Enhanced chitosan flocculation for microalgae harvesting using electrolysis

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Abstract

Charge neutralization is a key operating mechanism for chitosan flocculation in microalgae harvesting. Alkaline conditions have been conventionally used to modify chitosan to create an increase in charge neutralization. However, it is often difficult to operate the chemical processes needed for this method, which also pose environmental risks. In this study, a facile and environmentally safe method to increase chitosan charge neutralization using electrolysis was proposed and tested in the harvest of microalgae. The results demonstrated that the electrolysis produced a charging effect on the chitosan and exhibited a significant positive relationship with current intensity (r^2 = 0.91, P < 0.05). When the electrolysis was operated at 0.2, 0.4, and 0.6 A, the charge neutralization of chitosan increased by 2.05, 4.99, and 10.86 mV/mg, respectively. As the charge neutralization increased, chitosan flocculation yielded a higher microalgae harvesting efficiency at a lower chitosan dosage. One of the possible mechanisms[1] is the deacetylation of the acetyl groups caused by electrolysis. This idea needs further study that includes identifying a chitosan structure change. This study proposed a novel strategy for modifying chitosan and its derivatives for increased flocculation in microalgae-based engineering.

Keywords: chitosan, microalgae, electrolysis, charge neutralization.

1. Introduction

Chitosan, the second-most abundant natural biopolymer, is mainly derived from the shells of shrimp and other crustaceans [1]. The free amino groups on the chitosan chain backbone lead to a positive surface charge and exhibit the potential for flocculation [2]. Chitosan has been widely used as an environmentally friendly flocculant for microalgae harvesting where charge neutralization is the key mechanism behind chitosan flocculation [3, 4]. Since chitosan has a weak surface charge, more chitosan can often be dosed [2]or other strong flocculants can be added [3]to achieve effective flocculation. For instance, polyaluminum chloride and *Moringa oleifera* have been used to assist chitosan flocculation for the removal of microalgae [5, 6]. The charge neutralization of chitosan is mainly controlled by the degree of deacetylation, which can be increased by a series of chemical reactions under alkaline conditions [7, 8]. However, it is often difficult to operate the chemical processes needed for this method, which also pose environmental risks due to the use of alkali conditions. Therefore, there is a need for a facile and environmentally safe method to increase the chitosan charge neutralization for microalgae harvesting.

In this study, a new method using electrolysis for the enhancement of chitosan flocculation in microalgae harvesting was reported. The use of non-sacrificial electrodes do not introduce added chemicals, and the process can be easily controlled by an electrical switch. The associated impacts of electrolysis on the medium were also explored to guide future practical applications.

2. Materials and methods

2.1 Chitosan modification using electrolysis

Chitosan was purchased from Qingdao Yunzhou Bioengineering Co. Ltd., China[4]. A non-sacrificial carbon electrode was used during electrolysis, which was purchased from Jinjia Metal Co., Ltd., China.[5] In this study, a potential was applied [6]during chitosan flocculation because tiny gas bubbles produced during the electrolysis process favor floc separation and allow for the precise evaluation of chitosan flocculation [9]. Detailed information about the electrolysis process is presented below.

2.2 Microalgae species and culture

In this study, *Chlorella vulgaris* (*C. vulgaris*) was chosen to test chitosan flocculation. *C. vulgaris* cells (FACHB-24) were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences,[7] and were cultured in the BG11 medium, which consisted of 500 mg/L Bicin, 100 mg/L KNO_3, 100 mg/L b-$C_3H_7O_6PNa_2$, 50 mg/L $NaNO_3$, 50 mg/L $Ca(NO_3)_2$•$4H_2O$, 50 mg/L $MgCl_2$•$6H_2O$, 40 mg/L Na_2SO_4, 20 mg/L H_3BO_3, 5 mg/L Na_2EDTA, 5 mg/L $MnCl_2$•$4H_2O$, 5 mg/L $CoCl_2$•$6H_2O$, 0.8 mg/L Na_2MoO_4•$2H_2O$, 0.5 mg/L $FeCl_3$•$6H_2O$, and 0.5 mg/L $ZnCl_2$. The batch cultures were conducted in an illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co., Ltd., China[8]) with continuous cool, white, fluorescent light of 2500 ± 500 lux. The light cycle consisted of 12 h on and 12 h off at a temperature of 30 ± 1 °C.

