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Removing specific extracellular organic matter from algal bloom water by Tanfloc flocculation: Performance and mechanisms

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ABSTRACT

Extracellular organic matter (EOM), which is pervasive in harmful algal bloom water, adversely affects human health and the treatment of such water. In this study, we used Tanfloc, a natural flocculant, in EOM treatment to study its flocculation performance on dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM), as well as its flocculation mechanism at various pH levels. The removal performance was stronger on bEOM than on dEOM, indicated by the higher dissolved organic carbon (DOC) removal rate and the lower Tanfloc dosage in bEOM. The high molecular weight (MW) organic components (> 100 kDa) and protein-like substances in bEOM were removed adequately, and the humic acid, fulvic acid, and soluble microbial-product-like substances, mainly in dEOM, were difficult to remove. Tanfloc could remove bEOM and dEOM satisfactorily, mainly by combining with protein and polysaccharide. Removability depended on water pH and occurred in the order pH 4.5 > pH 8.0 > pH 10.5. Tanfloc could flocculate EOM effectively, even at pH 10.5. Charge neutralization and electrostatic patching and bridging were the main mechanisms at pH 4.5, 8.0, and 10.5, respectively. The hydrogen bonds and hydrophobic interaction were conducive to removing specific EOM. The C-O/C-N functional groups in bEOM had stronger interaction with Tanfloc compared with dEOM; consequently, the removal of bEOM was superior. Our results provide guidance to improve the flocculation efficiency on EOM in harmful algal blooms in the effort to reduce the attendant environmental risks.

1. Introduction

Nutrient enrichment in natural water bodies has led to a worldwide increase in eutrophication and has become a significant environmental issue [1,2]. In addition to the problems caused by the excessive reproduction of cyanobacteria, such as *Microcystis aeruginosa* (*M. aeruginosa*), the algal organic matter (AOM) they produce adversely affects the health of humans, animals, and the ecosystem [3,4]. AOM consists of extracellular organic matter (EOM) and intracellular organic matter (IOM), with EOM released from algal cells by diffusion, whereas IOM is released from senescent algal cells during cell lysis [5]. EOM can be divided into dissolved extracellular organic matter (dEOM) that dissolves in the culture medium, and bound extracellular organic matter (bEOM) that adheres to the cell surface [6,7], dEOM and bEOM are collectively called specific EOM for convenience. Removing algal cells removes IOM adequately as well. However, algae can release EOM to water during all their stages of growth and reproduction, which could increase the levels of disagreeable taste and odor (T&O) compounds, dissolved organic carbon (DOC), and assimilable organic carbon (AOC), and promote the formation of disinfection by-products (DBPs) [8,9]. Accordingly, finding effective methods to deal with EOM is crucial to reducing the attendant environmental risk.

Flocculation has become an important water treatment technology because of its high efficiency and low cost and it is applied widely in algal and EOM removal [10]. Water pH is a crucial factor in the flocculation of EOM, and can affect its removal performance [11]. Obviously, water pH can affect the surface charge of cells as well as the flocculant [12,13]. Furthermore, pH can also influence the removal efficiency of cells [14]. EOM is the medium through which the flocculant comes in contact with cells and pH can therefore influence the binding between the flocculant and cells, thereby influencing EOM removal [11]. The flocculation of EOM is influenced acutely not only by pH but also by the type of flocculant used. Henderson [15] found that the removal efficiency of aluminum sulfate for AOM reached 55% for *M. aeruginosa* at pH = 5, whereas it decreased markedly to 18% using ferric chloride at the same pH value.

Chemical flocculants have disadvantages that have restricted their being applied extensively. Firstly, their pH application range is narrow.

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Nomenclature

AOC	assimilable organic carbon
AOM	algal organic matter
bEOM	bound extracellular organic matter
DBPs	disinfection by-products
dEOM	dissolved extracellular organic matter
DOC	dissolved organic carbon
EEM	excitation-emission matrix
EOM	extracellular organic matter
FTIR	Fourier transform infrared spectroscopy
GPC	gel permeation chromatography
IOM	intracellular organic matter
MW	molecular weight
R_h	hydrodynamic radius
SD	standard deviation
SEM	scanning electron microscopy
SUVA	specific ultraviolet absorbance
T&O	taste and odor
TOC	total organic carbon

Pivokonsky [11] reported that the proteins in cellular organic mater (COM) could be removed adequately by ferric flocculant at pH 4–6, whereas the removal ability decreased significantly when pH > 7. However, water pH tends to be weak alkaline during cyanobacteria bloom occurrences [12]. Secondly, it is difficult to remove residual Fe or Al in the treated water and this could pose risks to the health of both humans and the environment [16]. Therefore, it is necessary to develop a non-toxic flocculant which can flocculate EOM effectively in a wide pH range.

Tannins are secondary metabolites of higher plants that derive from the bark, wood, leaves, and other organs and tissues of the plants. They belong to a class of natural products known as plant phenolics and are the fourth most abundant forest by-products after cellulose, lignin, and hemicellulose [17]. As tannins are negatively charged, they are not used generally in their natural state to remove anionic pollutants and have to be altered chemically before they can be applied as flocculants [18]. Tanfloc is produced when an amino group is introduced to the tannin chemical structure [19]. In contrast with chemical flocculants that pose health risks to humans, Tanfloc is a biodegradable and non-toxic flocculant. It has been used in treating algal bloom water, showing significant capacity for flocculation of algal cells [12,20–23]. In addition, it has good flocculation capacity for EOM, as Tanfloc combines easily with protein-like and polysaccharide-like substances—the main component of EOM. Wang [18] found that quaternary ammonium-salt-modified tannin (Q-TN) that has the same modifying method as Tanfloc were able to settle large numbers of EOM, including simple aromatic proteins and protein-like substances. However, their study focused on removing EOM in general (not specific EOM) and disregarded the differences in the composition and structure of, e.g., dEOM and bEOM. Furthermore, the study was restricted to the removal of major organic species, such as protein and humic acid. There is a lack of comprehensive evaluation of the removal performance of Tanfloc on specific EOM, as well as the flocculation mechanism on EOM or specific EOM. Therefore, we conducted such a comprehensive analysis, using *M. aeruginosa*, a prevalent algal blooming species, and we extracted the specific bEOM and dEOM for use in various analyses.

To the best of our knowledge, this is the first comprehensive study on the removal ability of a natural flocculant for EOM or specific EOM in treating water with high levels of algae. We expect the results of this investigation to provide guidance to improve the flocculation efficiency on EOM in harmful algal blooms with a view to reducing the attendant environmental risks.

2. Materials and methods

2.1. Materials

2.1.1. Algal culturing

We obtained *M. aeruginosa* FACHB-905 from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China). The algal culture was incubated in BG11 medium at 25 ± 1 °C with 2 000 lx illumination for a light/dark photoperiod of 12/12 h and harvested on day 16 of the stationary growth phase.

