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The effects of dissolved and bound extracellular organic matter on polyaluminum chloride and chitosan flocculation during algae harvesting or removal

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ABSTRACT

Excessive addition of chemical agents would endanger human health and increase the operation and maintenance costs during algae harvesting or removal. Here, the two types of extracellular organic matter (EOM), i.e. dissolved EOM (dEOM) and bound EOM (bEOM) stratified from Chlorella pyrenoidosa were investigated for their influence on the flocculating efficiency of algae cells when using chitosan and polyaluminum chloride (PAC). Chitosan was more effective in flocculation than PAC, with an optimal flocculating concentration of 40 mg/L versus that of 1000 mg/L using PAC. In contrast, the consumption of chitosan and PAC decreased to 10 and 200 mg/L after extracting dEOM, and 6 and 40 mg/L after extracting bEOM, respectively. Therefore, the dosage of the chemical agents and the charge density were significantly affected by the EOM, especially the dEOM. From the results, our research suggest that extracting EOM from algae cells might be an effective approach for reducing the cost of flocculation for collecting algae.

Keywords: Microalgae; EOM; Chitosan; Polyaluminum chloride; Flocculation efficiency

1. Introduction

Algae which pose harmful impact on natural environment and human health are becoming a great social concern [1]. In particular, drinking water quality and water treatment processes are adversely affected, mainly due to algae toxin pollution and interference of algae with physical and/or chemical water purification processes [2]. However, algae are pretty useful in many fields. Not only can they absorb greenhouse gas like CO_2 and treat wastewater, but also serve as a raw material for bio-energy, food supplements and chemical production [2–4]. Accordingly, collecting algae is becoming ever more important. To realize commercial-scale production in algal production industry, finding an energy-efficient and effective way to concentrate and collect algae will be beneficial to environmental sustainable [5]. The present regular algal concentrating processes are coagulation, flocculation, flotation, centrifugation, filtration (both screen and membrane) and gravity sedimentation. Among these methods, flocculation is one of the most promising methods because it can gather algae biomass in a much shorter time without mechanical energy [6]. When flocculating microalgae, algae cells with a negative charge can be neutralized by multivalent cationic ions such as aluminum and ferric ions. These cationic ions can quickly flocculate algae cells and form flocs, which can be easily separated from water.

Flocculation is suitable for collecting algae, but during the process, chemical or biological agents must be added for improving the effectiveness of coagulation/flocculation [7]. Excessive agents in the algae media have an important influence on several aspects of water quality, including the potential for harmful disinfection by-products (DBPs) of the extracellular organic matter (EOM) released by the algae [8,9]. The chemical agents are harmful to human health and increase the operation and maintenance costs as well. Therefore, gaining satisfactory algae collection with a minimal amount of flocculating agent is the environmental friendly result of the flocculation method. The

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required dosage of the flocculating agents is affected by several factors, including pH, salinity, agent type, et al. [10]. Garzon-Sanabria, et al. [11] reported that for a given $AlCl_3$ density, a pH value below 5.0 has a better efficiency for *Nannochloris oculata* flocculation than a pH value below 7.0, and the best condition was 0.0016 ng of $AlCl_3$/cell with a pH of 5.3. Moreover, Xu, et al. [12] concluded that the optimal chitosan dosage (approximately 10 mg per gram algae dry weight) is determined primarily by the cell concentration rather than by the cell age, lipid content or medium composition. It is necessary to study these factors to find the ideal flocculation approach.

Among the factors, extracellular organic matter (EOM) greatly increase the usage of flocculants. The EOM released by the algae cells contains many types of organic matter, including proteins, polysaccharides and humic-like substances. The microalgae surface is negatively charged, and the cells carry EOM to maintain a stable dispersed state [13]. Garzon-Sanabria, et al. [14] studied the effects of algogenic organic matter (AOM, also called EOM) and sodium chloride on algae flocculation efficiency and found that AOM was the main cause of the higher flocculant demand. $AlCl_3$, the studied flocculant, was relatively efficient at 50 mg/L in the absence of AOM and required a 3-fold greater dosage to achieve 90% removal in the presence of AOM. When using modified spent yeast (MSY) flocculant to harvest *Chlorella Vulgaris*, the presence of AOM increased the required dosage of flocculant (51 mg MSY/g biomass) compared with a complete mineral medium with phosphorus and without AOM (12 mg MSY/g biomass) [15]. Although many similar studies have proved that EOM inhibits flocculation and causes a decrease in efficiency, there is no thorough analysis of the mechanism. EOM mainly consists of dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM), which are different parts released by algae cells. Some studies have been conducted on the two types of EOM, but very little research has focused on the variable influence of dEOM and bEOM on flocculation efficiency [12,13,14].

Our previous study [16] has found that the dEOM and bEOM have different components and characteristics, which may affect the efficiency of flocculation differently. Based on the results, in this study, the dEOM and bEOM stratified from *Chlorella pyrenoidosa* were studied, and two types of flocculants, polyaluminum chloride (PAC) and chitosan, were used for algae flocculation. PAC is a traditional inorganic flocculant, and chitosan, which is produced by alkaline deacetylation of chitin, is a representative organic flocculant [12]. Some studies have been conducted to investigate the effects of dEOM on flocculation [14,17], but the difference between the effects of dEOM and bEOM is still unknown. Thus, in this study, the effects of dEOM and bEOM on flocculation were compared by measuring the flocculant dose and algae clarification rates. The reduction of EOM was also measured using analytical methods to obtain their protein and polysaccharide contents, which are the main components of EOM. Moreover, the algae flocs were observed by taking microscopic and SEM images, and the flocculation mechanism was assessed under different conditions. We hope to reveal the mechanisms of EOM affecting flocculation by breakdown of EOM constituents and thereby provide some valuable information about flocculation during algae harvesting or removal.

