An Eco-Friendly Harvesting of Microalgae Using Combination of Microbial Flocculant and Chitosan in Simulated Eutrophic Water

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Abstract

Cyanobacteria usually occurred in eutrophic waters, but the harvesting of microalgal biomass by flocculation was still facing a major technological and economic challenge. In order to solve this problem, the combination of microbial flocculant (MBF) and chitosan was used to flocculate the biomass of *Microcystis aeruginosa.* The addition sequence of MBF and chitosan had an important influence on flocculation, and the best flocculation method was as follow: MBF (5 mL/L) was added firstly into algal culture, then the chitosan (30 mg/L) was added, MBF (5 mL/L) was added again at last. In this way, all the algal cells aggregated together, and the algal flocs floated on the surface of solution and the algal flocs could be harvested by net (0.15 mm) easily, which exhibited the highest separation efficiency of 98.33 %,the lowest total phosphorus of 0.74 mg/L and neutral pH (6.61) of supernatant. Zeta potential measurement confirmed the flocculation mechanism was charge neutralization. Microscopic observation revealed that some bubbles attached to the algal flocs which increased buoyancy of flocs. Some advantages above proved that combination of chitosan and MBF was a promising technology to harvest cyanobacteria.

Keywords: *Microcystis aeruginosa; microbial flocculant; chitosan; flocculation; zeta potential*

Introduction

Microalgae can survive and bloom within a broad range of environmental conditions, some species are harmful such as *M.aeruginosa* and *Anabaena sp*. and some species are useful such as *Chlorella sorokiniana* and *Chlorella vulgaris*(Li et al., 2008; Xu et al., 2013). Microalgae are attracting much attention as sources of fuel and diverse other products in response to energy and food crisis in the future, so some technologies are developed to harvest algae which are rich in triacylglycerols and lipid(Chatsungnoen and Chisti, 2016; Misra et al., 2016). At present, a global environmental epidemic of cyanobacteria blooms are posing serious threat to aquatic life, fishindustry, local tourism, and water quality in lakes, rivers and reservoirs, it is also threating our drinking water safety(Guo, 2007; Li et al., 2015). Cyanobacteria is not valued product, so some in-situ emergency methods for controlling

cyanobacteria blooms are developed to inhibit or disrupt growth of cyanobacteria such as algicide(Jones and Orr, 1994), sedimentation(Pan et al., 2012), ultrasonic(Zhen et al., 2017) and so on, the destination of these method just make algae disappear in short time, they do not improve eutrophication state index. Zhu studied the influence of algal bloom degradation on nutrient release at the sediment-water interface, the results showed that the decay of algal blooms released N and P to the waterbody(Zhu et al., 2013). Some researches proved that cyanobacteria blooms promote phosphorus release of sediment which accelerated the P pollution in the water and provided nutrient for growth of algae(Brookes and Carey, 2011; Xie et al., 2003). In our previous research, it was proved that cyanobacterial blooms stimulated phosphorus release from the sediment to the overlying water and phosphorus was assimilated by *M.*aeruginosa(Hao et al., 2016a), so we believed that harvesting of cyanobacteria might be a suitable method to eradicate cyanobacterial blooms under controlled external nutrient input. However harvesting the microalgal biomass is particularly challenging given the small size of the cells (5–20 μm) and the relatively low biomass concentration in the culture medium (0.5–5 g L^{-1}), so the low-cost and easy-dewatering harvesting of microalgal biomass is considered at first(Vandamme et al., 2015). Besides, some eutrophic waterbodies such as reservoirs, lakes and rivers are drinking water sources, so the environmental-friendly technologies of harvesting algae must be developed to ensure minimal impact on subsequent processes of drinking water treatment (Ghernaout et al., 2010).

In order to solve these problems, combination of chitosan and MBF are explored to flocculate *M.aeruginosa*. New technology is developed to bring all algal cells together to form big algal flocs, and make algal flocs float on the surface of solution which facilitates harvesting. Besides, the main objective in this study is to propose optimum flocculation condition on *M.aeruginosa* with the combination of chitosan and MBF and discover flocculation mechanism and floating mechanism involved it.

