Enhanced removal of intracellular organic matters (IOM) from *Microcystis aeruginosa* by aluminum coagulation

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ABSTRACT

Algal organic matter (AOM), especially intracellular organic matter (IOM), increases the levels of color and taste & odor, and promotes the formation of disinfection by-products (DBPs). This study isolated the IOM from *Microcystis aeruginosa* into the fractions with different molecular weight (MW), i.e., >100 kDa, 30–100 kDa, 10–30 kDa, and 3–10 kDa, and characterized them by techniques of dissolved organic carbon (DOC), UV absorbance and Excitation-emission matrix (EEM) fluorescence spectroscopy. After that, the removal efficiency of these IOM fractions by aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$, alum) at different Al doses and pH were compared. The removal efficiency of IOM, as indicated by DOC, UV_{254} , and OD_{680} , increased consistently with higher Al doses, and the maximum removal was determined to be 99.7% for OD_{680} , 51.4% for UV_{254} , and 38.7% for DOC by alum at 5 mg/L. pH also showed effects, and the maximum removal was achieved at pH 6.5 with the maximum removal of 42.3% for DOC and 61.5% for UV_{254} . The higher UV_{254} removal as compared to that of DOC indicated the superiority of alum to remove the aromatic IOM species. EEM spectra indicated the higher protein content within these IOM fractions, and the formation of complexes between Al and proteins involved in their removal. IOM with higher MW showed more significant removal of both DOC and UV_{254} than that with lower MW, and the sweep flocculation mechanism played an important role. Alum coagulation is effective and available for IOM removal in case of algae bloom and minimize its adverse effects on water safety thereafter.

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1. Introduction

In China eutrophication is widely-occurred in many lakes and reservoirs and has received great concern in the past decades [1], and in 2007 the water supply in Wuxi City was even cut off due to the algae bloom in Taihu Lake. The algae cells at extremely high concentrations adversely affect the water treatment processes such as coagulation, sedimentation, and filtration [2,3]. Additionally, the seasonal growth of cyanobacteria such as *Microcystis aeruginosa* (*M. aeruginosa*) causes the release of toxins and taste & odor substances and increases the health risks of drinking water accordingly. The algal organic matter (AOM) is another family of organics to be well concern. AOM consists of extracellular organic matter (EOM) and intracellular organic matter (IOM), and EOM is released from algal cells by diffusion whereas IOM is from

senescent algal cells during cell lysis. In detail, AOM mainly includes the components of proteins, peptides, humic acid, amino sugars, and polysaccharides [4,5], among which the proteins concentrations are the highest. Meanwhile, some researchers found that the tryptophan (protein-like) was higher than others components in the reservoir in China, and the proportion of protein-like was above 65% [6]. The natural apoptosis of algae cells results in AOM release. Additionally, in drinking water treatment, the exposure of algae cells to chemical oxidants such as chlorine and potassium permanganate also contributes to the elevated AOM concentrations [7].

The release of AOM increases the levels of color, dissolved organic carbon (DOC), and assimilable organic carbon (AOC), and promotes the formation of disinfection by-products (DBPs) [8,9]. Additionally, AOM at high concentrations is reported to inhibit coagulation process [10], and its different components on coagulation have been investigated before. Bernhardt et al. (1987) first reported the effects of AOM on destabilization step and the coagulation efficiency thereafter [11], and the elevated coagulant doses may improve coagulation to some extent but at the expense of

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increased cost and sludge production. Tomoko et al. (2007) reported that both EOM and cellular organic matter (COM) disturbed the flocculation of suspended kaolin with polymer aluminum chloride (PACl), and the non-proteinous substances within EOM may play an important role [12]. Additionally, EOM mainly acts as non-ionic polymers or anionic polyelectrolytes and shows effects thereafter [13,14]. Basically, the effects of AOM on coagulation may include two aspects. Firstly, AOM at low levels may improve coagulation by the formation of inter-particle bridges or its adhesion to particles surface. However, AOM at extremely high levels shows inhibitive effects on coagulation by increasing the negative charge on particles surface [15]. Additionally, some proteins such as metalloenzymes have high affinity towards metal ions [16], and their formation of complexes with coagulant reduces the coagulant doses available for colloids destabilization and deteriorates coagulation accordingly [17]. In membrane filtration process, AOM may also show adverse effects and significantly accelerate membrane fouling. Qu et al. (2012) reported that the reversible and irreversible fouling of ultrafiltration (UF) membranes by extracellular organic matter (EOM) from cyanobacterial cells, and the serious flux decline was attributed to the deposit of macromolecular organics such as proteins and polysaccharides on membranes [18,19].

