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Microalgae harvesting by flotation using natural saponin and chitosan

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HIGHLIGHTS

- Biosurfactant (saponin) and chitosan were used in microalgae harvesting.
- Very effective separation for microalgae cells, polysaccharide, and protein.
- The process can be applied in the integrated microalgae-based biorefinery.

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ABSTRACT

This study aims to investigate the harvesting of microalgae by dispersed air flotation (DiAF) using natural biosurfactant saponin as the collector and chitosan as the flocculant. Two types of microalgae, *Chlorella vulgaris* and *Scenedesmus obliquus*, were used in this study. It was observed that saponin was a good frother, but not an effective collector when used alone for flotation separation of algae. However, with the pre-flocculation of 5 mg/L of chitosan, separation efficiency of >93% microalgae cells was found at 20 mg/L of saponin. Removal efficiency of >54.4% and >73.0% was found for polysaccharide and protein, respectively at 20 mg/L of saponin and chitosan each. Experimental results show that DiAF using saponin and chitosan is effective for separation of microalgae, and algogenic organic matter (AOM). It can potentially be applied in the integrated microalgae-based biorefinery.

1. Introduction

In the face of fossil fuels shortages and curbs on greenhouse gas emissions, the potential for biodiesel production from microalgal lipids and for CO_2 mitigation due to photoautotrophic growth of microalgae has been recognized (Yen et al., 2013). Microalgae are receiving increasing attention worldwide as an alternative and renewable source for energy production (Pragya et al., 2013). Algae can assimilate CO_2 photoautotrophically or mixotrophically, and are a perfect candidate for CO_2 sequestration and greenhouse gas reduction. On the other hand, seasonal algae bloom can cause adverse effects on water treatment processes, such as shortening of filter run, formation of disinfection by-products, production of several toxins, and unpleasant taste and odor. Therefore, harvesting or removal of algae from water is an important issue for both energy and environment viewpoints (Hung and Liu, 2006; Nguyen et al., 2013).

Harvesting microalgae poses a challenging task to engineers because microalgae are small-size microorganisms suspended in water (Lam and Lee, 2012). The harvesting process is of particular interests because it accounts for significant energy consumption and cost can be high as the mass fractions in the broth are generally low, while electrostatic repulsion between negatively charged cells and excess amount of algogenic organic matters (AOM) render separation difficult (Lam and Lee, 2012; Pragya et al., 2013; Tran et al., 2013). Efficient harvesting of biomass from either open pond or photobioreactor is essential for mass production of biodiesel from microalgae. The major techniques currently applied in microalgae harvesting include centrifugation, gravity sedimentation, flocculation, filtration, flotation, and integrated techniques (Pragya et al., 2013). Flotation is an effective method for algae separation from water that takes advantage of their natural characteristics of relatively low density and self-floating tendency (Garg et al., 2012). Other advantages of flotation include: rapid reaction time, small footprint, flexibility, and moderate operational cost (Chen et al., 1998; Henderson et al., 2008). Some collectors (or frothers) are used to facilitate the flotation separation process. Chemically synthesized collectors, such as sodium dodecylsulfate (SDS) and N-cetyl-N-N-N-trimethylammonium bromide (CTAB) have been employed, as well as natural substance like methylated egg ovalbumin (Chen et al., 1998; Maruyama et al.,

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2009; Henderson et al., 2010; Coward et al., 2013). CTAB can be used for simultaneous harvesting and cell disruption (Huang and Kim, 2014). Compared with synthetic surfactants, biosurfactants have advantages because they are natural compounds, which generally are more biodegradable and less toxic (Rahman and Gakpe, 2008). Saponin is a representative plant-derived biosurfactant. It consists of a heterogeneous group of sterol glycosides and triterpenoid glycosides that are present in a wide range of plant species that are distributed throughout the bark, leaves, stems, roots and even flowers (Chen et al., 2008). Saponin has been used to modify bentonite for methylene blue and malachite green adsorption (Kurniawan et al., 2011,2012). It can also be used in soil washing to remove phenanthrene and heavy metals (Chen et al., 2008; Zhou et al., 2013).