2.3 Chitosan flocculation test

The flocculation system consisted of a flat stirring paddle (Zhongrun Water Industry Technology Development Co., Ltd., China[9]) for mixing and two round carbon electrode plates for the electrolysis. The carbon electrode plates had an effective surface area of 38.7 cm^2 and a thickness of 0.2 cm, which were horizontally installed with a gap of 2 cm between the two plates. [10]The electric current was supplied by a direct current power supply (DF1730SL5A, Ningbo Zhongce Dftek Electronics Co., Ltd., China[11]). The schematic diagram of the flocculation system is presented in Fig. S1.

C. vulgaris was collected during the exponential growth phase was used in the flocculation test. The initial cell concentration was set to 3.63×10^{10} cells/L, and 0.5 L of readily prepared *C. vulgaris* solution was transferred to the flocculation cell. A chitosan stock solution (2 g/L) was prepared as follows: 1 g chitosan was added to 0.5 L distilled water and stirred. After chitosan was added, the microalgae solution was stirred at 200 rpm for 2 min and at 40 rpm for another 10 min, during which the electrolysis was performed. The chitosan dosages were set to 0 mg/L, 2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L, 10 mg/L, 12 mg/L, and 15 mg/L, and the current[12] was set to 0.0 A, 0.2 A, 0.4 A, and 0.6 A. After flocculation, the microalgae solution stood for 10 min, and then water samples were carefully collected from an outlet placed 2 cm above the carbon electrode plate to calculate the cell number using an Axioskop 2 MOT Plus microscope (Carl ZEISS, Germany[13]). All the tests were conducted in triplicate using a raw microalgae solution pH of 8.6.

2.4 Data collection and analysis

The increases in microalgae surface charge and flocculation efficiency were calculated to evaluate the charging effect of the electrolysis process on chitosan. The surface charge of microalgae cells was characterized using a Zetasizer 2000 (Malvern Co. United Kingdom[14]). The chitosan flocculation efficiency for microalgae was calculated as (initial cell concentration - sample cell concentration) / initial cell concentration × 100 %. Medium nutrients (phosphate, ammonium, and nitrate) were measured according to the Chinese Monitoring Analysis Method of Water and Wastewater [10]. The medium's pH and temperature were measured using Yellow Springs Instruments [15](Yellow Springs, Ohio, USA[16]) before and after chitosan flocculation.

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 Tukey's least significant difference procedure. All statistical calculations were performed using the SPSS (v22.0) statistical package [17]for personal computers. The level of significance was $P < 0.05$ for all tests.

3. Results and discussion

Microalgae have been widely used as animal feed, healthcare products, and food additives due to their rich lipids, proteins, and many functional components [11, 12]. The challenge is finding a safe and cost-effective method to harvest microalgae. Chitosan is considered an environmentally-friendly microalgae flocculant due to the biodegradability and nontoxic properties [2]. Charge neutralization is an essential mechanism for operating chitosan flocculation because it eliminates the energy barrier for floc aggregation [3, 13]. However,

