Harvesting of Chlorella Vulgaris Biomass by Using Different Bio-Flocculants: A Comparison Study

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Abstract

In the present study, several natural flocculants were extracted from natural resources to harvest Chlorella vulgaris. Jar tests were carried out to evaluate the flocculation efficiencies of the extracted flocculants in harvesting C. vulgaris cells. Chitosan and maize seeds were proven to be effective flocculants as they achieved more than 80 % efficiency after one(1) hour and four (4) hours of sedimentation time, respectively. Under the optimum flocculation conditions of pH 7, 2 mg/L dosage, 120 rpm of stirring speed for chitosan; and pH 3, 450 mg/L of dosage, 15 rpm of stirring speed for maize seeds flocculant; the flocculation efficiencies up to 97.9 % and 84.7 % are able to achieved, respectively. A scale-up study was done to test the flocculation ability of chitosan and maize seeds extracts to harvest 1 L of C. vulgaris. Approximately 97 % and 82 % of flocculation efficiencies were achieved by chitosan and maize seeds flocculants, respectively, under their optimum flocculation conditions. Comparatively, chitosan offer superiority in flocculating C. vulgaris than maize seeds extract in terms of floc size, dosage and sedimentation time.

Keywords: natural flocculant, chitosan, maize seed, microalgae, harvest

Introduction

As the issue of the impending energy crisis arises in recent years, exploration and development agendas on renewable energy sources have been greatly emphasized. Microalgae-to-fuel technology is one of the most researched energy alternatives to reduce the dependency on fossil fuels. Microalgae biomass posse enormous prospective as feedstock candidate for biodiesel production due to their substantial lipid content [1]-[3]. Certain microalgae species, such as *Chlorella* sp., *Nannochloropsis* sp. and *Neochloris oleoabundans* can accumulate the copious amount of lipid up to 60 % of their cell composition, which is relatively comparable to oil-bearing crops [3]-[4]. Apart from that, numerous oleaginous microalgae species grow at a rapid rate with higher biomass productivity than other common fuel crops like palm oil, corn, and oilseed rape [5]. Moreover, microalgae cultivation do not compete for arable lands as they can flourish in saline seawater and even on marginal lands where conventional agriculture is unfavorable [5]. In a broader context, utilization of microalgae biomass for biodiesel production has the following benefits: (1) bio-remediation of wastewater as microalgae cells can assimilate nitrogen and phosphorus sources from wastewater for growth; (2) mitigation of carbon dioxide in the atmosphere; and (3) production of value-added by-products, e.g. proteins, polysaccharides, biopolymers, hydrogen gas and the residual biomass can be converted into fertilizers and animal feed after lipid extraction through biorefinery schemes [6].

However, there are some major obstacles for commercializing microalgae biomass as alternative feedstock for biodiesel production. The harvesting process before oil extraction can be difficult and attribute approximately 20 % to 30 % of the total biomass production costs [4],[7]. Microalgae cells are small in size and usually dispersed in diluted medium with concentration not more than 1 g/L [7]. The situation is worsened by the colloidal stability of microalgae in the suspension which results in low harvesting efficiency [8]. Various solid-liquid separation techniques have been investigated with the aim to concentrate or dewater the microalgae slurry in an effort to make oil extraction possible at low cost [9]. Harvesting of microalgae biomass through gravitational sedimentation or screening alone is not feasible since while energy-intensive centrifugation technique is too expensive for low-value products like biodiesel [9]. Coupling of flocculation and sedimentation processes seem to be a better harvesting method, in consideration of cost factor and biomass recovery efficiency [9]. Generally, flocculation occurs when the microalgae cells with negatively charged surfaces are destabilized by oppositely charged flocculants through several mechanisms, such as charge neutralization, bridging, and electrostatic path [10]. The size of the flocs formed can be increased through mixing

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for even distribution of flocculants that can lead to more collisions, bonding and agglomeration of particles in the suspension. In order to further enhance the flocculation efficiency, the characteristics of flocculants, such as molecular weight, ionic strength, particle size and the process parameters like pH, flocculant dosage and stirring speed can be greatly manipulated [11].

Chemical flocculants such as alum, ferric chloride and synthetic polyacrylamide (PAM) polymers are effective option to harvest microalgae cells [12]. However, besides the requirement for high dosage in large scale operation, their toxic nature can contaminate the residual biomass after lipid extraction which limits their potential at commercial scale [13]. Microalgae sludge containing harmful residual flocculants are unfavorable for downstream usage, e.g. processing into animal feed and high-valued compounds like cosmetics, nutritional supplements and pharmaceuticals products in a biorefinery-based production [14]. As a result, the sustainability of microalgae biomass production is significantly reduced when the contaminated culture medium is non-recyclable.