2.1.2. Tanfloc

We obtained Tanfloc powder from TANAC S.A. (Montenegro, Brazil). This is a water-extracted, plant-based tannin, with a flavonoid structure. The Tanfloc production process is proprietary, but it is known that similar products have been synthesized by the Mannich base reaction [19]. Detailed information on Tanfloc (Figs S1–S3) is provided in the Supplementary Information. We prepared a stock solution of Tanfloc at 1 g L^{-1} by dissolving the powder in deionized water and stirring with a magnetic stirrer at 200 rpm for 30 min.

2.2. Extraction of dEOM and bEOM

The algal cultures in the stationary phase were collected and re-suspended to a final cell density of 10^6 cells mL^{-1} to simulate algal bloom [24]. Subsequently, they were centrifuged at 4000 × *g* and 4 °C for 15 min, using a high-speed refrigerated centrifuge (H2050R-1, Xiangyi, Hunan, China). Afterward, the supernatant was filtered through a 0.45-μm glass fiber membrane (Xin Ya Purification Equipment Co. Ltd., Shanghai, China), and the filtrate was named the dEOM solution [6,7]. Next, the bEOM solution was obtained by centrifuging the bEOM-attached algae solution at 10000*g* and 4 °C for 15 min and subsequently filtering the supernatant through the 0.45-μm glass fiber membrane [6,7].

2.3. Flocculation experiment

Flocculation tests were conducted using a programmed jar test apparatus at 25 ± 1 °C room temperature (ZR4-6, Zhongrun Water Industry Technology Development Co., China). The experiments were carried out in 600-mL glass beakers, each containing 500 mL dEOM or bEOM solution. After adding a certain volume of Tanfloc stock solution, the solutions were mixed intensively for 2 min at 300 rpm for uniform dispersion of the Tanfloc, during which the pH values of the solutions were respectively set up at three gradients (4.5, 8.0, 10.5) for dEOM or bEOM using 0.1 M NaOH or 0.1 M HCl, as required. Three pH conditions, namely pH 4.5, 8.0, and 10.5, were set up to represent water pH that was lower, equal to, and higher, respectively, than the zero potential point of Tanfloc. The temperature, zeta potential and other characteristics of dEOM and bEOM solutions are presented in Table S1. The solutions were mixed gently for 20 min at 50 rpm to allow floc formation, and subsequently left for 30 min to settle. Afterward, the supernatant samples were collected and filtered through the 0.45-μm fiber membrane to determine the DOC concentration, UV absorbance at 254 nm (UV_{254}), excitation-emission matrix (EEM), protein and polysaccharide concentration, MW distributions, and zeta potential. The DOC removal rate in dEOM and bEOM was determined from Eq. (1) and expressed as a percentage. The calculation of protein and polysaccharide removal rates were similar to that of DOC. The floc samples were collected for scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) analyses.

DOC removal rate (%)
= [(initial DOC concentration - supernatant DOC concentration)
/initial DOC concentration] × 100 (1)

2.4. Analyses

2.4.1. DOC, UV_{254}, and SUVA

We measured the DOC of the supernatant dEOM and bEOM samples before and after flocculation using a total organic carbon (TOC) analyzer (Model 1030, OLAnaltica, USA). We measured UV_{254} using an ultraviolet-visible (UV/VIS) spectrophotometer (TU-1901, Persee, China). We calculated SUVA as in Eq. (2) [25]:

$$SUVA = UV_{254}(cm^{-1})/DOC(mg/L) \times 100 \quad (2)$$

2.4.2. Excitation-emission matrix

The supernatant samples of dEOM and bEOM before and after flocculation were collected for EEM analysis. Each sample was analyzed by fluorescence spectrometry (F-7000, Hitachi, Japan). The excitation wavelengths were increased from 200 to 450 nm in 5-nm steps. The emission spectra were recorded from 200 to 550 nm in 1-nm increments. The excitation and emission slits were maintained at 5 nm. The scanning speed was set to 12000 nm min^{-1} [26].

2.4.3. Gel permeation chromatography

The MW distributions of the supernatant samples of dEOM and bEOM before and after flocculation were analyzed with a gel permeation chromatograph (GPC) (LA-20 AD, Shimadzu, Japan), equipped with a Shodex Ohpak SB-805 HQ gel chromatography column (Phenomenex Inc., California, USA). We used polystyrene glycol as the standard sample to draw a calibration curve because of the high solubility of dEOM and bEOM [5]. The reproducibility of the MW fractionation was admissible, with MW deviations of less than 5% in duplicate.

2.4.4. Scanning electron microscopy

The dEOM, bEOM, and their floc samples were preserved for SEM analysis. The samples were dehydrated through a series of ethanol solutions and dried with a vacuum drier. The specimens were mounted on copper stubs, coated with gold, and examined with SEM (S-4800, Hitachi, Japan) at 5 kV.

2.4.5. Fourier transform infrared spectroscopy

The freeze-dried dEOM, bEOM, and their floc samples were mixed with KBr at a mass ratio of 1:100 and formed into pellets for FTIR measurement, respectively (Tensor 27, Bruker). The infrared spectra were collected from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1}, using 32 scans.

2.4.6. Other analytical methods

The hydrodynamic radius (Rh) of Tanfloc and the zeta potential were measured with a Malvern Zetasizer Nano ZSP analyzer (Malvern, UK). Protein and polysaccharide concentration were determined by the bicinchoninic acid (BCA) and phenole-sulfuric method respectively (Beyotime Biotechnology Co. Ltd., Shanghai, China). All the experiments were conducted in triplicate and the data were expressed as means ± standard deviation (SD) using the Origin v. 9.0 (OriginLab Corp., Northampton, MA, USA). All of the parameters were compared across treatments with one-way ANOVA using SPSS v.17.0 (IBM Analytics, Armonk, NY, USA), and the statistical significance levels were set to $P < 0.05$.

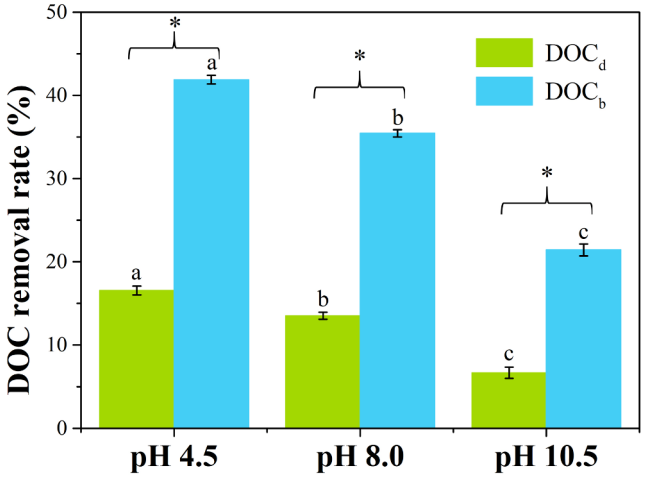

Fig. 1. DOC removal rates of dEOM and bEOM after flocculation in water pH 4.5, 8.0, and 10.5. Significant differences ($P < 0.05$) between the treatment means were represented by different letters or (*).

