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To cite this article: Jianchao Hao, Bin Lian, Ruanbao Zhou, Huifen Liu, Lijun Shi & Congqiang Liu (2015): Microbial flocculant combined ferric trichloride facilitates floating aggregation of *Microcystis aeruginosa* for efficient removal, *Desalination and Water Treatment*, DOI: [10.1080/19443994.2015.1108236](https://doi.org/10.1080/19443994.2015.1108236)

To link to this article: <http://dx.doi.org/10.1080/19443994.2015.1108236>



Published online: 29 Oct 2015.



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Microbial flocculant combined ferric trichloride facilitates floating aggregation of *Microcystis aeruginosa* for efficient removal

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Received 13 February 2015; Accepted 11 October 2015

ABSTRACT

A combination of microbial flocculant (MBF) and ferric trichloride (FeCl_3) was applied to aggregate and harvest algae. The orthogonal experiment was designed to optimize conditions of flocculation. Mechanism of flocculation was observed through determining the zeta potential and observing the morphology of algal floc using stereo and scanning electron microscope. The results showed that the optimum combination of flocculants was 0.175 g of FeCl_3 with 10 mL MBF per liter, which exhibited the highest flocculation efficacy (95.12%) and lowest chemical oxygen demand (10.44 mg/L). The flocculation mechanism was charge neutralization, where the MBF first adhered and coated algae. Due to its high affinity to iron-hydroxy ions and long-chain molecules, the MBF attracted positively charged hydroxyl ions generated by FeCl_3 hydrolysis and interacted with the iron-hydroxy ions to form a larger floc by bridging. The coat made of MBF formed a thin film which protected algae from being destroyed, and trapped the oxygen released from photosynthesis, making the cell more buoyant to float on the surface of water. Findings of this study provided a potentially practical and efficient method for harvesting unicellular algal cells.

Keywords: Cynaobacteria; *Microcystis aeruginosa*; Flocculation; Algal harvesting; Microbial flocculant

1. Introduction

Cyanobacterial blooms in aquatic systems (i.e. lakes, rivers, and seas) have become a global environmental

concern. Blooms of such species negatively affected water quality, aquatic ecosystems, fisheries, ecological landscapes, and public security by threatening the safe supply of drinking water [1,2]. Thus, developing environmentally acceptable strategies to control on-going

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harmful cyanobacterial blooms events are urgently needed [3]. It is widely known that cyanobacteria assimilate phosphorus and nitrogen in the process of growth, so harvesting cyanobacteria from the water body of algal blooms could decrease the concentration of nutrient elements of algal polluted water from the perspective of mass conservation which inhibit cyanobacterial blooms [4,5]. Therefore, the efficient harvesting of cyanobacteria is important to control cyanobacterial blooms [6].

Current methods used to harvest microalgae include: centrifugation, filtration, and flocculation/coagulation. Centrifugation seems to be the most efficient, yet cost-prohibitive method, especially in processing large volumes of culture [7]. Filtration technology, on the other hand, is an essential process in algae-liquid separation, but it is only useful in recovering large species such as *Spirulina*. Small unicellular taxa such as *Microcystis* spp. may pass through the conventionally used filter, which reduces filtration efficiency [8]. Also, some flocculants and coagulants such as hydrolyzing metal salts [9,10], chitosan [11], clay minerals, and modified clays [12] can aggregate microalgae. Hydrolyzing metal salts, such as aluminum, iron and their polymers, could destroy the cells [13] making it difficult to harvest small and loose flocs of algae [14]. Moreover, chitosan has a high cation density charge and has been recommended as an environmentally friendly coagulant, but the floc is loose and flocculation time is long which limits its application [15]. The use of nontoxic and modified clays to flocculate algae seems to be one of the most promising methods in controlling algal blooms [16,17]. However, using the clays, the flocs of microalgae sink to the bottom which releases and returns N, P, and organic compounds into the overlying water for decomposition. Therefore, all the current methods being used for flocculation have advantages and are also subject to some limitations.

Previous studies demonstrated that microbial flocculant (MBF), prepared from *Bacillus mucilaginosus*, could adsorb heavy metals and flocculate suspended substances in mine tailings [18–20]. Upon adding FeCl_3 , the positively charged iron-hydroxy ions produced by the hydrolysis of FeCl_3 adsorb negatively charged MBF by electrostatic interaction [21]. The combination of MBF and FeCl_3 posits great potential for the flocculation of algae in algal polluted water such as harmful cyanobacterial blooms. Blooms of *Microcystis aeruginosa* are a major causative organism in many cyanobacterial harmful algal blooms (CyanoHABs) reported in China [16]. It produces toxins (microcystin) which cause animal and human poisonings or health risks [22], and thus, become a major

