Coagulation performance and floc properties of *Microcystis aeruginosa* in the presence of humic acid

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**ABSTRACT**

Coagulation removal of algae in raw water could be significantly affected by humic acid (HA). A series of jar-tests were conducted to investigate the coagulation performance with polyaluminum chloride and floc properties of *Microcystis aeruginosa*, a unicellular cyanobacteria, in the presence of HA. Meanwhile the coagulation mechanism was explored through the measurement of zeta potential of the supernatant. The results showed that an optimal removal efficiency of chlorophyll-a (Chl-a) was obtained at pH 8.0 with a low concentration of HA (2 mg/L) and at pH 6.0 with a high concentration of HA (8 mg/L). The floc structure was more compact and bigger-sized and the calculated fractal dimension (*D*<sub>f</sub>) was larger at maximum coagulation efficiency. The variation of *D*<sub>f</sub> was consistent with that of Chl-a removal efficiency under the same coagulation conditions. Charge neutralization was inferred to be the dominant mechanism to remove algal cells with low concentration of HA, while charge neutralization, gathering and the bridging process worked together to remove algal cells with a high concentration of HA. These results provide insight on how to achieve an optimal removal efficiency of algae in the presence of different concentrations of HA in water treatment.

**Key words** | algae, coagulation, fractal dimension, humic acid, raw water

**INTRODUCTION**

At present, mass propagation of algae in raw water causes a series of problems in drinking water treatment (*Liu et al.* 2011); for instance, the production of unpleasant tastes and odors, the yields of toxins from blue-green algae, e.g. microcystins from *Microcystis aeruginosa* (*M. aeruginosa*), and the formation of disinfection byproducts (DBPs), e.g. trihalomethanes and haloacetic acids during the water chlorination process (*Pan et al.* 2006). The coagulation process is a commonly used method in water treatment (*Chen et al.* 2009). Furthermore, enhanced coagulation is recommended by US EPA as an optimal method to control DBP precursors, e.g. natural organic material (NOM) and algae, at the first stage in performing the D/DBP rule (*Wang et al.* 2007). Generally, adjusting pH and coagulant dosage have been utilized to enhance coagulation removal efficiency. However, coagulation of algal cells is difficult due to their widely variable physical and chemical characteristics, such as complex cell morphologies, cell motility, variable surface charge and the ability to release extracellular organic matter (EOM) (*Henderson et al.* 2008a). Moreover, there are some other environmental factors which would significantly influence algal removal in water treatment. Humic acid (HA), a typical constituent of NOM, has been generally regarded as the main precursor of DBPs in water treatment.

It is reported that presence of HA might dramatically enhance the growth of algae to some extent by acting as an organic ligand by complexation with iron to increase the solubility of iron, which was an essential ion for algae (*Lee et al.* 2009). In the presence of humic substances, Fe(III) or Al(III)–humic substance complexes are labile and soluble in water, and these complexed forms rapidly dissociate and release iron or aluminum ions to meet the equilibrium conditions (*Liu & Millero* 2002). Despite the complex interaction between HA, algae and coagulant,
there are few reports about coagulation efficiency at removing algae in the presence of HA. What is more, most reported studies (Henderson et al. 2010) show achievements about coagulation being used to remove algae in laboratory culture systems which are different from the natural aquatic environment. Therefore, further investigation of the influence of HA in the coagulation process of algae seems essential.

The characteristics of flocs formed by aggregating, such as floc size, floc growth rate as well as floc strength and recovery ability after being broken, caused a critical effect on the solid/liquid separation process (Yu et al. 2015). It has been well-known that aggregates formed during the coagulation process exhibit a fractal characteristic, implying that they are self-similar and scale invariant (Gregory 1997). Fractal dimensions ($D_f$) can reflect the fracture surface roughness (Yu et al. 2015). Thus, the characteristics of floc fractal dimensions in the coagulation process can be used to evaluate coagulation efficiency (Wang et al. 2015).

Based on the above background, *M. aeruginosa*, a unicellular cyanobacteria, was selected as the target algae in this paper since it is one of the typical unicellular blue-green algae and is generally observed in eutrophic raw water (Qu et al. 2012). As the most practical flocculant in drinking water treatment, polyaluminum chloride (PAC) was used in the following coagulation jar-tests. The major objectives of this study were to: (1) evaluate the coagulation efficiency of the algae in the presence of HA through determination of Chl-a removal efficiency, residual turbidity and floc structure, and calculation of floc fractal dimension; (2) analyze the corresponding mechanism during the coagulation process by determination of zeta potential of the supernatant.