3. Results and discussion

3.1. Removal performance of Tanfloc on dEOM and bEOM

3.1.1. Variations in DOC concentration and SUVA

After separating the dEOM and bEOM released by the *M. aeruginosa* cells from the culture and cell surface, we investigated their removal by Tanfloc (Fig. 1). The DOC concentrations of dEOM and bEOM were represented respectively by DOC_d and DOC_b. As shown in Fig. 1, the DOC_d removal rate was significantly ($P < 0.05$) lower than that of the DOC_b at the same pH value after flocculation. For example, the DOC_d removal rate was 16.6%, whereas the DOC_b removal rate increased to 41.9% at pH 4.5. This indicated that the removal ability of Tanfloc on bEOM was superior compared to that on dEOM; a result comparable to that of Tang [27]. These authors studied the removal effect of polyaluminum chloride (PAC) on dEOM and bEOM and found that the DOC removal rate of dEOM was less than 10% at pH ≈ 8.0, whereas that of bEOM reached up to 30%. Moreover, both the DOC_d and DOC_b removal rates of PAC were lower than that of Tanfloc. The DOC_d and DOC_b removal rates of Tanfloc at pH 8.0 reached 13.5% and 35.4% respectively, indicating that Tanfloc displayed a stronger removal ability on EOM compared with PAC.

The DOC_d and DOC_b removal rates decreased significantly ($P < 0.05$) with the increasing pH after flocculation. For example, the DOC_b removal rate at pH 4.5 (41.9%) was higher than it was at pH 10.5 (21.4%). This demonstrated that pH could affect the flocculation of dEOM and bEOM, with the order of the removal effect being the acid condition (pH 4.5) > weak alkaline condition (pH 8.0) > strong alkaline condition (pH 10.5). Notably, although a chemical flocculant could flocculate EOM in acid and neutral conditions, its removal ability was extremely poor in alkaline conditions. [11] used ferric flocculant to flocculate the proteins in COM, and found that they could be well removed at pH 4–6, but the removal ability decreased significantly when pH > 7. However, in our experiment, an increased dosage of Tanfloc was able to remove EOM even under alkaline conditions. The DOC_b removal rate of 21.4% was obtained even at pH 10.5 when Tanfloc dosage increased to 60 mg/L.

After flocculation, some EOM remained in the water, indicating that Tanfloc could remove EOM only partially. This could be ascribed to Tanfloc itself being organic matter; therefore, the measured DOC concentration could be lower than the actual amount of residual EOM was, resulting that a lower DOC removal rate was obtained. Additionally, some organic substances, such as humic acid-like substances, were difficult to remove with Tanfloc [28]. Wang [18] also found that the DOC removal rate of Q-TN on EOM was approximately 17.4% (< 100%).

SUVA can reflect the proportion of unsaturated organic matter in

the total organic matter and can characterize the aromaticity of water qualitatively. Generally, a higher SUVA value indicates more unsaturated hydrophobic organic matter in water [29,30]. Here, $SUVA_d$ and $SUVA_b$ were used to represent the SUVA value of dEOM and bEOM, respectively. As shown in Table 1, the $SUVA_b$ value was significantly ($P < 0.05$) less than the $SUVA_d$ value for all three pH values before flocculation. This indicated that dEOM could contain more organic matter with strong UV absorbability compared with bEOM.

Table 1. The SUVA values of dEOM and bEOM before and after flocculation in water pH 4.5, 8.0, and 10.5.

The values of both $SUVA_d$ and $SUVA_b$ decreased slightly ($P > 0.05$) after flocculation, with the decrement value of $SUVA_d$ being slightly lower than that of $SUVA_b$ at the same pH value. For instance, in pH 4.5, the decrement of the $SUVA_d$ value (0.06) was lower than was that of the $SUVA_b$ value (0.12) after flocculation. This indicated that although Tanfloc showed no obvious removal of aromatic compounds, it could remove those in bEOM relatively more easily. Generally, some unsaturated hydrophobic organic matter remained in the water after flocculation. Furthermore, with an increasing pH value, the values of both $SUVA_d$ and $SUVA_b$ decreased slightly ($P > 0.05$). For example, the decrement in the $SUVA_d$ value was 0.06 at pH 4.5, decreasing slightly to 0.02 at pH 10.5 after flocculation. This showed that the removal effect of pH on aromatic compounds was negligible.

3.1.2. Variations in fluorescent and non-fluorescent organic matter

Fluorescence EEM is a rapid, selective, and sensitive technique to distinguish organic compounds in water [31]. Here, EEM was used to determine variations in the fluorescent organic matter before and after flocculation. Five major fluorescence peaks were referred to as T1 (tryptophan-like), T2 (aromatic-like), A (humic-like), C (fulvic-like), and S (soluble microbial-product-like). Their excitation/emission wavelengths were located at 260–290 nm/305–335 nm (peak T1), 220–280 nm/310–349 nm (peak T2), 360 nm/440 nm (peak A), 270 nm/430–445 nm (peak C), and 310–340 nm/360–370 nm (peak S), respectively [6,32,33]. Both tryptophan-like and aromatic-like substances belong to the protein-like substances. As shown in Fig. 2, only the response values of peak T1 and T2 existed in bEOM, indicating that the composition of bEOM was simple, with the protein-like substances being the main components. Produced by algal metabolism process, they could be released to the extracellular domain and could subsequently adhere to the outer wall of cells [7]. The response values of peak T1 were relatively high and those of peak S, A, and C were lower in bEOM. This indicated that the components of dEOM were complex, with the tryptophan-like substances being the main components in dEOM. Additionally, it contained various humic and fulvic acid, and soluble microbial-product-like substances. This could be ascribed probably to organic matters in bEOM, particularly aromatic-like substance, being easily converted to other substances (including humic acid, fulvic acid and soluble microbial-product-like substances) by microorganisms with the help of microbial enzymes [7,34,35].

As shown in Fig. 2, the response value of peak T1 and T2 decreased obviously after flocculation. This indicated that the protein-like substances in bEOM were removed satisfactorily. Furthermore, the response value of peak T1 obviously decreased, whereas those of the other peaks were almost unchanged. This indicated that the tryptophan-like substance in dEOM was removed adequately, but the humic acid, fulvic acid, and soluble microbial-product-like substances were difficult to remove. Similarly, Wang [18] found that the removal rates of Q-TN for tryptophan-like and aromatic-like substances reached 78.8% and 100%, respectively, but were less than 30% for humic or fulvic acid.