chitosan often has a weak surface charge (< +6.0 mV, Fig. S2) and more often needs to be dosed[18] to achieve effective flocculation. When chitosan was used alone for microalgae flocculation, the zeta potential of microalgae cells slowly increased from –9.2 to –0.9 mV as the chitosan dosage increased from 0 to 15 mg/L (Fig. 1A). After electrolysis was performed, the increase of the microalgae cell surface charge accelerated and this effect was further enhanced as the current intensity was increased. When the currents of 0.2, 0.4, and 0.6 A were applied, the zeta potential of the microalgae cells increased to +7.3, +8.4 and +10.6 mV, respectively, at a chitosan dosage of 15 mg/L. However, this effect disappeared in the absence of chitosan, and the zeta potential value remained stable at –9.4 mV at a chitosan dosage of 0 mg L^{-1} (Fig. 1A). This indicated that the electrolysis produced a "charging" effect on chitosan and increased its charge neutralization for flocculation. The charging effect of electrolysis on chitosan was significantly and positively correlated with current intensity ($P < 0.05$), and the charge neutralization of chitosan was increased by 2.05, 4.99 and 10.86 mV/mg at 0.2, 0.4 and 0.6 A, respectively (Fig. 1B). As the chitosan charge neutralization increased, microalgae harvesting improved, and the use of higher current intensity yielded a higher harvesting efficiency at a lower chitosan dosage. When electrolysis was performed at the 0.2, 0.4 and 0.6 A, the microalgae harvesting efficiency reached 33.2%, 59.6%, and 63.5% at chitosan dosages of 6.0, 4.0, and 2.0 mg/L, respectively. In contrast, the addition of chitosan without electrolysis only achieved a microalgae harvesting efficiency of 16.9% (Fig. 2).

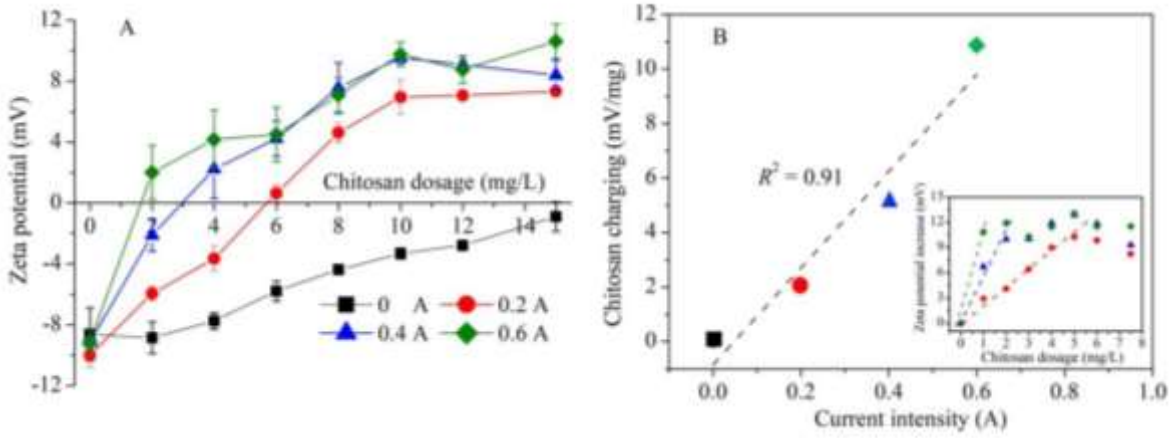

Fig. 1. The effect of electrolysis on the zeta potential of (A) microalgae and (B) chitosan as a function of chitosan dosage and current intensity (data shown is mean ± SD, n = 3).

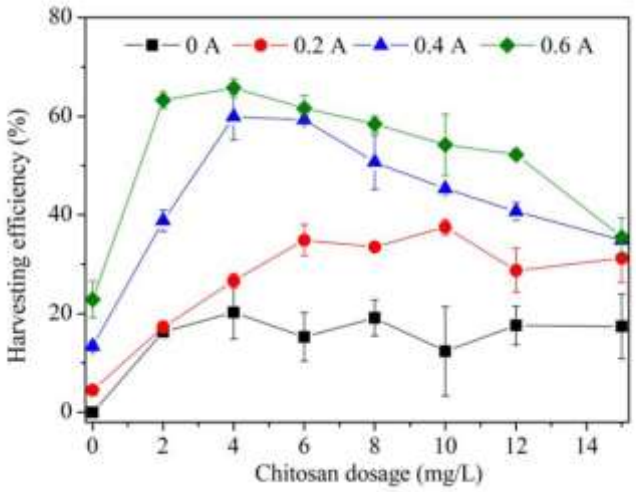

Fig. 2. The microalgae harvesting efficiency using chitosan (data shown is mean ± SD, n = 3).