It is of crucial importance to enhance the removal algal cells and IOM in drinking water treatment processes, the IOM released during pre-oxidation or hydraulic shearing of algae cells might exist throughout the whole water treatment. Therefore, research specifically on IOM, rather than the whole algae cell, might be more advisable to evaluate the DBP formation. Furthermore, IOM, as high protein compounds, may have a great potential to produce N-containing DBPs [3,20]. Among the unit processes of the conventional water treatment processes, i.e., coagulation, sedimentation, filtration, and disinfection, coagulation is the best unit to achieve this objective. The enhanced removal of algae cells and IOM by coagulation may also minimize the unfavorable effects of the sedimentation and filtration. As for membrane filtration process, the enhanced removal of IOM by coagulation prior to membrane filtration is also beneficial to membrane performance. To achieve the enhanced removal of IOM by coagulation, it is of crucial importance to illustrate the removal behaviors of IOM with different molecular weight distribution. However, rare attention has been attributed to this to the best of our knowledge.

In this study, we extracted IOM from *M. aeruginosa* cells and separated them into the fractions of >100 kDa, 30–100 kDa, 10–30 kDa, and 3–10 kDa by molecular weight (MW) fractionation method. After that, these fractions were carefully characterized by dissolved organic carbon (DOC), UV, and Excitation-emission matrix (EEM) fluorescence spectroscopy, and their removal behaviors by the widely-used aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$) were studied. We proposed to analyze and evaluate the coagulation efficiency for different molecular weight (MW) fractionation of IOM using aluminum at different doses and pH ranges. Furthermore, the purpose to this work is to investigate the different contents of IOM by the FRI technique and study the effects of fluorescent intensity in the presence or absence of aluminum for all fractions of molecular weight, and the dominant mechanisms involved in were proposed accordingly.

2. Materials and methods

2.1. *Aeruginosa* cultivation and intracellular organic matter(IOM) extraction

M. aeruginosa (strain FACHB-905) was obtained from Wuhan Institute of Hydrobiology, Chinese Academy of Sciences. *M. aeruginosa* was kept in a 500-mL conical flask containing 250 mL *M. aeruginosa* medium using two a fluorescent lamp (6000 lx) with a cycle of light (14 h) and dark (10 h) at 25 °C. The *M. aeruginosa* cells in a steady-state growth were used for IOM extraction.

On the 16th day of the cultivation period, *M. aeruginosa* cells were collected by centrifugation at 6000 r/min for 30 min and the supernatant was abandoned. After that, *M. aeruginosa* cells were washed two times and re-suspended in Milli-Q water, and were then centrifuged at 6000 r/min for 20 min. The obtained cells were crushed by the ultrasonic grinder for 60 min and then suspended in 20-mL Milli-Q water. The suspension cells suffered from freezing/unfreezing cycles to facilitate the destruction of cyanobacterial cells and achieve the entire release of intracellular organic matter (IOM) without surface retained organic matter (SOM). The frozen sample with *M. aeruginosa* cells were thawed at room temperature. After that, the sample was filtered through a 0.22-μm membrane filter to remove the residual solids and the obtained filtrate was referred to as intracellular organic matter (IOM) [21].

The samples retrieved immediately through 0.45 μm membrane, as the membrane is the organic matter dissolved organic matter (DOM). All the solutions were isolated using different filter cut-off sizes (3,10,30, and 100 kDa), which is consist of regenerated fiber membranes (Millipore Co., USA) with ultrafiltration cups placed in a centrifuge (Model 8200, Amicon Corp., Beverly, MA), as concentration factor 6:1, nitrogen pressure through the membrane: 0.03 MPa–0.25 MPa. Then samples were stored in a TEFLON (PTFE) sealed glass with 4 °C in a refrigerator, respectively.

2.2. Ultrafiltration fractionation of IOM

The samples retrieved immediately through 0.45 μm membrane, as the membrane is the organic matter dissolved organic matter (DOM). All the solutions were isolated using different filter cut-off sizes (3,10,30, and 100 kDa), which is consist of regenerated fiber membranes (Millipore Co., USA) with ultrafiltration cups placed in a centrifuge (Model 8200, Amicon Corp., Beverly, MA), as concentration factor 6:1, nitrogen pressure through the membrane: 0.03 MPa–0.25 MPa. Then samples were stored in a TEFLON (PTFE) sealed glass with 4 °C in a refrigerator, respectively.