To verify the removal effect on the protein-like substances and investigate variations in the non-fluorescent organic matter, changes in protein and an important non-fluorescent organic matter - polysaccharide before and after flocculation were studied. As shown in Fig. S4, the removal rates of protein and polysaccharide reached up to 53.4% and 45.0% in bEOM at pH 4.5 respectively, and their removal rates in dEOM at pH 4.5 were 31.8% and 26.1% separately, indicating that protein and polysaccharide, especially in bEOM, could be well removed by Tanfloc. Moreover, the removal rates of protein and polysaccharide, regardless of bEOM and dEOM, were higher than DOC removal rate. For instance, the removal rates of protein and polysaccharide in bEOM at pH 8.0 were 42.0% and 36.3% respectively, which were higher than that of DOC_b (35.4%). So it could be inferred that Tanfloc removed bEOM and dEOM, mainly by combining with protein and polysaccharide. In summary, the removal ability of Tanfloc on bEOM was stronger than it was on dEOM. The protein-like substances and polysaccharide consumed more Tanfloc and displayed a negative effect on flocculation; however, Tanfloc could flocculate and precipitate most of the protein-like substances and polysaccharide. This finding provides guidance for the treatment of algal bloom water.

The decrement in the response value of the characteristic peaks with different pH values in bEOM or dEOM was in the order pH 4.5 > pH 8.0 > pH 10.5. Both the protein and polysaccharide removal rates in bEOM and dEOM also followed the same order, and increased significantly ($P < 0.05$) with the increasing pH. Moreover, the protein and polysaccharide removal rates in bEOM were 27.8% and 22.0% respectively even at pH 10.5. These confirmed the DOC analysis that pH could affect flocculation. The removal ability of Tanfloc on EOM decreased with increasing pH but it could flocculate EOM effectively even under alkaline conditions.

3.1.3. Variations in MW distribution

The GPC analysis provided insight into the MW distribution variation of dEOM and bEOM before and after flocculation. As shown in Fig. 3(a) and (b), the MW distributions of dEOM and bEOM were mainly in the range > 100 kDa to < 1 kDa. Previous studies found that the MW distribution range of organic matter in EOM was as follows: high MW substances, including phycocyanin and polysaccharides (> 100 kDa), humic-like substances (< 20 kDa), and low MW substances, including amino acids, microcystin, chlorophyll, aldehydes, and hydrocarbons (< 1 kDa) [5,25,26,36]. It is obvious that compared with dEOM, the high MW organic matter content in bEOM was higher and the low MW organic matter content in bEOM was lower. This could be ascribed probably to some high MW organic matter, secreted by the *M aeruginosa* cells and attached to the cell surface, being degraded to low MW substances by extracellular enzyme [37]. Additionally, most humic-like substances, the main component in dEOM, were low MW substances [38].

As shown in Fig. 3(d) and (e), The peak intensity of high MW organic matter (> 100 kDa) decreased significantly in bEOM and dEOM after flocculation, whereas that of the low MW organic matter (< 1 kDa) decreased only slightly. This obviously indicated that the removal ability of Tanfloc was satisfactory on high MW substances but poorer on low MW substances. Tang [27] found that PAC could flocculate low MW substances effectively; however, we found that they were difficult to remove completely with Tanfloc. This is probably because PAC could be hydrolyzed into colloid ions released into the water, which had strong adsorption capacity on small molecules such as

Table 1
The SUVA values of dEOM and bEOM before and after flocculation in water pH 4.5, 8.0, and 10.5.

	Before flocculation		After flocculation	
	$SUVA_d$ ($m^{-1} mg^{-1} L$)	$SUVA_b$ ($m^{-1} mg^{-1} L$)	$SUVA_d$ ($m^{-1} mg^{-1} L$)	$SUVA_b$ ($m^{-1} mg^{-1} L$)
pH 4.5	1.58 ± 0.03^a	1.03 ± 0.02^b	1.52 ± 0.02^a	0.91 ± 0.01^b
pH 8.0	1.63 ± 0.02^a	1.05 ± 0.03^b	1.58 ± 0.01^a	0.95 ± 0.02^b
pH 10.5	1.75 ± 0.01^a	1.10 ± 0.01^b	1.73 ± 0.01^a	1.06 ± 0.02^b

Notes: $SUVA_d$ = SUVA value of dEOM; $SUVA_b$ = SUVA value of bEOM. Significant differences ($P < 0.05$) between the treatment means were represented by different letters.