**MATERIAL AND METHODS**

**Cultivation of *M. aeruginosa***

*Microcystis aeruginosa* (serial number was FACHB-469) was acquired from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences, inoculated in axenic BG-11 medium (Qu et al. 2012), and placed in a biochemical incubator under fluorescent light (25 ºC, 2,000 lux, 12 h light/12 h dark).

**Preparation of synthetic water sample**

*Microcystis aeruginosa* cells growing to the late exponential phase were collected by centrifugation (3,000 rpm/min, 5 min) and then were diluted with 0.5% NaCl solution to optical density of the cell suspension 0.070 at 680 nm. The cell concentration and the Chl-a concentration were $1.827 \times 10^6$ cell/mL and 67.87 mg/L, respectively (Daisuke & Shingo 2011). The zeta potential of the water sample containing algae was $-16.4 \pm 0.5$ mV.

**Reagents**

Stock solution of PAC was prepared at a concentration of 5.0 g/L using solid PAC salt (Shenzhen Zhongrun Water Purification Materials Co. Ltd, Guangdong, China) with 50% w/w Al$_2$O$_3$ and basicity of 90%.

One gram of HA (Shanghai Jufeng Chemical Technology Co. Ltd, Shanghai, China) was dissolved in 1 L 0.01 mol/L NaOH and mixed for 60 minutes with a magnetic stirrer and then the suspension was filtered twice by a 0.45 μm filter membrane. The HA stock solution was prepared as 1 g/L for later use.

The pH of synthetic water samples containing HA and *M. aeruginosa* was adjusted by 0.1 mol/L NaOH and 0.1 mol/L HCl.

**Jar-test coagulation experiments**

Jar-test coagulation experiments were executed with a six-paddle stirrer (ZR4–6, Shenzhen Zhongrun Water Purification Materials Co. Ltd, Guangdong, China). One litre of synthetic water sample was placed in an organic glass beaker and stirred for 2 minutes at 50 rpm in order to homogenize. Then it was rapidly stirred for 2 minutes at 200 rpm, and PAC solution was added to each sample, followed by slow stirring for 10 minutes at 50 rpm, and a quiet settling for 15 minutes. The supernatant sample was collected from 3 cm below the water surface for the assay of the following indexes.
Analytical methods

Residual turbidity (the turbidity of supernatant sample) and removal efficiency of Chl-a were detected to indicate removal efficiency of *M. aeruginosa* in the coagulation process.

The residual turbidity of supernatant was determined by a scatter turbidity instrument (YJ-A, Shenzhen Zhongrun Water Purification Materials Co. Ltd, GuangDong, China).

For the assay of Chl-a, a 200 mL water sample was filtrated through a 0.45 μm filter membrane and then the attached chlorophylls were extracted in 10 mL of 90% acetone at 4 °C for 24 h in darkness. After extraction, the optical density of the supernatant was measured at 664 and 630 nm with a spectrophotometer respectively; meanwhile, a solution of 90% acetone was used as a blank. The concentration of Chl-a can be calculated according to the following formula:

\[
\text{Chl-a (mg/L)} = 10 \times (11.47 \times \text{OD}_{664\text{nm}} - 0.4) \\
\times \text{OD}_{630\text{nm}})/0.2. \tag{1}
\]

The zeta potential of supernatant after settling was analyzed with a Zetasizer (3000Hsa, Malvern Instruments, UK).

Floc structure and floc fractal dimension

According to fractal geometry theory, the dimension can be calculated using different methods and the box-counting method is used in this study. The dimension for floc was described using the following equation:

\[
D_f = \lim_{L \to 0} \frac{-\log (N)}{-\log (L)} \tag{2}
\]

where \(D_f\) is the box-counting fractal dimension, \(N\) is the quantity of squares used to cover the scan emission micrograph (SEM) floc image and \(L\) is the inverse of the box size. The experimental and calculative process protocol includes the following steps.

The floc sample distributed in the bottom of the beaker was quick-frozen at −80 °C through an ultra-low temperature refrigerator (MDF-382E (N), Sanyo, Osaka, Japan) and then the frozen floc sample was put in a vacuum-freeze dryer (LGJ-10, Beijing Songyuanhuaxing Technology Develop Co. Ltd, Beijing, China) for 24–48 h with sublimated ice (*Wang et al. 2007*). The dried floc sample was collected with hermetic bags and stored in a desiccator for later use. The SEM images of freeze-dried samples were obtained on a scanning electron microscope (JSM6610LV, JEOL, Japan). According to the box-counting method, the fractal dimension of each floc sample can be calculated by a program software named FractalFox (Version 2.0, University of Memphis, USA) through a SEM image.

