



Biodegradable branched cationic starch with high C/N ratio for *Chlorella vulgaris* cells concentration: Regulating microalgae flocculation performance by pH

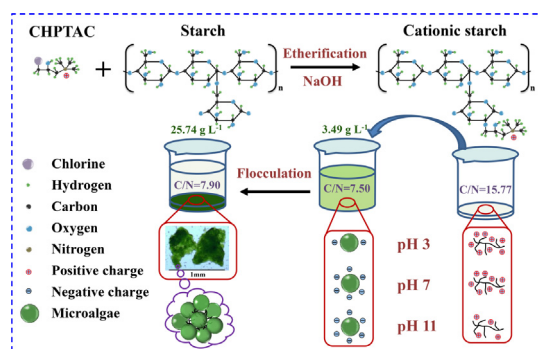
Yun Huang^{a,b}, Chaoyang Wei^{a,b}, Qiang Liao^{a,b,*}, Ao Xia^{a,b}, Xun Zhu^{a,b}, Xianqing Zhu^{a,b}

^a Key Laboratory of Low-grade Energy Utilization Technologies and Systems, Chongqing University, Ministry of Education, Chongqing 400044, China

^b Institute of Engineering Thermophysics, School of Energy and Power Engineering, Chongqing University, Chongqing 400044, China



GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Microalgae harvesting
Cationic starch
Flocculation
pH
Zeta potential

ABSTRACT

To improve the carbon to nitrogen (C/N) ratio of harvested microalgae biomass for better producing biogas by fermentation, biodegradable cationic starch with high C/N ratio were synthesized to harvest *Chlorella vulgaris*. The impact of pH was also studied as the zeta potential of both microalgae and cationic starch would change with pH. Results indicated the cationic starch can harvest above 99% of the microalgae and the C/N ratio can rise from 7.50 to 7.90. The zeta potential of microalgae always kept negative and presented a trend of descending firstly and then upgrade. The maximum microalgae biomass flocculation capacity of 1 g cationic starch was 8.62 g with the help of self-flocculation at pH 3. The concentration of flocs formed at pH 11 was 25.74 g L⁻¹ and the diameter was 0.553 mm which was much larger than the flocs formed at pH 3 (0.208 mm).

1. Introduction

Energy crisis and global warming are two primary global concerns of restricting social sustainable development. Technologies for simultaneously solving the two issues are in urgent demand. Microalgae are photosynthetic microorganisms which can utilize solar energy and

convert CO₂ to organic chemicals such as sugars, proteins and lipids (Mahdy et al., 2016; Shengyi et al., 2015). Moreover, microalgae also can handle the problem of water pollution with high ammonia nitrogen and COD (Chang et al., 2018). Thus, microalgae cultivation has been regarded as a promising approach to deal with the harmful effects of the energy crisis, global warming and water pollution (Milano et al., 2016).

* Corresponding author at: Institute of Engineering Thermophysics, School of Energy and Power Engineering, Chongqing University, Chongqing 400044, China.
E-mail address: lqzx@cqu.edu.cn (Q. Liao).

The utilization of microalgae biomass is critically dependent on the biomass concentration (or conversely the water content). Whereas, the microalgae biomass concentration cultivated under photoautotrophic condition at large scale (e.g., outdoor open raceway ponds and closed photobioreactors) is usually less than 1 g L^{-1} and it is too low for direct utilization (Barros et al., 2015; Pires et al., 2017). Consequently, it makes microalgae harvesting necessary before the utilization of microalgae biomass. While, due to the high energy consumption (20–30% of the total biomass production cost), microalgae harvesting has already become the main bottleneck of the microalgae biomass utilization (Japar et al., 2017; Wei et al., 2017). Current microalgae harvesting methods mainly contain centrifugation, filtration, flotation and flocculation. Among these methods, flocculation is a relatively low cost method for harvesting microalgae at large scale (Vandamme et al., 2013). While, the drawback of flocculation is the difficulty of separation the additives from the microalgae biomass. For instance, the common flocculants in the flocs like aluminium sulfate, ferric sulphate and polyacrylamide are hard to remove out (Japar et al., 2017). To solve this problem, non-poisonous additives can be chosen and used as the feedstocks with microalgae biomass together for later energy conversion. An energy conversion method with high efficiency is anaerobic digestion, during which biogas can be achieved (Xia et al., 2015). In addition, the anaerobic digestion requires a suitable carbon to nitrogen (C/N) ratio (about 15.6) for the feedstocks. The feedstocks with low or high C/N would inhibit the hydrogen producing bacteria to efficiently produce fermentative hydrogen (Xia et al., 2014). Whereas, the C/N ratio of microalgae biomass was too low due to the high protein content and relatively low carbohydrate content. Hence, flocculants with high carbon content could be used to improve the C/N ratio and the mixture could be used as the feedstocks for later energy conversion.

Starch is long chained hydrocarbons with linear polysaccharide glucose monomers and high carbon content (Toor et al., 2011). Additionally, starch is also an ideal feedstock for anaerobic digestion (Zhang et al., 2014). Starch can be considered as one of the most suitable skeleton materials for eco-friendly flocculants due to the numerous surface active groups and long chain structure. Cationic starch, as a derivative of starch, is prepared by addition of quaternary ammonium groups to starch. The biodegradability of cationic starch was studied by Rutiaga et al. (2005) and the result indicated that it degraded well and took about 1100 h for 100% biodegradation in soil. The potential toxicity of cationic starch to microalgae was also explored. Peng et al. (2017) pointed out that cationic starch had no immediate toxicological effects on the viability of the microalgae and similar study was reported by Vandamme et al. (2010). Cationic starch has proven to be an efficient flocculant to harvest microalgae (Leteliergordo et al., 2014). Leteliergordo et al. (2014) used cationic starch (Greenfloc 120) to harvest fresh water microalgae *Chlorella protothecoides*. The results show that there was little difference between the harvesting efficiency of pH 4.0, 7.7 and 10. Similar conclusion was also reported by Vandamme et al. (2010), and the microalgae species used were *Scenedesmus* and *Parachlorella*. Besides, Peng et al. (2017) studied the effect of starch sources and microalgae species. The results show that various starch sources functioned similarly and the dosage used were 89 and 119 mg g^{-1} for *C. pyrenoidosa* and *B. braunii*, respectively. Most of these studies were focused on the harvesting efficiency, effect of pH and the microalgae concentration. Furthermore, charge neutralization as the major mechanism involve in microalgae flocculation is related to the zeta potential of both microalgae and flocculants, and the zeta potential will change with pH (Singh and Patidar, 2018). While, studies focused on the flocculation process and mechanism of pH affecting flocculation combined with zeta potential were few, not to mention the study combined with the C/N ratio for later utilization.

