Sustainable harvesting of microalgae by coupling chitosan flocculation and electro-floatation

Lin Zhu[†], Hui Xu[‡], Weijie Guo[§], Jianghua Yu[†], Wenqing Shi[†,*]

[†]Jiangsu Collaborative Innovation Center of Atmospheric Environment and Equipment Technologies, Jiangsu Key Laboratory of Atmospheric Environmental Monitoring & Pollution Control, School of Environmental Science & Engineering, Nanjing University of Information Science & Technology, Nanjing 210044, China

[‡]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[§]Basin Water Environmental Research Department, Changjiang River Scientific Research Institute, Wuhan 430010, China

[*]Corresponding author: Wenqing Shi (shiwenqing2005320@126.com)

Abstract. Microalgae have been widely used as animal feeds, healthcare products and food additives; however, it still remains a challenge to harvest them safely and effectively from growth medium. In this study, we tested a green method for microalgae harvesting by coupling chitosan flocculation and electro-floatation. Results demonstrated that microalgae can be preliminarily flocculated into unstable flocs by chitosan, and then floated by electro-floatation, during which chitosan was charged by electrolysis and activated to flocculate microalgae, producing a positive priming effect. It is possibly amino groups of chitosan were positively charged by electrolysis, increasing the charge neutralization ability for microalgae flocculation. Thus, the use of stronger current yielded a higher harvesting efficiency at a lower chitosan dosage. At the current intensity of 0.2, 0.4 and 0.6 A, microalgae harvesting efficiency reached a maximum of 33.2%, 59.6% and 63.5%, and the optimal chitosan dosage was 6.0, 4.0 and 2.0 mg L^{-1}, respectively. The use of edible chitosan and inert carbon electrodes makes it possible to harvest microalgae biomass safely for food or healthcare use and achieve sustainable utilization of culture medium, which will be beneficial to microalgae-based engineering.

Keywords: microalgae harvesting, chitosan flocculation, electro-floatation, sustainable utilization, microalgae-based engineering

Introduction

In recent years, microalgae have been widely used as animal feeds, healthcare products and food additives.[1,2] Microalgae are more nutritious than traditional animal and aquatic feed like millet, grams and other small fishes in terms of protein, omega 3 fatty acids and carotenoids content.[3,4] Several kinds of antioxidant compounds have been extracted from microalgae to protect against oxidative stress, a cause of many diseases and aging.[1,5] However, it still remains a challenge to find an effective and especially safe method for microalgae harvesting.

Many physical and chemical methods have been tested to harvest microalgae, including sedimentation, centrifugation, filtration, chemical flocculation and electro-flocculation. Sedimentation is simple but time-consuming and only suitable for harvesting large-size microalgae, since most of microalgae have similar density to water, negative surface charge, and thereby low settling velocities.[6] Centrifugation and filtration are rapid but less cost-effective, limiting their applications at large scale.[7,8] Mata *et al*. (2010) considered that filtration consumes large amounts of membranes and pumping energy, especially for harvesting unicellular small microalgae cells.[8] Chemical flocculation and electro-flocculation are rapid and effective, but residual chemical flocculants in the harvested biomass pose potential health risks and limit their utilizations as food and healthcare products.[9] So far, there are few safe and effective technologies for microalgae harvesting.

Chitosan is the second-most abundant natural biopolymer, which is mainly derived from the shells of shrimp and other crustaceans.[10,11] It has received widespread applications in food and pharmaceutical industries due to its biological compatibility, biodegradability, and nontoxic

properties.[12,13] Chitosan has positively charged amine groups and thereby exhibits flocculation potentials for negatively charged microalgae.[14] Pan *et al.* (2011) used chitosan to flocculate microalgae cells and then sink them with the aid of soil particles, achieving effective harmful microalgae removal in Lake Taihu, China.[15] Electro-floatation is a separation process in which solids are separated from liquid by micro-bubbles produced from electrode surfaces.[16] It has been widely used to remove floatable materials including activated sludge, oil, surfactants, and flocculants etc.[17,18] The use of non-sacrificial electrodes in electro-floatation will not introduce chemical flocculants.[19,20] Hence, if microalgae are preliminarily flocculated into unstable flocs by chitosan, and then floated by electro-floatation using non-sacrificial electrodes, it is possibly to harvest microalgae biomass safely and effectively.