1. Materials and methods

1.1The algae and its culture

M.aeruginosa (FACHB469) was purchased from the freshwater algae pool at the Institute of Hydrobiology of the Chinese Academy of Sciences (CAS). The *M.aeruginosa* grew in the BG11 culture medium until the algal density is about 6×10^7cells/mL(OD_{680} is about 1.0), and the culture was kept under 2200 lx light intensity for 12/12 light dark cycle and 24 ± 1°C, in a BG11 culture medium(Hao et al., 2016b).

1.2 Microbial flocculant

B. mucilaginosus K02(Genbank database accession number HM579819)was chosen for the production of MBF(Mo and Lian, 2011).The preparation of MBF was same to previous study(Hao et al., 2016b).

1.3 Preparation of chitosan solution

Chitosan was produced by Kelong Chemical Co.Ltd, Chengdu, China. 1.0g chitosan was mixed in 100mL 1% hydrochloric acid solution with continuous stirring until the chitosan is completely dissolved, then the solution was diluted to 1.0L using deionized water(Rashid et al., 2013a).

1.4 Flocculation of M. aeruginosa byMBF, chitosan,and theirs combination

100mL algal culture was added into 250 mL conical flask. MBF, chitosan, and theirs combination was added to conical flask to flocculate algae as follows:□:1 mL MBF was added to algal culture and mixed for 5 min at 25 °C and 140 rpm in incubator shaker, then the mixture was allowed to stand for 10 min; □:3 mg chitosan was added to algal culture and mixed for 5 min at 25 °C and 140 rpm in incubator shaker, then the mixture was allowed to stand for 10 min; □:1 mL MBF was added to algal culture and mixed for

1 min, then 3 mg chitosan was added to algal culture and mixed for 5 min at 25 °C and 140 rpm in incubator shaker, at last the mixture was allowed to stand for 10 min; □: 0.5 mL MBF was added to algal culture and mixed for 1 min, then 3 mg chitosan was added to algal culture and mixed for 5 min , then 0.5 mL MBF was added and mixed for 1 min, at last the mixture was allowed to stand for10 min. At last flocculation efficiency of different treatments were directly observed.

1.5The influence of adding sequence of MBF and chitosan on flocculation

100mL algal culture was added into 250 mL conical flask. Four adding sequences of MBF and chitosan were chose to flocculate algae as follows: □: different dose of chitosan was added into algal culture, the mixture was shaken for 5 min at 25 °C and 140 rpm in incubator shaker, then MBF (10ml/L) was added into mixture and mixed for 1 min, at last, the mixture was allowed to stand for 10 min(CS-MBF); □: adding sequence of MBF and chitosan was contrary with CS-MBF(MBF-CS); □: MBF (10mL/L) and different dose of chitosan were premixed and then the mixture was added into algal culture, the mixture was shaken for 5 min at 25 °C and 140 rpm in incubator shaker, at last, the mixture was allowed to stand for 10 min(MBFCS); □:MBF (5mL/L) was added into algal culture and mixed for 1 min, then different dose of chitosan was added and mixed for 5 min, then MBF was added and mixed for 1 min, at last, the mixture was allowed to stand for 10 min(MBF-CS-MBF).The clear solution of different treatments was sampled by injector and optical density (OD_{680}) and pH of solution was determined to evaluate flocculation efficiency, The separation efficiency (%) was calculated according to the Rashid's method (Rashid et al., 2013a).

1.6 The flocculation and floating mechanism

Zeta potential of solution was determined and the morphological characteristic of algal floc was observed by stereomicroscope for the optimum flocculation method.