threat to public safety and security. The presence of this species in many freshwater bodies and the alarming increase in the frequency of reported blooms, make it as ideal model to test the efficiency of some flocculants. In this study, we demonstrated the use of combined MBF and FeCl_3 to flocculate *M. aeruginosa*, this method could aggregate *M. aeruginosa* together, and the cyanobacterial floc is not only big and dense but it could trap gas released from cells which makes the floc more buoyant, so that the floc of *M. aeruginosa* can be harvested by net without centrifugation and filtration. Meanwhile, optimum condition of flocculation was determined by orthogonal experiment, and flocculation mechanism was studied by determining the zeta potential and observing structure and morphology of floc via stereo and scanning electronic microscopes (SEM). Results from this study not only provided both theoretical and a practical bases for controlling cyanobacterial blooms, but also developed a new approach for harvesting other microalgae.

2. Materials and methods

2.1. Algal culture

M. aeruginosa (FACHB469) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences in Wuhan, Hubei Province, China. Cultures were subcultured in BG11 culture medium [17] and grown in $24 \pm 1^\circ\text{C}$ chambers illuminated with 2,200 lx light intensity at 12 h/d.

2.2. Bacterial flocculant

B. mucilaginosus K02 (accession number HM579819) was chosen for the production of MBF [19]. The bacterium was chosen since it is commonly used as plant growth-promoting rhizobacteria in soil and has been used widely as a biofertilizer in China, India, and other countries [23,24], and thus would not cause biological pollution.

The bacterium was inoculated in a 200-mL medium, made up of 10.0 g sucrose, 0.3 g yeast extract, 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 1.0 g K_2HPO_4 , 1.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.5 g CaCO_3 dissolved in 1 L distilled water, and incubated at 30°C on a shaker incubator (140 rpm) for 3 d. This served as the starter culture which was inoculated at 10% volume in 200 mL nitrogen-free medium (1 L medium with dissolved 5 g sucrose, 1.50 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g CaCO_3 , 5.0 mg FeCl_3 , and 1.0 g glass powder), and incubated at 30°C on a shaker (140 rpm) for 7 d [19]. The liquid with its viscosity (NDJ-5s digital viscometer, China) of 400 to 500 mPa s was used directly as MBF.

2.3. Flocculation of *M. aeruginosa* using MBF, FeCl₃, and their combination

Different set-ups were used to test the efficiency of different concentrations of flocculants against the test algae. Specifically, 200 mL of *M. aeruginosa* culture (about 6.0×10^7 cells/mL) was placed in a 250 mL flask and added with either MBF (2, 4, 6, 8, and 10 mL) or FeCl₃ (7, 14, 21, 28, 35, and 42 mg), and mixed uniformly at 30°C in the shaker (140 rpm) for 5 min. The mixture was allowed to stand for 30 min before flocculation effect was observed. Flocculation of *M. aeruginosa* with a combination of MBF and FeCl₃ was also tested which was done by first adding 2 mL of MBF in 200 mL of *M. aeruginosa* culture and then, mixed uniformly, after which, 35 mg of FeCl₃ was added, mixed uniformly again at 30°C in the shaker (140 rpm) for 5 min and allowed to stand for 30 min before being finally observed for the flocculation effect.

2.4. Orthogonal flocculation experiment

An L₂₅ (5⁶) orthogonal experimental design (three replicates) was used to determine the optimal flocculation dosage of MBF (2, 4, 6, 8, and 10 mL, factor A) and FeCl₃ (7, 14, 21, 28 and 35 mg, factor B) (see Table 1). First, the different dosages of MBF were added into separate flasks containing 200 mL of cyanobacterial culture and incubated for 5 min at 30°C and 140 rpm. Then, different dosages of FeCl₃ were added according to the orthogonal experimental design, and further incubated for additional 5 min at 30°C and 140 rpm. After letting stand for 30 min, the clear water was withdrawn by injector to test for flocculation efficiency, chemical oxygen demand (COD) (COD was measured by titration with acidic potassium permanganate [25]), and pH value (pH 211, HANNA Instruments, USA). Flocculation efficiency was calculated as follows:

Table 1

Result of flocculation orthogonal experiment. Factor A: MBF added (mL/200 mL culture); Factor B: FeCl₃ added (g/200 mL culture)