Carbohydrates from microalgae are now regarded as a class of high-value bioactive compounds with potential applications in food, cosmetics, textiles, emulsifiers, and clinical drugs (Yen et al., 2013). Commonly used inorganic coagulants may contaminate biomass and bring adverse effects toward the final product quality, especially if some bioactive compounds are included (Lam and Lee, 2012; Yen et al., 2013). Therefore, natural polymers, such as cationic cassis gum has been studied for algal biomass harvesting (Banerjee et al., 2014). In addition, chitosan has been used as an effective flocculant for algal harvesting (Tran et al., 2013; Vandamme et al., 2014). Chitosan has several advantages when compared with conventional metal salt coagulant: high cationic charge density, long polymer chain, non-toxic and biodegradable. Its use in algal biomass harvesting causes little problems for subsequent application of the recovered biomass and the recycling of the culture medium (Xu et al., 2013). Chitosan is also considered as one of the most promising materials in the coagulation/flocculation process for water treatment (Bhatnagar and Sillanpaa, 2009).

We utilized saponin and chitosan in flotation separation of two types of microalgae in the current study with the aim to develop a novel process for microalgae harvesting from water. To our best knowledge, this is the first study that utilized both biosurfactant and biopolymer in algal separation and harvesting.

2. Methods

Chlorella vulgaris was isolated from a shrimp pond located in southern Taiwan (Tran et al., 2013). It was cultured in the Bold's Basal Medium (BBM). Prior to transferring to a photobioreactor (PBR) as the inoculum, *C. vulgaris* was grown in a flask at 36 °C for 10 days under a light intensity of 6800 lux. The PBR was a 2-L glass vessel illuminated with an external light source (PLS-LAX500) mounted on both sides. Carbon dioxide (5.72% by volume) was continuously supplied at a flow rate of 25 mL/min. It was operated under a stirring rate of 239 rpm. The liquid sample was also collected from the sealed glass vessel with respect to time to determine algae cell concentration. *Scenedesmus obliquus* was cultured in a modified version of Detmer's Medium (DM). After pre-culture in 200-mL flasks, the *S. obliquus* cells were inoculated into a 2-L PBR with an inoculum concentration of 15 mg/L. *S. obliquus* was grown in the PBR at 28 °C for 12 d under a light source (PLS-LAX500) at the intensity of 6800 lux with a stirring speed of 300 rpm.

Protein analysis was performed using a modified Lowry method with bovine serum albumin as the standard solution. Polysaccharide analysis was performed using Anthrone method with glucose as the standard solution (Nguyen et al., 2013). Each sample was measured 3 times and the average value was taken. Turbidity was measured using a turbidity meter (Hach 2100P). Zeta potential was analyzed by a zeta meter (Malvern Zetasizer 2000). Particle size distribution before and after coagulation–flocculation was examined using a small-angle light scattering instrument (Malvern Mastersizer 2000).

Dispersed air flotation (DiAF) experiments were conducted using a flotation column with inside diameter of 5 cm and 47 cm in length (Nguyen et al., 2013). There is a lipped side arm at 5 cm from the top of the column as the foam discharge port, a gas sparger (pore size 10–16 μm, Merck) at the bottom of the column, and a side arm with stopcock for sampling. Nitrogen gas passes through a pressure regulator (Norgren), a flowmeter (J & W), and a humidifier (Merck) before flows into the column. N-cetyl-N-N-N-trimethylammonium bromide (CTAB, Merck) and saponin were used as collector, respectively. Saponin was extracted from dried fruit of a wild plant Sapindus rarak DC, which is a tall tree commonly found in Asia and Africa. The extraction procedures consist of crushing, sieving, hot water extraction, centrifugation, and vacuum drying. It has functional groups of hydroxyl, ester carbonyl, aromatic, and alkane in aliphatic and alicyclic structure (Kurniawan et al., 2011,2012). Measured amounts of stock solutions of collector, and algae were added to a 500-mL volumetric flask, and placed on a stirrer (Corning). The pH was adjusted with 0.5 M NaOH and 0.5 M HNO_3. Steady flow rate of N_2 (80 mL/min) was adjusted before 200 mL of suspension was transferred to the flotation column. The duration of flotation was 20 min. For DiAF with pre-flocculation, stock solution of chitosan was made by dissolving 100 mg of chitosan (MW: 100 k–300 k g/mol, Acros) in 10 mL of 1% HCl solution and mixed with a stirrer at 100 rpm for 30 min. Saponin and chitosan were added into 100 mL microalgae suspension in a 100 mL beaker by pipette, it was stirred at 100 rpm for 3 min before flotation experiments.