2. Results

2.2Flocculation of M. aeruginosa by MBF, chitosan,and theirs combination

Flocculation experiments using only MBF and Chitosan, and the combination of the both were carried out to evaluate flocculation effect of *M.aeruginosa* (Fig.1). Fig.1a showed that algal cells were small and uniformly distributed in the culture solution. Chitosan could flocculate *M.aeruginosa* through charge neutralization, the small algal flocs dispersed in the solution, so we did not see clearly the interface between algal flocs and solution (Fig.1b). Fig.1c showed that only MBF did not flocculate *M.aeruginosa* because *M.aeruginosa* and MBF charged negative. The combination of chitosan and MBF could flocculate *M.aeruginosa*, but adding sequence of the two flocculating materials had important influence on flocculation. If the MBF was first placed into *M.aeruginosa* solution and mixed well, then chitosan was added into solution, the algal cells aggregated to form small flocs, but some flocs floated on the surface of water and others precipitated into the bottom of water which brought some difficulties to harvest (Fig.1e). When MBF was added before and after Chitosan for twice, we found an interesting phenomenon that algal cells aggregated together and the algal flocs floated on the surface of water (Fig.1d), so the algal flosc could be harvested by net (150μm). Flocculation experiment clearly indicated that adding sequence of chitosan and MBF had important influence on flocculation, so we further studied adding sequences of MBF and chitosan on flocculation of *M.aeruginosa*.

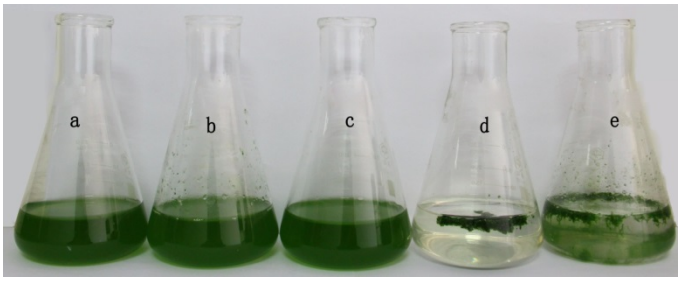

Fig.1. Images of different treatments. a: control; b: chitosan noly；c:MBF only；d:adding MBF first, then adding chitosan and adding MBF at last; e:adding MBF first, then adding chitosan

2.3 Flocculation of M.aeruginosa with combination of chitosan and MBF

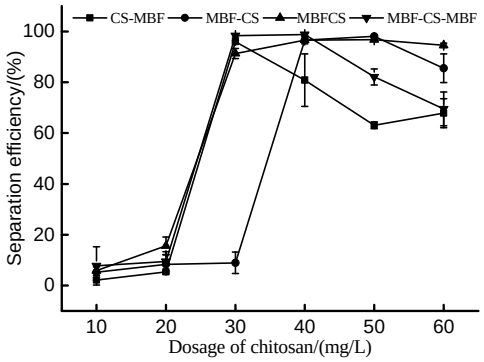

Fig.2. Separation efficiency of *M. aeruginosa* with different combination of MBF and chitosan

Fig.2 showed the influence of different adding sequence of MBF and chitosan on the separation efficiency, the results demonstrated that different adding sequence of MBF and chitosan had great influence on separation efficiency. The separation efficiency with adding sequence of CS-MBF was lower than 10% when the usage of chitosan was below 30 mg·L^{-1}, separation efficiency was highest when the usage of chitosan was 30 mg·L^{-1}, separation efficiency decreased when the usage of chitosan exceeded 30 mg·L^{-1}. Adding sequence of chitosan and MBF was reversed(MBF-CS), the separation efficiency was 95.56% and 98.09% respectively when the usage of chitosan was 40mg/L and 50mg/L, and separation efficiency decreased in the others concentrations. MBF and chitosan were premixed and the mixture was used as flocculant (MBFCS), the separation efficiency was higher than 90% when the usage of chitosan was above 30 mg·L^{-1} and low separation efficiency at low concentration of chitosan. The last flocculation method (MBF-CS-MBF) was that MBF was added into algal solution by twice and chitosan was added at the intermediate. The separation efficiency first increased and then decreased with the increase of chitosan, The separation efficiency was 98.33% and 98.74% respectively when the usage of chitosan was 30mg·$L^{-1}$and 40mg·L^{-1} which was highest among the four flocculation methods.