Trial no.	Factors		Experimental error				COD (mg/L)	Flocculation efficiency (%)	pH
	A	B	eC	eD	eE	eF			
1	1 (2 mL)	1 (7 mg)	1	1	1	1	400.2	9.17	7.36
2	1	2 (14 mg)	2	2	2	2	443.7	34.51	6.96
3	1	3 (21 mg)	3	3	3	3	139.2	57.50	6.75
4	1	4 (28 mg)	4	4	4	4	48.72	94.63	6.24
5	1	5 (35 mg)	5	5	5	5	10.44	95.12	5.00
6	2 (4 mL)	1	2	3	4	5	408.9	9.89	7.31
7	2	2	3	4	5	1	443.7	39.93	6.94
8	2	3	4	5	1	2	269.7	68.52	6.58
9	2	4	5	1	2	3	93.96	94.81	6.11
10	2	5	1	2	3	4	48.72	95.21	5.22
11	3 (6 mL)	1	3	5	2	4	478.5	11.47	7.47
12	3	2	4	1	3	5	452.4	29.67	7.32
13	3	3	5	2	4	1	256.7	51.63	7.14
14	3	4	1	3	5	2	87.00	82.88	6.78
15	3	5	2	4	1	3	52.20	95.17	6.13
16	4 (8 mL)	1	4	2	5	3	435.0	13.41	7.39
17	4	2	5	3	1	4	469.8	29.77	7.15
18	4	3	1	4	2	5	374.1	55.78	6.89
19	4	4	3	5	3	1	125.3	83.60	6.72
20	4	5	2	1	4	2	59.16	95.21	5.96
21	5 (10 mL)	1	5	4	3	2	452.4	15.76	7.15
22	5	2	1	5	4	3	382.8	31.07	6.88
23	5	3	2	1	5	4	226.2	51.81	6.50
24	5	4	3	2	1	5	100.9	88.30	6.20
25	5	5	4	3	2	1	59.16	95.17	5.51

$$\theta = \frac{C_0 - C_s}{C_0} \times 100$$

where θ is the flocculation efficiency (%), C_0 is the initial cyanobacterial density (cells/mL), and C_s is the cyanobacterial density after flocculation (cells/mL).

Note: the cyanobacterial cells were counted under a Motic digital microscope [26]. The pH value was also monitored in this experiment, although it was not chosen as an evaluation index to determine optimum conditions. Previous reports showed that the pH induced by hydrolyzing metal salts exerted a critical influence on the flocculation of algae [9,27].

2.5. Mechanism of flocculation

To understand the mechanism underlying the flocculation, microalgal flocs generated using the different flocculation methods were observed further by scanning electronic microscope (SEM, JSM-6510LV, Japan) and stereo microscope (Leica-M165FC, Germany). Samples were prepared for SEM following the methods described by Hao et al. [28]. First, the floc was affixed onto adhesive tapes supported by metallic disks, air dried, and then covered with a thin, electric conductive platinum film before being examined under SEM. Algal culture and flocs was also placed in Petri dishes for direct observation under stereo microscope.

The zeta potential of samples was also analyzed using a zeta meter (Zetasizer Nano ZS90, Malvern Instruments, UK) which combines electrophoresis and laser Doppler velocimetry.

2.6. Flocculation of *M. aeruginosa* in aeration tank

Water quality was also assessed after flocculation to help determine conditions with optimized flocculation. This was done by placing 2 L of *M. aeruginosa* culture in a 4 L aeration tank and then MBF (20 mL) was added, while simultaneously being aerated for 5 min to mix the mixture uniformly. Then FeCl_3 was added to the tank while aerating for another 5 min and the mixture was allowed to stand for 30 min in room temperature. Concentration of phosphorus, COD, and Fe were also measured after flocculation. Specifically, phosphorus level was determined by the molybdenum blue colorimetric method [29] while the total Fe digested by hydrochloric acid and hydroxylamine hydrochloride, was determined by phenanthroline spectrophotometric method [30].

2.7. Data analysis

Range analysis and analysis of variance (ANOVA) [31] were done to test for significant difference between sample and set-ups, and to determine the optimal conditions for flocculation and their magnitudes.

3. Results and discussion

3.1. Flocculation of *M. aeruginosa* by MBF, FeCl_3 , and their combination

Flocculation experiments using only MBF and FeCl_3 , and a combination of the both were carried out to evaluate the flocculation effect of *M. aeruginosa* with different flocculants. Some images of flocculation effect are shown in Fig. 1. Generally, MBF did not flocculate the algae (Fig. 1(b)) because MBF and *M. aeruginosa* are both negatively charged and they do not attract each other through charge neutralization, while FeCl_3 tests and the combination of both varied accordingly. Wyatt et al. [9] showed that a minimum concentration of ferric chloride is required to overcome the electrostatic stabilization of the algae. In this study, results demonstrated that the algae could be aggregated into flocs if the dosage of FeCl_3 was between 0.105 and 0.175 g/L solution (Fig. 1(d)), but the floc was so small that it was difficult to filter, in addition to it precipitating in the bottom of the flask which brought difficulty to harvest by salvage. *M. aeruginosa* could not form floc when dosage of FeCl_3 was lower than 0.105 g/L, while the algae turned yellowish when dosage of FeCl_3 was higher than 0.175 g/L because the hydrolysis of Fe^{3+} can produce H^+ and acid environment might destroy the algal cells. A combination of MBF and FeCl_3 also showed signs of flocculation of *M. aeruginosa*. In fact, the algae aggregated together and the flocs floated on the surface of the water (Fig. 1(c)). As suggested in earlier findings, MBF might facilitate Fe^{3+} -induced algal flocculation which was tested using the orthogonal experimental design.