In this study, we proposed a new method for edible microalgae harvesting by coupling chitosan flocculation and electro-floatation using carbon electrodes, and tested it at different operation conditions. The surface charge of microalgae cells was measured to explore the underlying mechanisms. The responses of growth medium to microalgae harvesting were also investigated to evaluate the feasibility of sustainable utilization of culture medium. The objective of this study is to develop a green method for the harvesting of edible microalgae.

Materials and methods

Microalgae species and culture

In this study, *Chlorella vulgaris* (*C. vulgaris*), an edible green microalgae species, was chosen to test the new harvesting method.[21,22] Here, *C. vulgaris* cells (FACHB-24) were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, and cultured in the BG11

77 medium, which consists of 500 mg L^{-1} Bicin, 100 mg L^{-1} KNO_3, 100 mg L^{-1} b-$C_3H_7O_6PNa_2$,
78 50 mg L^{-1} $NaNO_3$, 50 mg L^{-1} $Ca(NO_3)_2$•$4H_2O$, 50 mg L^{-1} $MgCl_2$•$6H_2O$, 40 mg L^{-1} Na_2SO_4, 20
79 mg L^{-1} H_3BO_3, 5 mg L^{-1} Na_2EDTA, 5 mg L^{-1} $MnCl_2$•$4H_2O$, 5 mg L^{-1} $CoCl_2$•$6H_2O$ and 0.8 mg
80 L^{-1} Na_2MoO_4•$2H_2O$, 0.5 mg L^{-1} $FeCl_3$•$6H_2O$ and 0.5 mg L^{-1} $ZnCl_2$. The batch cultures were
81 conducted in an illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co., Ltd.,
82 China) with continuous cool white fluorescent light of 2500 ± 500 lux on a 12 h light and 12 h
83 darkness regime at the temperature of 30 ± 1°C.

84 **Microalgae harvesting system**

85 The microalgae harvesting system consists of a flat stir paddle (Zhongrun Water Industry
86 Technology Development Co., Ltd., China) for mixing during chitosan flocculation and two
87 round carbon electrode plates (Jinjia Metal Co., Ltd., China) for electro-floatation (Fig. 1). The
88 carbon electrode plate has a surface area of 55.4 cm^2 and a thickness of 0.2 cm, which was
89 horizontally installed at the bottom with a gap of 2 cm between the two plates. There are 85
90 small round holes on each carbon electrode plate to allow gas bubbles freely pass it during
91 electrolysis, such that the effective surface area was 38.7 cm^2. The electric current was supplied
92 by a direct current power supply (DF1730SL5A, Ningbo Zhongce Dftek Electronics Co., Ltd.,
93 China).

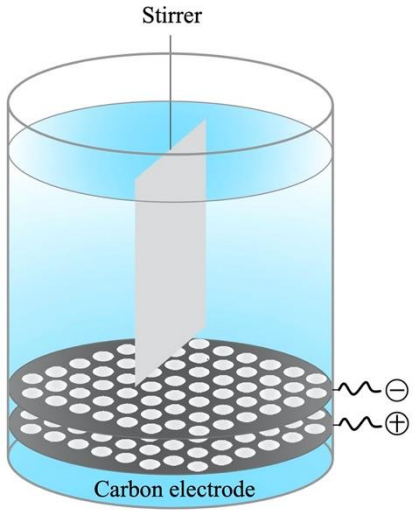

Fig. 1. The schematic diagram of microalgae harvesting system.

Microalgae harvesting test

C. vulgaris culture at the exponential growth phase was used in the microalgae harvesting test. The initial cell concentration was set to 3.63×10^{10} cells L^{-1}. 0.5 L of readily prepared *C. vulgaris* solution was transferred to the harvesting cell. Water-soluble chitosan was purchased from Qingdao Yunzhou Bioengineering Co. Ltd., China. Prior to the test, a chitosan stock solution (2 g L^{-1}) was prepared as follows: 1 g chitosan was added to 0.5 L distilled water and completely diluted by stirring. After chitosan was added, the microalgae solution was stirred at 200 rpm for 2 min and 40 rpm for another 10 min; electro-floatation was started in the last 5 min during chitosan flocculation. The microalgae solution was allowed to stand for 10 min, and then water samples were carefully collected from an outlet 2 cm above the carbon electrode plate to enumerate the cell number using an Axioskop 2 mot plus microscope (Carl ZEISS, Germany). The microalgae harvesting efficiency was calculated as (initial cell concentration-sample cell concentration)/initial cell concentration × 100%. In the test, the chitosan dosage

was set to 0, 2, 4, 6, 8, 10, 12 and 15 mg L^{-1}, and the current density was set to 0, 0.2, 0.4 and 0.6 A. All the tests were conducted in triplicate at the raw microalgae solution pH of 8.6.