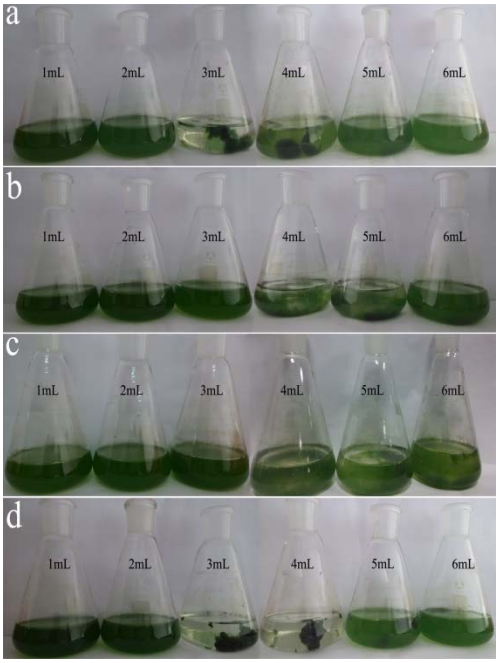

Fig.3. Images of flocculation of *M. aeruginosa*with different combination of MBF and chitosan. a: CS-MBF; b: MBF-CS; c: MBFCS; d: MBF-CS-MBF

Separation efficiency was only an evaluation index used to indicate whether algal cells could aggregate together, but it did not tell you whether the algal floc was easy to harvest in the subsequent. The images of flocculation effect were showed in Fig.3, the flocs not only had a big difference in size and shape under the best separation efficiency in the different flocculation methods, but also the interface between the floc and solution varied greatly. Among the four flocculation methods, the interface was clear between algal flocs and solution under the best separation efficiency with flocculation method of MBF-CS-MBF (Fig.3d), all the algal cells aggregated together and the algal flocs could be harvested by net (0.15mm), so MBF-CS-MBF was the best flocculation method.

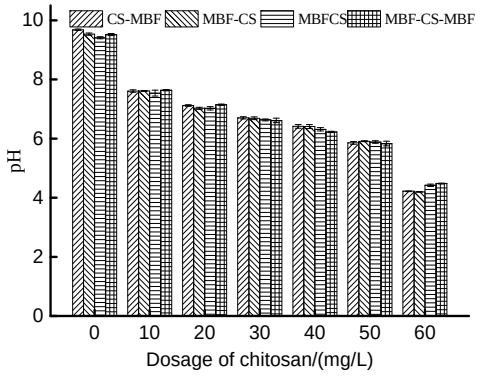

Fig.4. Change of pH after flocculation of *M. aeruginosa* with different flocculation methods

The change of pH with different treatment was showed in Fig.4. The pH of solution after flocculation decreased with the increase of chitosan for the all flocculation methods, the pH had no significant difference at the same usage of chitosan for different treatments. The chitosan dissolved in the acid solution which resulted in decrease of pH of solution. The pH of solution after flocculation of *M. aeruginosa* was 6.61 at the chitosan concentration of 30mg/L with flocculation method of MBF-CS-MBF which reached the national standards for the wastewater discharge and the environmental quality of surface water.

Algae assimilated a lot of phosphorus during growth. The change of TP after flocculation of algae was showed in Fig.5. The TP was highest with the flocculation method of the MBFCS, the TP of supernatant first increased and then decreased with the increase of chitosan concentration. The TP had no significant difference when the chitosan concentration was below 30mg/L for the flocculation method of MBF-CS, then the total phosphorus decreased. The TP first decreased and then increased with increase of chitosan concentration with the flocculation methods of CS-MBF and MBF-CS-MBF, the phosphorus removal was above 80.00% at the chitosan concentration of 30mg/Land 40mg/L. On the basis of above analysis, we know that the phosphorus was assimilated and stored in the algal cells, once algal cells were harvest, TP of suspend would have a sharp decrease.

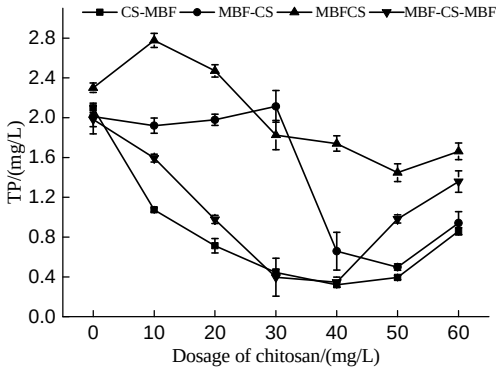

Fig.5. Change of TP after flocculation of *M. aeruginosa* with different combination of MBF and chitosan