A few concerns regarding the environmental impacts have to be deliberated while choosing flocculants to harvest microalgae biomass. The ideal flocculants chosen should, of course, demonstrate high efficiency in aggregating microalgae cells, cheap in price when applied at large scale, do not contaminate the biomass and non-toxic to allow recycling or reuse of the culture medium [7]. Chitosan, a cationic linear biopolyaminosaccharide that extracted from natural resources such as crustaceans shells or cell walls of some fungi, has been frequently studied in recent years as organic flocculants in treating turbidity of water [15]. Following the trend, abundant reviews focusing on the potential of plants' seeds to be used as natural flocculants due to their low cost and toxicity were documented. Seeds of papaya (*Carica papaya*), roselle (*Hibiscus sabdariffa*), jatropha (*Jartropha curcus*) and etc. were found to be effective in reducing water turbidity in many research works [16]-[19]. More interestingly, maize (*Zea mays*) seeds, *rambutan* (*Nephelium lappaceum*) seeds and dragon fruit (*Hylocereus undatus*) foliage had been studied and were reported to be potent tools in turbidity removal [20]–[22]. Since most of the active ingredients of natural flocculants are derivatives of proteins and polysaccharides with positive charges, long chains or high molecular weights, they associate to cause flocculation via adsorption, charge neutralization and interparticle bridging mechanisms [18]. Nevertheless, chitosan was found to result in minimum sludge volume index (SVI) and turbidity of real textile mill wastewater as compared to wastewater treated using *Moreinga oleifera* seed powder and maize seed powder [23].

As microalgae harvesting being conceptually similar to wastewater treatment or turbidity reduction process, natural flocculants can be exploited as potential substitutes over chemical flocculants, which are proven to be effective at large scale [24]. One of the most widely used natural flocculants in wastewater treatment, *Moringa oleifera* was proven for its efficiency in flocculating *C. vulgaris* [25]. Therefore, in this study, different flocculants were extracted from natural resources, such as seeds of *rambutan*, papaya, maize, as well as dragon fruit foliage and subsequently investigated for their effectiveness in harvesting *C. vulgaris* via flocculation with chitosan as a benchmark. Flocculation parameters such as pH of the medium, flocculant dosage, and stirring speed were also studied with respect to the flocculation efficiency of each flocculant.

Materials and methods

2.1 Cultivation of microalgae

C. vulgaris was supplied by Prof. Lee Keat Teong (School of Chemical Engineering, Universiti Sains Malaysia). The microalgae preserved and grown in Bold's Basal Medium (BBM) consisting of the following components: (1) 10 mL per L of culture medium with chemicals as follow, $NaNO_3$ (25 g/L), $CaCl_2.2H_2O$ (2.5 g/L), $MgSO_4.7H_2O$ (7.5 g/L), K_2HPO_4 (7.5 g/L), KH_2PO_4 (17.5 g/L), NaCl (2.5 g/L) and (2) 1mL per L of culture medium with EDTA anhydrous (50 g/L), KOH (31 g/L), $FeSO_4.7H_2O$ (4.98 g/L), H_2SO_4 (1ml), H_3BO_3 (11.4 g/L), $ZnSO_4.7H_2O$ (8.82 g/L), $MnCl_2.4H_2O$ (1.44 g/L), MoO_3 (0.71 g/L), $CuSO_4.5H_2O$ (1.57 g/L), $Co(NO_3)_2.6H_2O$ (0.49 g/L). All the chemicals used for microalgae growth and preservation was obtained from Sigma-Aldrich. The initial pH of the medium was adjusted to 6.8. Following the step, the culture was grown in 100 mL Erlenmeyer flask containing 50 mL medium with surrounding temperature ranging from 25-28°C. The culture was continuously aerated with compressed air and exposed to the illumination of cool white fluorescent light (Philip TL-D 36W/865, the light intensity of 60-70 µmol $m^{-2}s^{-1}$) [26].

2.2 Preparation of natural flocculants

Medium molecular weight chitosan was purchased from Sigma-Aldrich. Fresh *rambutan*, papaya and maize were purchased from local markets whereas dragon fruit foliage was collected from smallholder in Perak. *Rambutan* and papaya seeds were isolated from fruits, washed and dried at the ambient temperature of 23-25°C for 5 days [21,22]. As for dragon fruit foliage, the thorns were removed and the foliage was washed, cut and dried at 80°C for 24 hours [27]. Maize seeds were oven-dried at 60°C for 24 hours [23]. The dried materials were then separately ground into a fine powder using the blender and sieved to the particle size of 0.5-1.0 mm. All the powdered samples were collected and preserved in air-tight bottles and stored in the refrigerator at 4°C for further use.

For each type of natural flocculants, 1 g of processed powder was suspended in 100 mL of 1.0 M sodium chloride (NaCl) solution at room temperature. The suspensions were stirred for 60 minutes to extract the active ingredients from the samples for flocculation

purpose [27]. The resulting suspension was then filtered through filter paper and the filtrate was used as natural flocculant for microalgae harvesting using flocculation jar tests. On the other hand, 1 mg/L of chitosan flocculant was prepared by dissolving 0.1 g of chitosan powder with 100 mL of distilled water [15].

2.3 Flocculation jar test

Flocculation of *C. vulgaris* using the natural flocculants and chitosan were conducted separately with the help of a jar test apparatus as displayed in Figure 1. The microalgae suspension was diluted with tap water to control the absorbance at 0.5 ± 0.05 Abs. 500 mL of the suspension at desired pH was filled in each 1 L beaker and placed onto the floc illuminator. Desired dosages of flocculants were added into the microalgae suspension with stirring speed set at 100 rpm for 5 minutes and followed by a slower mixing speed at 60 rpm for the next 25 minutes to keep the flocs suspended [28]. All tests were carried out at room temperature. The suspensions were finally left to settle under the influence of gravity for an hour. All experiments were carried out in triplicates with ±5 % error and average values were reported.