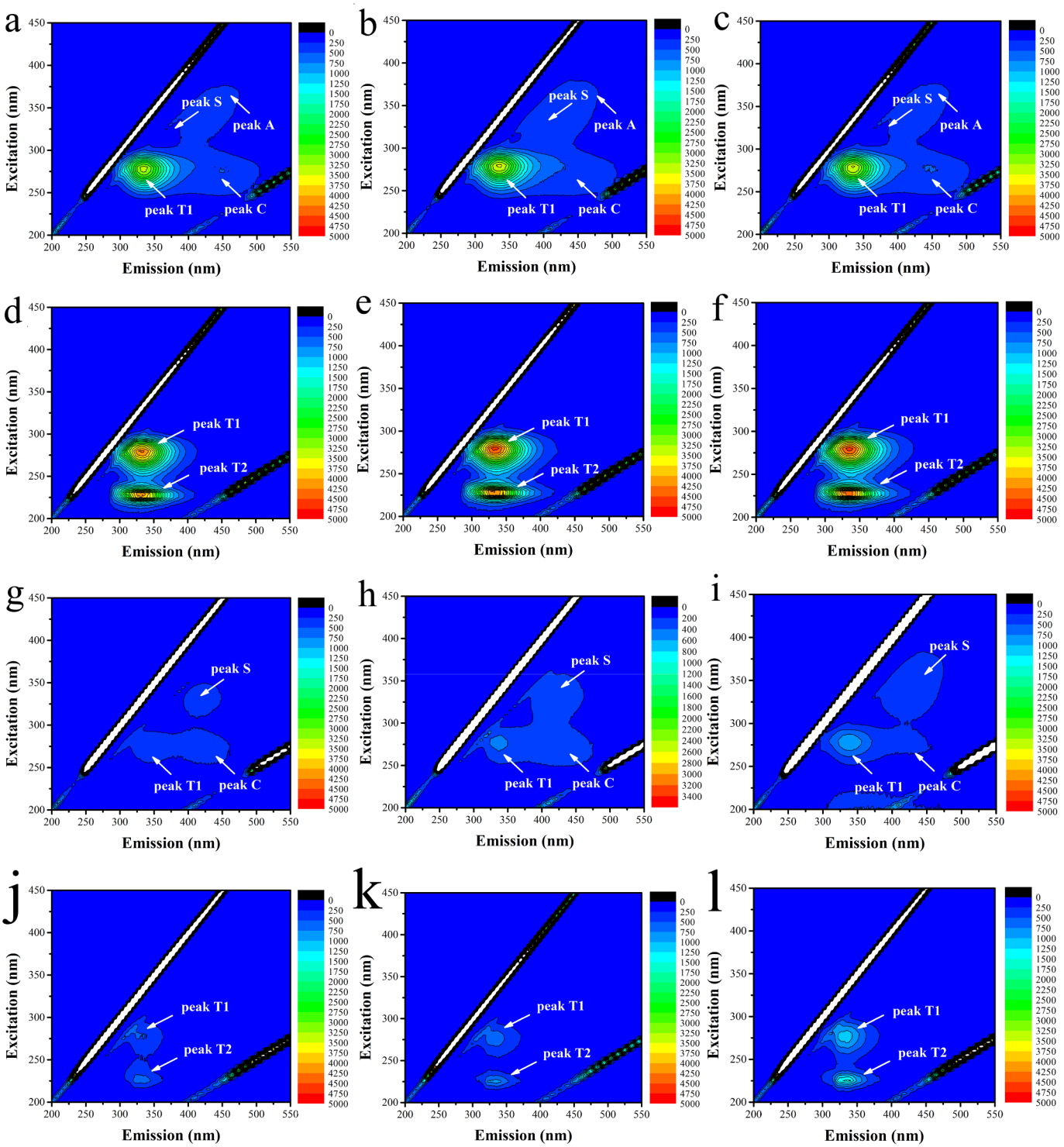

Fig. 2. EEM spectra of dEOM and bEOM: (a) $dEOM_{4.5}$, (b) $dEOM_{8.0}$, (c) $dEOM_{10.5}$, (d) $bEOM_{4.5}$, (e) $bEOM_{8.0}$, and (f) $bEOM_{10.5}$ before flocculation in water pH 4.5, 8.0 and 10.5; (g) $dEOM_{4.5}$, (h) $dEOM_{8.0}$, (i) $dEOM_{10.5}$, (j) $bEOM_{4.5}$, (k) $bEOM_{8.0}$, and (l) $bEOM_{10.5}$ after flocculation in water pH 4.5, 8.0, and 10.5.

microcystin, whereas the adsorption capacity of Tanfloc on these substances was weaker [39]. Furthermore, the decrement in peak intensity of organic matters in bEOM was higher than it was in dEOM after flocculation, indicating that bEOM was easier to remove. It could be ascribed mainly to the differences in the components. As shown in Fig. S5, the main components in bEOM, especially protein, belonging mainly to high MW substances with a long chain structure, whereas the main components in dEOM, such as humic acid and fulvic acid, were mainly low MW substances. Considering that the results of Section 3.1.2 showed that the protein-like substances could be removed more easily by Tanfloc compared with the humic-like substances, this showed that bEOM could be removed more effectively. In addition, the proportion of high MW organic matter in bEOM was obviously higher than that in dEOM. Further, many high MW substances with strong hydrophobicity, including phycocyanin, some microbes and enzymes (< 0.45-μm), could be easily precipitated and removed during flocculation process [7]. Therefore, a better removal performance was obtained in bEOM compared with dEOM.

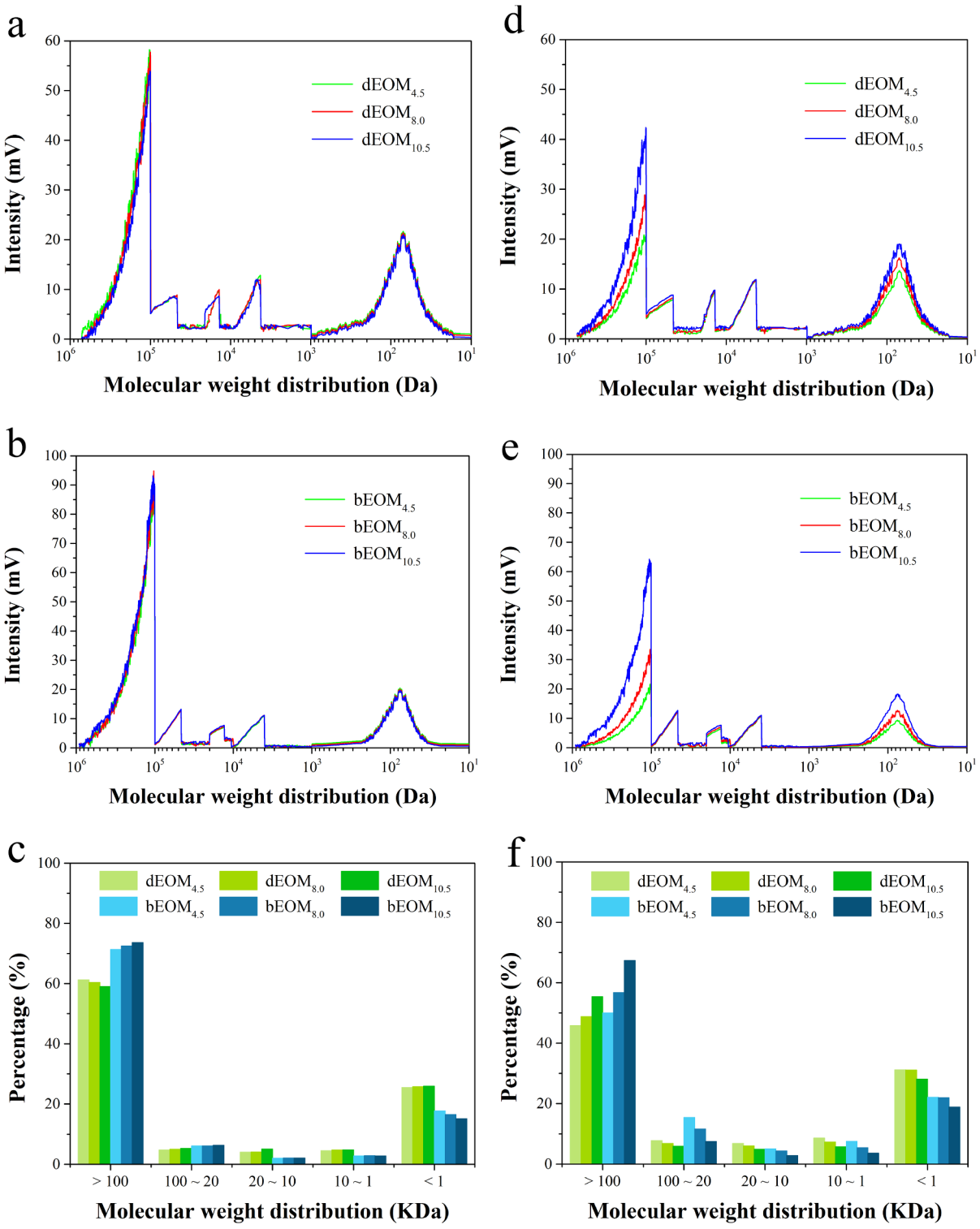

Fig. 3. MW distributions of (a) dEOM, (b) bEOM and (c) the percentage of molecular weight distribution before flocculation in water pH 4.5, 8.0, and 10.5; (d) dEOM, (e) bEOM and (f) the percentage of molecular weight distribution after flocculation.

The decrements of peak intensity in the MW substances for bEOM or dEOM were in the order pH 4.5 > pH 8.0 > pH 10.5. This indicated that the removal ability of Tanfloc for high MW substances decreased gradually with the increasing pH. Notably, Tanfloc could remove organic matter efficiently in broad MW ranges, even under alkaline conditions, indicating that Tanfloc has a wide pH application scope in EOM treatment. Furthermore, it could be inferred that the residual DOC concentration after flocculation derived mainly from low MW substances (< 1 kDa).