2.4 Flocculation and floating mechanism of M. aeruginosa by combination of MBF and chitosan

The flocculation method of MBF-CS-MBF gave the lowest TP and high separation efficiency. To understand the mechanism involved in flocculation, the stereo microscope was also utilized to observe the structure and morphology of floc at the optimum condition. Due to the small size of of *M.aeruginosa* (3-7μm) and their colloidal stability in the solution, so microalgal cells was distributed uniformly in algal culture(Fig.6a). The same observations were seen with the algal cells when MBF was attempted to flocculated algae (Fig.6b). The chitosan could aggregate the algae, but the flocs were loose which brought difficulty to directly harvest (Fig.6c). The combination of MBF and chitosan could aggregate algae and the size of algal floc was bigger than floc with chitosan only (Fig.6d). The best flocculation images (Fig.6e) demonstrated that all algal cells aggregated together to form big flocs. An interesting phenomenon was that there were some bubbles in the floc, which increased buoyancy of the algal floc.

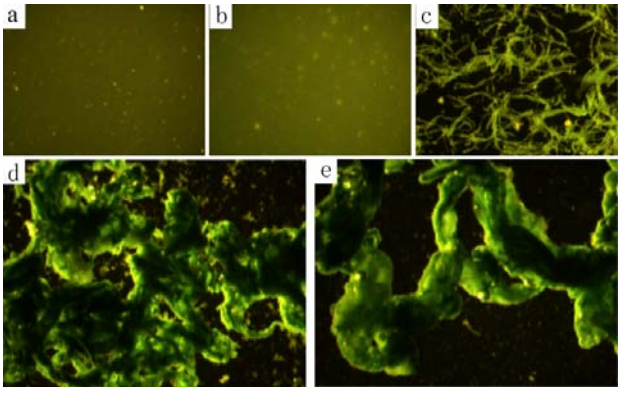

Fig.6. Stereo microscope photographs of different treatments. original algal culture(a); 100 mL algal culture containing 0.5 mL MBF(b), 3mg chitosan(c); 0.5mLMBF + 3mg chitosan (d), 0.5mLMBF + 3mg

chitosan+0.5 mL MBF (e).

Fig. 7 showed the zeta potential of different treatments. The zeta potential of original algal culture was -18.20±0.71 mV, suggesting the algal cells charged negative. The zeta potential of BG11 medium (100 mL) containing 0.5 mL MBF was -30.5+0.28 mV, suggesting that MBF was more negative than algal culture. The zeta potential of algal culture (100mL) containing 0.5mL MBF was -16.76±0.75 mV, which charged lower negative than algal culture and MBF solution. Chitosan could flocculate *M. aeruginosa* and zeta potential was -4.35+0.83 mV, which demonstrated the flocculation mechanism was charge neutralization. The zeta potential of algal culture (100mL) containing 0.5 mL MBF and 3 mg chitosan was 5.03+0.06 mV, which was higher than algal culture (100mL) containing only 3 mg chitosan. The zeta potential of algal solution with flocculation method of MBF-CS-MBF was 3.77+0.10mV which was lower than that of algal culture containing 0.5 mL MBF and 3mg chitosan.

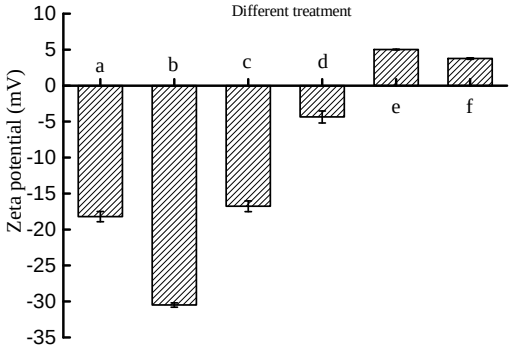

Fig.7. Zeta potential of different treatments. original algal culture(a); BG11medium(100mL) containing 0.5mL MBF(b); 100mL algal culture containing 0.5mL MBF(c), 3mg chitosan(d); 0.5mL MBF+3mg chitosan (e), 0.5mL MBF+3mg chitosan+0.5mL MBF (f).