3.2. Results from the orthogonal experiment of flocculation

Results of the orthogonal experiment (Table 1) showed that the range of COD varied from 10.44 to 478.5 mg/L, flocculation efficiency from 9.17% to 95.21, and pH from 5.00 to 7.39, respectively. The results showed that the dosage of factor A and factor B exerted a stronger influence on the flocculation of *M. aeruginosa* at different levels. Mean values of k_{ji} for different factors at different levels and ranges of R_j are shown in Table 2. COD is a measure of oxygen

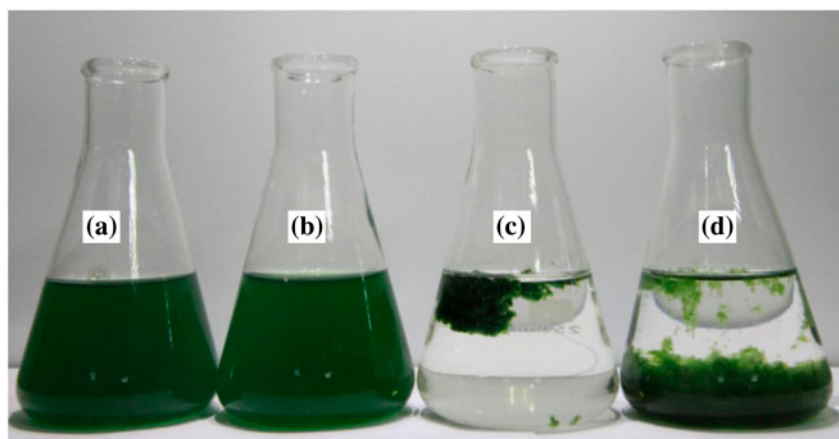


Fig. 1. Images of different treatments. *M. aeruginosa* culture, control (a), MBF only (b), combination of MBF and FeCl_3 (c), and FeCl_3 only (d).

Table 2
Range analysis of orthogonal experiment

Evaluation index	Evaluation parameters	Factors	
		A	B
COD (mg/L)	k_{j1}	208.5	435.0
	k_{j2}	253.0	438.5
	k_{j3}	268.0	255.9
	k_{j4}	292.7	91.18
	k_{j5}	244.3	45.94
	R_j	84.22	392.5
Flocculation efficiency (%)	k_{j1}	58.19	11.94
	k_{j2}	61.67	32.99
	k_{j3}	54.16	57.05
	k_{j4}	55.55	88.84
	k_{j5}	56.42	95.18
	R_j	7.51	83.24
pH	k_{j1}	6.46	7.34
	k_{j2}	6.43	7.05
	k_{j3}	6.97	6.77
	k_{j4}	6.82	6.41
	k_{j5}	6.45	5.56
	R_j	0.54	1.78

depletion effect of a waste contaminant, the smaller the COD value the lower the pollution level. In this study, results showed that the best combination was A_1B_5 with the lowest COD for the MBF at 2 mL per 200 mL culture ($K_{A1} = 208.5$ mg/L) and FeCl_3 at 35 mg per 200 mL culture ($K_{B5} = 45.94$ mg/L). The range (R_j) indicated the significance of the effect of each factor and a larger R_j meant that the factor had a greater effect on the flocculation. The R_B value

(392.5 mg/L) was higher than R_A (84.22 mg/L), thus, FeCl_3 had a greater impact on COD than MBF. Flocculation efficiency is an evaluation index used to indicate algal removal. The flocculation efficiency for each level was clearly distinct (see Table 2). The best combination was A_2B_5 with the highest flocculation efficiency for MBF at 4 mL per 200 mL culture ($K_{A2} = 61.67\%$) and FeCl_3 at 35 mg per 200 mL culture ($K_{B5} = 95.18$). FeCl_3 had a greater impact on flocculation efficiency than MBF because R_B (83.24%) was higher than R_A (7.51%). Notably, the optimal condition for COD was A_1B_5 with 95.12% flocculation efficiency and 10.44 mg/L COD, and the optimal condition for flocculation efficiency was A_2B_5 with 95.21% flocculation efficiency and 48.72 mg/L COD. The results above showed that there were no significant differences ($p > 0.05$) in flocculation efficiency between A_1B_5 and A_2B_5 , but the COD under condition of A_2B_5 was 4.7 times higher than that of A_1B_5 . These further suggested that A_1B_5 could be the optimal flocculation condition since COD was the strict control index for the discharge of waste water.