2.5 Optimum pH for flocculation

The pH values of *C. vulgaris* suspension were varied using 1 M HCL and 1 M NaOH. The pH of the medium was adjusted within the range of pH 2 to pH 12 whereas the dosage of flocculants and stirring speed were fixed.

2.6 Optimum dosage of flocculants

To investigate the effect of flocculant dosage on flocculation of *C. vulgaris*, different dosages ranging from 1 to 800 mg/L were injected to the microalgae suspension with optimized pH of the medium and desired stirring speed were kept constant.

2.7 Optimum stirring speed

At optimum pH and flocculant dosage, the microalgae suspension were mixed at 100 rpm for 5 minutes. The effects of stirring speed on the flocculation performance of *C. vulgaris* were examined by varying the stirring speeds (15, 30, 45, 60, 90 and 120 rpm) for 25 minutes. The flocculation and sedimentation process were then observed for its efficiency record.

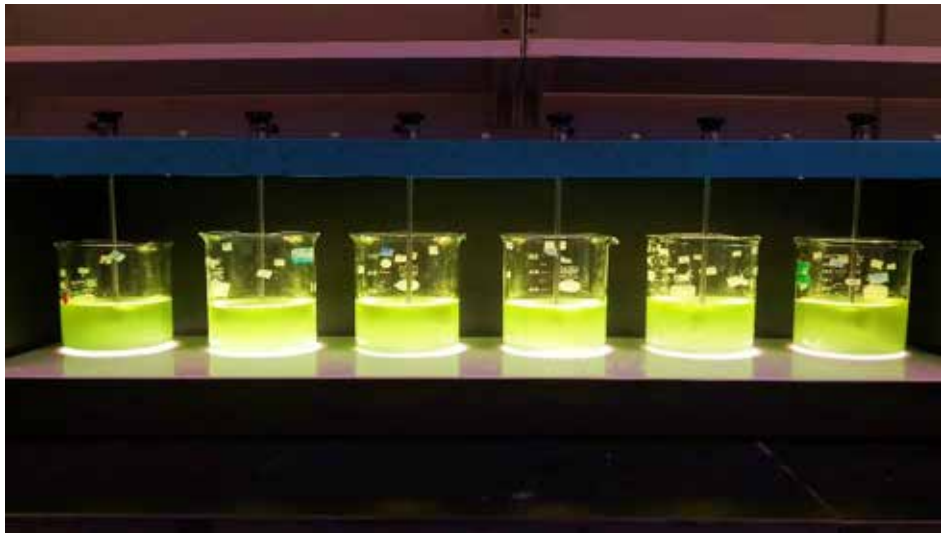


Figure 1 Flocculation jar test apparatus

2.4 Determination of flocculation efficiency

To evaluate the flocculation efficiency, 2 mL of microalgae sample, before and after flocculation, was withdrawn from the middle of microalgae suspension to measure its optical density at 684 nm, using a UV-Vis spectrophotometer [29]-[31]. The flocculation efficiency was calculated as follows:

$$E = \frac{C_b - C_a}{C_b} \times 100\ \% \quad (1)$$

where E is the flocculation efficiency, C_b and C_a are the optical density of the microalgae sample before and after flocculation, respectively.

2.8 Scale-up study: Comparisons of flocculants effectiveness

To further investigate the effectiveness of flocculants to harvest a larger volume of microalgae biomass at optimized conditions, flocculation jar tests were scaled-up using 1 L of *C. vulgaris* suspension.

3. Results and Discussions

3.1 *Rambutan* seeds, papaya seeds and dragon fruit foliage

Natural flocculants extracted from *rambutan* seeds, papaya seeds and dragon fruit foliage failed to flocculate *C. vulgaris* cells

at various pH and dosages despite their proven flocculating properties to treat kaolin water, river water, and concentrated latex effluent.

3.2 Chitosan and maize seeds

3.2.1 Effects of pH

The pH values of the medium had significantly influenced the flocculation efficiency of *C. vulgaris* as shown in Figure 2. Fixing the chitosan dosage at 2.5 mg/L, the optimum pH was observed to be 7, attaining about 83 % of flocculation efficiency. At pH 6, the flocculation process was considered to be effective as it achieved 73 % of flocculation efficiency after 1 hour of settling time. At pH 7, visible flocs were formed after 3 minutes of stirring. The flocs were slightly larger and the number of flocs formed was much more as compared to those formed at acidic and alkaline conditions. As reported by Rashid et al. [32], the pH of medium affected the surface charges of microalgae cell, making it highly positive at low pH and highly negative at high pH. This supports the results of the present study where acidic pH (pH 2) and basic pH (pH 9 and pH 11) were generally ineffective for flocculation using chitosan. In acidic medium, the highly protonated microalgae cells and the positively charged chitosan flocculant particles repelled each other, causing the flocculation to be ineffective. When the medium was too alkaline, repulsion occurred among the highly negative charged microalgae cells [32].