2. Materials and methods

2.1. Algae cultivation

C. pyrenoidosa (green algae, FACHB-9) was purchased from the Institute of Hydrobiology at the Chinese Academy of Sciences in China. *C. pyrenoid*osa was cultured in a basal medium under controlled ambient conditions at 25°C and a 14 h light/10 h dark cycle with illumination ranging from 2000 lx to 5000 lx over time (GZX-300BS-III, CIMO Co., Shanghai, China). 1.0 g L^{-1} of glucose was added in the basal medium to imitate the production of industrial algae biomass. Axenic conditions were maintained throughout the experiment. The micro algae was cultured for about 20 days to reach the stationary phase. To prevent sedimentation of algae, all conical flasks were placed on magnetic stirring plates (YG-60W, Fujian, China) and stirred at 250 rpm for 30 s twice a day. The pH values of the collected algae broth varied from 7.3 to 7.6 between different batches but were generally stable at approximately 7.5. In addition, there was an auto trophic medium without glucose for auto trophic growth of algae.

2.2. EOM extraction

According to the previous study, centrifugation at 10000 g for 10 min and heating at 70°C for 20 min were the best conditions for extracting dEOM and bEOM, which may lead to high extraction efficiency and low cell lysis [16]. During the stationary phase when glucose was consumed, the algae broth was diluted with a phosphate-buffered solution (10-fold dilution to maintain the osmotic equilibrium and pH) to approximately 2.0×10^7 cell mL^{-1} with a hemocytometer (XB-K-25, China), in which no EOM was extracted. The original culture matrix with both dEOM and bEOM was named Medium I. Six hundred milliliters of Medium I was centrifuged at 10000 g for 10 min, and the pellet was resuspended to the same volume. This solution from which the dEOM had been eliminated was named Medium II. Six hundred milliliters of Medium II was heated at 70°C for 20 min, and the previous step was repeated. The solution from which both the dEOM and bEOM had been eliminated was named Medium III.

2.3. Preparation of PAC and chitosan solution

Chitosan (Sigma-Aldrich, USA) was purchased in powder form and it was soluble in neutral water. Then, 1.0 mg/ mL of chitosan stock solution was prepared according to the following steps. One hundred milligrams of chitosan powder were weighed into a glass beaker and continuously mixed with 10 mL of 0.1 M HCl solution until the chitosan was completely dissolved. The solution was then diluted to 100 mL with water [18].

PAC was synthesized in the lab from $AlCl_3$ powder (Sigma-Aldrich, USA), and 40.0 mg/mL of PAC stock solution was prepared.

2.4. Flocculation and harvesting

Media I, II and III were all tested at different chitosan (2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 mg/L) and PAC concentra-

tions (20, 40, 60, 80, 100, 200, 400, 600, 800 and 1000 mg/L). For the flocculation of Medium I by chitosan, 10 beakers of 100 mL volume were prepared, and a 50-mL algae solution from Medium I and a measured volume (for a certain concentration) of chitosan stock were added into each beaker. A standard jar test procedure was then applied to assess the flocculation performance. The following steps consisted 2 min of fast mixing at 150 rpm to ensure the complete solubility of the flocculant and 10 min of slow mixing at 20 rpm to promote aggregation. The medium samples were then transferred into a 50-mL gravimetric cylinder for algae settling. To monitor the sedimentation, the optical density at 680 nm was measured periodically for 3 h at 5 cm below the top of the gravimetric cylinder [19]. Demineralized water was used as a reference to measure the optical density. The biomass recovery efficiency was calculated as follows:

$$\text{Recovery}\% = \frac{OD_{680(t0)} - OD_{680(t)}}{OD_{680(t)}} \times 100\% \quad (1)$$

where $OD_{680(t0)}$ is the optical density at time zero and $OD_{680(t)}$ is the optical density of the sample at time t.

The same processes were performed to test the other flocculants and media. Each flocculation experiment was repeated at least twice for consistency and accuracy.

2.5. Analytical methods

The pre-flocculant treatment zeta-potentials of each sample were determined by a Malvern ZetaSizer 2000 (Malvern, UK) at pH 7 [20]. The individual and flocculated algae cells were observed under an optical microscope (Olympus CX41, Japan) [21] and then freeze-dried to obtain the SEM images (Hitachi S4800, Japan). The polysaccharides concentration was measured using the anthrone–sulfuric acid method, and the protein concentration was measured using a modified Lowry method. DOC was measured using a total organic carbon analyzer (TOC-V_{CPH}, Shimadzu).

3. Results and discussion

3.1. The influence of EOM type on flocculant dosage

The influence of EOM type on the flocculation efficiency of PAC is shown in Fig. 1. A significant influence on the optimum dosage of PAC was caused by the presence and type of EOM. In the flocculation of Medium I (with both dEOM and bEOM), there was a linear correlation between the flocculation efficiency and PAC dosage: flocculation efficiencies were enhanced by higher contents of PAC. In general, PAC had a low flocculation efficiency when there was dEOM and bEOM; the required dosage for over 90% clarification of algae was 1000 mg/L. In contrast, the efficiency at low PAC concentrations (less than 600 mg/L) declined with increasing settling time, indicating that flocs which were loosely packed and easy to be resuspended were generated by the use of PAC in the medium.