Herein, the present study aims to explore the change of C/N ratio during the harvesting process and the mechanism of pH affecting flocculation. To fulfill this purpose, synthesis conditions of cationic starch, zeta potential of microalgae and cationic starch at different pH

were investigated. Besides, the relationship of microalgae biomass concentration and cationic starch consumption dosage was also explored. Moreover, the effects of pH on the consumed dosage, flocculation capacity and the C/N ratio during the harvesting process were also studied.

2. Materials and methods

2.1. Synthesis of cationic starch

The sweet potato starch purchased from local Wal-Mart was produced by Chongqing Jiaxian Foods Co., Ltd., Chongqing, China. (3-Chloro-2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) with mass concentration of 60% was obtained from Aladdin Chemistry Co., Ltd. The cationic starch was synthesized by using CHPTAC to substitute part of the hydroxyl groups on starch chains with NaOH as the catalyst (Haack et al., 2002). Briefly, 0.51 g NaOH, 2.49 mL CHPTAC and 2.80 mL de-ionized water were mixed together for 10 min with temperature of 4°C . 5.0 g sweet potato starch was dissolved in the mixture and stirred thoroughly for 5 min. Then, the mixture in the flask was placed in a water bath with variable temperature (60, 70, 80, 90°C) and time (2, 3, 4, 5 h). After the reaction, cationic starch in the flask was washed with 80% ethanol for three times, and the targeted precipitate was collected and dried in a vacuum oven (BGZ-70, Shanghai Boxun Co., Ltd., China) at 50°C for 6 h. The obtained cationic starch was milled to fine powder before use.

2.2. Experimental setup and operation

Cationic starch was first dissolved in hot de-ionized water (80°C) with magnetic stirring (524G, Shanghai meiyongpu instrument manufacturing Co. Ltd., China) and then cooled to the room temperature. The microalgae harvesting experiments were conducted by adding the cationic starch solution to the microalgae suspensions with variable microalgae biomass concentration (0.353, 0.706, 1.412, 2.823 g L^{-1}) and pH (3, 5, 7, 9, 11). The microalgae suspensions used for harvesting experiments was freshwater *Chlorella vulgaris* FACHB-31 (*C. vulgaris*) that cultivated 7 days in the modified BG11 (BG11-M) medium (Sun et al., 2016) with 5% CO_2 (v/v) bubbling and $90 \mu\text{mol m}^{-2} \text{ s}^{-1}$ continuous illumination. After the addition of cationic starch solution, the microalgae suspension was intensively mixed (500 rpm) for 20 min. Subsequently, the suspension was allowed to settle for 5 min. In order to estimate the harvesting efficiency, samples (3.5 mL) were collected in the middle of the clarified zone and the absorbance was measured at an absorbance of 680 nm using a UV–Visible spectrophotometer (T6 New century, Persee, China). The harvesting efficiency was calculated as Eq. (1) (Vandamme et al., 2016):

$$HE = \frac{OD_i - OD_f}{OD_i} \quad (1)$$

where OD_i and OD_f represent the initial and final microalgae suspension optical density measured at 680 nm.

The concentration of microalgae biomass and flocs were measured gravimetrically. Briefly, the sampled suspension was centrifuged (LYNX 4000, Thermo Fisher Scientific, America) at 8000 rpm for 10 min and then dried at 85°C to a constant weight. Different concentrations of microalgae were achieved by quantifiably adding the superfluous supernatant of the centrifuged microalgae suspension according to the measured biomass concentration and then mixing the solution well. The different pH microalgae suspensions were obtained by adjusting the pH with 1 M HCl and NaOH. The pH was measured by using the pH meter (FG2, MettlerToledo, Switzerland). The flocs size was measured by an inverted fluorescence microscope (ix81, Olympus Corporation, Japan).

2.3. Analytical methods for characteristics of cationic starch and *C. vulgaris*

The total nitrogen in the starch was determined using elemental analysis (HACH Company, Germany) and the degree of substitution (DS) of cationic starch was calculated as Eq. (2) (Hansel et al., 2014):

$$DS = \frac{M_{AGU} \times N\%}{100(M_N) - M_{CEA} \times N\%} \quad (2)$$

where M_{AGU} represent the molar mass of an anhydroglucose unit of starch (162.15 g/mol), M_N represent the molar mass of nitrogen (14.01 g/mol) and M_{CEA} represent the molar mass of the cationizing agent (151.6 g/mol).

Zeta potential measurements of *C. vulgaris* and cationic starch at different pH were conducted with a Zetasizer Nano (ZS90, Malvern, UK). The changes of functional groups and chemical composition were conducted by a Fourier transform infrared spectroscopy (FTIR) analyser (Nicolet iS5, ThermoFisher, USA). The carbon and nitrogen content was measured by a total organic carbon/total nitrogen (TOC/TN) analyzer (Multi N/C 2100S, Analytik Jena AG, Germany).

