et al. [9] report that DON cannot be effectively removed in the conventional water treatment process, causing the increase in disinfectant/oxidant consumption, formation of disinfection by-products (DBPs) and membrane fouling. Typically, algae are rich in organic nitrogen (org-N), which contributes to the formation of N-DBPs whose genotoxicity and carcinogenicity are higher than that of regular DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs) [1–3]. Current research focus on forming mechanism of N-DBPs during chlorine disinfection of algae containing water, commonly using the algae cells in exponential growth period. However, the release and the characteristics of AOM are statistical variable within the different growth period of algae. Also, research about the transformation mechanism of different nitrogen forms, especially DON, is rarely found.

Recently, the correlation between the characteristics of DON deriving from AOM and the precursors of N-DBPs has been paid more attention, while the results remain uncertain. H. Chang et al. [10] found that the characteristics rather than the amounts of DON materials dominate NDMA yields. Lee et al. [11] indicated that the nitrogen content of DOM affects N-DBP yields in chlorination, and the DOC/DON ratios could be applied to estimate the approximate formation. The yield of N-DBPs (DCAcAm and DCAN) is positively correlated with the concentration of DON [12]. Moreover, organic matter characteristics, such as molecular size, hydrophobicity, organic nitrogen content, and aromatic content, play important roles in the formation of DBPs during chlorination [13].

As acknowledged, it is necessary to provide a clear mechanism to control the formation of N-DBPs based on the research of the correlation analysis among the characteristics, concentration of DON and its N-DBPs formation potential. To realize the characteristics of algae organic matter, several methods have been reported in the literature. This includes UV-visible absorbance, fluorescence excitation emission matrix (EEM), high-pressure size exclusive chromatography (HPSEC) coupled with UV/fluorescence/DOC detection (or ultrafiltration membrane separation), and those using the Fourier transform infrared (FTIR) spectrophotometer, solid state 13C NMR spectroscopy and Pyrolysis-GC-MS [2,3,14]. Most researches focused on characteristics of AOM during the exponential phase or stationary phase of algae [1–4], less is known about the characteristics variation of AOM in different growth periodicity of algae. Henderson et al. [15] reported that the EOM of *Microcystis aeruginosa* was dominated by polysaccharides and proteins, and its MW (molecular weight) distribution was characterized by a bimodal distribution with 55% > 30 kDa and 38% < 1 kDa. However, Gao [4] reported that MW distributions of IOM and EOM follow a trimodal distribution pattern. MW fractions of IOM in the ranges of <1 kDa (chlorophyl, algaetoxin, amino acids and other small molecules), 40–800 kDa, and >800 kDa (mostly phycocyanin and carbohydrates) were 27%, 42%, and 31% of the total DOC, respectively. So far, no conclusion had yet been made on that issue.

The objectives of this study are: (1) provide a comprehensive understanding of the transformation of different nitrogen in the growth of algae; (2) study the variation of NH_4^+-N, NO_2^--N, NO_3^--N and DON concentration during algae cultures; (3) characterize physic-chemical properties of EOM and IOM; (4)analysis the hydrophilic property of IOM and EOM extracted in decline phase; (5) compare their potential in forming N-DBPs in chlorine disinfection.

2. Materials and methods

2.1. Algae cultivation procedure

M. aeruginosa (blue-green algae, Collection No. FACHB-905) was purchased from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). *M. aeruginosa* cells in exponential phase were inoculated in 750 mL BG11 medium, contained in 1000 mL flasks. The algal cells were maintained in an incubator at 298 K, with an illumination at 1500–2000lx under a 12 h light/12 hdark (12L/12D) regimen. There was not medium supplement during the culture. Cell populations were measured by counting at least 100 cells in triplicate using a light microscope (Olympus) and hemocytometer, determining the growth curve of *M. aeruginosa* in special culture conditions described above and the specific time horizon of exponential, stationary and decline growth phases.

2.2. Procedures for IOM and EOM separation

To characterize the EOM and IOM of algae in different growth phases, centrifugal process was applied. Different algal cells are taken during exponential (1–5 d), stationary (6–33 d) and decline (34–131 d) growth phases and centrifuged by using Fichal centrifuge (TDL-4A, Shanghai) at 4000 rpm for 20 min to separate EOM from the cell. The supernatant was then filtered by 0.45 μm PES membrane filter; the filtrate obtained was referred to as EOM solution. The algal cells separated after the centrifugation were mixed with purified water, and was subjected to three freeze/thawing cycles. Finally, the cells suspension was filtered through 0.45 μm PES membranes. The organic matters in the filtrate were referred to as IOM [4].