The effect of electrolysis on the medium's properties was also evaluated in this study. No significant changes in the pH of the medium (P > 0.05), nor the temperature (P > 0.05) or conductivity (P > 0.05) were detected, and the properties remained stable at 7.0, 2.4 mS/cm and 19.6°C, respectively (Table S1). The insignificant variations of the medium's physical properties were possibly due to the low current intensity applied in this work. As for the

medium's nutrients, the phosphate concentration did not show significant changes ($P > 0.05$) and remained in the range of 4.57 to 4.90 mg/L across the tested chitosan and current values (Table S1). The latter observation was most likely the result of using non-sacrificial electrodes because sacrificial electrodes typically reduce phosphate concentration as reported previously [14]. However, there was a significant increase in ammonium[19] concentration ($P < 0.05$), which was further enhanced by the higher current values. The ammonium concentration remained stable below 0.2 mg/L with chitosan flocculation alone and increased to 1.0 mg/L, 1.6 mg/L, and 2.2 mg/L when electrolysis was performed at the currents of 0.2 A, 0.4 A, and 0.6 A, respectively (Fig. 3). This ammonium increase was attributed to the transformation from nitrate to ammonium according to our additional experiments[20] (Fig. S3). During electrolysis, nitrate reduction (nitrate + $10H^+ + 8e^-$ = ammonium + $3H_2O$) [21]occurred at the cathode, producing ammonium [15]. The shift of nitrate to ammonium may be beneficial to medium reuse in microalgae-based engineering because ammonium is generally preferred by microalgae relative to nitrate [16], but may hinder microbial nitrogen removal in wastewater treatment since ammonium should be re-transformed to nitrate by nitrification [17].

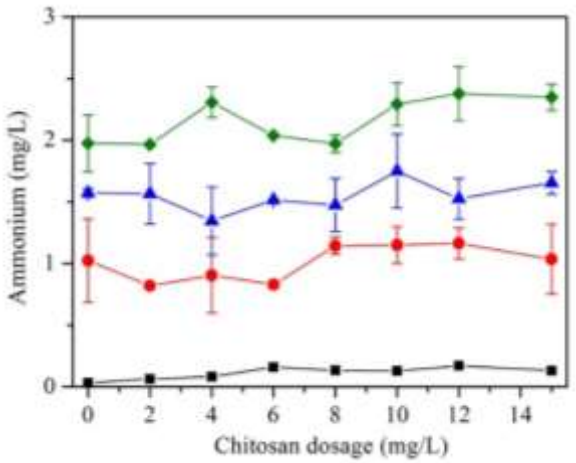

Fig. 3. The effect of electrolysis on the medium's ammonium concentration during chitosan flocculation (data shown is mean ± SD, n = 3).

4. Conclusions

This study proposed a new method for increasing chitosan charge neutralization for microalgae harvesting using electrolysis. The results demonstrated that electrolysis produced an effective "charging" effect on chitosan, exhibiting a significantly positive relationship with current intensity. As the chitosan charge neutralization increased, microalgae harvesting was also improved, i.e. an increase of the harvesting efficiency and a simultaneous decrease in chitosan dosage. Therefore electrolysis proved to be a simple and efficient way to enhance chitosan flocculation for microalgae harvesting. The process was easily controlled by an electrical switch, and the use of non-sacrificial electrodes did not introduce additional chemicals, making it possible to harvest microalgae biomass safely and effectively for food use [18]. One of the possible mechanisms investigated for the chitosan charge neutralization was the degradation of chitosan acetyl groups caused by the electrolysis [19, 20]. Further studies are needed to identify the chitosan structure change using other techniques, such as infrared spectroscopy [21], and for the evaluation of the cost. Moreover, it is worthy to note the associated impacts of electrolysis on the medium's nutrients in practical applications.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights apply to this study.

CRediT Author Contributions Statement

Lin Zhu: Investigation, Writing - original draft. **Gang Pan:** Writing - review & editing. **Hui Xu:** Methodology, Writing - original draft. **Weijie Guo:** Methodology, Writing - review & editing. **Jianghua Yu:** Conceptualization, Writing - review & editing. **Wenqing Shi:** Conceptualization, Writing - review & editing, Supervision.

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