In this study, the microalgae harvesting experiment was duplicated at least twice to eliminate the individual influences. Additionally, all of the experiment data shown are reported as mean values and standard deviations (in the figures) or standard errors (in the tables).

3. Results and discussions

3.1. Determination of the cationic starch synthesis conditions

As shown in Fig. 1, both of the cationic starch substitution degree and harvesting efficiency were investigated to determine the optimum conditions of cationic starch synthesis. The results show that there was a positive correlation between the harvesting efficiency and the substitution degree except NaOH/etherifying agent mole ratio (a) and etherifying agent content (b). That is to say, with the increasing etherifying agent content, more hydroxyl groups on starch chains could

be substituted by positively charged groups, which would attach the electronegative microalgae cells by charge neutralization (Leteliergordo et al., 2014). Both of the harvesting efficiency and substitution degree increased generally with the suitable reaction conditions, then reached the peak, and finally decreased with the going too far conditions. While, as for the NaOH/etherifying agent mole ratio (a) and etherifying agent content (b), the harvesting efficiency increased firstly and then decreased with the rising substitution degree. This may be explained by the unreacted etherifying agent which was difficult to wash away by ethyl alcohol as the cationic starch gelled together. Therefore, in the process of cationic starch synthesis, the attention should be paid not only to the substitution degree but also to harvesting efficiency. As Fig. 1 indicated, the optimum conditions for the synthesis of cationic starch were 50% etherifying agent content, 1.4 mol ratio of NaOH to etherifying agent, 80 °C reaction temperature and 4 h of reaction time, respectively.

3.2. Characterization of cationic starch and *C. vulgaris*

To verify the cationic starch, FTIR of native sweet potato starch and synthesized cationic starch were obtained in the spectrum range of 4000–400 cm^{-1} (E-supplementary data). And the new peaks attributed to bending of C–H in CHPTAC (Chang et al., 2014) appeared at 1476 cm^{-1} , which demonstrated the successful synthesis of cationic starch. In addition, the adsorption bands appearing at 3447 cm^{-1} and 2932 cm^{-1} are attributed to O–H and C–H stretch vibration. The strong peaks at 1417 cm^{-1} , 1155 cm^{-1} and 1023 cm^{-1} are due to bending of C–H, stretching of C–O and C–N, respectively. The characteristic absorption band occurred at 1644 cm^{-1} was originates from tightly bound water present in the starch molecules (Kar et al., 2013).

The formation of microalgae flocs was driven by electrostatic interactions, which was only related to the zeta potential and increased with the rise of the absolute value of zeta potential, when the ionic strength remained the same (Nabweteme et al., 2015). Whereas, the zeta potential would change with the pH of the suspension (Noh et al.,

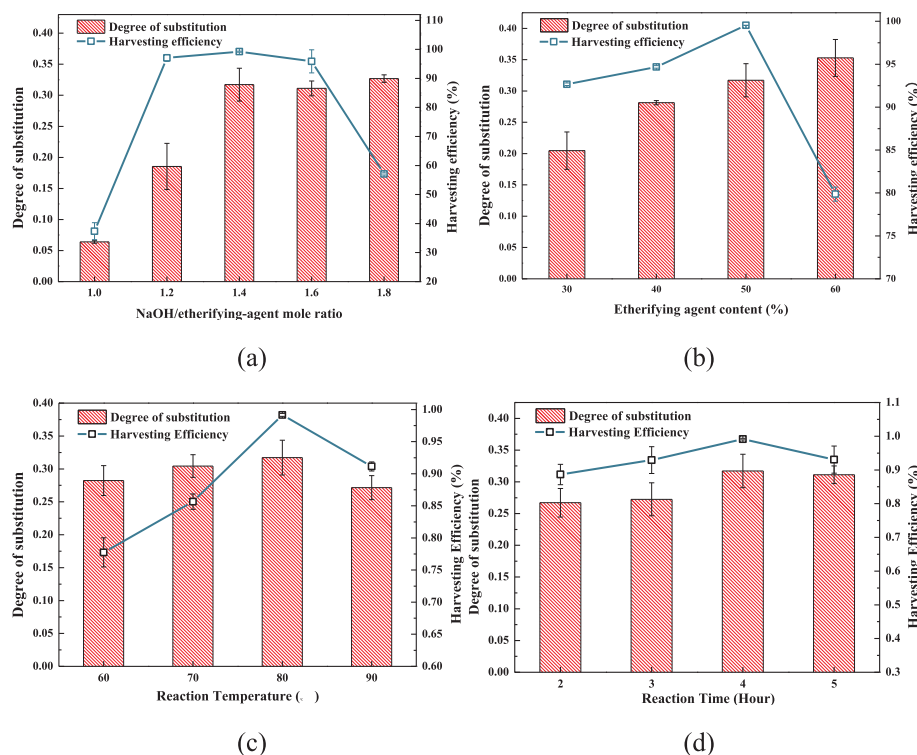


Fig. 1. The effect of (a) mole ratio of NaOH to etherifying agent; (b) etherifying agent content; (c) reaction temperature and (d) reaction time on degree of substitution and harvesting efficiency.

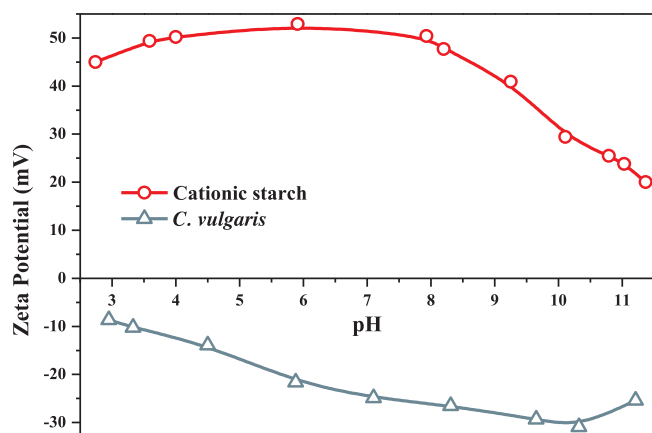


Fig. 2. The zeta potential of cationic starch and *C. vulgaris*.