2.3. Analytical methods

Some water quantity parameters, such as DOC, UV254 and SUVA, are measured to indirectly reflect the variation of compounds in water (Table 1). DOC was measured by Shimadzu TOC-VCSH analyzer (high-temperature combustion at 720°C; non-dispersive infrared detection). UV absorbance at 254 nm (UV254) was determined by spectrophotometer (DR5000, Hach) using a 1 cm quartz cell, and SUVA was calculated as UV254/DOC. NH_4^+-N, NO_2^--N, NO_3^--N was measured through salicylate-hypochlorite method, N-(1-naphthyl)-ethylenediamine photometric method, UV spectrophotometry method respectively [16]. Total dissolved nitrogen (TDN), the sum of NH_4^+-N, NO_2^--N, NO_3^--N and DON, was measured through alkaline potassium persulfate digestion-UV spectrophotometric method [17]. DON was quantified as the difference between TDN and the dissolved inorganic nitrogen [17–19].

Table 1
Properties of EOM and IOM from different growth phases

Sample (cm^{-1})		UV254 (mg/L)	DOC (L/mg·m)	SUVA (mg/L)	DON	DON/DOC
Exponential phase	EOM	0.14	10.36	1.34	n.d[a]	n.d
	IOM	0.24	27.94	0.87	1.47	0.053
Stationary phase	EOM	0.24	27.60	0.88	n.d	n.d
	IOM	0.30	16.73	1.79	4.16	0.25
Decline phase	EOM	0.43	39.62	1.10	n.d	n.d
	IOM	0.13	5.52	2.34	0.53	0.10

[a] n.d: not determined

2.4. Molecular weight (MW) fractionation

To characterize the MW distribution of AOM in different growth phases, it was fractionated into six groups after filtration by a series of PES UF membranes (Mosu, Shanghai) with MW cut-offs of 100, 30, 10, 5 and 1 kDa in terminal stirred cell (MSC-300, Shanghai). Prior to filtration, the membranes were flushed with Milli-Q water to ensure that DOC in the leachate is less than 0.1 mg/L. High purity nitrogen (99.999%) was applied to pressurize the filtration process (0.15 MPa), and the leachate was collected and stored at 278 K before analysis. The percentages of DON, DOC and UV254 in each MW fraction were calculated via the method described above [9,20,21].

2.5. Separation of hydrophilic and hydrophobic compositions

In separation of hydrophilic and hydrophobic compositions, following procedure are needed: adjust pH of the water samples, filtrate by 0.45 μm glass fiber filter, add 2 mL high concentrated HCl, and pass the samples through the DAX-8 and XAD-4 resin adsorbent columns at flow rate of 1 mL/min. The composition in effluent is regarded as hydrophilic organic matter. Eluting the DAX-8 and XAD-4 with half volume of water samples 0.1 M NaOH at the flow rate of 1 mL/min, the resulting solution mainly contains strongly hydrophobic organic matter. Eluting the DAX-8 and XAD-4 with ultrapure water at the same flow rate, the resulting solution contains weakly hydrophobic organic matter.

2.6. EEM fluorescence spectroscopy

Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy (F-7000 FL Spectrophotometer, Hitachi, Japan) is applied to characterize algae organic matter. Excitation (Ex) wavelength was set from 200 to 550 nm at 5 nm scanning intervals, meanwhile emission (Em) wavelengths from 220 to 650 nm at the same intervals. The excitation and emissions slits were set at 5 nm, with the scanning speed of 60,000 nm/min [20,22].

2.7. Measurement of formation potential of nitrogenous disinfection by-products(N-DBPFP)

The experimental procedure for the determination of DBPFP refers to the method given by Xie et al. [13]. First, adjust the water pH to about 7.0. Second, take 120 mL water sample into a 100 mL blue cap reagent bottle, and a certain volume of sodium hypochlorite solution at a ratio of mass concentration to Cl_2/DOC more than 5. The reaction is carried it in a biochemical incubator at 298 K for 7 days in the dark. Finally, sodium bisulfite was used to terminate the reaction, and then the sample can be used for N-DBPs analysis.

2.8. Measurement of nitrogenous disinfection by-product

Disinfection by-product such as dichloroacetonitrile, trichloroacetonitrile and trichloronitromethane were measured by liquid-liquid extraction gas chromatographic method combined with GC-ECD analysis, which was based on USEPA 551.1. The instrument used for analysis is Shimadzu GC2010PLUS equipped with CD-5 separator column (30 m × 0.25 mm, 0.25 μm, GAEQ-521511). The heating process of separator column are as follows: heat up the separator column to 308 K and hold for 6 min; heat up the column to 373 K at a rate of 10 K/min and hold for 5 min; heat up the column to 493 K at a rate of 40 K/min and hold for 2 min. The injection mode is a splitless injection (instantaneous splitless). High purity nitrogen was used as carrier gas. The temperature at the injection port and the detector were 473 K and 563 K, respectively. The tail-blowing flow is 30 mL/min. The injection volume is 1 μL, the column flow is 1 mL/min. The average linear velocity of the carrier gas flow is about 25 cm/s.