The surface charge of microalgae cells was characterized using a Zetasizer 2000 (Malvern Co. United Kingdom). To study the responses of culture medium to microalgae harvesting, medium nutrients (phosphate, ammonium and nitrate) were measured according to Chinese Monitoring Analysis Method of Water and Wastewater;[23] medium pH and temperature were measured using a Yellow Springs Instruments (Yellow Springs, Ohio, USA) before and after microalgae harvesting.

Cost evaluation

The cost of microalgae harvesting was estimated by summing flocculants and energy costs per unit of harvested microalgae biomass as follows:

$$Cost = (Cost_{floccluation} + Cost_{eletrofloatation})/W_{biomass} \quad (1)$$

$$Cost_{floccluation} = Cost_{chitosan} + Cost_{mixting\ energy} \quad (2)$$

$$Cost_{eletrofloatation} = Cost_{eletrolysis\ energy} \quad (3)$$

$$Cost_{chitosan} = C * v * a \quad (4)$$

$$Cost_{mixing\ energy} = P * T * b \quad (5)$$

$$Cost_{eletrolysis\ energy} = U * I * t * b \quad (6)$$

$$W_{biomass} = v * \beta * \theta * \sigma \quad (7)$$

where C is the chitosan dosage, mg L^{-1}; v is the volume of microalgae solution, L; a is the chitosan price, which is 0.03 USD g^{-1} ; P is the stirrer power, which is 40 W; T is the stirring time, h; b is the electric power price, which is 0.08 USD $(kWh)^{-1}$; U is the electrolysis voltage,

V; I is the current intensity, A; t is the electrolysis time, h; β is the initial microalgae concentration, cell L^{-1}; θ is the microalgae harvesting efficiency, %; σ is the weight per microalgae cell, 32×10^{-12} g $cell^{-1}$.

Data analysis

One-way analysis of variance (ANOVA) was employed to test the statistical significance of differences between treatments. Post-hoc multiple comparisons of treatment means were performed using the Tukey's least significant difference procedure. All statistical calculations were performed using the SPSS (v22.0) statistical package for personal computers. The level of significance was $P < 0.05$ for all tests.

Results

Surface charge properties of chitosan and *C. vulgaris* cells

The isoelectric point of chitosan was pH 9.7, making it possess net positive charges under most microalgae culture conditions. The zeta potential of chitosan kept above +1.5 mV in the wide pH range of < 9.0, and then decreased to nearly zero at pH 9.7. In contrast, the zeta potential of *C. vulgaris* cells kept below zero in the pH range of > 2.5 (Fig. 2).

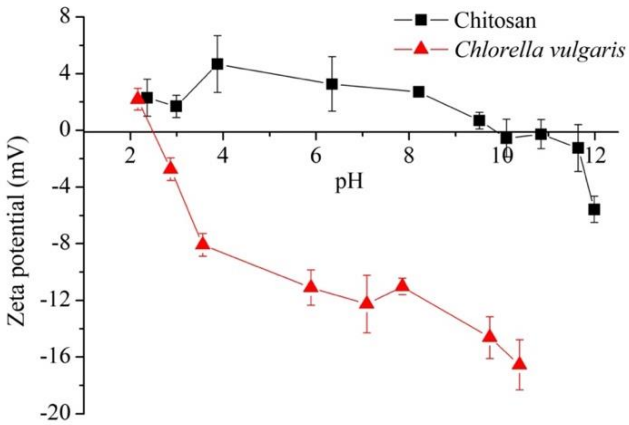

Fig. 2. The surface charge properties of chitosan and *C. vulgaris* cells. Error bars indicate standard deviations.