2018). Besides, there were precipitation of divalent cations in alkaline conditions. In order to explore the correlation between pH and zeta potential, the zeta potential of *C. vulgaris* and cationic starch with different pH (3, 5, 7, 9 and 11) were investigated (Fig. 2). As data indicated, the zeta potential of *C. vulgaris* was always negative and presented a general trend of descending firstly and then upgrade. To be specific, the zeta potential of *C. vulgaris* decreased gradually from -8.64 mV (pH 3) to -30.9 mV (pH 10.3). When the pH exceeded 10.3, the zeta potential increased rapidly to -25.4 mV (pH 11.21). Contrary to the trend of *C. vulgaris*, the zeta potential of cationic starch always kept positive and offered a general trend of gradually upgrade and then descending rapidly. The zeta potential of cationic starch ranged from 20 mV (pH 11.4) to 52.9 mV (pH 5.9). And it remained relatively steady from pH 2.74 (45 mV) to pH 9.25 (40.9 mV), which meant that cationic starch could work over a broad pH range.

3.3. Relationship between biomass concentration and cationic starch dosage

The harvesting efficiency of microalgae suspensions with different biomass concentration (0.353 , 0.706 , 1.412 and 2.823 g L⁻¹) was shown in Fig. 3(a). As data indicated, all these suspensions with different biomass concentration could be harvested above 95% and the highest harvesting efficiency could reach 98.7%. The cationic starch consumption dosage for achieving 95% harvesting efficiency increased with the rising concentration of microalgae suspension. It was because there was more surface charge needed to be neutralized at the higher

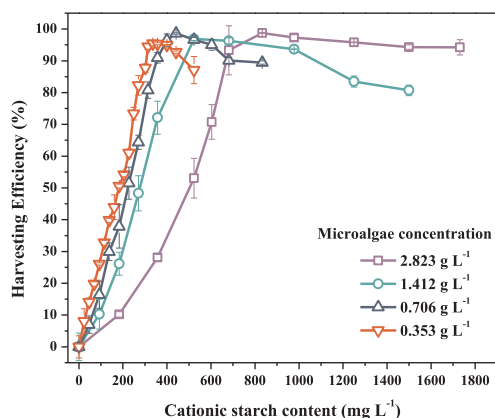
biomass concentration (Granados et al., 2012). Furthermore, when the cationic starch consumption dosage increased, the harvesting efficiency of all microalgae suspensions increased firstly and then decreased. In the first upgrade stage, the dosage of cationic starch was not enough to attach on the entire cell surface. Each cationic starch polymer chain only could attach on few cells with flat patch conformation, and lots of the cells might be free from the attachment (Zhou and Franks, 2006). During the later descending tendency, the dosage of cationic starch was excessive and tended to attach fully on cell surface. The cationic starch formed a layer of polymer that contained positively charged surface. And the positively charged surface could make the aggregate re-stabilize in suspension due to electrostatic repulsion between the polymer-coated cells (Ahmad et al., 2011), which was known as electrosteric stabilization (Fritz et al., 2002). Moreover, the descending trend was evident when the microalgae biomass concentration was low. Therefore, the dosage of cationic starch should meet the optimum concentration in order to achieve a higher harvesting efficiency.

The optimal dosage was defined as the lowest cationic starch consumption dosage for achieving 95% removal of the microalgae cells from the suspension. As for microalgae suspension with 0.353 g L⁻¹ biomass concentration, above 95% of the biomass could be removed by flocculation with 325.41 mg L⁻¹ cationic starch. While, for the microalgae concentration of 0.683 g L⁻¹, 1.366 g L⁻¹ and 2.733 g L⁻¹, the optimal dosage were 388.19 g L⁻¹, 510.61 g L⁻¹ and 729.10 g L⁻¹, respectively. The correlation between microalgae biomass concentration and optimal dosage of cationic starch was presented in Fig. 3(b). It indicated that the correlation equation was fitted well and about 168.24 mg cationic starch was needed to harvested 1 g microalgae biomass in 1 L microalgae suspension. In addition, there was a Y-intercept which meant 272.84 mg cationic starch was needed if the microalgae biomass concentration was 0 g L⁻¹. This might be caused by the extracellular polymeric substances (EPS) in the microalgae suspension, which would consume some of the cationic starch (Vandamme et al., 2012).

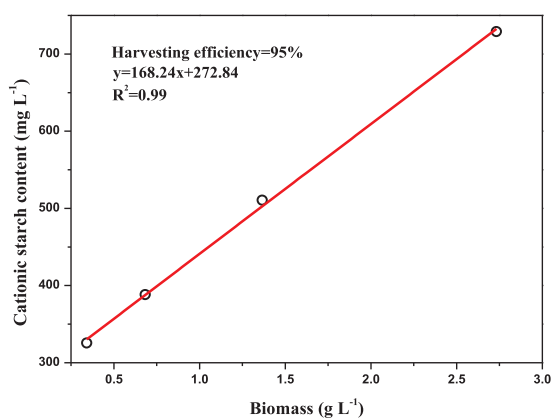
3.4. Effects of pH on the flocculation performance of cationic starch

3.4.1. Effects of pH on the consumption of cationic starch

So as to verify the stability of cationic starch harvesting efficiency with different pH, various concentration of cationic starch were used to harvest microalgae suspensions with 2.89 g L⁻¹ biomass and different pH of 3, 5, 7, 9, 11 (Fig. 4a). As depicted, all these suspensions with different pH can be harvested above 99%. The harvesting efficiency increased rapidly at pH 3 and 11. While, as for pH 5, 7 and 9, the



(a)



(b)

Fig. 3. The harvesting efficiency of cationic starch on *C. vulgaris* with different biomass concentration (a) and the correlation between them for 95% harvesting efficiency (b).