3. Results and discussion

3.1. Algal growth

The algae were cultured in a batch reactor without adding supplementary medium during the whole growth periodicity. Changes in algae concentration are used to distinguish the four growth phases: lag, exponential, stationary, and decline phase (Fig. 1). The lag phase of *Microcystis aeruginous* growth in this experiment lasted approximately 5 days, during which no significant changes in cell number were observed. The exponential phase lasted about 28 d following the lag phase. Dramatic increases in algal concentration were found during exponential phase. The stationary phase, with relatively adequate nutrition in the medium,

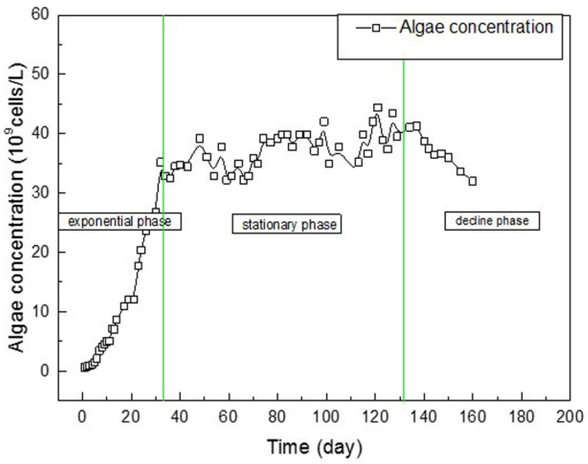

Fig. 1. Batch growth curves for *Microcystis aeruginous* (single column fitting image).

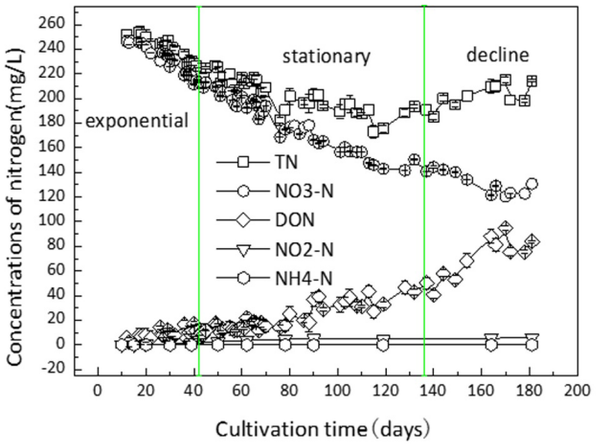

Fig. 2. Variation of different nitrogen during the growth of *Microcystis aeruginous* (single column fitting image).

lasted a long period from 34th day to 131th day, after which the cell numbers began to decrease and entered into the decline phase. This is quite conflicting with Yang et al.'s [23] study that the four growth stages of *Microcystis aeruginous* are 0–3 d, 4–22 d, 23–28 d, >28d, respectively. The growth curve gives an reference for further study on the characteristics of AOM in different growth stages of *Microcystis aeruginous*.

3.2. Transformation of different forms of nitrogen during algae growth

Variation of different forms of nitrogen in the cultivation medium during the growth was studied, and the results are shown in Fig. 2. It can be seen from the figure that the nitrate concentration of the solution decreased during the growth of algae (Fig. 2) from about 250 mg/l at the initial to 120 mg/l at the end of this study. The concentration of nitrite and ammonia was so low that can hardly be detected during the exponential growth phase, and then rose up slowly in the stationary and early decline stage (Fig. 2). The increase of nitrite concentration can be ascribed to the autotrophic denitrification under dark conditions, while the increase of ammonia can be ascribed to the decomposition of intracellular and extracellular compounds. The total nitrogen (TN) concentration of the solution was decreased gradually from about 250 mg/l to 180 mg/l in the exponential growth phase and the early stationary phase. It got stable in the late stationary phase and showed a slow increase trend in the final decline growth phase from 190 mg/l to 220 mg/l (Fig. 2).

After the analysis of the TN and TIN (Total Inorganic Nitrogen), the variation of DON concentration became the key to this study. It can be seen that, the DON concentration stayed stable in the exponential and the early stationary growth phase (Fig. 3). In the late stationary and the declining growth phase, the DON concentration in the solution rose apparently, and its concentration kept at about 80 mg/l at the end of the study.