There were different variations of flocculation efficiency in Media I and II at the same PAC contents. In Media II, dEOM was removed and only bEOM adhered to the cell surface. At low concentrations (20–60 mg/L), higher flocculation efficiencies were realized in Medium II than in Medium I at the same PAC dosage. The efficiency in Medium II was increased rapidly as the dosage increased and reached over 90% at 200 mg/L after 3 h of deposition. The clarity rapidly increased from 12.8% at 20 mg/L to 75.2% at 80 mg/L,

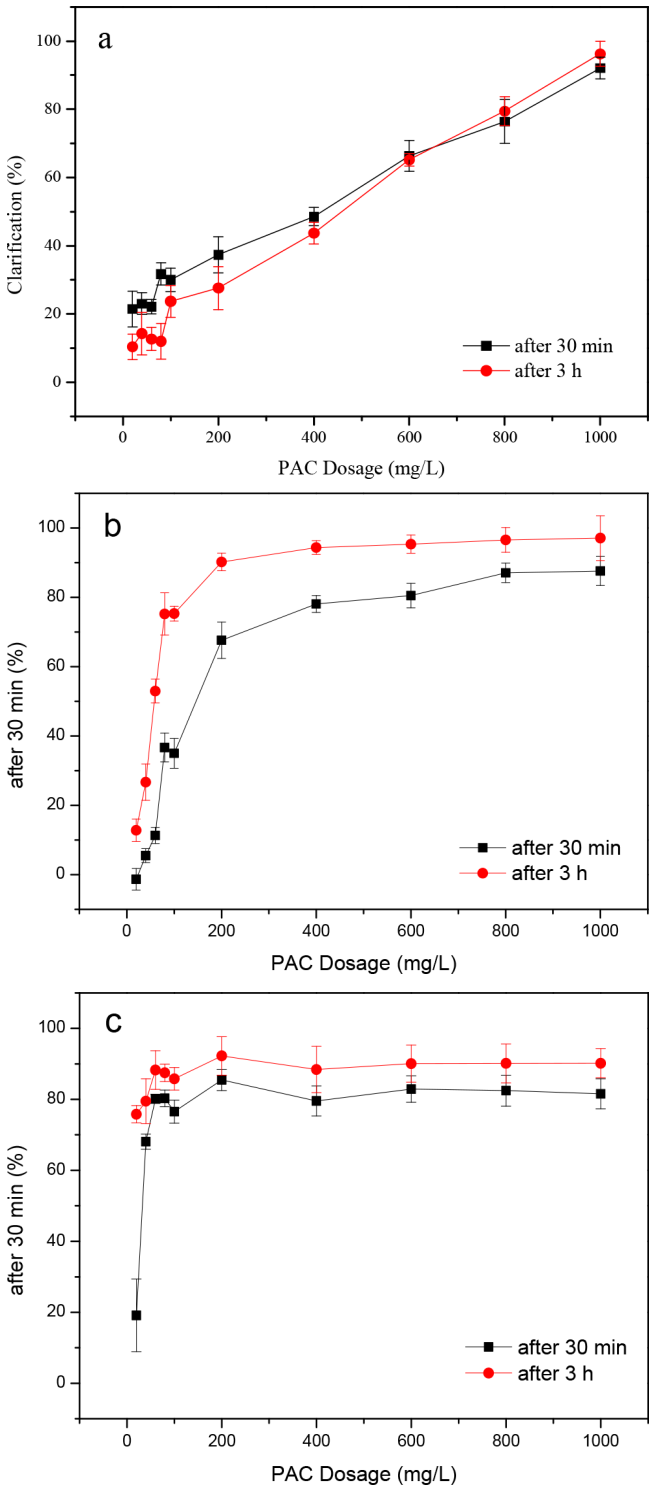


Fig. 1. The influence of EOM type on the flocculation efficiency of PAC. (a). Medium I with both dEOM and bEOM; (b). Medium II with only bEOM; and (c). Medium III with no EOM.

while it slowly increased from 75.2% at 80 mg/L to 97.1% at 1000 mg/L. However, the dosage of PAC required for Medium II to reach 90% clarity was 200 mg/L, which was much less than the 1000 mg/L required in Medium I. These results suggested that the existence of dEOM can affect the flocculation efficiency of PAC and result in a great increase in the optimum PAC dosage. In addition, for Medium II, the same phenomenon could not be observed at low PAC concentrations. This demonstrated that in flocculation with PAC, dEOM is the primary factor that results in the flocs being loosely packed and thus easily resuspended.

For flocculation in Medium III, which contained neither dEOM nor bEOM, a different trend was observed: the dosage differences had little influence on the efficiency. The clarities were all higher than 75% except at 20 mg/L dosage with a 30 min deposition. At 60 mg/L, the clarity was 90.1% and was maintained at approximately 90% at higher concentrations. The optimum dosage for PAC flocculation in Medium III decreased from 200 mg/L to 60 mg/L, indicating that flocculation was impeded by the presence of bEOM and the dosage required for effective collection was also increased by it.

Flocculation by chitosan exhibited some differences from PAC (Fig. 2). First, the dosages of chitosan required for each medium were approximately an order of magnitude lower than that of PAC, indicating that chitosan had better ability to promote algae flocculation [11]. Second, the difference in clarity between 30 min and 3 h of deposition was negligible compared with PAC flocculation, indicating that chitosan had a higher flocculation speed. Third, when the chitosan dosage exceeded a certain concentration in Media II and III, the clarification rapidly declined. This may be due to charge neutralization (CN). Excess positive charge carried by high chitosan is attached to the surface of algae cells, so that the algae cells regain dispersion stability [22], which indicated that chitosan may have a different flocculation mechanism than PAC. However, there are some properties in common between flocculation by PAC and chitosan. For example, the optimum dosages of chitosan for Media I, II and III were 40 mg/L, 10 mg/L and 6 mg/L, respectively. The downward trend was similar to PAC flocculation. This similarity indicated that dEOM and bEOM can both hinder the flocculation process by chitosan and increase the required flocculant dosage. Garzon-Sanabria, Ramirez-Caballero, Moss and Nikolov [14] studied the influence of AOM and salt concentration on 5 types of flocculants and reported that AOM was the main cause of the increased flocculant dosage requirement. This finding indicates that the flocculation process was severely inhibited by EOM.