3.2. Flocculation mechanism of Tanfloc on dEOM and bEOM

Water pH can affect the removal performance of Tanfloc on dEOM and bEOM, probably because of the different flocculation mechanisms in different pH conditions. Here, the changes in the Zeta potential and SEM images of flocculation were investigated to illuminate the flocculation mechanisms.

3.2.1. Zeta potential

From the DOC concentration–dosage profiles at pH 4.5, 8.0, and 10.5, the optimal dosages for bEOM were 20, 30, and 60 mg/L, respectively. With the increasing pH, the residual DOC concentration corresponding with every optimal dosage also increased. However, the flocculation windows in the three pH conditions did not narrow and the flocculation performance was satisfactory, particularly in acid conditions. Additionally, as the morphology of Tanfloc in water influenced the flocculation performance significantly, we also investigated the pH dependence of Rh (Fig. S6).

At pH 4.5 (Fig. 4(a)), the positive charges of Tanfloc resulted in larger Rh because of electrostatic repulsion. Both the positive charges and the stretched conformation structure of Tanfloc were conducive to enhancing the removal efficiency and reducing the optimal dosage [40]. Furthermore, the zeta potential of the supernatant was close to zero when the optimal removal dosage of bEOM was reached; however, the zeta potential became the opposite with an overdose of Tanfloc. This indicated that charge neutralization was the main mechanism in the acid condition [41]. The negatively charged bEOM was attracted, neutralized, and coated by the positively charged Tanfloc. After being covered thoroughly, the destabilized bEOM, with nearly zero surface charge, aggregated continually to form large flocs. In addition, Tanfloc could be combined with protein through a "glove–hand" reaction [42]. The hydrophobic groups, such as gallic acid in the polyphenol molecule of Tanfloc, were close to the protein because of the hydrophobic interaction and entered "the hydrophobic bag" of protein, where leucine, valine, phenylalanine, and other amino acid residues were concentrated. Subsequently, the phenol, as a hydrogen donor, interacted with the polar groups in the protein, including carboxyl, peptide, hydroxyl, and the guanidyl groups [42]. The hydrogen bonds and hydrophobic interaction existed simultaneously, which was beneficial to the multipoint combination between Tanfloc and protein. A hydrophobic layer formed between the protein molecules, which induced the protein molecules to gather and eventually to precipitate. The combination of Tanfloc with polysaccharide was similar to the above, but Tanfloc could not combine with the humic-like acids in this way [42,43]. Accordingly, this explained why the EEM analysis indicated that protein could be removed satisfactorily but it was difficult to remove the humic-like acids.

At pH 8.0 (Fig. 4(b)), owing to the loss of the net charge, the Tanfloc molecular chain became curly and short, leading to a smaller Rh. Furthermore, the zeta potential of the supernatant was less than zero at the optimal dosage, whereas it became the opposite with an overdose of Tanfloc. This indicated that electrostatic patching was the main mechanism in this instance [40]. In the experiment, the negatively charged bEOM attached itself to the positively charged Tanfloc molecular chain. This resulted in the development of negatively charged areas on the surface of Tanfloc. When the charged Tanfloc molecular chains moved close together, a simultaneous attachment of oppositely charged areas occurred. However, the flocculation effect of electrostatic patching on bEOM was usually weaker in comparison with that of charge neutralization. This could explain the DOC analysis indicating that the removal ability of Tanfloc on bEOM in weak alkaline conditions was weaker than in acidic conditions. In addition, the special function, i.e., the "glove–hand" reaction between Tanfloc and protein or polysaccharide was helpful to bEOM removal.

At pH 10.5 (Fig. 4(c)), a high Rh was reached again, probably because the Tanfloc molecular chain became stretched because of the increase in negative charges. However, the negative charges caused greater electrostatic repulsion between the Tanfloc molecules and bEOM, eventually resulting in inferior flocculation performance, i.e., much higher optimal dosage, as well as DOC residues. Furthermore, the zeta potential of the supernatant after flocculation was far from zero, regardless of the increase in the dosage, because of the shortage of positive charges. However, in this instance, Tanfloc still showed some removal ability for bEOM. This could be explained by the bridging mechanism [40,41]. Numerous negative bEOM molecules were absorbed simultaneously onto the stretched Tanfloc molecule chains by Van der Waals force and the special adsorption between Tanfloc and protein or polysaccharide, including hydrogen bonds and hydrophobic interaction with hydraulic agitation. Consequently, numerous different bEOM molecules were connected by the long chains of the Tanfloc, with larger flocs forming. Notably, the electrostatic repulsion was not likely to destroy the adsorption forces. The surface charges of bEOM were screened partially by the Tanfloc molecules, leading to the increase in the zeta potential, but not beyond zero. However, the Tanfloc MW was not high enough and its removal ability for bEOM decreased slightly in strong alkaline water. This resulted in inferior flocculation performance when the bridging effect was the main flocculation mechanism. Accordingly, this explained the DOC analysis indicating that the

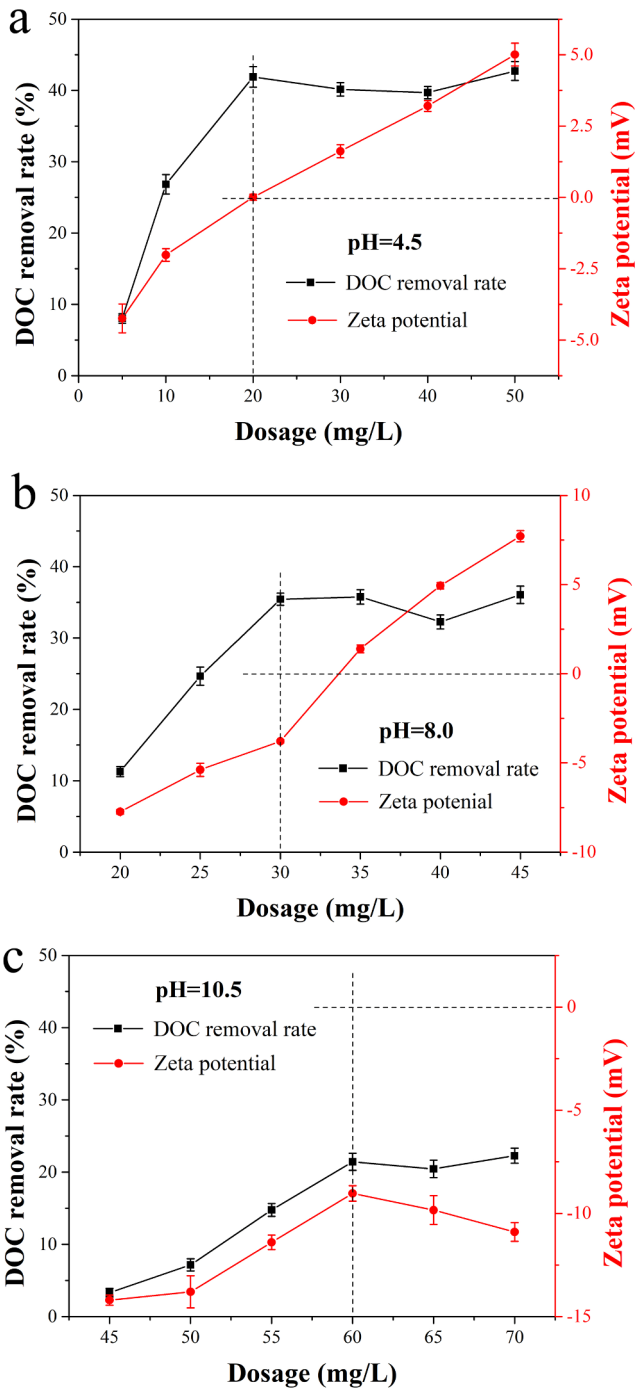

Fig. 4. DOC removal rates and zeta potential of bEOM as a function of Tanfloc dosage after flocculation in water (a) pH 4.5, (b) pH 8.0, and (c) pH 10.5.

decrement in DOC concentration after flocculation in a strong alkaline condition was lower than it was in acidic or weak alkaline conditions.