3. Results and discussion

3.1. Removal of microalgae cells, polysaccharide and protein

Characteristics of the two microalgae suspensions are shown in Table 1. *C. vulgaris* suspension was in neutral pH and *S. obliquus* suspension was slightly alkaline. Both had relatively low protein and high polysaccharide contents. Microalgae cells are negatively charged, though both showed distinctly different zeta potential values. Saponin and CTAB were used as the collector and frother in separating microalgae by flotation process. As can be seen in Fig. 1**a**, flotation separation efficiency increased with CTAB dose and resulted in very good separation (>93.7%) of both microalgae cells at CTAB dose of 60 mg/L. The dose was higher than 20 and 30 mg/L as reported in literature (Coward et al., 2013), while comparable to that of our previous work (Nguyen et al., 2013). The difference could attribute to influences of algogenic organic matters (AOM) that varied with culture condition. When saponin was used, the separation efficiency was limited and did not increase much as saponin dose increased, and only 22.5% removal was found at a dose of 100 mg/L. It indicated that CTAB was more effective for separating microalgae cells than saponin. The removal efficiency was similar for both microalgae when either CTAB or

Table 1
Characteristics of microalgae suspension.

Parameter	*C. vulgaris*	*S. obliquus*
pH	6.89 ± 0.4	7.77 ± 0.05
Absorbance	1.48 ± 0.2	2.6 ± 0.2
Protein (mg/L)	6.65 ± 0.06	10.88 ± 0.49
Polysaccharide (mg/L)	107.2 ± 0.1	122.4 ± 0.1
ζ-potential (mV)	−30.3 ± 2.4	−13.43 ± 0.4