3.2. Protein and polysaccharide clarity during algae flocculation

EOM, a mixture of negatively charged organic matters, contains high-molecular-weight proteins, polysaccharides and other materials that could consume a certain amount of flocculant and lead to an increase in the required dosage in Media I and II [22]. In this study, the dissolved organic carbon (DOC) and protein and polysaccharide concentrations in the dEOM and bEOM (Table 1) were measured, along with the influence of proteins and polysaccharides on the flocculant dosage. The clarity of proteins and polysaccharides following the addition of PAC or chitosan

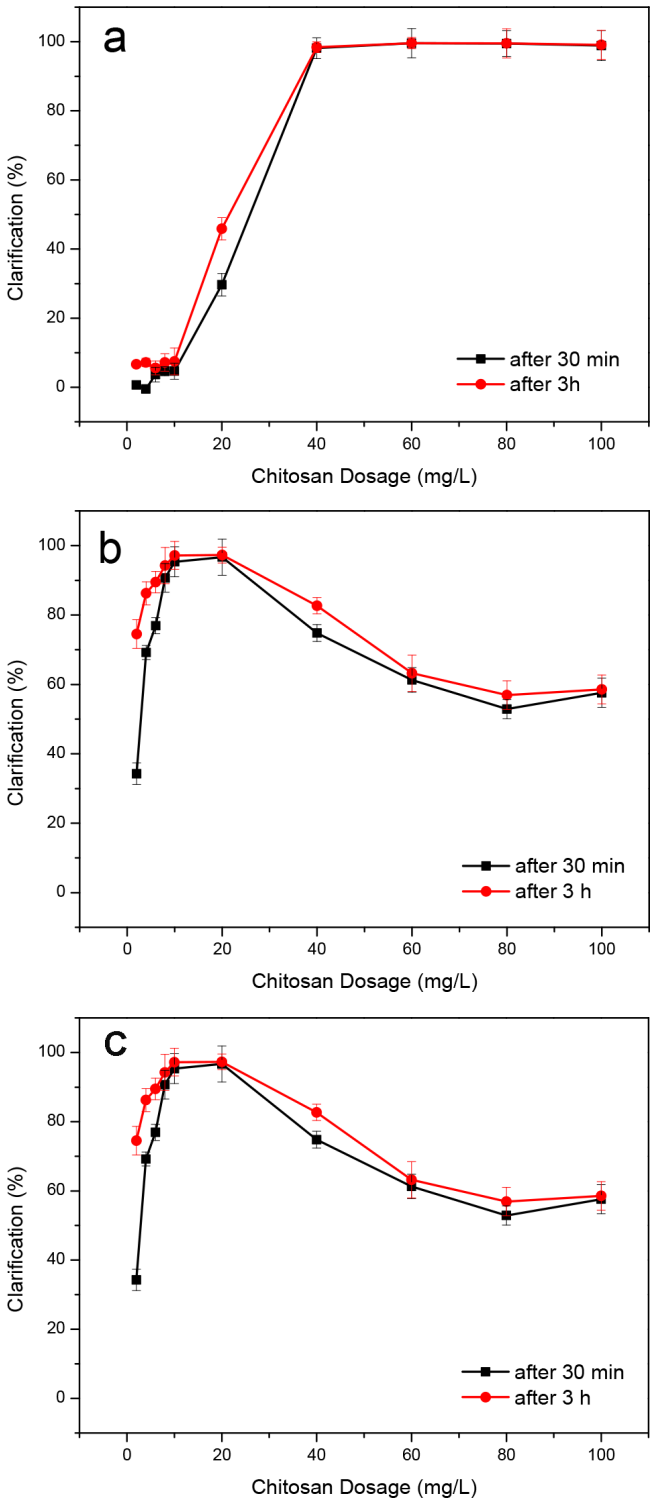


Fig. 2. The influence of EOM type on the flocculation efficiency by chitosan. (a). Medium I; (b). Medium II; and (c). Medium III.

agent is shown in Figs. 3 and 4, respectively. As illustrated in Figs. 3a and b, both PAC and chitosan had a flocculation effect on the proteins in Media I and II. The difference was that the flocculation efficiency gradually increased with the dosage of PAC and chitosan in Medium I but peaked

Table 1
The concentrations of DOC, proteins and polysaccharides, and the zeta potentials of the dEOM and bEOM from Chlorella pyrenoidosa

EOM fraction	dEOM	bEOM
DOC (mg L^{-1})	340.91 ± 5.12	73.40 ± 2.01
Proteins (mg L^{-1})	116.21 ± 7.89	174.06 ± 6.64
Polysaccharides (mg L^{-1})	39.64 ± 3.64	13.88 ± 1.32
Zeta potential (mV)	–21.20 ± 4.30	–15.40 ± 2.07

Note: The values represent the mean ± standard deviation (n = 3).

in clarity at a relatively low concentration in Medium II. This finding may be because more protein was contained in Medium I than in Medium II due to the existence of bEOM. The fact that proteins can interfere with flocculation has been demonstrated by some recent investigations [23]. Moreover, charge neutralization between the proteins and the flocculant, which is similar to that between the algae cells and the flocculant, can also explain the decline in clarity after the peak. The high clarification rate at a certain dosage suggests that proteins, as a type of organic matter in the algae medium, can consume the added flocculant and thus increase the dosage required for high algae-collecting efficiency.

Figs. 4a and b show the polysaccharide clarity at different flocculant dosages. Although the polysaccharide clarity was lower than that of proteins at the same dosage, they had the same variation trends, indicating that the polysaccharides in dEOM and bEOM were also a type of organic matter that can consume flocculant and lead to higher flocculant requirements. The presence of EOM in the medium may increase the required dosage because proteins, polysaccharides and other organic matter consume the flocculant during algae cell flocculation. In corroboration of our findings, Barros, Gonçalves, Simões and Pires [7] have suggested that proteins form complexes with the metal ions of most chemical coagulants and polysaccharides (with negatively charged carbonyl groups) and interact with the positively charged coagulants, making them unavailable for micro algal flocculation.