3. Discussions

Chitosan was a natural biopolymer material produced by the deacetylation of chitin, it is non-toxic, bio-degradable, wide availability of raw material and positive charge which made it as a potent flocculant for aggregating microalgae and suspended particles in the water(Renault et al., 2009). Although only chitosan could flocculate algae by charge neutralization at the optimum chitosan dose of 30 and 40mg/L, but there are some disadvantage as follows: Ⅰ Algal flocs was small and precipitated at the bottom, after decanting the supernatant, the volume of slurry was one tenth of initial biomass density (Rashid et al., 2013b), this slurry was further dewatered by a centrifuge machine；Ⅱ flocculating process consists of two stages, one was adsorption between algae and chitosan by charge neutralization, and another was bridging action among algal flocs, so flocculation time was longer. It was reported that chitosan needs a mixing time of at least 20 min to coagulate 99.0% of the *Chlorella sp*(Ahmad et al., 2011), but it cost at least of 40 min to flocculate microalgae in our preliminary research. The above merits inhibit the application of chitosan in the practice. In order to solve these problems, the new flocculation method was developed to harvest micoalgae using combination of microbial flocculant (MBF) and chitosan. Chitosan was also attached to solid materials (soil, sand and coal fly ash) to facilitate precipitation of algae, this technology had been used in practice(Pan et al., 2012; Yuan et al., 2016). Rashid(Rashid et al., 2013a) found an interesting phenomenon that some algal cells moved upward and stay at the top immediately after addition of chitosan at low mixing. Our previous research had proved that MBF combined ferric trichloride facilitated floating aggregation of *M.aeruginosa* (Hao et al., 2016b)，but ferric trichloride might bring second pollution, so the non-toxic natural polymers of MBF and chitosan was used to aggregate M.

aeruginosa and made the algal floc move upward. Among of the different influent factors, adding sequence had an important influence on separation efficiency. Fig.1 showed the images of flocculation with different treatments, chitosan alone could aggregate algal cells to form small flocs in 10 min, and the algal flcos suspended in the solution, so there was no clear interface between algal floc and solution (Fig.1b). Fig.1d showed that all the algal flocs floated on the surface of water and had a good dewatering effect. In order to understand the effect of adding sequences on flocculation effect, four kinds of adding sequences were designed in the following experiments (CS-MBF, MBF-CS, MBFCS, MBF-CS-MBF).

According to our previous experiment (Hao et al., 2016b), the usage of MBF was same (10 mL/L) among the different flocculation methods. Chitosan was added into the algal culture first with the flocculation method of CS-MBF, positive charge chitosan could adsorb negative charge algae to form small positive algal flocs by charge neutralization, then MBF was added to aggregate small positive algal flocs by trapping and bridging. Flocculation efficiency seemed good from images of flocculation at the chitosan concentration of 30 mg/L and 40mg/L(Fig. 3a), but it would take a long time because flocculation of algae with chitosan was the controlling step. If we reversed the adding sequence of chitosan and MBF (MBF-CS), separation efficiency was high at the chitosan concentration of 40 mg/L and 50mg/L, but the flocculation efficiency was bad from the images of flocculation (Fig. 3b), MBF was added in the algal culture before chitosan, the chitosan would first reacted with MBF to form positive charge particles, then the positive charge particles aggregated algal cells, so algal flocs was small and suspended in the solution. In order to prove this hypothesis, chitosan and MBF were premixed (MBFCS) and then the mixture was used to flocculate alage, chitosan reacted with MBF to form solid material, so the floc was small and loose(Fig. 3c). MBF-CS-MBF was the best flocculation method from aspects of separation efficiency and size of flocs(Fig.3d). MBF and algal cells charged negative, so MBF did not aggregate algal cells. However, MBF could adhere to the surface of algae by chemical bond such as -COO and –OH which improved flocculation of algae in the subsequence, some studies have reported that some oxidizers could stimulate algal cells to secret desirable amount of EOM which aiding coagulation of algae with polyaluminium chloride(Wang et al., 2013). MBF could replace oxidizer as an assistant flocculant which impeded second pollution induced by chemical materials. Once MBF adhered to the algae, the algal cells were easy to bond with chitosan by neutralization charge, so flocculation time was short. The MBF was added into mixture at last, it could link small flocs to form big flocs by netting and bridging, the algal floc could be harvested by net, so MBF-CS-MBF was the best flocculation and the optimum usage of chitosan was 30mg/L.