As shown in Fig S7, the variations in the DOC concentrations and zeta potential of the supernatant in dEOM with an increasing Tanfloc dosage were similar to those in bEOM, indicating that the flocculation mechanism on dEOM could be the same as that on bEOM. This is probably because despite its poor removal ability on humic and fulvic acid, Tanfloc removed EOM, regardless of bEOM or dEOM, mainly by combining with protein and polysaccharide. Additionally, the removal ability on bEOM was obviously superior to that on dEOM. The reason was probably that the protein-like substances were the vital component for bEOM and the efficient removal of protein therefore aided in removing most bEOM. However, as regards dEOM, there were many other substances in addition to the protein-like substances, such as humic acid. Therefore, the removal of protein played a limited role in the overall removal of dEOM.

3.2.2. Scanning electron microscopy

We further explored the flocculation mechanism by conducting SEM to study the surface morphology of the bEOM and dEOM flocs. As shown in Fig. S8, the surface morphology of the bEOM and dEOM flocs differed obviously. The bEOM flocs showed a lumpy structure, with a smooth and compact shape but a slight wrinkle on the surface. The dEOM flocs showed a loose and irregular granular structure, with some pores on their rugged and curved surface. Furthermore, at pH 4.5 and 8.0, the flocs produced from both bEOM and dEOM displayed a homogeneous and regular structure, with a relatively small size and dense distribution. However, the EOM flocs displayed a loose net-like structure at pH 10.5, with an irregular surface and a larger size. This proved our speculation that the flocculation mechanism on EOM was different in different water pH levels. In acid or weak alkaline conditions, electrostatic attraction (including charge neutralization and electrostatic patching) was the main mechanism. The positive Tanfloc could combine with the negative EOM and form homogeneous and tight microflocs by electrostatic interaction. However, there was no compact and cross-linked structure between the microflocs; therefore, loose small flocs formed in the end. In strong alkaline conditions, by means of the high MW properties and special adsorption function, Tanfloc connected numerous EOM molecules with the bridging effect, resulting in the formation of a cross-linked network structure and tighter and larger flocs. However, no obvious long-chain flocs appeared in the SEM images because the Tanfloc MW was not high enough.

3.3. Interaction of Tanfloc and dEOM/bEOM

The flocculation experiment showed that Tanfloc had different removal efficiencies on dEOM and bEOM, which could be related to the intensity and mode of the interaction between dEOM, bEOM, and Tanfloc. We conducted FTIR spectroscopy analysis of dEOM, bEOM, Tanfloc, and their flocs to identify the changes in their composition and functional groups after flocculation (Fig. 5 and Table 2).

A broad absorption band at 3400–3458 cm^{-1} was observed in all

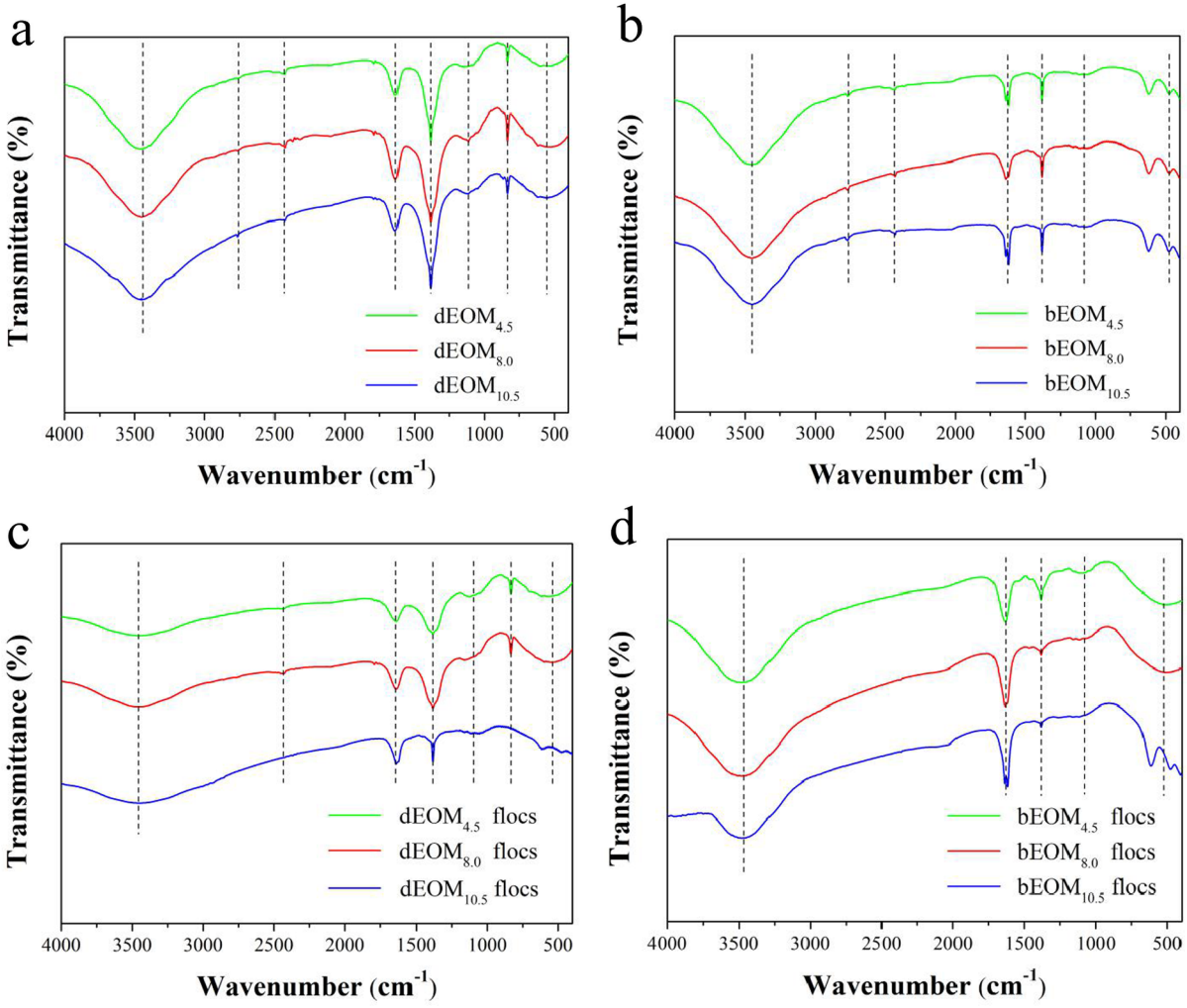

Fig. 5. FTIR spectra of (a) dEOM, (b) bEOM, (c) dEOM flocs, and (d) bEOM flocs recovered from water with pH 4.5, 8.0 and 10.5.