2.3. Coagulation experiments

The raw water was prepared by suspended Kaolin (23,000–02, Kanto, Tokyo, China) in the presence of IOM from *M. aeruginosa*. pH value was adjusted to sodium 1 M hydroxide (NaOH) or 1 M hydrochloric acid (HCl). The characteristic of raw water is as follow: OD_{680} was with 0.03 cm^{-1} , DOC with 8.4 mg/L, UV_{254} with 0.09 cm^{-1} , pH with 7.0, turbidity with 30 NTU, and hydronium intensity with 3.3 mM.

Coagulation tests were performed with 500 mL sample in 800 mL beakers and conducted on a programmable jar tester (MY3000-6, MeiYu, China). After dosing ($\text{Al}_2(\text{SO}_4)_3$) in the initial 20 s rapid mixing at 250 rpm, the samples were rapidly mixed at 200 rpm for 2 min and then at 40 rpm for 15 min, consecutively. After 30-min sedimentation, the supernatants (200 mL) were carefully collected to avoid the suction of precipitated solids. Solution pH was analyzed by a 720 A pH meter (Thermo Orion, USA) and adjusted to 7.0 by 1 M HCl and/or 1 M NaOH during rapid mixing period, All experiments were triple.

The absorbance at 680 nm (OD_{680}) and at 254 nm (UV_{254}) of the collected supernatants were measured by a U-3010 Spectrophotometer (Hitachi Co., Japan), and the DOC concentrations were measured by a TOC-VCPh total organic carbon analyzer (Pheonix8000, Tekmar-DOHRMANN, USA). All samples were filtered through a 0.45 μm membrane except for OD_{680} .

2.4. Excitation-emission matrix (EEM) fluorescence spectroscopy

In recent years, Excitation-emission matrix (EEM) fluorescence spectroscopy has been commonly used to characterize dissolved organic matter (DOM) in water treatment [22]. In our study, the composition of IOM in the supernatant was analyzed with 3D-EEM by F-2500 spectrofluorometer (HITACHI, Tokyo, Japan) after being filtered through 0.45 μm membranes. The 3D-EEM spectra

were constructed by scanning emission spectra as a function of excitation wavelength. An excitation wavelengths from 200 to 400 nm in 5 nm steps were used, and emission wave lengths from 290 to 550 nm in 5 nm steps were measured. Milli-Q water was used as a blank sample. Each EEM complexes of emission scans from a single sample recorded at incrementing excitation wave wavelengths and arranged in a grid (excitation–emission-intensity), the FRI technique was used in analyzing drinking water samples [23], which was developed to integrate the area beneath EEM spectra. According to Wen, the volume (φ_i) was expressed by

$$\varphi_i = \sum_{ex} \sum_{em} I(\lambda_{ex}\lambda_{em}) \Delta\lambda_{ex} \Delta\lambda_{em}$$

where λ_{ex} is the excitation wavelength interval (taken as 5 nm), λ_{em} is the emission wavelength interval (taken as 0.5 nm), and $I(\lambda_{ex}, \lambda_{em})$ is the fluorescence intensity at each excitation–emission wavelength pair. Within each of the five FRI regions, but in our study, there are three main regions to be attention, that is to say, in general, firstly, peaks at shorter excitation wavelengths (<250 nm) and shorter emission wavelengths (<350 nm) are related to simple aromatic proteins (PR) [23], secondly, humic acid-like organics (HA) is the peaks at longer excitation wavelengths (>280 nm) and longer emission wavelengths (>380 nm) [23], and the last peaks at shorter excitation wavelengths (<250 nm) and longer emission wavelengths (>350 nm) are related to fulvic acid-like materials (FA)(Region III) [23]. Therefore, in this study the FRI technique was applied to characterize all fractions of molecular weight of IOM transformation.

Fluorescence intensity (FI) is determined by Mc Knight et al. (2001) [24], which is the ratio of degrees defined as emission with 450 nm fluorescence intensity and emission with 500 nm fluorescence intensity at excitation with 370 nm. FI can be used to distinguish organic matter from terrigenous sources (surface runoff and soils).

or biogenic (bacterial, algae-derived), and it has a negative correlation with the aromaticity of humics [25], Mc Knight et al. [24] reported that FI(450/500) of the biological source was higher, In this study, FI(450/500) are above 1.9.