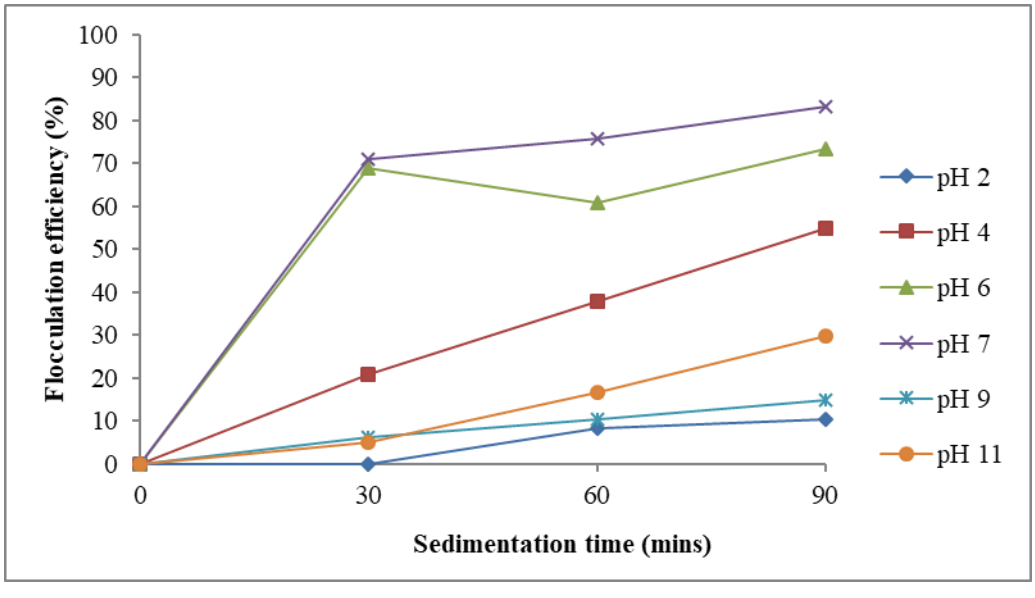


Figure 2 Effects of different pH on flocculation efficiency of *C. vulgaris* using chitosan at flocculant dosage and stirring speed of 2.5 mg/L and 60 rpm, respectively

On the other hand, using 450 mg/L maize seeds flocculant, flocculation was found to be only effective at pH 3 as shown in Figure 3. It was observed that very tiny flocs formed after 15 minutes of stirring at pH 3, however, the flocculation efficiency increased very slowly. After 1 hour of sedimentation, the flocculation efficiency reached about 50 %. By extending to 4 hours of sedimentation, the maize seeds flocculant was able to reach flocculation efficiency of 82.9 %. In comparison with chitosan, maize seeds flocculant required an additional of 4 folds of settling time to achieve above 80 % of flocculation efficiency.

3.2.2 Effects of flocculant dosage

In Figure 4, low chitosan dosages of 1 to 3 mg/L were found to be effective as the flocculation efficiencies reached above 75 % after 30 minutes of stirring. The optimum chitosan dosage in harvesting *C. vulgaris* was 2 mg/L, achieving approximately 86.5 % of flocculation efficiency after 1 hour of sedimentation. At 2 mg/L dosage, visible flocs were formed and observed after 2 minutes of stirring. Nevertheless, flocculant dosage of 5 mg/L and above were less effective (flocculation efficiencies of below than 30 %) even after 1 hour of settling time. This was probably due to the smaller size of flocs formed as compared to the large flocs formed at low chitosan dosage of 1 and 2 mg/L. At dosages above 20 mg/L, the flocculation of microalgae cells was hindered due to overdose. With a low dosage of cationic chitosan, the negative charges on the cell surfaces of *C. vulgaris* could be neutralized, thus causing the cells to agglomerate and form flocs. However, when overdosing occurred, the extra flocculant particles could encapsulate the cell surfaces of microalgae and caused reversion of charges to re-stabilize the suspension, preventing them to aggregate together [33].

Keeping the pH and stirring speed at constant, maize seeds flocculant was added to the microalgae suspension at dosages of 200, 300, 400, 450, 500, 600, 700 and 800 mg/L. As shown in Figure 5, it was evident that flocculant dosage ranging from

400 mg/L to 600 mg/L was effective to harvest *C. vulgaris* with flocculation efficiencies of more than 80 %. 450 mg/L of maize seeds flocculant was found to result in the highest flocculation efficiency, about 83.9 % at pH 3. At a higher dosage of 800 mg/L, the flocculation efficiency decreased drastically to about 31 %. Similar to the results discussed earlier for the effect of chitosan dosage, overdosing would reduce the flocculation efficiency as the microalgae cells were re-stabilized by the repulsion of charges on their surfaces.

3.2.3 Effects of stirring speed

The effects of stirring speed on the flocculation efficiency of *C. vulgaris* using chitosan flocculant are displayed in Figure 6. The flocculation efficiency increased with the increase of stirring speed. At medium and high stirring speeds (more than 45 rpm), the flocculation efficiencies were above 70.0 % after 30 minutes of stirring. Increasing the stirring speed to 120 rpm had resulted in the highest flocculation efficiency of 97.9 %. At the high stirring speed of 90 rpm and 120 rpm, the flocs formed were smaller than