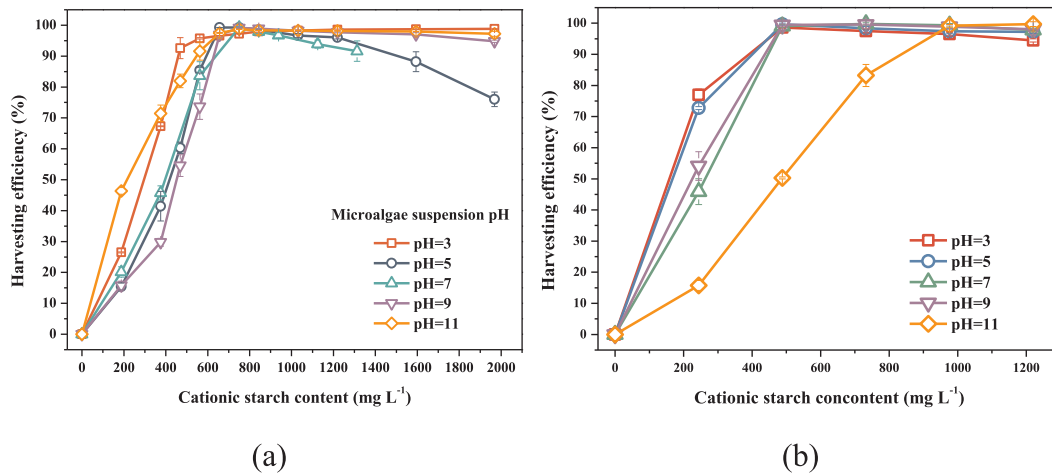


Fig. 4. The harvesting efficiency of different dosage cationic starch in BG11-M medium (a) and de-ionized water (b) at different pH.

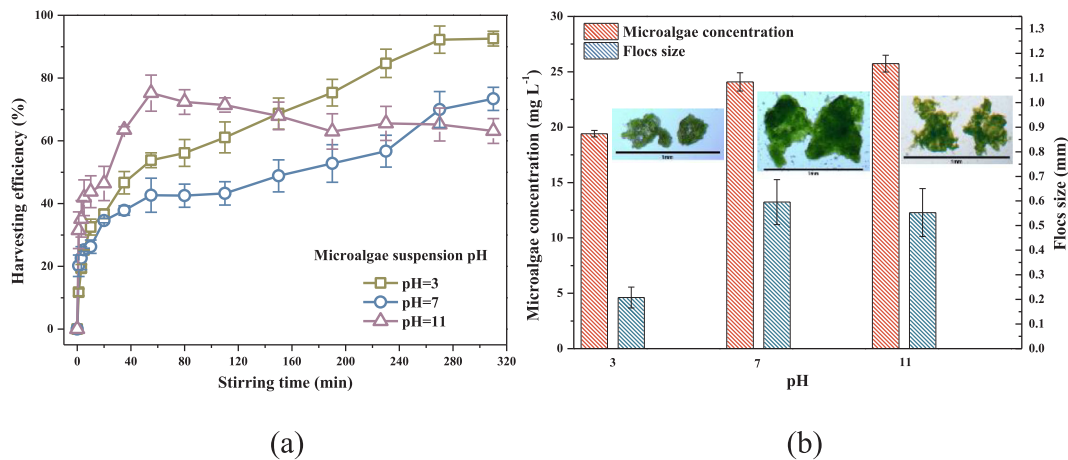


Fig. 5. The harvesting efficiency of insufficient cationic starch with different stirring time (a), flocs concentration and size for 5 min settling (b) at different pH.

Table 1
the flocculation performance of cationic starch at different pH.

pH	Microalgae concentration		Harvesting efficiency	Flocculation capacity of 1 g cationic starch	Microalgae C/N	Flocs C/N	Supernatant C/N
	Initial	Harvested					
	g L ⁻¹		%	g			
3	3.49	19.41	92.57	8.62	7.50	7.81	9.25
7	3.49	24.09	73.38	6.83		7.83	9.05
11	3.49	25.74	75.21	7.00		7.86	8.98

C/N: The ratio of carbon to nitrogen.

upward trend were less pronounced. With regard to pH 3, this was mainly because the zeta potential of *C. vulgaris* at pH 3 was -8.64 mV and it was the closest to 0 comparing to other pH (Fig. 2). And this would induce a part of microalgae to settle down as the attractive van der Waals forces overcame these electrostatic repulsion forces (Liu et al., 2014). These results corresponded well with the previous findings of Henderson et al. (2008).

As for pH 11, this was caused by the alkaline flocculation which was mainly induced by the precipitation of divalent cations in the microalgae suspension (Şirin et al., 2012; Wu et al., 2012; Yang et al., 2016). To prove this, the alkaline flocculation was eliminated by harvesting the microalgae and re-suspending them in de-ionized water. The results of cationic starch harvesting microalgae in de-ionized water at different pH were shown in Fig. 4(b). The experimental data indicated that the

harvesting efficiency of pH 11 was the lowest. When the harvesting efficiency of pH 3, 5, 7 and 9 reached 98.60%, 99.61%, 99.06% and 99.42%, the harvesting efficiency of pH 11 was only 50.31% and it was almost the half of the others. This was mainly caused by the disappearance of alkaline flocculation and the low zeta potential of both *C. vulgaris* and cationic starch. Specifically, the zeta potential of cationic starch at pH 11 (24.01 mV) was also almost the half of the others (45–52.9 mV) and the zeta potential of *C. vulgaris* at pH 11 (-26.70 mV) was close to the ones of pH 7 (-24.46 mV) and pH 9 (-27.91 mV). For pH 3 and 5, the weakened electrostatic repulsion made the harvesting efficiency rise faster than pH 7 and 9. In addition, the gap of the optimal dosage of cationic starch between pH 3, 5, 7, 9, 11 for BG11-M and pH 3, 5, 7, 9 for de-ionized water was narrow, which indicated that cationic starch could work over a broad pH range.