As acknowledged, algae cell proliferate are accelerated in exponential growth phase, and the cell number rose

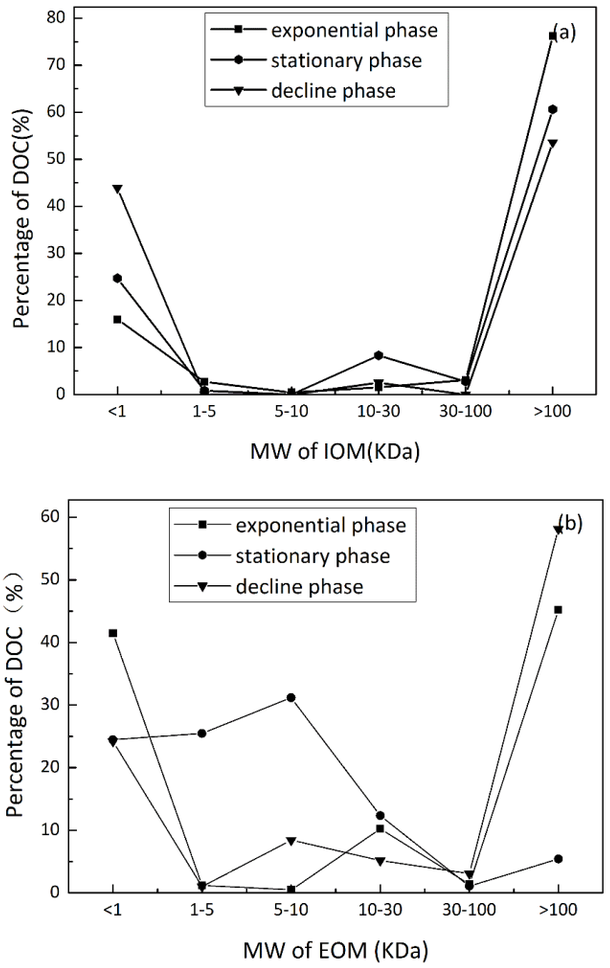

Fig. 3. Transformation of MW fractions of IOM and EOM with the growth stages (single column fitting image) (a) the MW distribution of IOM in different phase; (b) the MW distribution of EOM in different phase.

sharply. As an important inorganic nutrient, nitrate was taken in a large amount in exponential growth phase for synthesizing cells, bringing a fast reduction in TN concentration in solution. In early stationary stage, algae cell pose highly activity, and take nutrients for organism's growth and maintenance. Meanwhile, the rate of cell synthesizing was faster than cells declining at this stage, so the nitrate uptake kept highly in the early stationary stage. Sequentially, the rate of cells synthesizing and declining got flat and quantities of dissolved organic nitrogen (DON) were released from cell death. So, the decline rate of TN was slowed down at the end of the stationary stage. In decline phase, the concentration of TN rose slightly, because plenty of IOM were released from apoptosis of algae cells.

3.3. Properties of EOM and IOM in different growth phases

Physicochemical properties of EOM and IOM can be firstly realized through analyzing some basic water quality parameters such as UV254, DOC, SUVA and DON. Their value in IOM and EOM solution were detected and are shown in Table 1.

As shown in Table 1, SUVA values for AOM (including IOM and EOM) were about 0.87–2.34 L/(m·mg), which is relatively low, showing that AOM has a low aromaticity and the proportion of hydrophilic organic compounds is large. Low SUVA value also reflects that humic acid, fulvic acid, macromolecule organic compound, unsaturated double bond and aromatic organic compounds take small proportion on constituent of AOM. SUVA value for IOM in exponential, stationary and decline phase were 0.87, 1.79 and 2.34 L/(m·mg) respectively, from which it can be drawn that aromatic organic and hydrophobic compounds in IOM increase gradually with the growth of algae. SUVA value for EOM in exponential, stationary and decline phase were 1.34, 0.88 and 1.10 L/(m·mg), demonstrating that the proportion of hydrophilic compound, which is difficult to be removed in conventional treatment, occupy a great proportion in EOM.

DOC/DON, reflecting the nitrogen content in AOM, are also measured and shown in Table 1. For IOM, the DOC/DON value in exponential, stationary and decline phase were 0.053, 0.25 and 0.10, respectively. Algae cells began to synthesize protein as intracellular energy storage substance when the nutrient in the medium was slightly scarce in stationary phase, and so the high content of organic nitrogen are observed. With the growth of algae, nutrient in medium is deficient during decline phase. Hence, the decomposition of intracellular energy storage substance by algae cells account for the decrease of percentage of nitrogen in IOM.