Results of ANOVA used to evaluate significant differences in the indices are shown in Table 3. It was interesting to note that Factors A and B had different effects on the evaluation indices. For the evaluation index of COD, it was clear that $F_{0.05} < F_A < F_{0.01}$ and $F_B > F_{0.01}$. When the F-value of each factor (F_j) and the critical value of the F-value (F_A) were compared, factor B seemed to be the prominent factor affecting the COD of water and factor A exerted less influence on COD after flocculation than factor B. Although MBF is mostly comprised of organic materials and raising its dosage would increase the COD of the water, most MBF successfully attached with the algal flocs and

was altogether removed when the flocs were collected. ANOVA for flocculation efficiency showed that $F_A < F_{0.05}$ and $F_B > F_{0.01}$. Factor B had stronger influence on flocculation efficiency while factor A had none. Indeed, MBF and the algal cells did not bond each other because they are both negatively charged with a zeta potential of -33.0 and -18.5 mV, respectively. However, FeCl_3 could generate positive iron-hydroxy ions through hydrolysis, which then adsorbed the negatively charged *M. aeruginosa* and MBF by charge neutralization. Taken together, FeCl_3 played a central role in *M. aeruginosa* flocculation and MBF may facilitate formation of big algal floc by bridging.

Ideally, the pH value of water after treatment should be as close to neutral as possible from the perspective of environmental protection, but the flocculation of algae induced by FeCl_3 was carried out in more acidic conditions. It was reported that carboxyl groups in the algae of *Chlorella zofingiensis* dominated the surface charge [9] and thus the pH required a threshold of 4.0 ± 0.3 for effective flocculation. This is the very reason why the pH value was not chosen as evaluation index in the flocculation. Based on range analysis of pH (Table 2), the value of R_B (1.78) was higher than R_A (0.54), suggesting that FeCl_3 had more influence on the pH of the culture than MBF, which might have been due to its hydrolysis. FeCl_3 hydrolysis produces H^+ and some iron-hydroxy ions such as $\text{Fe}(\text{OH})^{2+}$, $\text{Fe}_2(\text{OH})_2^{4+}$, $\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})_3$, and FeOOH [32,33]. The pH of the solution after flocculation was 5.00 under optimum conditions of A_1B_5 (Table 1) which was higher than the critical pH reported by

Wyatt et al. [9], but ANOVA results (Table 3) indicated that both factors A and B were important in controlling the pH of the solution. The reason was that FeCl_3 was an ideal reagent capable of both inducing algal flocculation by neutralization and introduction of an acidic environment. In contrast, other acidic chemicals such as hydrochloric acid and sulfuric acid did not induce algal flocculation although they decreased pH of solution. In addition, the pH of A_1B_5 , A_2B_5 , A_3B_5 , A_4B_5 , and A_5B_5 were 5.00, 5.22, 6.13, 5.96, and 5.51, respectively, with increased amounts of MBF at the same addition of FeCl_3 of 35 mg per 200 mL algae culture. The tendency of the pH in culture increased first then decreased with increasing amount of MBF, implying that MBF carried negatively charged functional groups such as $-\text{OH}$ and $-\text{COO}^-$; these functional groups not only aggregated iron-hydroxy ions, but also combined with H^+ to accelerate the hydrolysis of Fe^{3+} and buffered the solution from acidifying rapidly. pH of solution decreased if the dosage of MBF was higher than 6 mL because MBF itself is acidic.

3.3. Flocculation mechanism of *M. aeruginosa* by combination of MBF and FeCl_3

The SEM images and stereo microscope photographs are shown in Figs. 2 and 3.

The combination of 10 mL/L MBF and 0.175 g/L FeCl_3 gave the highest flocculation efficiency of 95.12% and the lowest COD of 10.44 mg/L. To understand the mechanism involved in flocculation, additional flocculation experiments using MBF alone, FeCl_3 alone, and their combination were carried out

Table 3
Analysis variance (ANOVA) of orthogonal experiment

Evaluation index	Source	SS	df	V	F	F_A	Significant level
COD (mg/L)	A	19,286.30	4	4,821.58	3.27	$F_{0.05}(4,16) = 3.01$	*
	B	682,983.45	4	170,745.86	115.98	$F_{0.01}(4,16) = 4.77$	**
	e	23,554.24	16	1,472.14			
	T	725,823.99	24				
Flocculation efficiency (%)	A	167.51	4	41.88	2.88	$F_{0.05}(4,16) = 3.01$	–
	B	25,390.67	4	6,347.67	436.87	$F_{0.01}(4,16) = 4.77$	**
	e	232.51	16	14.53			
	T	25,790.69	24				
pH	A	1.26	4	0.32	8.00	$F_{0.05}(4,16) = 3.01$	**
	B	9.40	4	2.35	58.75	$F_{0.01}(4,16) = 4.77$	**
	e	0.64	16	0.04			
	T	11.30	24				

*Difference is significant at the 0.05 level.