pH was a monitoring index for discharge wastewater. Chitosan was soluble in the dilute acid and insoluble in the water, so it had important influence on pH of water after flocculation of alage. Rashid's research proved that chitosan hydrochloride had best flocculation on *C. vulgaris (Rashid et al., 2013a)*, so hydrochloride was used to dissolve chitosan in our experiment. The pH of solution had no significant difference at the same level of chitosan which proved that adding sequence of MBF and chitosan had no influence on pH. However, the pH of solution decreased with increase of chitosan usage, which showed that proton (H^+) first reacted with alkaline materials in the algal culture and then the protonated amino groups of chitosan interacted with negatively charged *M.aeruginosa* via charge neutralization**.** In order to prove the hypothesis above, pH of alkaline algal solution (pH ≈10.00) was adjusted to about 8.00 first, and then MBF and chitosan were added to flocculate *M.aeruginosa* according to flocculation method of MBF-CS-MBF, The result showed that the usage of chitosan was low. pH not only influenced separation efficiency and usage of chitosan, but also it decided whether the water could discharge and was reused directly after flocculation of *M.aeruginosa*, so pH of water after flocculation should be kept in neutral.

Wyatt researched the critical conditions for ferric chloride induced flocculation of freshwater algae, the result showed that the pH threshold for effective flocculation was 4.0±0.3 (Wyatt et al., 2012). My previous research found that the optimum pH was about 5.30 by combination of MBF and ferric chloride(Hao et al., 2016b). The pH was about 6.50 at optimum flocculation conditions with flocculation method of MBF-CS-MBF which was near neutral and met the national standard of environmental quality for surface water.

The indexes of separation efficiency and pH corresponded to the feasibility of flocculation technology, but phosphorus removal was highly focused because it was limited factor for eutrophic water body. In order to inhibit algal blooms, it was proved as an effective method to reduce phosphorus load of water body such as controlling exogenous phosphorus input, sediment environmental dredging and phosphorus removal by some absorbing materials (Brookes and Carey, 2011). Hao's research showed that algal growth could stimulate phosphorus release from sediment and it was assimilated by algae(Hao et al., 2016a), if algae was flocculated and collected by environmental technology, the internal phosphorus pollution would purified and algal blooms would be controlled. However, phosphorus removal by collecting algae was neglected because there was no effective and inexpensive technology to aggregate microalgae. The flocculation technology in our research was approved to harvest microalgae, Fig.2 and Fig.5 showed that high separation efficiency was corresponding to low phosphorus concentration, which proved the phosphorus was assimilated by *M.aeruginosa* and stored in the cell. The total phosphorus content of dry algal flocs was 1.28%±0.01% at the optimum condition with flocculation method of MBF-CS-MBF, so total phosphorus decreased after harvesting algae. Algae was cultured in the BG11 culture and phosphorus was not the limited index for algal growth, so there was phosphorus residual after harvesting algal flocs.
The combination of 10 mL/L MBF and 30 mg/L chitosan gave the high separation efficiency of 98.33 %, and neutral pH of solution after flocculation of algae. However, flocculation mechanism and float mechanism were not clear, so algal flocs were observed by stereo microscope and zeta potential of culture solution was determined in the every stage of flocculation process. Scanning electron microscopy (SEM) was widely used to observe the structure and morphology of materials, but preparation of sample might destroy structure of algal floc. To understand the mechanism involved in flocculation, the stereo microscope was utilized to observe the structure and morphology of floc because the samples could be directly observed by stereo microscope. The stereo microscope photographs of algae and algal floc were showed in Fig.6. *M.aeruginosa* was a single-celled organism with the diameter of about 3-7μm, so algal culture had a uniform distribution in the solution (Fig.6a). The same observations were seen with flocculation of MBF on algal cells (Fig.6b) which proved that MBF did not flocculate algae. There were a lot of researches reported that chitosan could flocculate algae by charge neutralization(Henderson et al., 2008; Kurniawati et al., 2014). Rahid found an interesting phenomenon when chitosan was used to flocculate *C. vulgaris*, some algal cells moved upward and stayed at the top at high chitosan dose (60-90 mg/L) and the others stayed at the bottom of solution (Rashid et al., 2013a). In our research, only using chitosan could flocculate algae, but we did not see floating algal floc and the flocs were loose in the bottom (Fig.6c). In order to obtain the big and floating algal flocs, MBF was used to improve separation efficiency for harvesting of *M.aeruginosa*. The algal floc was bigger than that with the only flocculant of chitosan when MBF was added in the algal culture before chitosan (Fig.6d), the formation of big algal floc had closed relationship with bridging and netting effect of MBF. Fig.6e showed that all algal cells aggregated together and the algal flocs floated on the surface of solution with the flocculation method of MBF-CS-MBF, the algal cells were wrapped with thin film like a pocket. The most interesting observation was that some bubbles in the flocs, which might be the oxygen released by algae for photosynthesis. To verify this