RESULTS AND DISCUSSION

Effect of pH in the presence of HA

In the majority of raw water, HA is typically presented to various degrees with a concentration range of 2–12 mg/L (*Ge et al. 2007*). Thus, in order to investigate the coagulation performance of *M. aeruginosa* in the presence of HA of different concentrations, 2 and 8 mg/L were appointed as a low and a high concentration of HA in this study, respectively.

Figure 1(a) shows the effect of pH on Chl-a removal efficiency and residual turbidity with the low concentration of HA (2 mg/L) when the PAC dosage was 25 mg/L. It was observed that Chl-a removal efficiency presented a parabola tendency at a pH value range from 5.0 to 9.0, which achieved a maximum Chl-a removal efficiency 87.1% at pH 8.0 and all above 85% in a pH range 7.0–8.0.

As shown in Figure 2(a), the zeta potentials of settling supernatant after coagulation decreased gradually and moved from the positive side to the negative side in the pH range 5.0–9.0. It achieved the isoelectric point in the pH range 7.0–8.0, which was in accordance with the optimal pH range for the coagulation process in Figure 1(a). This result indicated that charge neutralization was the dominant mechanism for *M. aeruginosa* removal by coagulation with a low concentration of HA (*Sun et al. 2012*). The dissolved HA presents negative electric charge due to the carboxyl and hydroxyl produced by its hydrolysis (*Zhang et al. 2008*), which will compete with algal cells to react with aluminum salt. However, because of the low concentration of HA in the tested water, the high positive electric charge of
aluminum complexes that result from hydrolysis of PAC will neutralize both HA and algal cells.

The effect of pH on Chl-a removal efficiency and residual turbidity with high concentration of HA (8 mg/L), when the PAC dosage was 25 mg/L, is shown in Figure 1(b). From the figure, the Chl-a removal efficiency also displayed a parabolic tendency at pH 5.0–9.0. But unlike Figure 1(a), the Chl-a removal efficiency achieved a maximum (74.0%) at pH 6.0 and was above 72.0% in a pH range 6.0–7.0. Compared with Figure 1(a), it can be observed that the removal efficiency of algae was less effective at high concentration HA than that at a low concentration HA.

Figure 2(b) illustrates the variation of zeta potential of water samples before and after coagulation. The zeta potential of settling supernatant after coagulation decreased gradually and moved from the positive side to the negative side. Isoelectric point was obtained in the range 5.0–6.0, which did not match the optimal pH range in coagulation in Figure 1(b). Thus, it was indicated that charge neutralization was not the exclusive mechanism to remove the algal cells. When the concentration of HA is relatively high in the water sample, PAC would react with HA first and algal cells next (Ma & Liu 2002). Moreover, according to the Derjaguin–Landau–Verwey–Overbeek theory, there are also electrostatic repulsions between the negatively charged HA and the negatively charged algae cells (Xu et al. 2014). Thus, the aggregation of algae could be significantly reduced in the presence of HA due to the electrostatic repulsion being stronger than the Van der Waals force (Kim et al. 2010). More HA would consume more PAC, whereas the electrostatic repulsion between HA and algal cells would be stronger. Therefore, the removal efficiency of algal cells would be decreased when increasing HA concentration. In addition to charge neutralization, the flocs formed by PAC and enough HA may first promote algal cell coagulation because of gathering and bridging contributing to the removal efficiency of algal cells (Ma & Liu 2002).

Effect of PAC dosage in the presence of HA

The influence of PAC dosage in the presence of low and high concentration HA is shown in Figure 5 (the pH value of water samples was 7.0). With a low concentration HA (2 mg/L), Chl-a removal efficiency increased, with PAC dosage increasing from 5 to 30 mg/L and declined at a PAC dosage higher than 30 mg/L (Figure 5(a)), achieving a peak value of 85% at 30 mg/L PAC. The variation of zeta potential in the process is shown in Figure 2(c). Zeta potential increased with the increasing of PAC dosage; the isoelectric point was acquired at a PAC dosage of approximately 30 mg/L. When the PAC dosage achieved 40 mg/L, excessive PAC produced a positive charge and thus resulted in charge reversal of the algal cell surface. Then a
restabilization phenomenon in the coagulation process appeared (Ahmad et al. 2011). Therefore, it tended to weaken flocculation of algal cells when PAC was dosed more than the optimum dosage.