3.4. Molecular weight (MW) distribution

Molecular weight (MW) distributions of IOM and EOM are shown in Fig. 3. The results show that the MW distributions were heavily affected by the growth phase of algae. The IOM displayed a bimodal distributions with a higher peak in >100kDa range and a lowerpeak in <1kDa. The MW fractions in the range of <1kDa, commonly recognized as chlorophyl, algaetoxin, amino acids and other small molecules, were about 16%, 25% and 44% in the exponential, stationary and decline phase, respectively. In contrast, the MW fractions in the range of >100kDa (commonly as phycocyanin and carbohydrates) during the whole growth periodicities were about 76%, 61%, 54%, respectively, presenting a decreasing trend.

For EOM, the MW distributions of the stationary phase were located mostly in the range of <30 kDa, accounting for 93% of the total DOC. Specially, the percent in the range of <1 kDa, 1–5 kDa, 5–10 kDa and 10–30 kDa were 24%, 26%, 31%, 12%, respectively. However, the MW distributions of EOM in the exponential and decline phase both presented bimodal distribution with a higher peak in the ranges of >100 kDa and a lower in <1 kDa, accounting for 41%, 45% and 24%, 58%, respectively.

The MW distributions of IOM and EOM in exponential and decline phase were similar, while quite different in stationary phase (Fig. 3). In exponential phase, the medium is rich in nutrients, and metabolic activity of algae cells was vigorous, so the properties of IOM and EOM were similar. The primary reason for the significant properties difference between IOM and EOM is the decomposition of IOM released from algae cells. During the process of decomposition, IOM transforms into organic matter with small molecular weight of 1–100 kDa, which is more suitable for cellular metabolism. Plenty of intracellular organic matter were released during the decline phase because of the autolysis of the algal cells, which leads the similarity between IOM and EOM in MW distribution in decline phase. It is commonly regarded that the presence of AOM would affect the coagulation process [27], while the compounds in water show differences due to the different stage of algae growth. Therefore, it is of great importance to understand the characteristics of AOM, which is released from different algae cell growth phase into the water.

3.5. Fluorescence spectroscopy for different AOM sample

EEM was applied to analysis the characteristics of IOM and EOM indifferent growth phase, and the results are shown in Table 2. It is commonly regarded that there are five regions in Fluorescence EEMs. Region I (Ex < 250 nm, Em < 330 nm) and Region II (Ex < 250 nm, 330 nm < Em < 380 nm) representing an aromatic proteins region, Region III (Ex < 250 nm,Em > 380 nm) presenting a fulvic acid-like region, Region IV (Ex > 250 nm, Em < 380 nm) presenting a soluble microbial by-product-like region and Region V (Ex > 250 nm, Em > 380 nm) presenting a humic acid-like region. Fluorescence in Regions I, II and IV can be referred to org-N compounds, and that in Regions III and V can be referred to org-C compounds [24].

From the table, it can be seen that the main fluorescence regions of EOM during the whole growth periodicity located in Region IV and Region V, mostly in region IV (Table 2), representing that the soluble microbial product-like materials (SMPs) dominant in EOM. The SMPs, including tyrosine-, tryptophan- and protein-like compositions, were mainly composed of rich org-N compounds, indicating that the EOM was rich in org-N. The results of EOM are in consistent with the existed literature [1,4]. The location of the peak in Region IV was consistently at the excitation/emission wavelengths (Ex/Em) of 275/330 nm in the whole growth periodicity with an increase of peak

Table 2
Fluorescence spectral parameters of different samples

Sample	Phase	Region I[a]		Region II		Region III		Region IV		Region IV	
		Ex/Em	Int.	Ex/Em	Int.	Ex/Em	Int.	Ex/Em	Int.	Ex/Em	Int.
EOM[b]	exp	n.d	n.d	n.d	n.d	n.d	n.d	275/330	2438	325/400	380
	sta	n.d	n.d	260/440	826	n.d	n.d	275/330	4147	350/440	630
	dec	n.d	n.d	n.d	n.d	n.d	n.d	275/330	4813	330/395	1617
IOM	exp	225/335	5051	270/440	1665	n.d	n.d	280/330	5057	365/445	965
	sta	225/330	3028	275/435	488	n.d	n.d	275/330	2696	355/440	163
	dec	225/330	2243	270/440	1304	240/435	881	275/330	1813	360/445	876

[a]Ex: excitation; Em: emission; Int: Characteristic peak intensity; n.d: not determined.
[b]exp: exponential phase; sta: stationary phase; dec: decline phase.

intensity (Table 2). In Region V, there was a change in the location of peaks (Table 2), indicating that there may be a structure change of humic acid-like materials in EOM during different phases [2]. However, the peak located at the Ex/Em of 260/440 nm appears indistinct (Table 2), while EEMs of EOM in the stationary phase displayed a peak intensity at that region. One reason for that could be the fluorescence of humic acid-like substances located at the Ex/Em of 260/440 nm was covered by the strong SMPs fluorescence.