**Difference is significant at the 0.01 level.

under their respective optimum conditions determined in the earlier sections. The images of algal flocs observed by SEM are shown in Fig. 2, where Fig. 2(b) shows that the crystalline substance condensed loosely and the algal cells anchored onto the surface of there, Fig. 2(a) showed that the crystalline substance condensed tightly and the algal cells anchored together onto their surface. However, sample preparation for SEM observation might break the structure of floc, so the stereo microscope was also 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 are shown in Fig. 3. *M. aeruginosa* is a single-celled organism with a diameter of about 3–7 μm , so algal culture looked like a solution (Fig. 3(a)). The same observations were seen with the cells that were attempted to be flocculated with MBF (Fig. 3(b)). In addition, although the hydrolyzing metal salts of FeCl_3 aggregated the algae, the flocs however were loose and did not settle down (Fig. 3(c)). A combination of MBF and FeCl_3 could aggregate algae and the algal flocs were big and dense (Fig. 3(d)), in which the algae were tightly stacked and evenly covered with thin film. The most interesting observation was the formation of some bubbles in the flocs, which could be due to the oxygen released by algae as a product of photosynthesis. The presence of such air pockets proved that the algal cells were not destroyed in the process of flocculation. Besides, the flocs kept afloat on the surface of water which made it easier to harvest. These evidences supported the previously postulated hypothesis that MBF first adhered to algal cell to form coated algae, then, due to MBF's high affinity to iron-hydroxy ions, MBF attracted positively charged hydroxyl ions generated by the hydrolysis of FeCl_3 . With MBF's larger surface area, it interacted with the iron-hydroxy ions to form a larger particle

that could trap gas released from the inside of the cells which then makes the floc more buoyant. Taking these results together, it was concluded that MBF can facilitate algal flocculation induced by FeCl_3 . MBF actually played an important role in forming big algal flocs and protecting the algal cells from destroying.

3.4. Zeta potential of different treatment

In order to support the hypothesis proposed above (on the role of MBF), the zeta potential in the different treatments were determined (Fig. 4). The zeta potential of original algal culture was -18.50 ± 0.28 , suggesting that the algae was mostly negatively charged. The zeta potential of BG11 medium (200 mL) containing 2 mL MBF was -30.00 ± 3.86 mV, lower than that of the algal culture alone, suggesting that indeed MBF was more negative than the algae. The more interesting observation was that the zeta potential of algal culture (200 mL) containing 2 mL MBF was -18.05 ± 0.78 mV which was higher than that of algal culture and BG11 medium containing MBF. This proved that algae and MBF interacted in the solution.

Using the FeCl_3 only could flocculate algae, and the zeta potential of algal culture (200 mL) containing 35 mg FeCl_3 was 2.91 ± 0.19 mV, suggesting that the prevalent mechanism for flocculation was charge neutralization. The zeta potential of algal culture containing MBF and 35 mg FeCl_3 (MBF added first then FeCl_3) was -0.86 ± 0.39 , which was close to the isoelectric point. The algal floc using the combination MBF and FeCl_3 was big and buoyant which might be due to a number of reasons: (1) *M. aeruginosa* covered with negatively charged MBF could combine with positive charged iron-hydroxy ions produced by hydrolysis of FeCl_3 by charge neutralization; (2) MBF was made up of longer chains of polysaccharide

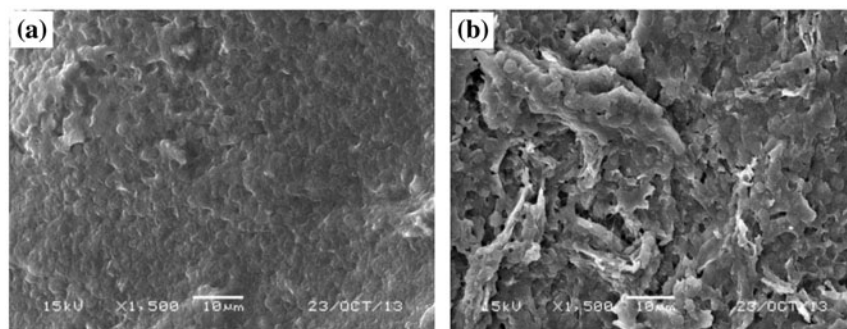


Fig. 2. SEM images of flocculated algae. Flocculated algae with MBF and FeCl_3 (a) and FeCl_3 (b).