Microalgae harvesting efficiency

The use of chitosan flocculation alone only achieved limited microalgae harvesting no matter what the chitosan dosage was. The microalgae harvesting efficiency reached 16.9% at the chitosan dosage of 2 mg L^{-1} and maintained stably at this value as the chitosan dosage further increased. Similarly, the use of electro-floatation alone could not effectively harvest microalgae either and the harvesting efficiency was only 4.5%, 13.4% and 22.9% at the current intensity of 0.2, 0.4 and 0.6 A, respectively. When chitosan flocculation and electro-floatation were used together, the microalgae harvesting was greatly improved by increasing harvesting efficiency and simultaneously decreasing the optimal chitosan dosage, and this effect was enhanced as the current density increased. When the current intensity of 0.2, 0.4 and 0.6 A was applied, microalgae harvesting efficiency reached a maximum of 33.2%, 59.6% and 63.5%, and the optimal chitosan dosage was 6.0, 4.0 and 2.0 mg L^{-1}, respectively. However, a remarkable decrease in microalgae harvesting efficiency was observed as chitosan was overdosed at the current intensity of 0.4 and 0.6 A, and the harvesting efficiency decreased to 35.2% at the chitosan dosage of 15 mg L^{-1} (Fig. 3).

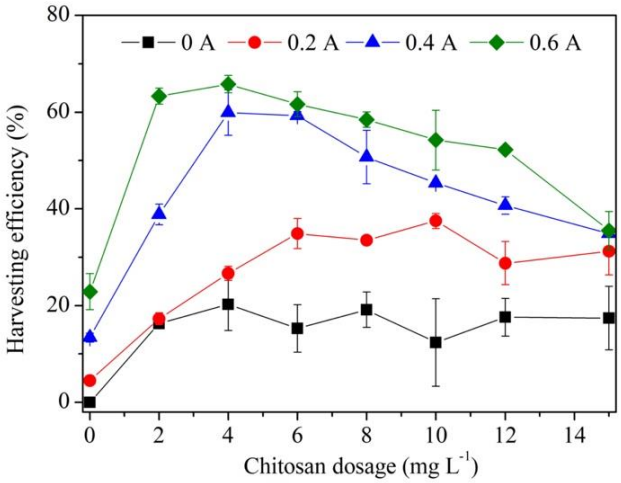

Fig. 3. Effects of chitosan dosage and current density on microalgae harvesting efficiency. Error bars indicate standard deviations.

Microalgae surface charge

After chitosan was added, the zeta potential of microalgae cells showed a remarkable increase. The zeta potential of microalgae cells was gradually increased from –9.2 to –0.9 mV as the chitosan dosage increased from 0 to 15 mg L^{-1}. The use of electro-floatation further increased the zeta potential of microalgae cells, indicating a charging effect, and this effect was enhanced as the current intensity increased (Fig. 4A). When the current density of 0.2, 0.4 and 0.6 A was applied, the zeta potential of microalgae cells was finally increased to +7.3, +8.4 and +10.6 mV at the chitosan dosage of 15 mg L^{-1}, respectively. The charging effect was further evaluated by subtracting the zeta potential of microalgae cells in presence of electro-floatation from the value in the absence of electro-floatation. It exhibited chitosan limitation at low chitosan dosages and current limitation at high chitosan dosages (Fig. 4B). As the chitosan dosage increased, the increase amplitude of zeta potential gradually increased and then reached an equilibrium; the use of higher intensity currents yielded a higher equilibrium value at a lower

chitosan dosage. At the current intensity of 0.2, 0.4 and 0.6 A, the increase amplitude of zeta potential of microalgae cells reached a maximum of 9.3, 11.0 and 11.5 mV, and the optimal chitosan dosage was 2, 4 and 8 mg L^{-1}, respectively. In contrast, in the absence of chitosan, there were no significant differences in zeta potential of microalgae cells among different intensity currents ($P < 0.05$), and the zeta potential values kept stably at –9.4 mV at the chitosan dosage of 0 mg L^{-1} (Fig. 4A).