postulated hypothesis, the flocculation experiments were conducted in the dark and light environment respectively, the algal floc floated on the surface of solution in the light environment and the bubbles attached to the surface of algal floc, but we did not see the same phenomenon in the dark environment. The presence of such bubbles also proved that algal cells were not destroyed in the process of flocculation, and the bubbles increased buoyancy of algal floc, so it kept afloat on the surface of water which made it easy to harvest.

Observation of stereo microscope provided the access to understand floating mechanism. However, the flocculation mechanism was not clear, so zeta potential of solution at different stage of flocculation was determined to explore flocculation mechanism. The zeta potential of original algal culture was -18.20±0.71mV, suggesting the algae was negatively charged. The zeta potential of BG11 medium (100mL) containing 0.5 mL MBF was -30.5+0.28 mV, suggesting MBF was more negative than the algae. The more interesting observation was that the zeta potential of algal culture (100mL) containing 0.5 mL MBF was -16.76±0.75 mV which was higher than that of algal culture and BG11 medium containing MBF. Zeta potential of algal culture containing MBF should be mostly negatively charged if MBF and algal cells existed independently in the solution, on the contrary, zeta potential was few negatively charged which proved that the MBF might combined with the algal cells. The combination of MBF and algal cells did not depend on the charge neutralization because both of them charged negative. A lot of researches showed that algae could secrete polysaccharide which attached to surface of algal cells(Ma et al., 2012; Wang et al., 2013). Ours previous studies proved that the main component of MBF was a polysaccharide secreted by *B. mucilaginosus*(Lian et al., 2008), so the combination of MBF and algal cells depended on viscous effect. Zeta potential of solution increased to 5.03+0.06 mV if chitosan was added after MBF, which was higher than that with chitosan only (-4.35+0.83mV), suggesting the combination of MBF and algal cells would decrease negative charged algal cells. The flocculation rate was also faster with combination MBF and chitosan than that with chitosan only, MBF was a polysaccharide which contained some negative charged functional groups such as –OH and COO- and chitosan contains positive charged functional groups -NH_3, So charge neutralization made it easy to bond together between chitosan and algal cells which had been attached by MBF. The zeta potential decreased from 5.03+0.06mV to 3.77+0.10mV and the size of algal floc was big (Fig.7f) when MBF was added last, which showed that flocculation mechanism included charge neutralization, bridging and netting action.

4. Conclusion

MBF-CS-MBF was the best flocculation method, all algal cells aggregated together and algal flocs float on the surface of the water which made it easy harvest by net (0.15 mm). Zeta potential measurement confirm that the flocculation mechanism is charge neutralization, the addition of MBF before chitosan accelerate the formation of algal flocs, MBF aggregate all algal flocs into together by bridging and netting effect when MBF was added again after chitosan. The flocculation with the combination of MBF and chitosan not only aggregate algal cells together, but also captured the oxygen released by algae for photosynthesis which causes the algal flocs float on the surface of supernatant. The total phosphorous content of dry algal floc was1.28% which proved that harvesting of algal cells could purify phosphorus pollution of eutrophic waterbody.

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