Figure 3(b) illustrates the effect of PAC dosage on coagulation efficiency with a high concentration of HA concentration (8 mg/L). It was observed that the Chl-a removal efficiency increased gradually with the raising of PAC dosage. The Chl-a removal efficiency achieved a highest value of 79.8% at a PAC dosage of 40 mg/L. The variation of zeta potential with PAC dosage which is illustrated in Figure 2(d) shows that zeta potential increased with increase of PAC dosage. However, the zeta potential presented a slighter rise with the increase of PAC dosage at 8 mg/L HA than that at 2 mg/L HA (Figure 2(c)), and still emerged at a negative value (−0.759 mV), which did not reach the isoelectric points at 40 mg/L PAC. A high concentration HA provided a more negative charge in tested

Figure 2 | The variation of supernatant zeta potential. (a) With 2 mg/L HA at different pHs (PAC dosage = 25 mg/L). (b) With 8 mg/L HA at different pHs (PAC dosage = 25 mg/L). (c) With 2 mg/L HA at different PAC dosages (pH = 7.0). (d) With 8 mg/L HA at different PAC dosages (pH = 7.0). The initial concentration of algae cells was OD$_{680}$nm = 0.070 ± 0.001, the initial concentration of Chl-a was 67.87 mg/L and the initial turbidity was 17.8 ± 0.1 NTU. The error bars were the standard deviations of average values (n = 2).
water and reacted with hydrolyzed metal cations and thus declining algal removal efficiency (Henderson et al. 2012).

**Flocs structure and fractal dimensions**

In the coagulation process, $D_f$ has been usually calculated to express the roughness and irregularity of flocs and evaluate coagulation efficiencies. Previous research work has shown coagulation efficiency to be proportional to the $D_f$ of floc (Mao et al. 2015). Figure 4 illustrates typical SEM images of four kinds of flocs in experiment. Figures 4(a) and 4(b) show the floc structure at initial pH 5.0 and 8.0, respectively, which represented the worst and the optimal coagulation performance in the presence of low concentration HA (2 mg/L). It can be observed distinctly that smaller size and distributed flocs are displayed in Figure 4(a), while Figure 4(b) reveals a compact structure and larger size flocs. In addition, Figures 4(c) and 4(d) show the floc structure which represented the worst and the optimal coagulation performance at pH 5.0 and 6.0 in the presence of a high HA concentration (8 mg/L), respectively. It can also be suggested that the flocs in Figure 4(d) are more compact and larger-sized than the flocs in Figure 4(c).

It is reported that flocs formed during the coagulation process exhibit a fractal characteristic, implying that they are self-similar and scale invariant (Zhao et al. 2012). Based on the floc morphology, a comparative study on the floc formation with different coagulation conditions was also carried out in terms of fractal dimension. A series of $D_f$ values for flocs are shown in Table 1, which presents the variation of $D_f$ at the different initial pH when HA concentration was low (2 mg/L) or high (8 mg/L) in algae-containing raw water, respectively. It can be seen that the $D_f$ value at initial pH 8.0 was larger than that at other initial pHs with 2 mg/L HA. Furthermore, the $D_f$ value at initial pH 6.0 was the largest in the pH range 5.0–9.0 in the presence 8 mg/L HA. This illustrated that the coagulation floc formed at pH 8.0 with 2 mg/L HA had a more compact structure because charge neutralization can contribute to a compact and dense floc significantly, as well as the structure of floc formed at pH 6.0 being more compact with 8 mg/L HA (Li et al. 2006). These data are consistent with the coagulation efficiency shown in Figures 1(a) and 1(b).

Table 1 also displays the $D_f$ values of flocs formed with different PAC dosage when the concentration of HA was low (2 mg/L) or high (8 mg/L) in algae-containing raw water. $D_f$ presented a maximum value with 30 mg/L PAC at 2 mg/L HA, therefore 30 mg/L PAC gave the most compact floc structure during the coagulation process. When PAC dosage increased to 40 mg/L, the excess coagulant would lead to charge reversal of algal cells in preventing flocs. In addition, the $D_f$ values (listed in Table 1) increased with the increasing of PAC dosage in the presence of 8 mg/L HA, which indicated that the floc structure became more
compact and denser. This was also consistent with the results discussed in Figures 2(a) and 2(b).

CONCLUSION

The results showed that an optimal Chl-a removal efficiency was obtained at pH 8.0 with a low concentration of HA by charge neutralization, and at pH 6.0 with a high concentration of HA by charge neutralization, gathering and the bridging process. The floc structure was more compact and bigger-sized and the calculated $D_f$ was larger at a more optimal coagulation efficiency. The variation of $D_f$ was consistent with that of Chl-a removal efficiency under the same coagulation conditions. These results provide insight on how to achieve an optimal
removal efficiency of algae in the presence of HA in raw water. Further studies should be focused on the removal mechanism of algal cells and their EOM in the presence of HA.

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