Table 2
Absorption peaks identified in the FTIR spectra of dEOM, bEOM, and their flocs.

Wavenumber (cm^{-1})	Function group	Compound	Reference
3400–3458	O—H/N—H stretching vibration	Organic matter/Tanfloc	[45,46]
2764	—CHO/—NH_2 stretching vibration	Organic matter/Tanfloc	[47]
2424–2440	P-H stretching vibration	Organic matter	[48]
1634–1640	C—O/C—N stretching vibration	Proteins	[49]
1384	C—H symmetric transformation vibration	Organic matter	[46]
1111–1147	C—O/C—N stretching vibration	Polysaccharides	[50]
834	C—H bending vibration	Organic matter	[48]

the flocs, which was caused by the O-H/N-H stretching vibration of the organic matter in dEOM and bEOM or the phenolic hydroxyl group in Tanfloc. The weak absorption peak at 2764 cm^{-1} in dEOM and bEOM was associated with the —CHO/—NH_2 stretching vibration in dEOM and bEOM. The weak absorption band in the range 2424–2440 cm^{-1} was caused by the P-H stretching vibration observed in the dEOM floc, but not observed in the bEOM floc. This indicates the presence of organic compounds containing P-H in dEOM, which could be removed adequately. The strong absorption peaks at 1634–1640 cm^{-1} and 1111–1147 cm^{-1}, caused by the C—O/C—N stretching vibration in proteins and polysaccharides, were observed in all the flocs respectively. The peak intensity in the bEOM floc was higher than it was in the dEOM floc, indicating that both proteins and polysaccharides could be removed adequately. Furthermore, the functional groups of C—O/C—N in bEOM had a stronger interaction with Tanfloc compared with that of dEOM. The strong absorption peaks at 1384 cm^{-1} caused by the C—H symmetric transformation vibration can be found in all flocs. The higher peak intensity of the dEOM floc could be ascribed to its higher organic matter content in comparison with that of the bEOM floc. The absorption peak at 834 cm^{-1} in the dEOM floc was associated with the C-H bending vibration in the organic matter.

With an increasing pH value, the intensity of the characteristic peak decreased gradually for both dEOM and bEOM flocs. This indicated that pH influenced the combination of Tanfloc and EOM and the interaction decreased when the pH value increased. Notably, there was no corresponding characteristic peak at 2424–2430 and 834 cm^{-1} in the dEOM flocs at pH 10.5. This could be ascribed to the organic compounds containing P-H not being removed adequately. Additionally, the absorption peaks of 526 cm^{-1} in dEOM and 480 cm^{-1} in bEOM appeared blue shifted after flocculation. This could be ascribed to various special structures forming after Tanfloc had combined with dEOM/bEOM. Further study is required for this specific structure.

3.4. Environmental implication

This study provided a relatively comprehensive and in-depth discussion of the flocculation performance and mechanism of Tanfloc in removing specific EOM, as well as the interaction of Tanfloc and dEOM/bEOM. The study showed that compared with the chemical flocculant, Tanfloc could flocculate EOM, particularly bEOM, effectively in a wide pH range (4.5–10.5). We introduced a safe and effective strategy to remove EOM that has guiding significance for reducing or avoiding the environmental risks associated with EOM. When treating a small area of algal bloom water, a lower Tanfloc dosage was needed and higher flocculation efficiency was achieved by lowering the water pH. Both dEOM and bEOM could be removed adequately using a Tanfloc dosage of only 25 mg/L at pH 4.5. When treating a large area of cyanobacteria bloom water with weak alkalinity (pH ≈ 8.5), EOM could be removed adequately by increasing the dosage. Dosages of 50 and 150 mg/L could flocculate both dEOM and bEOM effectively at pH 8.0 and 10.5, respectively. Our previous study indicated that high algal removal efficiency (98.9%) could be achieved with a Tanfloc dosage of 10.42 mg L^{-1} [12]. The Tanfloc dosage required for efficient EOM treatment is therefore adequate to remove algae cells.

Tanfloc is relatively cheap at ~$0.0161 US when treating 1 tonne of algal bloom water. Although the price is higher than that of PAC ($0.0026 US $tonne^{-1}$), it is far lower than that of another flocculant, namely, chitosan ($0.1667 US $tonne^{-1}$) [6]. Furthermore, Tanfloc is harmless to the water environment. Gutiérrez [44] assessed the potential toxicity of Tanfloc based on the quantum yield measurements for freshwater microalgae and reported that doses up to 50 mg L^{-1} presented no adverse effects. Therefore, Tanfloc has significant application potential in the treatment of algal bloom water. However, it must be noted that Tanfloc has various shortcomings relative to the chemical flocculant. Tanfloc itself is organic matter; therefore, when it is used for EOM removal, some organic matter could remain in the water after flocculation. Moreover, the removal ability of Tanfloc on low MW organic matter, one of the sources of DBP precursors, is weak. In future, more pH gradients should be set up to further investigate the removal performance on EOM and specific EOM in the pH range of 4.5–8.0 or 8.0–10.5.

4. Conclusions

The removal performance of Tanfloc on bEOM was stronger than on dEOM, proven by an obviously higher DOC removal rate and lower Tanfloc dosage in bEOM. The protein-like substances, mainly from bEOM, including tryptophan-like and aromatic-like substances, were treated adequately, but the humic acid, fulvic acid, and soluble microbial-product-like substances, mainly in dEOM, were difficult to remove. The high MW substances (> 100 kDa) were removed easily, whereas various low MW substances (< 1 kDa), derived from both dEOM and bEOM, remained in the water after flocculation. Tanfloc removed EOM, regardless of bEOM and dEOM, mainly by combining with protein and polysaccharide, with the removability dependent on water pH. An adequate flocculation performance was achieved in acidic and weak alkaline water, with charge neutralization and electrostatic patching, respectively, the main mechanisms. However, a relatively poor flocculation performance was observed because of the bridging mechanism in strong alkaline water, although large amounts of specific EOM could be removed adequately even in this instance. Additionally, the hydrogen bonds and hydrophobic interaction between Tanfloc and protein or polysaccharide were conducive to specific EOM removal. The functional groups of C-N/C-O in bEOM, presenting stronger interaction with Tanfloc compared with dEOM, were responsible for the superior removal of bEOM.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2018.11.008.

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