In contrast, EEMs of IOM showed different distributions. The most intense peak of IOM occurred in Region I (Ex/Em, 225/330–335 nm), followed by Region IV (Ex/Em, 275–280/335 nm) (Table 2). Region I and Region IV represent org-N compounds, including aromatic proteins, tyrosine-, tryptophan- and protein-like compositions. The other two distinct peaks of IOM occurred in Region V and Region III (Table 2), representing humic acid-like and fulvic acid-like substances are rich in org-C. Along with the growth of algae, the peak intensity at E_X/E_M of 275–280/330 nm and 225/330–335 nm decreased (Table 2). This might be attributed to the release of IOM to solution, transforming from EEMs fluorescence of IOM in Region I and Region IV to that of EOM in Region III and Region V. It is indicated that the fluorescence intensity located in the region V and III (representing humic acid-like and fulvic acid-like substances) have a high correlation with some disinfection by-products formation potential (DBPFP) (including THMFP and HAAFP), and the correlation coefficient is over 0.93 (Hao, et al. 2012).

3.6. Hydrophilic property of AOM in decline phase

It is commonly acknowledged that strong hydrophobic organic matter (HPO) in surface water mainly includes of humic acid and fulvic acid, while weakly hydrophobic organic matter (TPI) includes polysaccharides, small molecule alkyl alcohols, aldehydes, ketones and other substances. Also, hydrophilic organic matter (HPI) mainly includes amino acid, small molecule alkyl carboxylic acid and amine. Due to complex composition in AOM in decline phase, it is of great interest to separate these organic matters according to hydrophobicity. Some water quality parameters of strongly hydrophobic organic matter, weakly hydrophobic organic matter and hydrophilic organic matter in EOM and IOM in the final stage of algae growth are shown in Table 3. It can be seen from the table that the percentages of strongly hydrophobic organic matter, weakly hydrophobic organic matter and hydrophilic organic matter in IOM extracted during late decline phase are 47.50%, 25.75% and 26.75%, respectively (Table 3), which indicates that humic acid and fulvic acid occupied in IOM extracted during late decline phase. The hydrophilic organic matter takes up 41.02% of EOM extracted in late decline phase, which reflects that large proportions of EOM are hydrophilic. DOC/DON ratio of AOM shown in Table 3 illustrate that organic nitrogen compounds take up a large percentage of IOM and weakly hydrophobic organic matter in EOM contains a small quantity of organic nitrogen.

The main fluorescence regions of strongly hydrophobic organic matter in EOM extracted during late decline phase located in Region I and Region IV (Fig. 5), showing that the aromatic protein and SMPs take up a large proportion. The Region V showed a characteristic peak of weakly hydrophobic organic of EOM and the Region IV presented a characteristic peak of hydrophilic organic of EOM. For IOM extracted during the late decline, characteristic peak of strongly hydrophobic organic was observed in Region I and Region IV (Fig. 5) and a minor fluorescence peak in Region III and Region V, while the characteristic peak of weakly hydrophobic organic located at the similar region to strongly hydrophobic organic with a weaker peak intensity. There was not only the characteristic peak of hydrophilic organic in IOM at Region I and Region IV and fluorescence peak at Region III with strong peak intensity but also the characteristic peak at Region V with weak peak intensity.

3.7. N-DBPs formation potential of AOM

The N-DBPs, such as dichloroacetonitrile, trichloroacetonitrile, chloropicrin and 1,1,1-trichloroacetone, formation potential of strongly hydrophobic organic matter, weakly hydrophobic organic matter and hydrophilic organic matter were measured, then divided by DOC of corresponding compositions. For EOM, the DCAN formation potential is about 1.13 μg/mg DOC, while the potential order is strongly hydrophobic organic (0.434 μg/mg DOC) > weakly hydrophobic organic (0.355 μg/mgDOC) > hydrophilic

Table 3
Water quality parameters for hydrophilic/hydrophobic organic matters in AOM

Substance	Composition	DOC	DOC Ratio	UV254	SUVA	DON	DOC/DON
		mg/L	%	cm^{-1}	L/m·mg	mg/L	
EOM	HPO	22.895	36.88	0.109	0.476	2.61	8.77
	TPI	13.715	22.1	0.024	0.175	0.03	457.17
	HPI	26.465	41.02	0.076	0.298	1.97	13.43
IOM	HPO	10.645	47.5	0.059	0.554	1.54	6.91
	TPI	5.77	25.75	0.011	0.191	1.09	5.29
	HPI	5.995	26.75	0.032	0.534	2.75	11.23