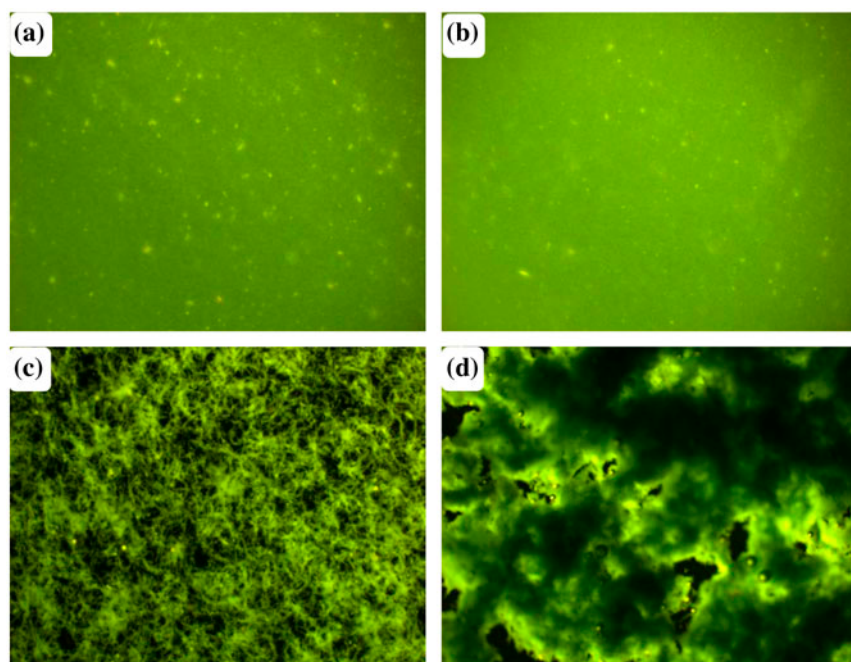


Fig. 3. Stereo microscope photographs of different treatments. *M. aeruginosa* culture, control (a), MBF only (b), and FeCl₃ only (c) and a combination of MBF and FeCl₃ (d).

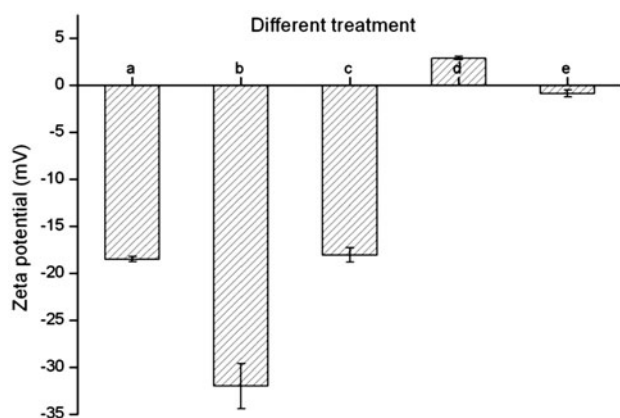


Fig. 4. Zeta potential of different treatment. Original algal culture (a), BG11medium (200 mL) containing 2 mL MBF (b), algal culture (200 mL) containing 2 mL MBF (c), algal culture (200 mL) containing 35 mg FeCl₃ (d) and algal culture (200 mL) containing 2 mL MBF and 35 mg FeCl₃ (e).

capable of cross linking with iron-hydroxy ions which then form big flocs; (3) algal photosynthesis was not inhibited during flocculation, and the produced oxygen formed bubbles adhered to floc surface, making the aggregation float on the surface of water. In comparison to algal flocculation with FeCl₃ alone, we know that MBF could protect algal cell from destruction which made solid-liquid separation easier.

3.5. Flocculation of *M. aeruginosa* in aeration tank

The last part of the study was an experiment conducted in 4 L aeration tank to determine the engineering feasibility of this study using the optimum conditions determined in previous sections (Fig. 5). Results showed flocculation efficiency of approximately 97.94%, and the color of algae remained dark green. The COD of the solution decreased from 511.7 to 44.28 mg/L (a 91.34% decrease). Due to the conversion of inorganic phosphorus to organic phosphorus by algal proliferation, the total phosphorus of the culture medium decreased from 5.25 to 0.90 mg/L. Approximately, 82.86% of phosphorus was assimilated for algal growth. The total Fe content decreased from 76.69 to 0.62 mg/L, suggesting that most of the Fe was used in the flocculation process. The pH of effluent was 5.30 after flocculation, lower than the normal pH of most water bodies at 7. To augment the pH of water and buffer the system, calcium hydroxide was added to adjust the pH of the effluent to 7, after which the Fe residues even dropped further to 0.09 mg/L. Since growth of algae could transfer much of the phosphorus and CO₂ into biomass, harvesting the algae could not only prevent endogenous phosphorus pollution, but also promoted atmospheric carbon sequestration.