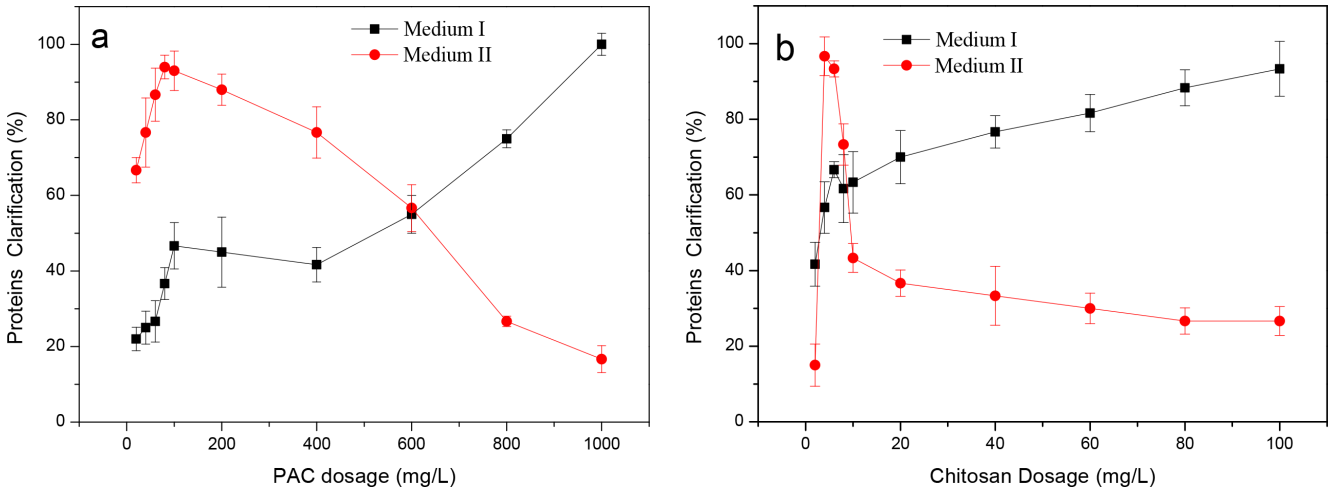


Fig. 3. Protein clarification at different dosages of PAC and chitosan. (a). PAC; (b). Chitosan.

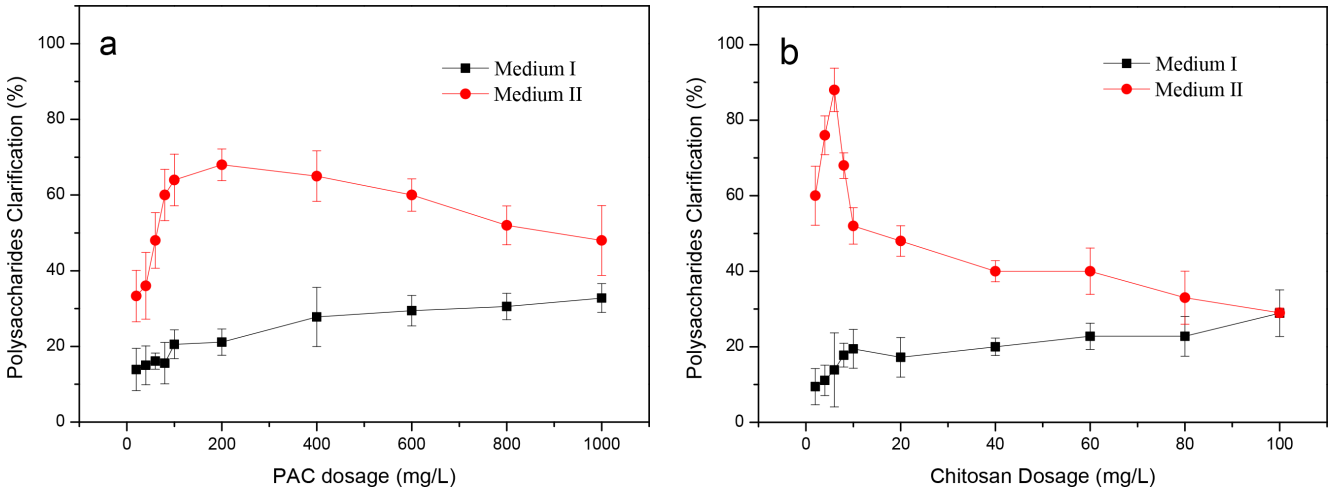
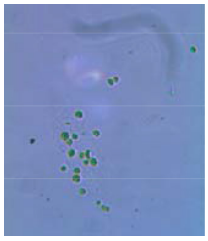
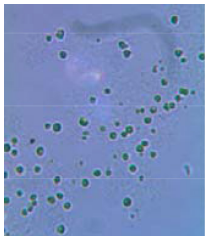
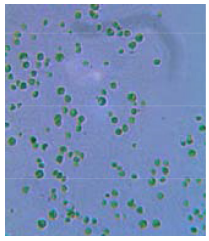
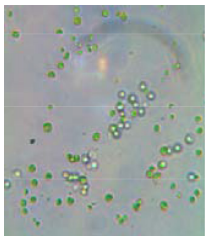
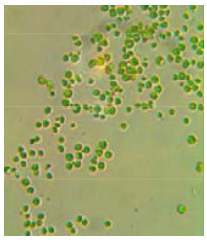
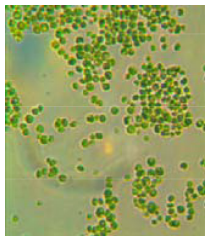
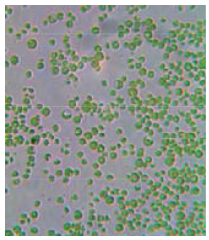
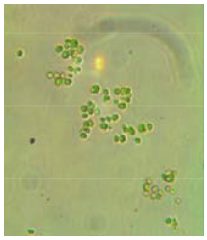
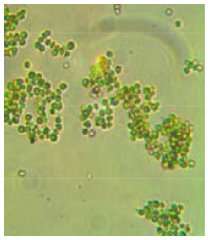
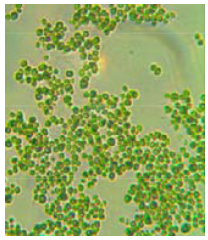
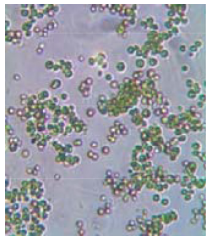
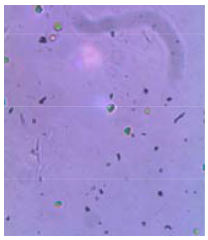
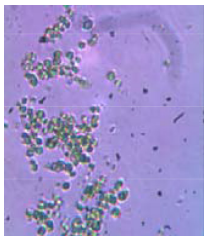
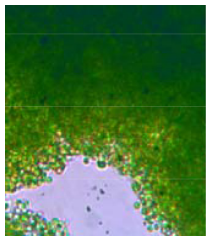
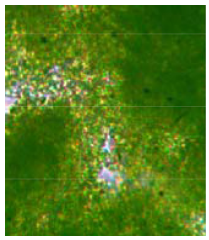
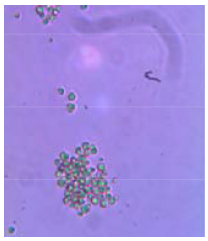
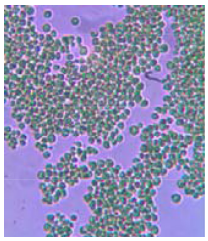
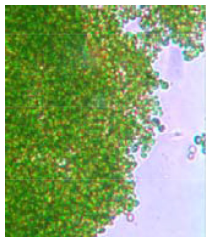
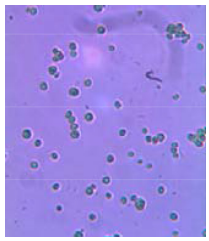
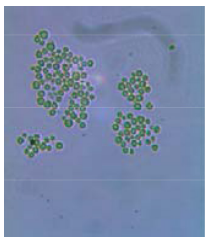
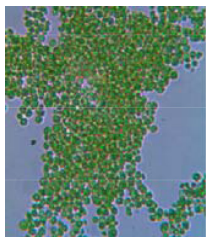
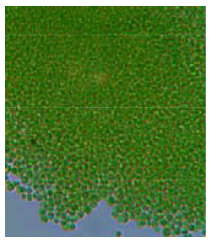
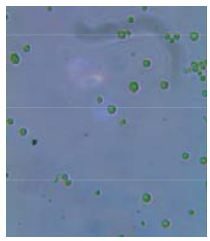