Table 2

The total carbon content, total nitrogen content and C/N ratio of starch, cationic starch, microalgae and the mixture.

	TC		TN		C/N
	(mg L ⁻¹)	(mg)	(mg L ⁻¹)	(mg)	
Starch	137.65		0.145		969.34
Cationic starch	147.60	6.20	9.36	0.39	15.77
Microalgae	1388.57	68.97	185.05	9.20	7.50
Mixture	1536.17	75.17	194.40	9.59	7.90

TC: The total carbon content.

TN: The total nitrogen content.

C/N: The ratio of carbon to nitrogen.

Mixture: The mixture of microalgae and cationic starch.

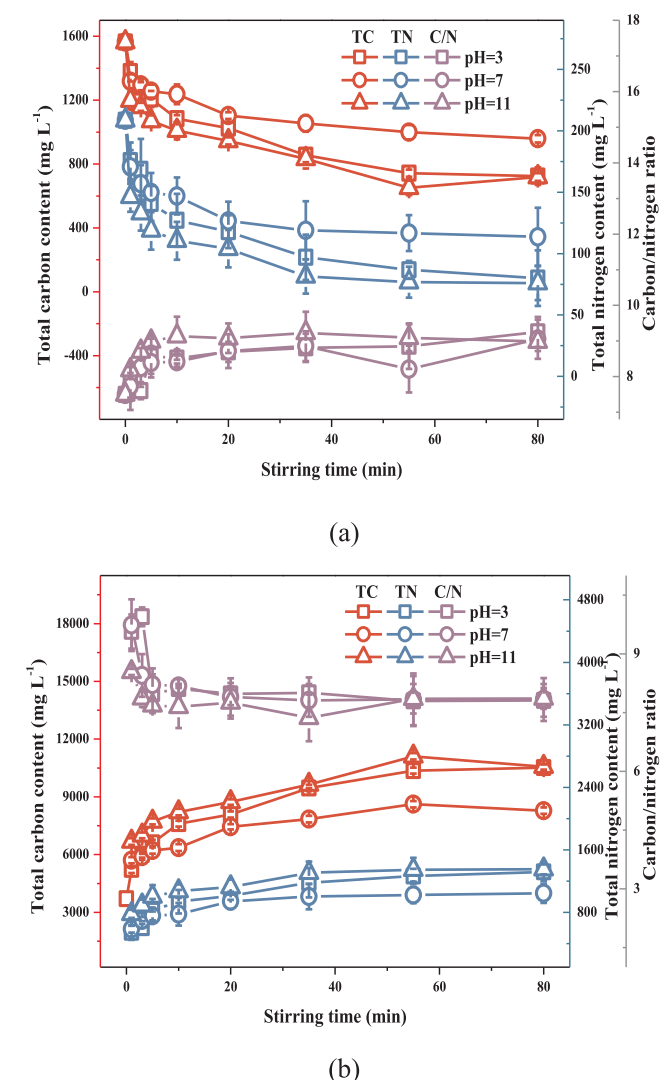


Fig. 6. The total carbon content, total nitrogen and C/N ratio of the supernatant (a) and flocs (b) during the flocculation process at different pH.

Furthermore, the huge gap of harvesting efficiency between pH 11 and the others in de-ionized water meant that the impact of zeta potential on the harvesting performance was great.

3.4.2. Effects of pH on the flocculation capacity of cationic starch

To explore the effect of pH on the flocculation capacity of cationic starch, insufficient dosage of cationic starch was added to the microalgae suspension with the concentration of 3.49 g L⁻¹ at pH 3, 7 and 11.

As shown in Fig. 5(a), the harvesting efficiency of pH 3 and 7 increased gradually with the extension of stirring time, and the highest harvesting efficiency achieved were 92.57% and 73.38%, respectively. While, the harvesting efficiency of pH 3 always kept ahead of pH 7 with the help of self-flocculation. Unlike to pH 3 and 7, the harvesting efficiency of pH 11 increased rapidly and then declined slowly with the extension of stirring time. The drop of harvesting efficiency might be caused by the disruption of the formed flocculants-cell aggregates by long-time stirring (Xu et al., 2011). During the increasing stage, the harvesting efficiency of pH 11 kept ahead of pH 3 and 7 with the help of alkaline flocculation, which was consistent with previous results (Fig. 4a). In addition, the corresponding flocculation capacity of 1 g cationic starch at pH 3, 7 and 11 were 8.62 g, 6.83 g and 7.00 g microalgae biomass, respectively (Table 1).

The effect of pH on the concentration and size of flocs with 95% harvesting efficiency and 5 min settling time were also investigated (Fig. 5b). As was shown by the graph, the flocs concentration of pH 11 was the highest and it was 25.74 g L⁻¹. This was mainly caused by the alkaline flocculation as there was additional sediment of divalent cations (Wu et al., 2012; Yang et al., 2016). The flocs concentration of pH 7 (24.09 g L⁻¹) was close to pH 11. While, the flocs concentration of pH 3 was the least and the value was 19.41 g L⁻¹. The reason for this was the reduction of the cells zeta potential which led to the reduction of electrostatic attraction interaction and the loose flocs. Additionally, the flocs size of different pH may support this explanation. As Fig. 5(b) revealed that the flocs size of pH 3 was 0.208 mm which was less than the size of pH 7 and 11, which were 0.596 and 0.553 mm.