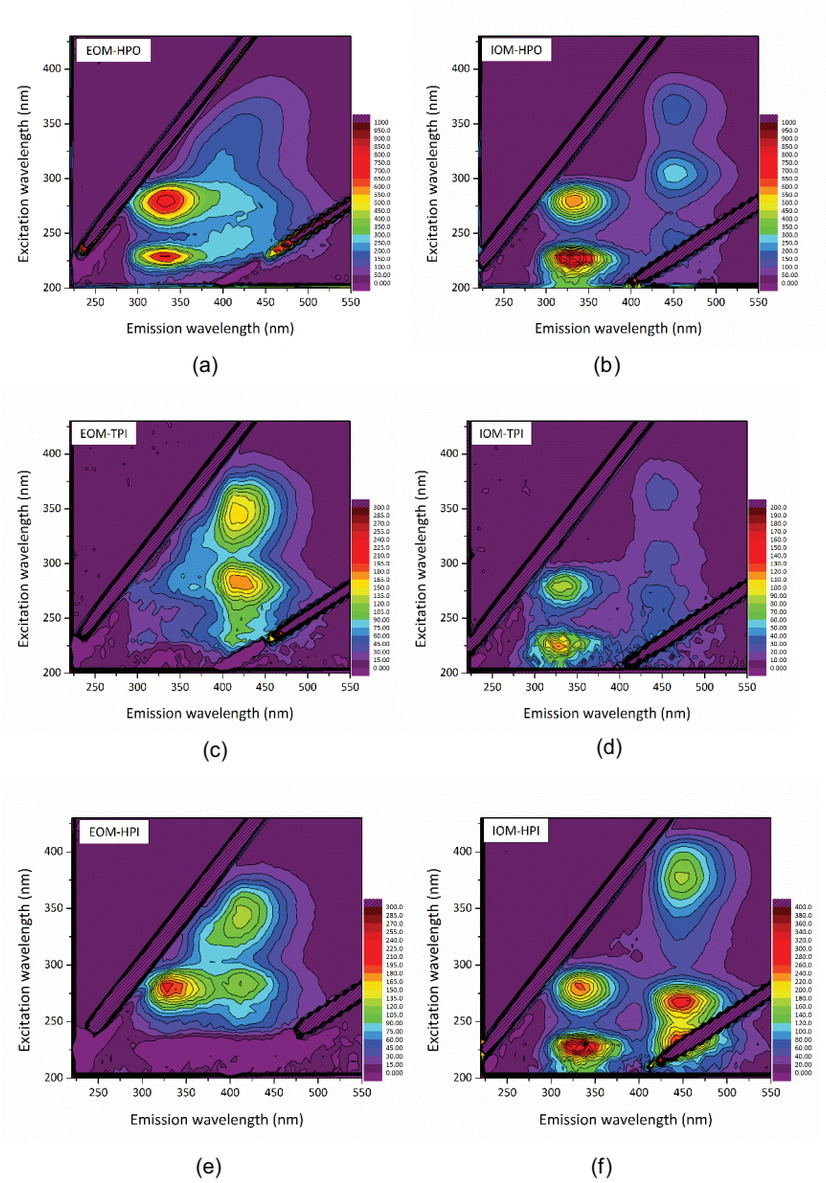

Fig. 4. Fluorescence EEMs for hydrophilic/hydrophobic organic matters in AOM obtained in the decline phase (2-column fitting image). (a) HPO in EOM; (b) HPO in IOM; (c) TPI in EOM; (d) TPI in IOM; (e) HPI in EOM; (f) HPI in IOM.