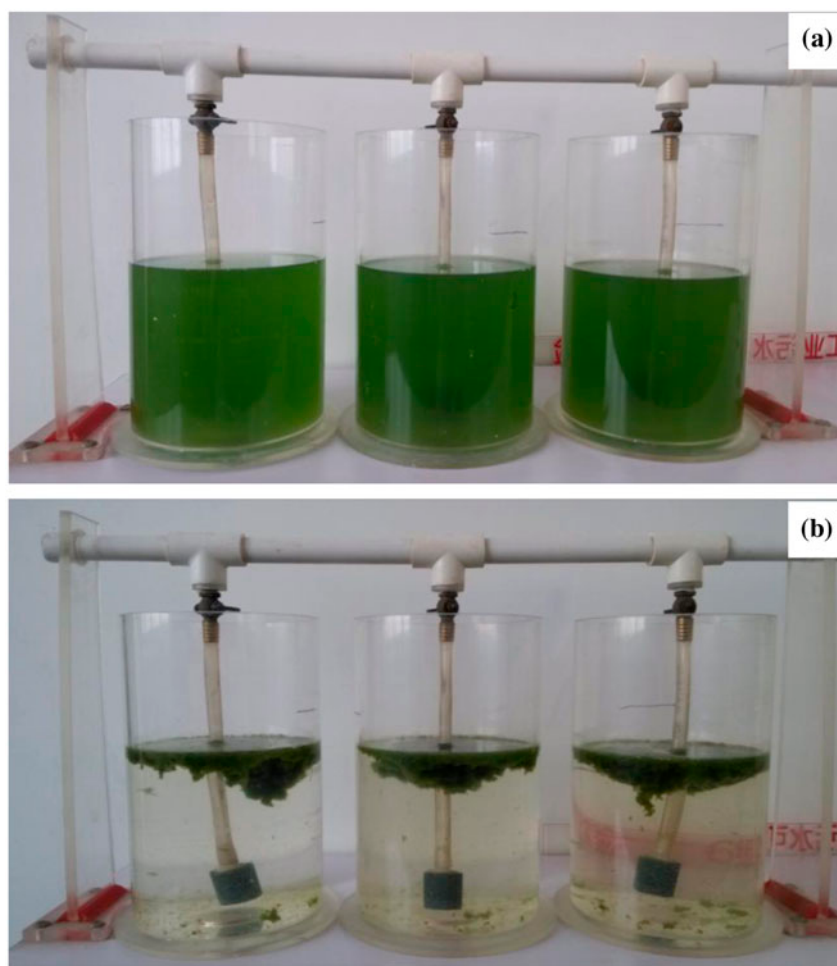


Fig. 5. Flocculation of *M. aeruginosa* in aeration tank control (a) and flocculated algae with MBF and FeCl_3 (b).

3.6. The cost–benefit analysis

The application of this harvesting method depended on treatment cost, including the reagents, preparation of MBF and operational costs, totaling to about $0.2\$/\text{m}^3$ which was able to harvest around 2.50 kg of dry algae. The advantages of this harvesting method include its simplicity, low cost, and easy solid–liquid separation, in addition to its ability to remove endogenous nutrients from the aquatic system.

Harvesting of algae by combining FeCl_3 and MBF was easy and cost-effective, but has not yet been directly used in natural water bodies due to the generated low pH and some Fe residues after treatment. In order to solve this problem, a transit pool was set up to harvest algae. Water containing algal blooms was transferred to the transit pool where it was treated with the combination of FeCl_3 and MBF. Lastly,

calcium hydroxide was added to adjust the pH of water and decrease Fe residues of the effluent.

4. Conclusions

MBF facilitated *M. aeruginosa* flocculation as induced by FeCl_3 by charge neutralization. The aggregate also trapped the oxygen produced during photosynthesis making the flocs more buoyant and convenient for their harvesting and removal. The optimum combination of the flocculants was 0.175 g FeCl_3 and 10 mL MBF per liter of algal culture with the highest flocculation efficiency (95.12%) and the lowest COD (10.44 mg/L). The flocculation experiment showed that the technology would have potential applications for harvesting of algae to prevent from algal blooms if the external phosphorus input was strictly controlled, this technology could also be

extended to harvest microalgal biomass for biofuel production.

Acknowledgments

This research was jointly supported by the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Tianjin Science and Technology Support Program (13ZCZDSF02600) and Tianjin City High School Science & Technology Fund Planning Project (Grant No. 20130513).

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