3.4.3. Effects of pH on the C/N ratio of the supernatant and flocs

To give guidance to anaerobic digestion, the TC, TN and C/N of starch, cationic starch, microalgae suspension, supernatant and flocs were investigated (Table 2, Fig. 6). As depicted in the graph, the component of native sweet potato starch was mainly carbon and the C/N ratio was 969.34. When the hydroxyl groups on starch chains were substituted by quaternary ammonium groups, the C/N ratio decreased to 15.77 and it was still large than the C/N ratio microalgae which was 7.50. During the flocculation process, the TC, TN of the supernatant at pH 3 and 7 decreased as the microalgae were the main source of carbon and nitrogen and they were settling. Besides, the C/N ratio of supernatant increased and the final C/N ratio was large than the microalgae suspension, and the reason was that there was unreacted starch in the supernatant. While, for pH 11, the increment of TC and TN and the fall of the C/N ratio after 55 min were resulted from the disruption of the formed flocculants-cell aggregates mentioned above (Xu et al., 2011). Similar to pH 3 and 7, the final C/N ratio of the supernatant was also large than microalgae.

With regard to the flocs, the TC, TN of the flocs at pH 3 and 7 increased with the accumulation of flocs. Meanwhile, the C/N ratio decreased when more microalgae were harvested. Similar to the supernatant, the TC, TN and C/N ratio of the flocs at pH 11 occurred a tipping point with the disruption of flocculation. Moreover, the final C/N ratio of the flocs was similar to the microalgae as the added flocculants was far less than the microalgae biomass. Nevertheless, the mixture of the supernatant and flocs could act as the feedstock for anaerobic digestion since the anaerobic digestion needed lots of water and there was unreacted starch in the supernatant. Additionally, the C/N ratio of the mixture was 7.90 which would to some extent improve the anaerobic digestion condition for hydrogen fermentation.

In conclusion to this, it becomes obvious that cationic starch was a promising technology combined microalgae harvesting with the later utilization of anaerobic digestion. Specifically, the C/N of mixture feedstocks could increase to 7.90 after the addition of cationic starch. What's more, the starch used to synthesize cationic starch could be replaced by the waste starch from flour processing plants. In this way, not only reduced the cost of microalgae harvesting process, but also effectively solved the environmental pollution caused by the discharge

of the waste. In addition, the wide resource of waste starch makes it very suitable for widespread application. While, the studies of synthesis cationic starch by waste starch were few. Therefore, to further reduce the cost of microalgae harvesting, the future work will focus on the synthesis cationic starch by waste starch.

4. Conclusions

The optimal synthesis conditions of cationic starch were determined and the maximum flocculation capacity of 1 g cationic starch was 8.62 g microalgae biomass with the help of self-flocculation at pH 3. While, without the help of divalent cations precipitation at pH 11, microalgae harvesting efficiency was almost the half of the ones at acid and neutral condition. The flocs formed at pH 3 was loose and small size compared to pH 7 and 11 due to the reduction of the electrostatic attraction. Additionally, the C/N ratio of the mixture was 7.90 and it will be beneficial for anaerobic digestion.

Acknowledgements

The authors are grateful for the financial support provided by the National Natural Science Funds for Young Scholar (No. 51606020), the State Key Program of National Natural Science of China (Grant No. 51836001), the Venture & Innovation Support Program for Chongqing Overseas and Returnees (No. cx2017019), and the Fundamental Research Funds for the Central Universities (2018CDXYDL0001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2018.12.072>.