3. Results and discussion

3.1. Characterization of IOM with different molecular weight

Table 1 illustrates the dominant characters of each IOM fraction with different molecular weight distribution. The DOC of different IOM fractions, i.e., >100 kDa, 30–100 kDa, 10–30 kDa, and 3–10 kDa, were determined to be 275.12, 24.05, 15.21, and 15.41 mg/L, and the DOC ratios of these fractions were calculated to be 82.6%, 7.2%, 4.5%, and 4.63% accordingly. The high DOC of >100 kDa fraction was relative to the high UV₂₅₄ and specific ultra-violet absorbance at 254 nm (SUVA₂₅₄), which were determined to be 0.14 cm^{−1} and 0.03 L/mg·m, respectively. Interestingly, the 30–

100 kDa fraction showed much lower DOC level as compared to that of >100 kDa fraction. However, the SUVA₂₅₄ of these two components was similar. It was noted that the <3 kDa fraction showed DOC of as low as 3.14 mg/L, and the contributive ratio to DOC was below 0.94%, 1–3 kDa fraction and <1 kDa fraction were lower for DOC than <3 kDa fraction, and all were ignored in this study thereafter.

EEM results indicate that the proteins-like organics outweighed the other two components of humic-like and fulvic acid-like species, and its contributive ratios to EEM intensity were above 50% for different IOM fractions (Table 1). This result was ascribed to the high protein levels within algae cells and was in accordance with what have reported before [26]. Comparatively, the intensity of Peak for the proteins-like species, of the >100, 30–100, 10–30, and 3–10 kDa fractions was determined to be 591.1, 268.4, 235.8, and 486.2 A.U., respectively. The humic-like substances mainly existed in the 10–30 and 3–10 kDa fractions whereas the fulvic acid-like species was in the MW range of 10–30 and 30–100 kDa. EEM fluorography of every MW fractions were shown in Fig. 1S (seen in supplementary data).

Table 2 compares the SUVA₂₅₄ and FI of different IOM fractions under acidic (pH = 4.5), neutral (pH = 7.5), and alkaline (pH = 9.5). The >100 kDa fraction showed the lowest FI among these IOM fractions. Comparatively, the 3–10 kDa fraction showed higher FI than the other two fractions of 30–100 kDa and 10–30 kDa in wide pH ranges. This might be indicative of the involvement of the IOM species with strong polar and/or double bond functional groups [27]. Comparatively, pH had much less effect on the fluorescence absorption of fulvic-like organics, which was shown in Fig. 2(seen in supplementary data).

Compared to the >100 kDa fraction, the SUVA₂₅₄ of the other three fraction (30–100 kDa, 10–30 kDa and 3–10 kDa) reduced under acidic (pH = 4.5), neutral (pH = 7.5), and alkaline (pH = 9.5). The SUVA₂₅₄ of the >100 kDa fraction showed the unstable FI among these IOM fractions under different pH, i.e. 7.1 with pH = 4.5, 4.6 with pH = 7.5, 4.8 with pH = 9.5.

3.2. Coagulation behaviors of aluminum towards IOM

3.2.1. Effect of aluminum doses

The absorption value of *M. aeruginosa* at 680 nm (OD₆₈₀) was reported to be positively correlated with algae cells density [28], and was indicative of the pigment-like organics such as chlorophylls and carotenoids [29]. Additionally, the organics with strong absorption at 680 nm released into solution during IOM extraction, and OD₆₈₀ was indicative of IOM levels thereafter. Fig. 1 shows the removal behaviors of IOM as indicated by DOC, UV₂₅₄, and OD₆₈₀ with elevated aluminum doses from 0.5 to 5 mg/L as Al, respectively. The removal efficiency of DOC, UV₂₅₄, and OD₆₈₀ increased consistently with higher aluminum doses, and the maximum removal was determined to be 99.7% for OD₆₈₀, 51.3% for UV₂₅₄, and 38.6% for DOC(Fig. 1a). The different removal efficiency of

Table 1

Main characters of each IOM fraction with different molecular weight.

MW	DOC ^a (mg/L)	UV ₂₅₄ ^b (cm ^{−1})	SUVA ^b (L/mg·m)	pH ^b	EEM Intensity ^c (A.U.)		
					Peak P	Peak H	Peak F
>100 kDa	275.1	0.14	0.03	5.9	591.1	25.2	11.2
30–100 kDa	24.1	0.06	0.01	6.2	268.4	75.2	102.3
10–30 kDa	15.2	0.05	0.01	6.8	235.8	105.8	124.8
3–10 kDa	15.4	0.09	0.02	6.9	486.2	172.5	20.3
<3 kDa	3.2	0.01	–	6.8	78.9	54.3	25.7

^a The original DOC before dilution for all fraction.

^b The measured value after dilution for the DOC diluted to 5 mg/L.

^c Peak P: Proteins-like, maxima at (220,345); Peak H: Humic-like, maxima at (375,450); Peak F: Fulvic acid-like, maxima at (225,455).