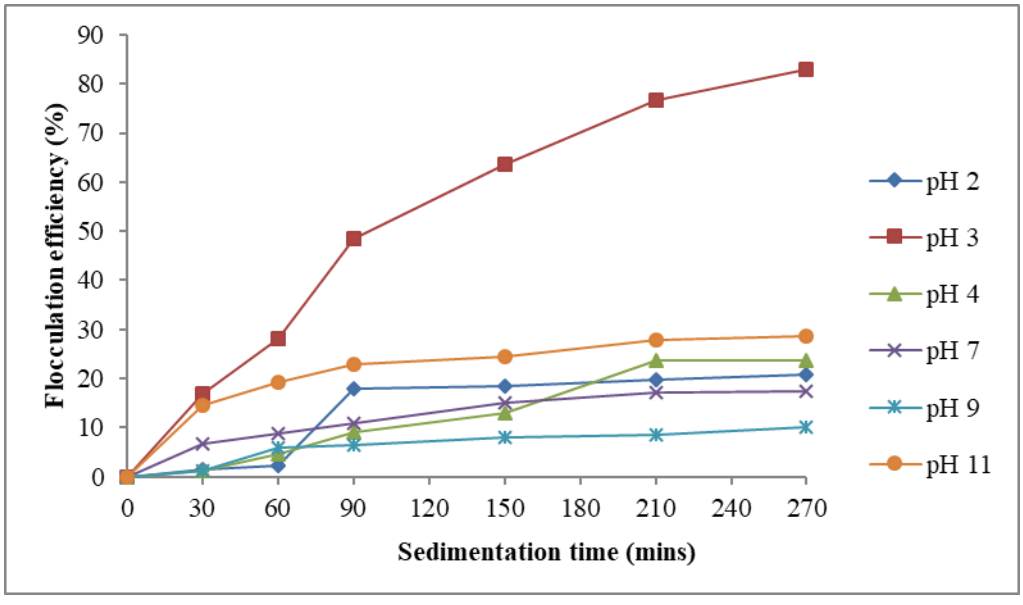


Figure 3 Effects of different pH on flocculation efficiency of *C. vulgaris* using maize seeds flocculant at flocculant dosage and stirring speed of 450 mg/L and 60 rpm, respectively

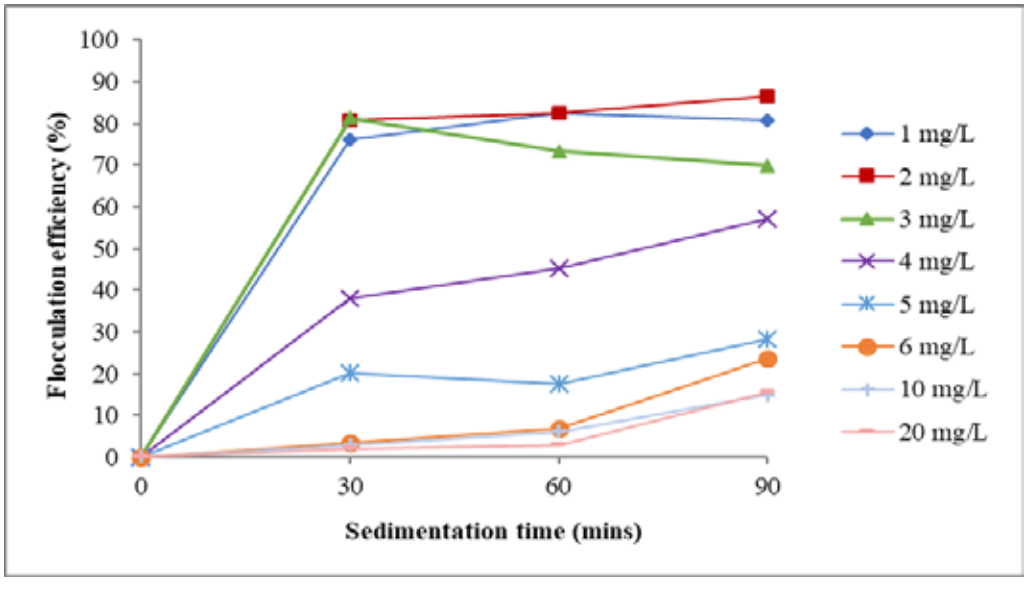


Figure 4 Effects of different dosages on flocculation efficiency of *C. vulgaris* using chitosan at pH value and stirring speed of 7 and 60 rpm, respectively.

those formed at a low stirring speed of 15 rpm and 30 rpm. High frequency of agitations had caused the flocs to break and unable to bind into large flocs. Nevertheless, more small flocs were able to form due to the increase in surface area of particles in contact and rate of charge neutralization with the increase of flocculants dispersion in the suspension [11], thus flocculation occurred more efficiently at high stirring speed.