Fig. 4. Polysaccharide clarification at different dosages of PAC and chitosan. (a). PAC; (b). Chitosan.

Table 2
Microscopic pictures (400×) at the low phase, the rising phase, the optimum phase and the steady or falling phase under different flocculation conditions

Efficiency	Low phase	Rising phase	Optimum phase	Steady or falling phase
Condition A: PAC to Medium I				/
	20 mg/L	400 mg/L	1000 mg/L	/
Condition B: PAC to Medium II				
	20 mg/L	60 mg/L	200 mg/L	800 mg/L
Condition C: PAC to Medium III				
	20 mg/L	40 mg/L	60 mg/L	200 mg/L
Condition D: Chitosan to Medium I				
	2 mg/L	20 mg/L	40 mg/L	80 mg/L
Condition E: Chitosan to Medium II				
	2 mg/L	6 mg/L	10 mg/L	40 mg/L
Condition F: Chitosan to Medium III				
	2 mg/L	4 mg/L	6 mg/L	20 mg/L

Note: in Condition A, there was no falling phase because the designed dosage did not exceed the range.

3.3. Microscopic analysis

At the end of the sedimentation phase, samples of the flocs formed from the micro algae cells were taken from the bottom of the cuvettes to obtain microscopic pictures with a CK41 Olympus microscope with a ×400 magnification. Table 2 lists these sample pictures at different flocculation efficiencies: the low phase, the rising phase, the optimum phase and the steady or falling phase. Algae cells are negatively charged and steadily suspended in media without outside interference. However, large particles, which were sufficiently heavy for efficient sedimentation, were formed by dispersive cells in response to addition of flocculants such as PAC and chitosan. This could be explained by the mechanism of CN. The larger and tighter the floc particles are, the higher the efficiency of the algae collection. However, the cell-formed particles would be changed to positively charged by excessive flocculant and re-suspended in media [22]. The CN mechanism can be effectively illustrated by condition F (chitosan flocculating Medium III): for a low dosage of added flocculant, the positive charge could not counter the negative charge of the algae surface in the system ,the particles formed were very small and loose, leading to a low flocculation efficiency (61%). When more agent was added, the negative charge was neutralized by sufficient positive charge, the particles grew larger and denser, and the clarity increased (79.7%). At the optimum dosage (6 mg/L chitosan), the formed particles enlarged nearly beyond the viewing field of the microscope. It was seen that all the cells in Medium III formed one particle and precipitated quickly. However, excessive positive charge was attached to the algae surface by excess floculant dosage (more than 20 mg/L in Condition F), making particles divide; the algae cells would be re-suspended in the medium leading to a low clarification rate because of positive charge. Compared with Condition F, Condition E, with bEOM present in the system, exhibited the same variation trend, but the optimum dosage was larger than that in Condition F. One reason was the flocculant consumption by bEOM. Another reason was related to the zeta potentials of the different media. As shown in Fig. 5, the zeta potential (ZP) of Medium I, which contains large amounts of dEOM and bEOM, was approximately –23 mV. When dEOM was removed, the ZP of Medium II rose to approximately –17 mV. In the case of Medium III, both dEOM and bEOM were removed, and the ZP increased further to approximately –14 mV. These data were consistent with our previous report [14], in which the dEOM and bEOM are both negatively charged, and the dEOM carries more negative surface charge than the bEOM. The ZP results showed that EOM not only consumed flocculant but also changed the ZP of the systems. A larger absolute ZP indicated a requirement for more positive charge. Thus, the required dosage of Condition E was higher than that of Condition F. For the same reason, the required dosage of Condition D was higher than that of Condition E. It is likely that the particles would also disperse at a certain point with more chitosan added in Medium I. Bridging and netting are also common mechanisms that operate during flocculation. However, Xu et al. have reported that the mechanism operating in chitosan-algal cell flocculation is more likely a combination of CN and static patch effects rather than bridging and netting [12].