Table 2
SUVA₂₅₄ and FI of IOM with different MW at pH 4.5, 7.5, and 9.5.

pH	MW (kDa)	SUVA ₂₅₄ (L mg ⁻¹ m ⁻¹)	FI
pH = 4.5	>100	7.07	1.96
	30–100	9.55	2.27
	10–30	8.66	2.41
	3–10	4.81	2.11
pH = 7.5	>100	4.56	2.23
	30–100	8.78	2.55
	10–30	7.96	3.03
	3–10	4.18	2.29
pH = 9.5	>100	4.76	2.25
	30–100	8.44	2.99
	10–30	9.27	2.47
	3–10	4.71	3.18

Fig. 1. (a) Removal IOM at different Al dose range; (b) Adsorption of IOM at different Al dose ranges using UV-Vis spectrometry [Experimental conditions: DOC of raw water = 8.45 mg/L].

OD₆₈₀, UV₂₅₄, and DOC at the same Al dose may be attributed to the different species involve in them. UV₂₅₄ was indicative of the aromatic compounds [30], whereas OD₆₈₀ represented the IOM with strong absorbance at 680 nm, which was mainly relative to the IOM with high molecular weight [31]. These results indicated that aromatic IOM are difficult to be removed by coagulation whereas the OD₆₈₀ with higher molecular weight are comparatively more easier.

Moreover, the UV-Vis absorption of IOM was the lowest to be 0.01 cm⁻¹ at 5 mg/L of aluminum (Fig. 1b). This may be first attributed to the significant removal of organics with strong absorption

at 254 nm by aluminum coagulation. Additionally, soluble Al ions also tended to form complexes with negatively-charged IOM [31]. Prior to Al coagulation, IOM showed two main absorption peaks in wave-number range of ultraviolet zone (i.e., 200–350 nm) and visible light zone (i.e., 600–700 nm), respectively. These two peaks decreased consistently with higher aluminum doses, and the lowest absorption was determined to be 0.05 cm⁻¹ in 200–350 nm and 0.01 cm⁻¹ in 600–700 nm at 5 mg/L of aluminum as Al. The removal of organics as indicated by absorbance in 200–350 nm was less than those in 600–700 nm.

Fig. 2 compares the ratios of OD₆₈₀ to UV₂₅₄ ($R_{OD680:UV254}$) and those of OD₆₈₀ to DOC ($R_{OD680:DOC}$) with elevated aluminum doses. It was observed that $R_{OD680:UV254}$ values were much lower than those of $R_{OD680:DOC}$ at the same Al doses, and more significant difference was observed at lower Al doses. This indicated that UV₂₅₄ was more difficult to be removed as compared to DOC. In addition, the fluorescence index (FI), i.e., the ratios of fluorescence absorbance at λ_{em} of 450 nm to that at 500 nm (λ_{ex} = 370 nm), increased from 2.2 to 2.5 with elevated Al doses from 0.5 to 5 mg/L, and this result was in accordance with the more significant decrease of aromatic substances with elevated Al doses. SUVA₂₅₄, the specific ultraviolet absorbance as the ratio of UV₂₅₄ to DOC, showed opposite trends with elevated Al doses as compared to that of FI (Fig. 2b). In other words, FI increased from 2.25 to 2.37 with elevated Al doses from 0.5 to 5 mg/L, while SUVA₂₅₄ decreased from 5.1 to 1.33 with elevated Al doses from 0.5 to 5 mg/L. The opposite behaviors between SUVA₂₅₄ and FI inferred that they represented

Fig. 2. (a) The ratios of OD₆₈₀ to UV₂₅₄ ($R_{OD680:UV254}$) and those of OD₆₈₀ to DOC ($R_{OD680:DOC}$) at different Al dose ranges; (b) the SUVA₂₅₄ and FI at different Al dose ranges (Experimental conditions: DOC of the raw water = 8.45 mg/L).

different species within IOM, and the stronger aromaticity was relative to the lower FI values [32].

The removal of SUVA_{254} and FI can hardly be improved by increasing Al doses range from 3 to 5 mg/L (Fig. 2b). This inferred that aluminum exhibited high affinity towards unsaturated C=C and C=O bonds within aromatic compounds with high SUVA, e.g., tryptophan-like aromatic proteins. Comparatively, the tryptophan-like aromatic proteins and dissolved microbial metabolites mainly existed in the hydrophobic fraction with high FI values [33]. Henderson et al. (2008) also reported that hydrophobic proteins and polysaccharides were the dominant AOM species [4]. It was reported that proteins were the main species in the released IOM from cyanobacteria, and some proteins such as metalloenzymes exhibited high affinity toward metal ions [34]. Hong et al. (2008) reported that the algal cells consisted of 82.1% proteins, and some proteins from cyanobacteria tended to form complexes with coagulants and deteriorated coagulation efficiency thereafter [35]. These studies supported the removal efficiency of different IOM species at different Al doses.