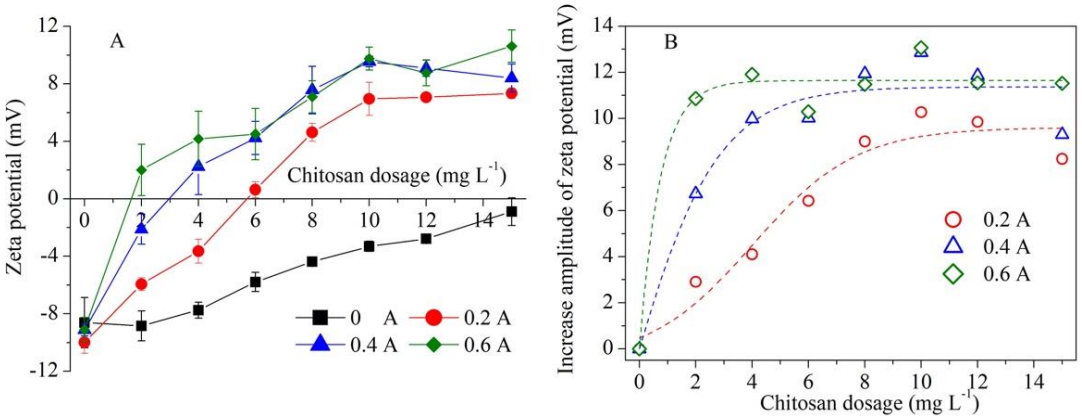

Fig. 4. Changes of microalgae cell surface change during microalgae harvesting. (A) Zeta potential of microalgae cells; (B) The charging effect of electro-floatation. Error bars indicate standard deviations.

Microalgae culture medium

Compared with chitosan flocculation alone, ammonium in culture medium was significantly increased after electro-floatation was introduced ($P < 0.05$), and the use of higher intensity currents yielded higher ammonium concentrations. After microalgae harvesting, ammonium concentration maintained stably below 0.2 mg L^{-1} in chitosan flocculation alone, and was increased to 1.0, 1.6 and 2.2 mg L^{-1} when electro-floatation was applied at the current intensity of 0.2, 0.4 and 0.6 A, respectively (Fig. 5A). In contrast, there were no significant changes in

other parameters observed after microalgae harvesting ($P > 0.05$). In all the tests, medium nitrate and phosphate maintained stably at 250.9 and 4.66 mg L^{-1} (Fig. 5B); medium pH, conductivity, and temperature maintained stably at 7.0, 2.4 mS cm^{-1} and 19.6°C, respectively (Table S2).

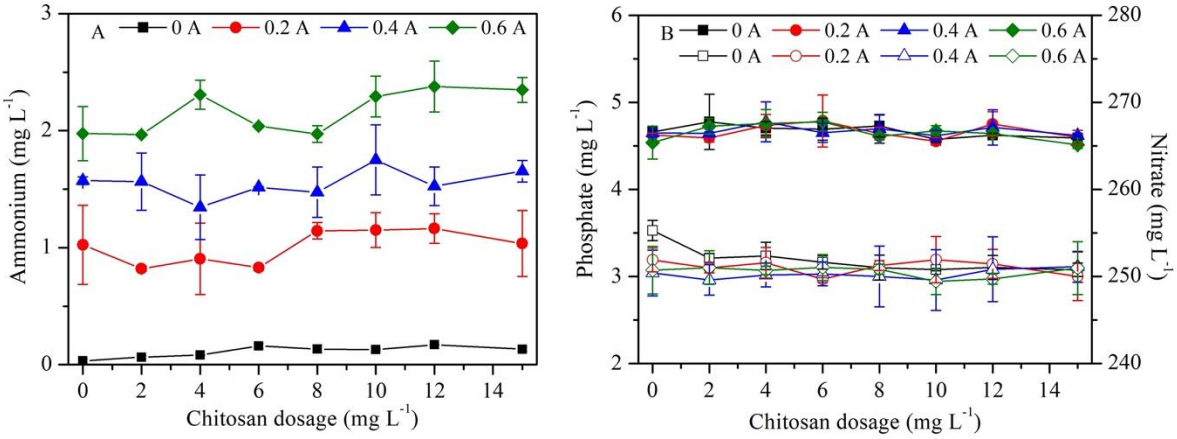

Fig. 5. Changes of nutrients in culture medium after microalgae harvesting. (A) Ammonium; (B) Phosphate and nitrate. Error bars indicate standard deviations.