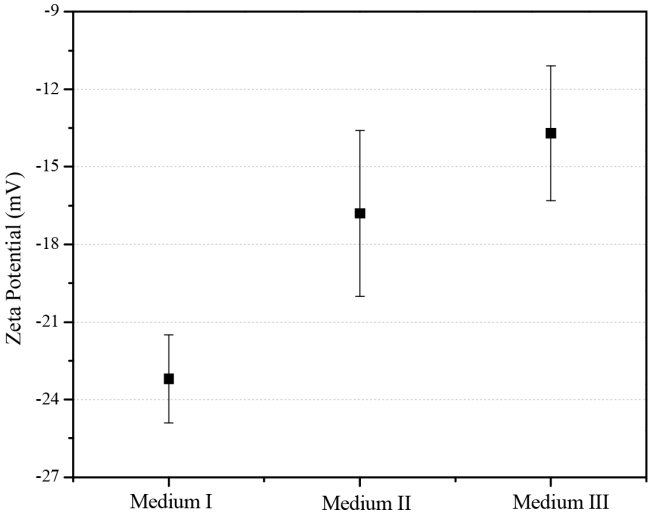
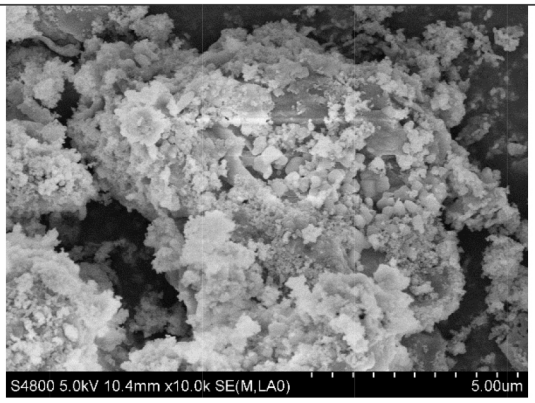
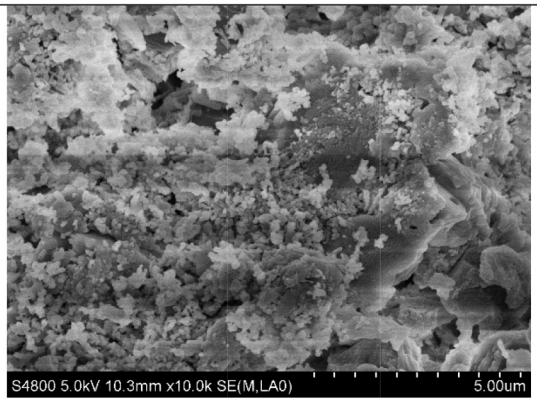
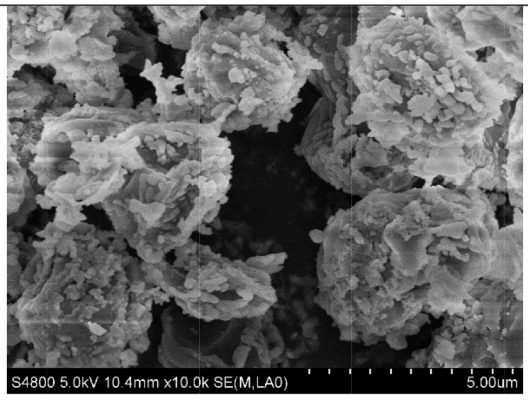
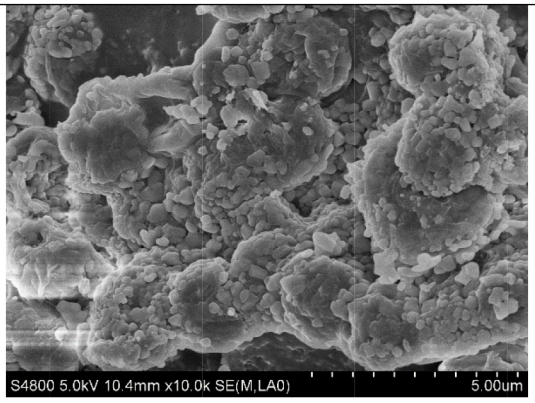
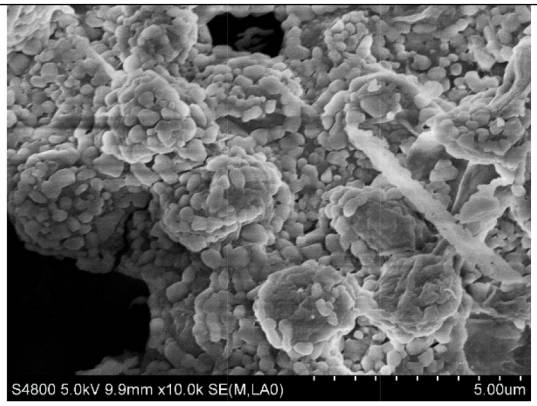


Fig. 5. The zeta potential (ZP) of Media I, II and III.

The optimum dosage in conditions A, B, and C gradually decreased in the same manner as with chitosan flocculation, potentially due to flocculant consumption by EOM and the influence of ZP. The CN mechanism can also be used to explain the flocculation performance of PAC. However, there were differences between the PAC- and chitosan-flocculated particles, suggesting that other mechanisms play an important role during PAC flocculation [5]. Specifically, in Condition C, as the PAC dosage increased, the cell particle size grew, similar to the chitosan-flocculated flocs. However, these particles did not appear to become denser. In contrast, the distances between the cells were larger than under the chitosan flocculation conditions, and they were not influenced by the increase in dosage. As a result, the flocs were very loose and in large volumes. In Conditions B and A, although the optimum dosage increased, the changing regulation of the floc patterns did not change greatly after a certain point. This phenomenon could be explained by the mechanism of sweep flocculation (SF). Garzon-Sanabria, Davis and Nikolov [11] have reported that SF, the entrapment of cell flocculation by amorphous aluminum hydroxide precipitate , was an important additional mechanism for PAC performance. This has also been demonstrated by subsequent studies [24,25]. Another reason the optimum dosage is increased in Media I and II is that EOM can sterically interfere with the aggregation process and complexes with the PAC flocculant [26].