For maize seed flocculants, the highest flocculation efficiency of 84.7 % was attained after 4 hours of sedimentation at 15 rpm. As in Figure 7, the results were found to be opposite of the findings for chitosan. For maize seed flocculant, low and medium stirring speed (< 60 rpm) resulted in flocculation efficiency of more than 80 % after 4 hours of sedimentation time. At the high stirring speed of 90 rpm and 120 rpm, the flocculation efficiencies were

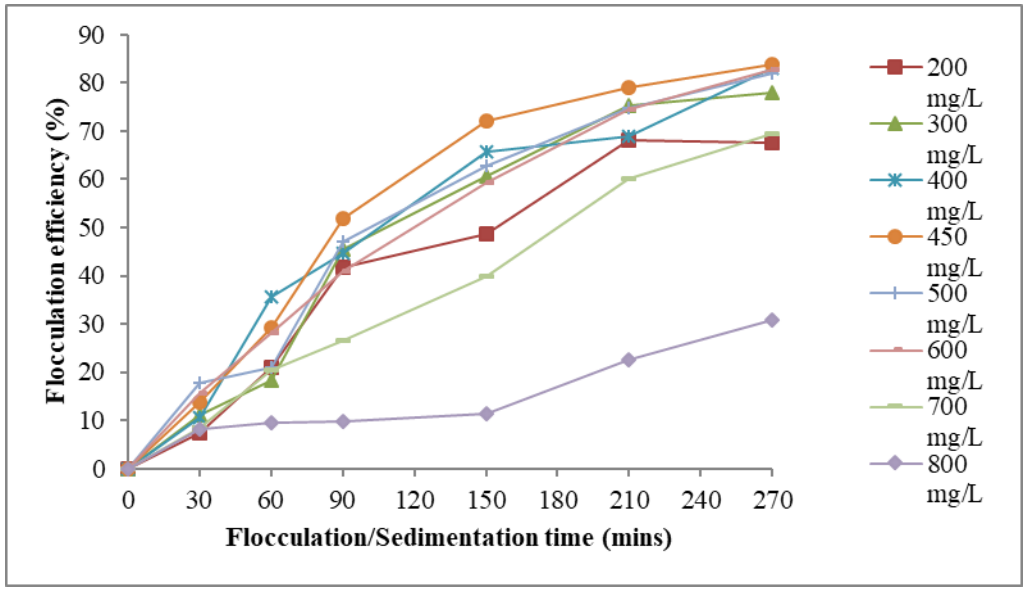


Figure 5 Effects of different dosages on flocculation efficiency of *C. vulgaris* using maize seeds flocculant at pH value and stirring speed of 7 and 60 rpm, respectively

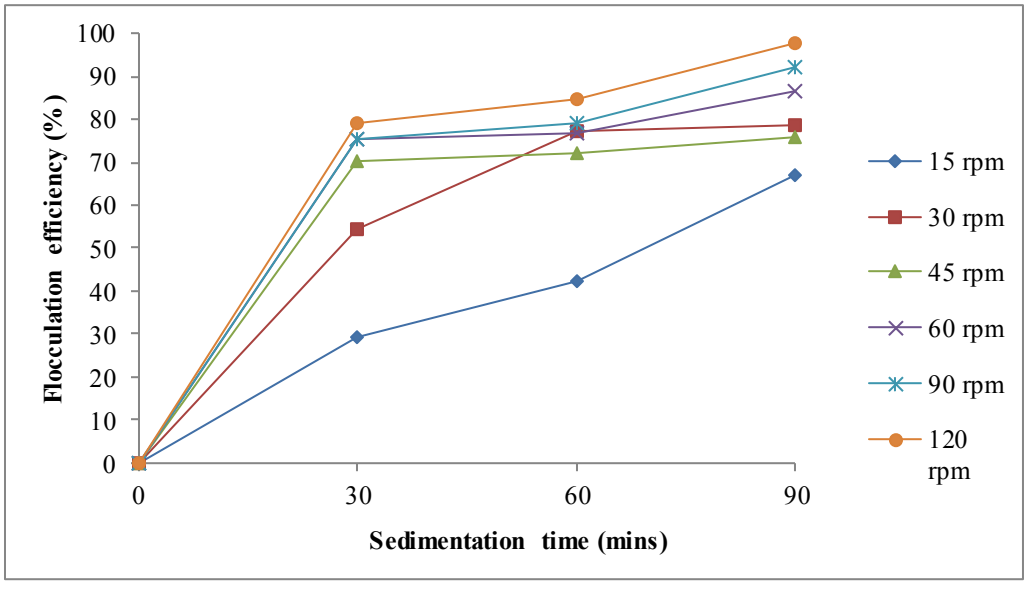


Figure 6 Effects of different stirring speeds on flocculation efficiency of *C. vulgaris* using chitosan at pH value and dosage of 7 and 2 mg/L, respectively

significantly reduced. This can be related to the observation where the size of the flocs produced decreased with the increase of stirring speed, possibly due to the high frequency of collisions. Since maize seeds flocculant formed very small flocs at optimum pH and dosage, high agitations would further break the flocs easily, thus decreasing the settling rate and causing the harvesting to be less effective via flocculation [11].

seeds flocculant, respectively. For chitosan, it was able to achieve 97.1 % of flocculation efficiency after 1 hour whereas 82.1 % was achieved for maize seeds flocculant after 4 hours sedimentation as shown in Figure 8.