Microalgae harvesting cost

Compared with chitosan flocculation (0.08×10^{-3} USD g^{-1} biomass), it costed much more to harvest microalgae using electro-floatation, reaching 2.83×10^{-3}, 2.76×10^{-3} and 3.30×10^{-3} USD g^{-1} biomass at the 0.2, 0.4 and 0.6 A, respectively. When chitosan flocculation and electro-floatation were used together, the cost could be greatly reduced, but exhibited a potential increase as the current intensity increased. The cost reached 0.41×10^{-3}, 0.64×10^{-3} and 1.18×10^{-3} USD g^{-1} biomass at 0.2, 0.4 and 0.6 A, respectively.

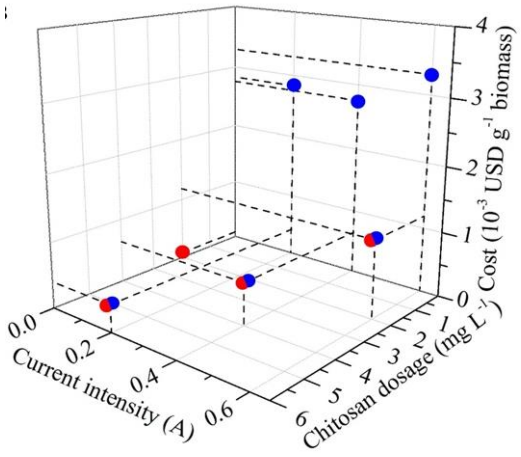

Fig. 6. The cost evaluation for microalgae harvesting using the coupled chitosan flocculation and electro-flocculation. The single red and blue circles indicated chitosan flocculation and electro-floatation were used alone, respectively. The combined red and blue circles indicated that chitosan flocculation and electro-floatation were used together.

Discussion

The synergistic effect of chitosan flocculation and electro-floatation

Microalgae particles have negative surface charge and often stably suspend in the solution with electrostatic repulsion.[24,25] Charge neutralization is an essential step in microalgae flocculation, which eliminates energy barrier for microalgae aggregation.[26-28] Chitosan is positively charged over a wide range of pH < 9.7, which made it obtain flocculation potential for negatively charged microalgae (Fig. 2). However, most of the formed microalgae flocs still suspended with the aid of buoyancy. The zeta potential of microalgae cells showed a remarkable increase as the chitosan dosage increase (Fig. 4). As a result, the use of chitosan flocculation alone yielded limited microalgae harvesting efficiency whatever the chitosan dosage was (Fig. 3). After electro-floatation was introduced, large amounts of tiny gas bubbles were produced,

carrying the flocs to water surface where they can be easily collected (Fig. S1). Microalgae harvesting efficiency were therefore remarkably increased when chitosan flocculation and electro-floatation were used together (Fig. 3). However, the use of electro-floatation alone could not effectively harvest microalgae either. This is because microalgae cells stably suspend with electrostatic repulsion, and it is difficult to float them by bubbles without preliminary flocculation.[29]

The positive priming effect of electro-floatation on chitosan flocculation

Chitosan was appointed to neutralize microalgae surface charge for microalgae flocculation (Fig.2 and Fig. 4). Surprisingly, after electro-floatation was introduced, the zeta potential of microalgae cells differed at the same chitosan dosage, depending on current intensity applied. The zeta potential of microalgae cells generally exhibited an increase as the current intensity increased, but this effect disappeared in the absence of chitosan (Fig. 4A). It indicated that electrolysis charged chitosan and increased its charge neutralization ability for microalgae flocculation, producing a positive priming effect. This explained that the use of higher current density yielded higher microalgae harvesting efficiency at a lower optimal chitosan dosage. There are a lot of amino groups on the chain of chitosan, which were possibly charged by electrolysis during electro-floatation.[30] At the low chitosan dosage, the receptor capacity was limited and the charging effect of electrolysis exhibited a chitosan limitation. As the chitosan dosage increased, chitosan limitation was transferred to current limitation (Fig. 4B), and the stronger current has a higher charging effect. However, excess loads of positive charges can cause microalgae cells positively charged and re-establish electrostatic repulsion.[31] Thus, a

247 remarkable decrease in microalgae harvesting efficiency was observed as chitosan was
248 overdosed at the high current intensity (0.4 and 0.6 A, Fig. 3).