3.2.2. Effect of pH

pH affects the hydrolysis of coagulants and the species distribution of IOM species, and affects the coagulation efficiency accordingly [36]. Fig. 3 illustrates the removal of DOC, UV_{254} , and OD_{680}

in wide pH range from 3.5 to 9.5 at Al dose of 2 mg/L. It was observed that their removal efficiency increased with elevated pH in pH range from 3.5 to 6.5, and the maximum removal efficiency was observed to be 42.3% for DOC and 61.5% for UV_{254} at pH 6.5. After aluminum coagulation at pH 6.5, the UV–Vis scan spectrometry in wave-number range from 250 to 700 nm was consistently below 0.01 cm^{-1} , and this supported that the organics concentrations were rather low. The elevation of pH to 7.5 improved OD_{680} removal and the maximum removal efficiency was as high as 99.5% accordingly. The further increase of pH inhibited their removal and the removal efficiency of DOC, UV_{254} , and OD_{680} decreased to 36.2%, 35.3%, and 71.8% at pH 9.5 (Fig. 3a). pH showed influence on aluminum hydrolysis and affected the removal efficiency of organic matter thereafter [37]. At pH above 6.5, aluminum ions transformed to the hydrolyzed polymer and/or colloid, and the particulate IOM with high MW, e.g., OD_{680} , may be removed by bridging and sweeping effects [37]. At lower pH of below 6.5, the polymeric and monomer aluminum were the dominant Al species, and the removal of IOM was achieved by the formation of complex, adsorption, bridging and co-precipitation, and these combined effects enabled the high removal of DOC and UV_{254} [38].

To further illustrate the mechanisms involved in, the values of $\text{ROD}_{680}:\text{UV}_{254}$ and $\text{ROD}_{680}:\text{DOC}$ were compared in wide pH range from 3.5 to 9.5 (Fig. 4a). $\text{ROD}_{680}:\text{DOC}$ values were observed to be higher than those of $\text{ROD}_{680}:\text{UV}_{254}$ in pH range from 3.5 to 7.5 (Fig. 4a), and it was inferred that UV_{254} was easier to be removed at low pH as compared to DOC. SUVA_{254} decreased gradually from 2.0 L/

Fig. 3. (a) The removal of DOC, UV_{254} , and OD_{680} in wide pH range from 3.5 to 9.5 at Al dose of 2 mg/L; (b) Adsorption of IOM in wide pH range from 3.5 to 9.5 at Al dose of 2 mg/L using UV–Vis spectrometry (Experimental conditions: DOC of raw water = 8.42 mg/L).

Fig. 4. (a) The values of $\text{ROD}_{680}:\text{UV}_{254}$ and $\text{ROD}_{680}:\text{DOC}$ in wide pH range from 3.5 to 9.5; (b) SUVA_{254} and FI with elevated pH from 3.5 to 9.5 (Experimental conditions: DOC of raw water = 8.42 mg/L).

mg m to 1.0 L/mg m, whereas FI increased from 1.6 to 2.3 with elevated pH from 3.5 to 9.5 (Fig. 4b). This result also indicated that the aromatic substances were easier to remove with elevated pH. Hoyer et al. (1985) investigated that molecular weight (MW) and the concentration of dissolved organic carbon (DOC), carbohydrates and uronic acid was highly variable, depending on both species and culture age, and also reported that the $SUVA_{254}$ was positively related with the aromaticity [39].

Table 3 illustrates the 3D-EEM results of residual IOM after aluminum coagulation (Al dose = 2 mg/L). After data integration by FRI method, it was observed that the integrated values of protein (PR) area decreased from 72.8% to 50.2%, whereas those of HA and FA areas respectively increased from 11.7% to 27.1% and from 16.4% to 22.7% with pH increasing from 3.5 to 9.5. This result indicated that the concentrations of Al-IOM complexed were highly pH-dependent and significantly affected the fluorescence intensity accordingly [40].