The SEM images of algae flocs at the optimum phase under different flocculation conditions are shown in Table 3. Combined with the microscope pictures, it is obvious that the floc particles of PAC and chitosan are different in morphology. The PAC-algal particles are loose with the indistinct boundary while the chitosan-algal particles are tighter with the clearly visible globular boundary. When PAC is used as flocculant, in Condition A, PAC mainly adsorbs algae cells through bridging. The particles are small and loose with a network structure, and the connection between the particles is not tight enough. When the dEOM was removed, in Condition B, The negative charge on the cell surface is greatly reduced and CN replaced bridging to play the major role. The particles are changed from dispersed to

Table 3
SEM images (10000×) at the optimum phase under different flocculation conditions

ConditionA （1000mg/L PAC to Medium I）	Condition D （40mg/LChitosan to Medium I）
	
Condition B （200mg/L PAC to Medium II）	Condition E （10mg/LChitosan to Medium II）
	
Condition C （60mg/L PAC to Medium III）	Condition F （6mg/LChitosan to Medium III）
	

individual agglomerate, which is more compact and regular than the network structure. In condition C, the particles are more closely connected when the negative charge on the cell surface is neutralized, difficult to distinguish the boundary. When chitosan is used as a flocculant, the main flocculation mechanism in this experiment is CN. The algal cells in Condition D are tightly bound. However, when the EOM was extracted, the particles in Condition E and F was larger and clearer than that in Condition D, with less flocculant to acheive the optimum phase. The particles of Con-

dition F are especially spherical and regularly distributed. The SEM images also showed that the removal of EOM is beneficial for producing more compact and stable flocs.

3.4. Reducing EOM in algae cultivation

The results show that EOM had an adverse impact on flocculation, which not only required more flocculant but also reduced the flocculation efficiency. Thus, it is necessary to reduce the content of EOM as much as possible in algae harvesting or removal by flocculation. Although removing EOM is conducive to flocculation, it is impractical to remove EOM for large scale collection of algae. In fact, during algae cultivation the change of culture conditions, such as temperature and growth mode, can influence the secretion of EOM. In our previous study, it was demonstrated that the capacity of EOM secretion had a declining trend with the increase in temperature from 15 to 35°C, mainly due to the adjustment of synthesis and overflow metabolism at low temperature [27,28]. In large scale cultivation, it is uneconomical to increase the cultivation temperature by heating. Thus, in algae cultivation, a high temperature should be maintained without extra cost for as long as possible to reduce EOM secretion. For example, warmer climates could be chosen to cultivate algae and the application of industrial waste heat to mass culture may be practical.

Moreover, the growth mode (heterotrophic growth or auto trophic growth) not only affects the growth rate of algae but also affects the secretion of EOM. When the cultivation in the auto trophic growth mode was on the 40th day, the algae concentration was 0.2 g/L. As shown in Fig. 6, when the concentration of auto trophic and heterotrophic algae was approximately 0.2 g/L the content of EOM was measured, but the content of d-polysaccharide at the heterotrophic growth stage was not measured, because at the heterotrophic stage there was a large amount of glucose which led to d-polysaccharide containing both polysaccharides and glucose. Fig. 6 shows that although there was no obvious difference between the content of bEOM (b-polysaccharide and b-protein) in heterotrophic growth and auto trophic growth, bEOM is slightly higher in the heterotrophic growth than in the auto trophic growth. However, the concentration of d-protein in heterotrophic growth was obviously higher than that in auto trophic growth. Thus, it can be concluded that the auto trophic growth of algae secretes less EOM compared with the heterotrophic growth.

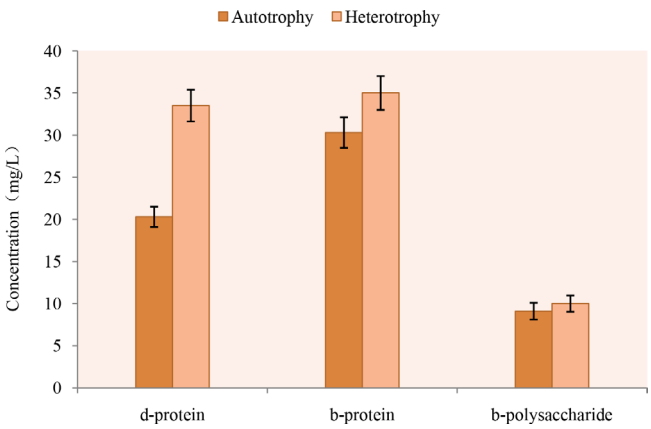


Fig. 6. The content of dEOM and bEOM in auto trophic and heterotrophic growth.

Both increasing the culturing temperature and auto trophic growth are beneficial to reduce EOM secretion. Therefore, in the collection process of algae by flocculation, to increase the flocculation efficiency and decrease the flocculant requirement, a high cultivation temperature should be maintained without extra cost. In addition, algae harvesting by flocculation is more suitable for the algae of auto trophic growth than heterotrophic growth.

4. Conclusion

Both dEOM and bEOM can greatly enhance the flocculant dosage in algae collection. EOM mainly contains proteins and polysaccharides that can consume a part of the flocculant, leading to a higher flocculant requirement. Both dEOM and bEOM are negatively charged, which can reduce the zeta potential of flocculation systems. Because CN is the main mechanism affecting chitosan flocculation, the reduced ZP results in a larger demand for positive charge. In contrast, with chitosan, SF is the dominant mechanism in PAC flocculation. The steric interference of EOM with the aggregation process also leads to the requirement for more PAC flocculant.

Acknowledgments

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