249 **Recommendations for future applications**

250 In the microalgae-based engineering, culture medium reuse can offer a promising strategy for
251 saving water and nutrients.[32,33] Because of the electrolysis, the physico-chemical properties of
252 culture medium may change after microalgae harvesting.[34,35] For instance, water temperature
253 may increase as waste heat releases. However, there were no significant changes in medium
254 pH ($P > 0.05$), temperature ($P > 0.05$) and conductivity ($P > 0.05$) after microalgae harvesting
255 in this study (Table S1), which is possibly due to weak electrolysis (low current intensity and
256 short electrolysis time). As for main nutrients, there was a significant increase in ammonium
257 ($P < 0.05$) (Fig. 5A). We attributed it to the transformation from nitrate to ammonium under
258 electrolysis according to our additional experiments (Fig. S2). During electrolysis, nitrate
259 reduction ($NO_3^- + 10H^+ + 8e^- = NH_4^+ + 3H_2O$) can occur at the cathode, releasing ammonium
260 to the medium.[36,37] Thus, the stronger current had a higher ammonium yield (Fig.5A and Fig.
261 S2). However, we did not detect the significant decrease in nitrate ($P > 0.05$, Fig. 5B) because
262 of the extremely high levels of nitrate in the BG11 medium (> 250 mg L^{-1}). The shift of nitrate
263 to ammonium can increase nitrogen bioavailability since ammonium is generally favored by
264 microalgae relative to nitrate.[38-40] In contrast, phosphate did not show significant changes after
265 microalgae harvesting ($P > 0.05$, Fig. 5B) due to the use of non-sacrificial electrodes, which
266 differs with the decrease of phosphate concentration by electrolysis using Fe/Al electrodes.[41,42]

Hence, nutrient bioavailability increase and other parameter stability (Fig. 5B, Table S2) make medium reusable for microalgae continuous culture.

In practical applications, cost is often another major concern for a technology. For the microalgae harvesting technology in this study, there was a trade-off between the harvesting efficiency and the cost, since the cost exhibited a potential increase as the current intensity increased (Fig. 6). It will be cost-effective to apply the low current intensity to harvest microalgae in the continuous system, and the remaining cells can benefit microalgae recovery. Despite the microalgae harvesting efficiency was greatly increased by coupling chitosan flocculation and electro-floatation (Fig. 3), it may be further increased by optimizing operation condition or screening other edible flocculants, such as *moringa oleifera* and tannin.[43,44]

Conclusions

Microalgae harvesting is a crucial step but still remains a challenge for microalgae-based engineering. This study proposed a green way to harvest microalgae by coupling chitosan flocculation and electro-floatation. Microalgae can be preliminarily flocculated into unstable flocs by chitosan, and then floated by electro-floatation; electro-floatation charged chitosan and activated it to flocculate microalgae, producing a positive priming effect. The use of edible chitosan and inert carbon electrodes makes it possible to harvest microalgae biomass safely and effectively for food or healthcare use and achieve the sustainable utilization of culture medium. Further studies are needed to optimize operation conditions to increase harvesting efficiency and reduce the cost.

Acknowledgments

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Supporting Information

The Supporting Information is available free of charge on the web.

The microalgae floc image; the effects of electrolysis using carbon electrodes on BG11 medium; changes of physical properties of growth medium after microalgae harvesting.

Notes

The authors declare no competing financial interest.

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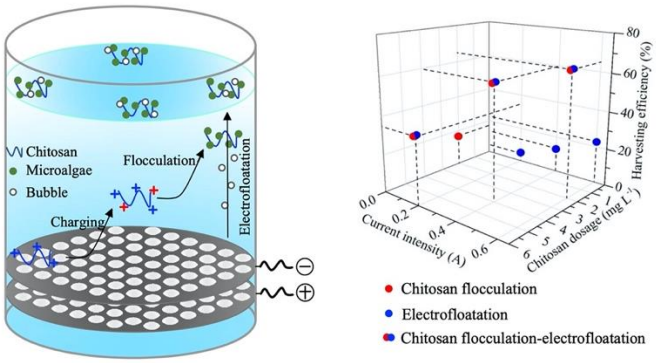

Synopsis: A green method for harvesting microalgae biomass will be beneficial to microalgae-based engineering for healthcare and food use.