Chitosan was a more effective flocculant to harvest *C. vulgaris* as compared to maize seeds extract as the dosage needed for

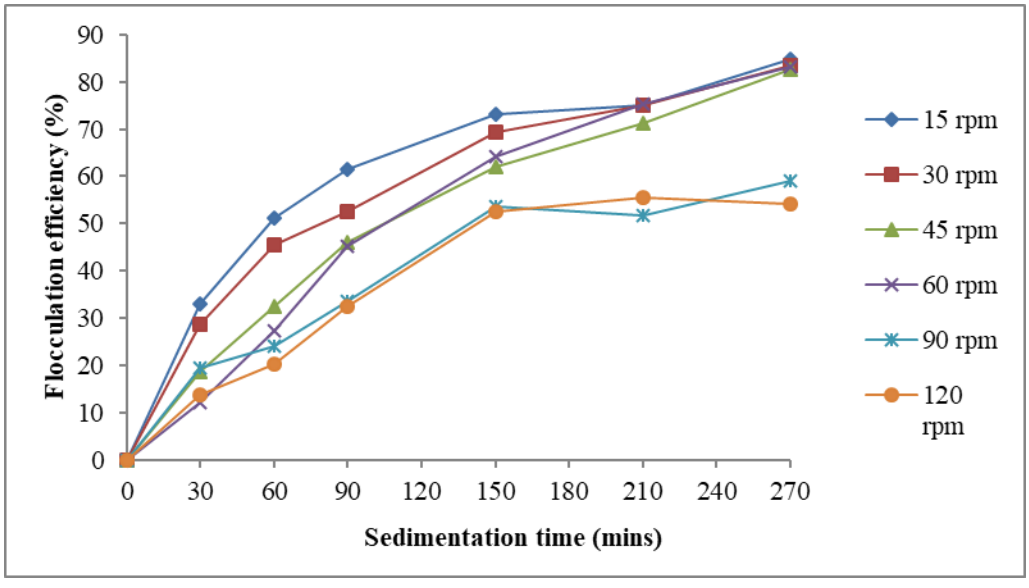


Figure 7 Effects of different stirring speeds on flocculation efficiency of *C. vulgaris* using maize seeds flocculant at pH value and dosage of 3 and 450 mg/L, respectively

Table 1 Optimum flocculation conditions for chitosan and maize seed flocculant

Optimum parameter	Chitosan	Maize seeds
pH	pH 7	pH 3
Flocculant dosage (mg/L)	2	450
Stirring speed (rpm)	120	15
Flocculation efficiency (%)	98	85
Flocculation time (hour)	1	4

3.4 Scale-up study: Comparison of chitosan and maize seeds flocculants under optimum flocculation conditions

The optimum flocculation conditions including pH, flocculant dosage and stirring speed to harvest I L of *C. vulgaris* using chitosan and maize seeds flocculant were tabulated in Table 1.

When the flocculation was scaled-up to harvest 1 L of *C. vulgaris* biomass under the optimized conditions, the results were comparable to the experimental test results using 500 mL of microalgae suspension. There were only minor differences of about 1 % and 3 % in flocculation efficiency for chitosan and maize

maize seeds flocculant was several hundred folds higher while the sedimentation time required was 4 times of that needed by chitosan. Besides that, the time taken by chitosan to produce flocs was only about 2 to 3 minutes as compared to maize seeds flocculant which took 15 to 20 minutes for the floc formation. The size of flocs formed by chitosan also was larger than flocs formed by maize seeds flocculant, as displayed in Figure 9. Figure 10 clearly showed the end results of *C. vulgaris* harvesting where the suspension become clear after flocculation and sedimentation process.

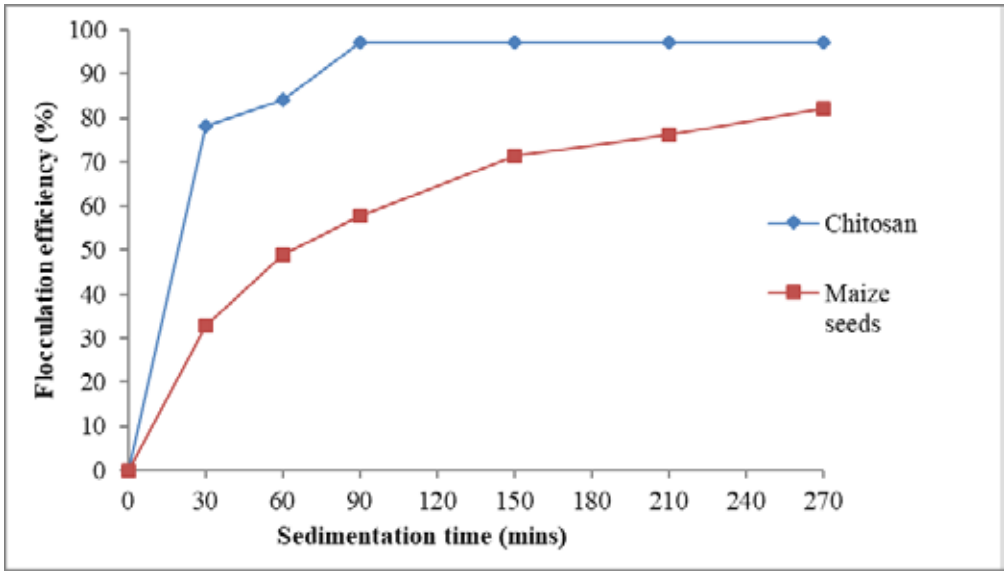


Figure 8 Comparison of flocculation efficiencies of *C. vulgaris* using chitosan and maize seeds flocculant