3.3. Removal behaviors of IOM with different MW by aluminum coagulation

In different water sources, the species of NOM is very different, due to the carbon cycle and water force conditions for experienced biochemical, physical, chemical process [41], for example, the nitrogen content is higher and the content of aromatic carbon and phenol is lower for algae, meanwhile with the process of rain-fall and other surface runoff, the DOC from soil flowed into the water of surface, which caused the species was significantly different between NOM and algae [42], causing removal behaviors was different. Therefore, to further illustrate the mechanisms involved in the removal of IOM by aluminum coagulation, the different IOM fractions, i.e., >100 kDa, 30–100 kDa, 10–30 kDa, and 3–10 kDa, were separately coagulated by aluminum at pH 4.5, 7.5, and 9.5, and their removal efficiency was illustrated in Fig. 5. It was observed that the IOM fraction with high MW was easier to be removed as compared to that with low MW. The removal efficiency of >100 kDa fraction was highest among these IOM fractions, and DOC removal was higher than that of UV_{254} for the same IOM fraction. As for >100 kDa fraction, the removal of DOC and UV_{254} was determined to be 31.5% and 53.2% at pH 4.5. At elevated pH of 7.5, the similar trends were observed. Meanwhile, as for the same IOM fraction, the removal of UV_{254} was observed to be higher than that of DOC. UV_{254} represented the negatively-charged aromatic compound with conjugate double-bond compounds [40], and it was more likely to be removed by the positive hydrolysis products. However, all fractions with different MW included the neutral and weak polar substances, and their removal by coagulation is relatively weak as compared to UV_{254} . At pH 9.5, the fractions of 30–100 kDa and 10–30 kDa showed lower UV_{254} removal than DOC. Quantitatively, the removal efficiency of 30–100 kDa fraction was observed to be 4.1% for UV_{254} and 20.5% for DOC, and that of 10–30 kDa fraction was determined to be 2.8% for UV_{254} and 5.6% for DOC. These results demonstrated that aluminum coagulation showed different removal behaviors towards the IOM with different MW. pH had significant effect on the removal of the organics with different MW, and the organics with lower MW was more

Fig. 5. Removal efficiency of UV_{254} , DOC of IOM at different pH ranges by aluminum coagulation for the different IOM fractions (Experimental conditions: DOC of the raw water for all MW fractions = 5 mg/L).

difficult to be removed by coagulation [43]. The removal of >100 kDa fraction was consistently high in wide pH range from 4.5 to 9.5. The macromolecular organic matter with high MW is more hydrophobic and is easier to be removed by chemical coagu-

Table 3

The contributive ratios of different species on total FI from 3D-EEM analysis after $Al_2(SO_4)_3$ coagulation in wide pH range from 3.5 to 9.5.

pH	PR(%)	HA(%)	FA(%)	PR/HA	PR/FA
3.5	72.83	11.71	16.35	6.15	4.42
4.5	71.88	11.57	16.55	6.22	4.34
5.5	69.43	12.61	17.97	5.51	3.86
6.5	59.42	20.06	20.52	2.96	2.89
7.5	52.43	24.49	23.07	2.14	2.27
9.5	50.22	27.12	22.65	1.85	2.22

lation as compared to that with low MW [44]. Interestingly, the 3–10 kDa fraction showed higher removal of DOC and UV₂₅₄ than the 10–30 kDa fraction did. This might be attributed to the higher content of dissolved organic matter and aromatic organics within 3–10 kDa fraction, i.e. for Table 1, the DOC and UV₂₅₄ of the 3–10 kDa fraction were higher than that of the 10–30 kDa. Generally, the charge neutralization by aluminum and the adsorption of IOM onto positive aluminum hydrolyzed precipitates may be the dominant mechanism involve in IOM removal.

The protein within AOM, e.g., the IOM from *M. aeruginosa*, were reported to be the main species to adversely inhibit coagulation [17,35]. This study indicated that protein was the main component as compared with HA and FA, and their concentrations varied for different IOM fractions. Additionally, the removal of the protein within different IOM fractions was pH dependent, and its removal efficiency varied from 70.8% to 50.2% in wide pH range from 3.5 to 9.5. All the IOM fractions with different MW showed higher portion of protein in comparison with HA and FA in wide pH range (Fig. 6). For example, the portions of protein, HA, and FA were respectively determined to be 44.9%, 24.6%, and 30.4% at pH4.5 for >100 kDa (Fig. 6a). The ratios of protein within all fractions decreased whereas those of FA increased with elevated pH from 4.5 to 9.5 (Fig. 6b–d), possibly due to the precipitation of proteins.