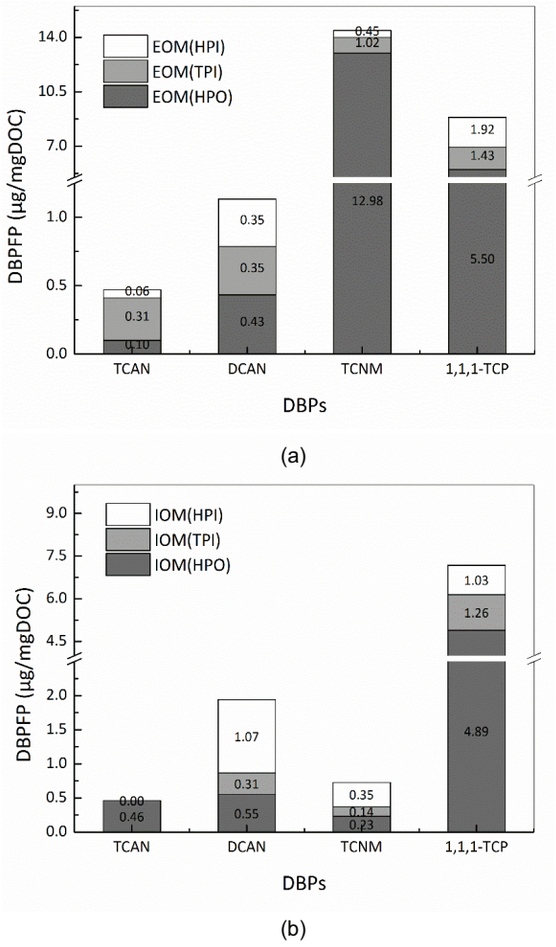

Fig. 5. DBPFP for hydrophilic/hydrophobic organic matters in AOM (single column fitting image) (a) hydrophilic/hydrophobic organic matters in EOM; (b)hydrophilic/hydrophobic organic matters in IOM.

organic (0.347 μg/mg DOC), the TCAN formation potential order is weakly hydrophobic organic (0.310 μg/mg DOC) > strongly hydrophobic organic (0.101 μg/mg DOC) > hydrophilic organic (0.059 μg/mg DOC). Then, the TCNM formation potential order is strongly hydrophobic organic (12.983 μg/mg DOC) > weakly hydrophobic organic (1.015 μg/mg DOC) > hydrophilic organic (0.451 μg/mg DOC), the 1,1,1-TCP formation potential order is strongly hydrophobic organic (5.495 μg/mg DOC) > hydrophilic organic (1.919 μg/mg DOC) > weakly hydrophobic organic (1.434 μg/mg DOC). For IOM, the DCAN formation potential order is hydrophilic organic (1.075 μg/mg DOC) > strongly hydrophobic organic (0.551 μg/mg DOC) > weakly hydrophobic organic (0.314 μg/mg DOC). After that, the TCNM formation potential order is hydrophilic organic (0.352 μg/mg DOC) > strongly hydrophobic organic (0.230 μg/mg DOC) > weakly hydrophobic organic (0.144 μg/mg DOC). The 1,1,1-TCP formation potential order is strongly hydrophobic organic (4.887 μg/mg DOC) > weakly hydrophobic organic (1.257 μg/mg DOC) > hydrophilic organic (1.032 μg/mg DOC) and TCAN was not detected in all components except for strongly hydrophobic organic, which is consistent with the results from the real drinking water and typical precursor compounds [25–27].

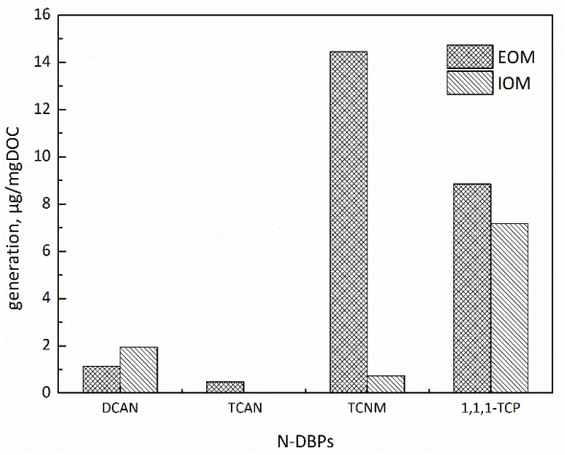

Fig. 6. Generation of N-DBPs for EOM/IOM(single column fitting image).

4. Conclusions

From the results shown above, some conclusion can be drawn as following: The concentrations of DON rise sharply in the late stationary phases. The MW of EOM displayed a unimodal distribution with a peak in the < 30 kDa in stationary phase. MW of IOM presented a bimodal distribution during the growth of algae with a higher peak in <1 kDa and a lower peak in >100 kPa. The fluorescence substance in EOM are mainly humic acid-like and soluble microbial by-product-like matters, while in IOM are mainly aromatic proteins and soluble microbial by-product-like matters. Hydrophilic organic matter accounts for the largest proportion of EOM in decline phase, but the strong hydrophobic organic matter in EOM is most likely produce N-DBPs (DCAN, TCAN, TCNM, 1,1,1-TCP) during disinfection process. For the IOM extracted during the late decline, strong hydrophobic organic matter takes up the largest proportion, but Hydrophilic organic matter is most likely produce N-DBPs (DCAN, TCNM, 1,1,1-TCP,) during disinfection process.

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