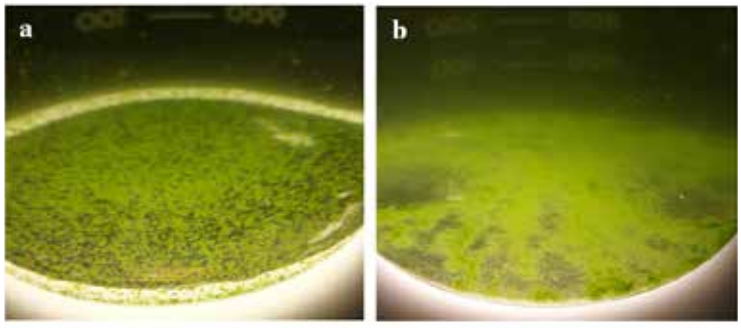


Figure 9 Flocs settled at bottom of beakers for (a) chitosan and (b) maize seeds flocculant after 1 and 4 hours of sedimentation time, respectively

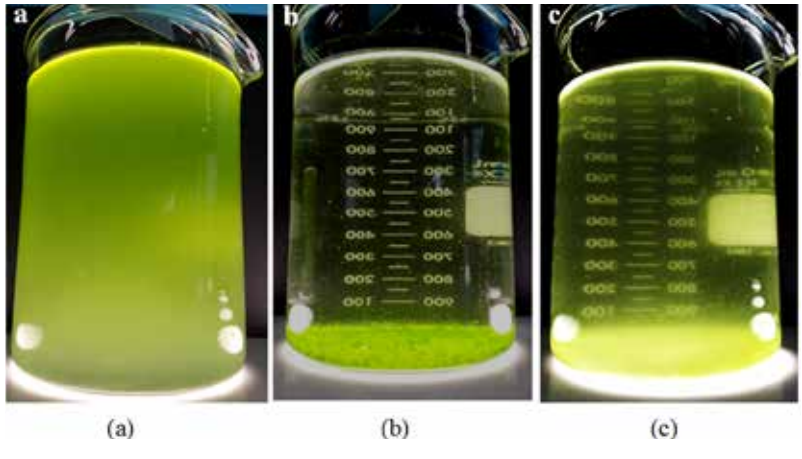


Figure 10 *C. vulgaris* sample (a) before harvesting, (b) harvested by chitosan after one (1) hour of sedimentation time and (c) harvested by maize seeds flocculant after four hours of sedimentation time

3.5 Comparison of flocculants from natural resources in harvesting *C. vulgaris* biomass

In this study, chitosan prepared using distilled water yielded higher flocculation efficiency to harvest *C. vulgaris* biomass than those reported in the literature. About 98 % of flocculation efficiency was achieved in present finding using only 2 mg/L of chitosan flocculant whereas 30 mg/L of chitosan prepared using citric acid, nitric acid, phosphoric acid and hydrochloric acid resulted in lower flocculation efficiency of 5.1, 54.5, 69.3 and 84.5 %, respectively [34]. Therefore, this experimental work serves as the cost-cutting protocol in excluding the involvement of chemicals to prepare chitosan as a flocculant to harvest microalgae cells. Latest literature collection on the potential of plant seed extracts as flocculants revealed that relatively high dosages about 1000 and 600 mg/L of *Moreinga oleifera* seeds and flour, respectively were required in order to recover *C. vulgaris* at 84 and 80 % of flocculation efficiencies, respectively, for both flocculants [13]. However, lower dosage (450 mg/L) requirement for maize seed flocculant to harvest *C. vulgaris* suspension up to 85 % of flocculation efficiency in the current study proved the effectiveness and feasibility of maize seed extract in microalgae harvesting. Application of flocculants from different natural resources to harvest *C. vulgaris* biomass was presented in Table 2.

Conclusion

Natural flocculants like chitosan and maize seeds were able to flocculate *C. vulgaris* effectively under optimized conditions. At pH 7, using 2 mg/L of chitosan had resulted in 98 % of flocculation efficiency with 120 rpm of stirring speed. Meanwhile, for maize seeds flocculant, about 85 % of flocculation efficiency could be achieved by setting the optimum flocculation parameters at pH 3, the dosage of 450 mg/L and stirring speed of 15 rpm. Flocculation process of *C. vulgaris* is greatly affected by the surface charges and interactions between flocculants and microalgae cells. In principles, the surface of microalgae cells is more positively charged in acidic medium and more negatively charged in basic medium; whereas, the dosages of flocculant added altered the surface charges of cells to prevent stabilization due to like-charges repulsion. Stirring speed too had influenced the interactions between particles in the microalgae suspension and caused flocs breakage. To summarize, cationic chitosan appeared to be a better natural flocculant than maize seeds in harvesting *C. vulgaris* biomass, by considering the dosage needed, size of the flocs formed, sedimentation time and flocculation efficiency as evaluation basis.

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Table 2 Comparison of flocculation performances of natural flocculants in harvesting *C. vulgaris* biomass

Natural resources	Flocculants	Dosage (mg/L)	Flocculation efficiency (%)	Reference
Crustacean shells	Chitosan in citric acid	30	5.1	[34]
	Chitosan in nitric acid	30	54.5	[34]
	Chitosan in phosphoric acid	30	69.3	[34]
	Chitosan in hydrochloric acid	30	84.5	[34]
	Chitosan in distilled water	2	98	This study
Plant seeds	*Moreinga oleifera* seeds	1000	84	[13]
	Moreinga oleifera seed flour	600	80	[13]
	Maize seeds	450	85	This study

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