Proteins were reported to have strong affinity with metalloids and alkaline metal ions, and the formation of complexes between metal and cyanobacterial proteins played an important role [45]. Fig. 7 compares the fluorescence intensity values of all IOM fractions with different MW before and after aluminum coagulation at different pH, and the significant effect was observed. At pH 4.5 and 7.5, the fluorescence intensity values of >100 kDa and 10–30 kDa fractions increased whereas those of 30–100 kDa and 3–10 kDa decreased after aluminum coagulation. This inferred that the fractions of >100 kDa and 10–30 kDa had greater affinity towards Al. At alkaline pH of 9.5, the fluorescence intensity values of all these fractions decreased after aluminum coagulation, and the most significant decrease was observed for the >100 kDa frac-

Fig. 7. The FI values of all IOM fractions with different MW before and after aluminum coagulation at different pH (Experimental conditions: DOC of the raw water for all MW fractions = 5 mg/L).

tion. This may be attributed to the lower affinity of this fraction towards the hydrolyzed aluminum as compared to Al-protein complexes, and the attachment onto the particle surfaces was inhibited thereafter [16]. It can clearly be seen that there were some organic acidic matters for >100 kDa, which has a lower affinity with hydrolyzate of aluminum as to complexes attached to the particle surface. (See Fig. 8)

3.4. Mechanisms of AOM removal by aluminum coagulation

It is widely-accepted that coagulation involves in four mechanisms of charge neutralization, adsorption, bridging, and sweep flocculation. aluminum was characterized by different Al species

Fig. 6. PR, HA and FA of IOM at different pH ranges for all the IOM fractions with different MW (Experimental conditions: DOC of the raw water for all MW fractions = 5 mg/L).

Fig. 8. Mechanisms of IOM removal by aluminum coagulation.

using Al Ferron method, and the detailed analysis procedure can be found in the literatures in our study [46,47], it was shown that the initial ratios of Al_a , Al_b , and Al_c within aluminum were determined to be 95.2%, 4.8%, and 0%. However, upon being dosed, the aluminum ions tended to rapidly transform to different species and ratios of monomeric aluminum (Al_a), polymeric species (Al_b) and $Al(OH)_3$ amorphous flocs (Al_c) [48], meanwhile, the hydrolysis products distribution were highly dependent on pH. It was reported that the removal of AOM with positively-charged coagulants mainly included two mechanisms of charge neutralization and sweep flocculation [37]. Comparatively, the IOM with higher MW was more easily removed by sweep flocculation than that with lower MW [30]. At acidic pH of 4.5, the aluminum mainly existed as positively-charged hydrolyzate such as Al^{3+} and $Al(OH)_2^+$ [37], therefore charge neutralization mechanism played the dominant role in the removal of the negative IOM with aromatic groups. Additionally, the combined effects of *in situ* Al_b formation involved in aluminum coagulation, and also benefited the removal of IOM by charge neutralization. At pH 7.5, the aluminum ions transformed to $Al(OH)_3$ by hydrolysis reactions, and the $Al(OH)_3$ with huge mesh structure tended to remove great amount of IOM via adsorption and sweep flocculation mechanisms [49].

The removal mechanism of IOM with different MW was also different. At the same pH and aluminum doses, the fractions of >100 kDa and 30–100 kDa showed higher removal efficiency than those of 10–30 kDa and 3–10 kDa, and the removal of the >100 kDa fraction was most significant among these fractions in wide pH range. These results indicated that the IOM with higher MW was easier removed by $Al(OH)_3$ precipitates, meanwhile the mechanisms of charge neutralization and sweep flocculation also involved in [50]. Especially at neutral pH of 7.5, the removal of total IOM was much higher, and the removal of the >100 kDa fraction contributed as high as 84.4% for UV_{254} and 45.8% for DOC of IOM removal. These results inferred that the high MW fractions may adsorb onto the flocs at neutral condition and the sweep flocculation was the main mechanism involve in IOM removal. For IOM fractions with lower MW, at acid pH of 4.5 the positively-charged aluminum hydrolyzate showed charge neutralization effect towards the IOM with negative charge.

4. Conclusions

This study extracted IOM from *Microcystis aeruginosa* and separated it by ultrafiltration to get IOM fractions with different MW, and the removal efficiency by alum coagulation was carefully investigated. Alum coagulation can effectively remove IOM and the maximum removal is determined to be 99.7% for OD_{680} , 51.3% for UV_{254} , and 38.6% for DOC. Generally the pigment-like

species as indicated by OD_{680} and the aromatic species as indicated by UV_{254} are removed in superiority as compared to other components. Additionally, alum exhibits high affinity towards unsaturated C=C and C=O bonds within aromatic compounds, e.g., tryptophan-like aromatic proteins, and the formation of complexes involves in and plays an important role in their removal by alum coagulation. The weak acidic pH, i.e., pH 6.5, is optimum for the interactions between alum flocs and IOM, and the observed IOM removal was maximal to be 42.3% for DOC and 61.5% for UV_{254} . The FI values of all IOM fractions with different MW vary greatly after alum coagulation and were highly dependent on pH, owing to the different reaction mechanisms involved in. Alum coagulation is an effective and low-cost strategy for the IOM control among conventional treatment processes in the treatment of algae-laden source water.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.seppur.2017.06.066>.

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