References

- Ahmad, A., Yasin, N.M., Derek, C., Lim, J., 2011. Optimization of microalgae coagulation process using chitosan. *Chem. Eng. J.* 173 (3), 879–882.
- Barros, A.L., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: a review. *Renew. Sust. Energ. Rev.* 41 (C), 1489–1500.
- Chang, H., Quan, X., Zhong, N., Zhang, Z., Lu, C., Li, G., Cheng, Z., Yang, L., 2018. High-efficiency nutrients reclamation from landfill leachate by microalgae *Chlorella vulgaris* in membrane photobioreactor for bio-lipid production. *Bioresour. Technol.* 266, 374–381.
- Chang, Y.J., Choi, H.W., Kim, H.S., Lee, H., Kim, W., Kim, D.O., Kim, B.Y., Baik, M.Y., 2014. Physicochemical properties of granular and non-granular cationic starches prepared under ultra high pressure. *Carbohydr. Polym.* 99, 385–393.
- Fritz, G., Schädler, V., Willenbacher, N., Wagner, N.J., 2002. Electrosteric stabilization of colloidal dispersions. *Langmuir* 18 (16), 6381–6390.
- Granados, M.R., Acien, F.G., Gómez, C., Fernándezsevilla, J.M., Molina, G.E., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresour. Technol.* 118 (118), 102–110.
- Haack, V., Heinze, T., Oelmeyer, G., Kulicke, W.M., 2002. Starch derivatives of high degree of functionalization. 8. Synthesis and flocculation behavior of cationic starch polyelectrolytes. *Macromol. Mater. Eng.* 287 (8), 495–502.
- Hansel, P.A., Guy Riefler, R., Stuart, B.J., 2014. Efficient flocculation of microalgae for biomass production using cationic starch. *Algal Res.* 5, 133–139.
- Henderson, R., Parsons, S.A., Jefferson, B., 2008. The impact of algal properties and pre-oxidation on solid-liquid separation of algae. *Water Res.* 42 (8), 1827–1845.
- Japar, A.S., Takriff, M.S., Yasin, N.H.M., 2017. Harvesting microalgal biomass and lipid extraction for potential biofuel production: a review. *J. Environ. Chem. Eng.* 5 (1), 555–563.
- Kar, B., Sahoo, H., Rath, S.S., Das, B., 2013. Investigations on different starches as depressants for iron ore flotation. *Miner. Eng.* 49, 1–6.
- Leteliergordo, C.O., Holdt, S.L., De, F.D., Karakashev, D.B., Angelidaki, I., 2014. Effective harvesting of the microalgae *Chlorella protothecoides* via bioflocculation with cationic starch. *Bioresour. Technol.* 167 (167C), 214–218.
- Liu, J., Tao, Y., Wu, J., Yi, Z., Gao, B., Yu, T., Li, A., Zhang, C., Zhang, Y., 2014. Effective flocculation of target microalgae with self-flocculating microalgae induced by pH decrease. *Bioresour. Technol.* 167 (3), 367–375.
- Mahdy, A., Fotidis, I.A., Mancini, E., Ballesteros, M., González-Fernández, C., Angelidaki, I., 2016. Ammonia tolerant inocula provide a good base for anaerobic digestion of microalgae in third generation biogas process. *Bioresour. Technol.* 225.
- Milano, J., Hwaichyuan, O., Masjuki, H.H., Chong, W.T., Mankee, L., Pingkwan, L., Vellayan, V., 2016. Microalgae biofuels as an alternative to fossil fuel for power generation. *Renew. Sust. Energ. Rev.* 58, 180–197.
- Nabweteme, R., Mi, Y., Kwon, H.S., Kim, Y.J., Hwang, G., Lee, C.H., Ahn, I.S., 2015. Application of the extended dlvo approach to mechanistically study the algal flocculation. *J. Ind. Eng. Chem.* 30, 289–294.
- Noh, W., Kim, J., Lee, S.-J., Ryu, B.-G., Kang, C.-M., 2018. Harvesting and contamination control of microalgae *Chlorella ellipsoidea* using the bio-polymeric flocculant α -poly-L-lysine. *Bioresour. Technol.* 249, 206–211.
- Peng, C., Li, S., Zheng, J., Huang, S., Li, D., 2017. Harvesting microalgae with different sources of starch-based cationic flocculants. *Appl. Biochem. Biotechnol.* 181 (1), 112–124.
- Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., 2017. Photobioreactor design for microalgae production through computational fluid dynamics: a review. *Renew. Sust. Energ. Rev.* 79, 248–254.
- Rutiaga, M.O., Galan, L.J., Morales, L.H., Gordon, S.H., Imam, S.H., Orts, W.J., Glenn, G.M., Nino, K.A., 2005. Mechanical property and biodegradability of cast films prepared from blends of oppositely charged biopolymers. *J. Polym. Environ.* 13 (2), 185–191.
- Shengyi, C., Chienya, K., Chen, T.Y., Chang, Y.B., Chiumei, K., Lin, C.S., 2015. Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. *Bioresour. Technol.* 184, 179–189.
- Singh, G., Patidar, S.K., 2018. Microalgae harvesting techniques: a review. *J. Environ. Manage.* 217, 499–508.
- Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J., 2012. Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *J. Appl. Phycol.* 24 (5), 1067–1080.
- Sun, Y., Liao, Q., Huang, Y., Xia, A., Fu, Q., Zhu, X., Zheng, Y., 2016. Integrating planar waveguides doped with light scattering nanoparticles into a flat-plate photobioreactor to improve light distribution and microalgae growth. *Bioresour. Technol.* 220, 215–224.
- Toor, S.S., Rosendahl, L., Rudolf, A., 2011. Hydrothermal liquefaction of biomass: a review of subcritical water technologies. *Energy* 36 (5), 2328–2342.
- Vandamme, D., Beuckels, A., Vadelius, E., Depaetere, O., Noppe, W., Dutta, A., Foubert, I., Laurens, L., Muylaert, K., 2016. Inhibition of alkaline flocculation by algal organic matter for *Chlorella vulgaris*. *Water Res.* 88, 301–307.
- Vandamme, D., Foubert, I., Fraeye, I., Muylaert, K., 2012. Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation. *Bioresour. Technol.* 124 (337), 508.
- Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K., 2010. Flocculation of microalgae using cationic starch. *J. Appl. Phycol.* 22 (4), 525–530.
- Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol.* 31 (4), 233–239.
- Wei, C., Huang, Y., Liao, Q., Fu, Q., Xia, A., Sun, Y., 2017. The kinetics of the polyacrylic superabsorbent polymers swelling in microalgae suspension to concentrate cells density. *Bioresour. Technol.* 249, 713.
- Wu, Z.C., Zhu, Y., Huang, W.Y., Zhang, C.W., Li, T., Zhang, Y.M., Li, A.F., 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresour. Technol.* 110 (2), 496–502.
- Xia, A., Cheng, J., Ding, L., Lin, R., Song, W., Zhou, J., Cen, K., 2014. Enhancement of energy production efficiency from mixed biomass of *Chlorella pyrenoidosa* and cassava starch through combined hydrogen fermentation and methanogenesis. *Appl. Energy* 120 (120), 23–30.
- Xia, A., Cheng, J., Murphy, J.D., 2015. Innovation in biological production and upgrading of methane and hydrogen for use as gaseous transport biofuel. *Biotechnol. Adv.* 34 (5), 451–472.
- Xu, L., Guo, C., Wang, F., Zheng, S., Liu, C.Z., 2011. A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresour. Technol.* 102 (21), 10047–10051.
- Yang, F.F., Xiang, W.Z., Fan, J.W., Wu, H.L., Tao, L., Long, L.J., 2016. High pH-induced flocculation of marine *Chlorella* sp. for biofuel production. *J. Appl. Phycol.* 28 (2), 1–10.
- Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. *Renew. Sust. Energ. Rev.* 38 (5), 383–392.
- Zhou, Y., Franks, G.V., 2006. Flocculation mechanism induced by cationic polymers investigated by light scattering. *Langmuir* 22 (16), 6775–6786.