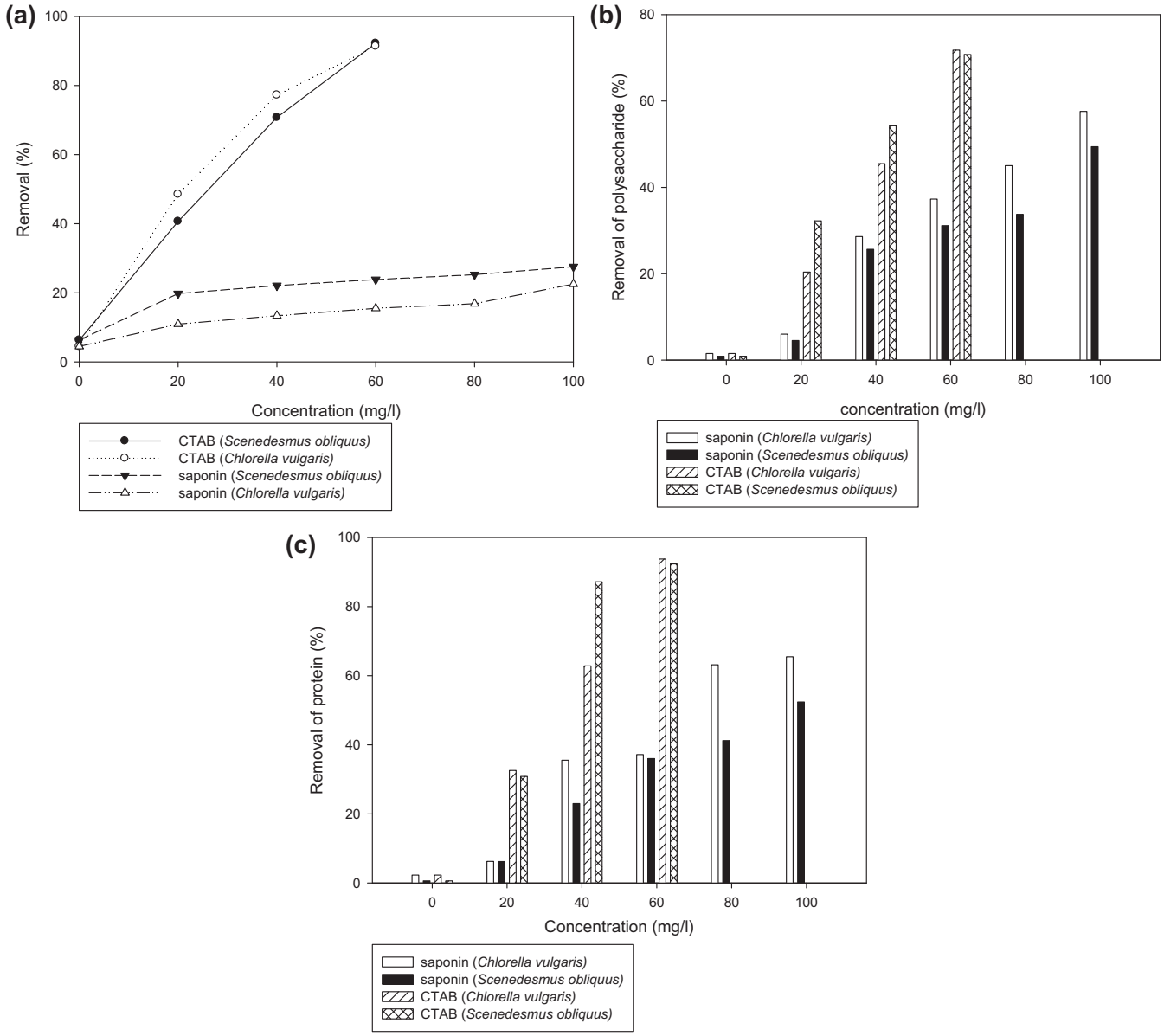

Fig. 1. Effect of collector dose of two different collectors on the separation of (a) microalgae cells; (b) polysaccharide; (c) protein.

saponin was used. It indicated that the characteristics of microalgae, including size and shape were not significantly related to the flotation separation efficiency (Henderson et al., 2008). Cationic CTAB is adsorbed onto the cell surfaces via electrostatic interactions, and then cells became more hydrophobic and easier to be separated from aqueous phase (Chen et al., 1998). Surface hydrophobicity of microalgae cells increased with increasing collector dose, and higher flotation separation efficiency was obtained when the surface became more hydrophobic (Garg et al., 2012). In addition, the flocculation brought about by CTAB adsorption is beneficial to flotation as well because larger flocs resulted in increased gas–solid collision efficiency (Lien and Liu, 2006). On the other hand, the adsorption of saponin onto algal cells lacked significant driving force due to its anionic character (Chen et al., 2008). Judging from experimental results, saponin was a good frother because abundant bubbles were generated. However, it was not a good collector when used alone.

Knowing that AOM will affect the separation of algal biomass, the separation of polysaccharide and protein was examined. Fig. 1b shows that 20.4% and 32.2% polysaccharide were removed at 20 mg/L of CTAB for *C. vulgaris* and *S. obliquus* suspensions, respectively. Lower removal efficiency was found at 20 mg/L of saponin, though efficiency increased as saponin dose became higher. Over 70% polysaccharide was removed at 60 mg/L of CTAB for both algae, while slightly lower efficiency was found when using saponin. As for protein, removal efficiency was higher than 90% when at 60 mg/L of CTAB, and ca.<40% for saponin at identical dose (Fig. 1c). Overall, CTAB was significantly better than saponin in separating polysaccharide and protein. This is in agreement with previous work that CTAB could induce separation of AOM (Nguyen et al., 2013).

Separation of algae by using fixed amount of saponin (20 mg/L) and various chitosan doses is shown in Fig. 2a. Flotation separation efficiency of both species of microalgae cells significantly improved when chitosan concentration varied from 0 to 20 mg/L. The amino groups of chitosan can react with the anionic amide group and carboxylic group on the algae cell surface via electrostatic interaction (Pranowo et al., 2013). It was apparent that the

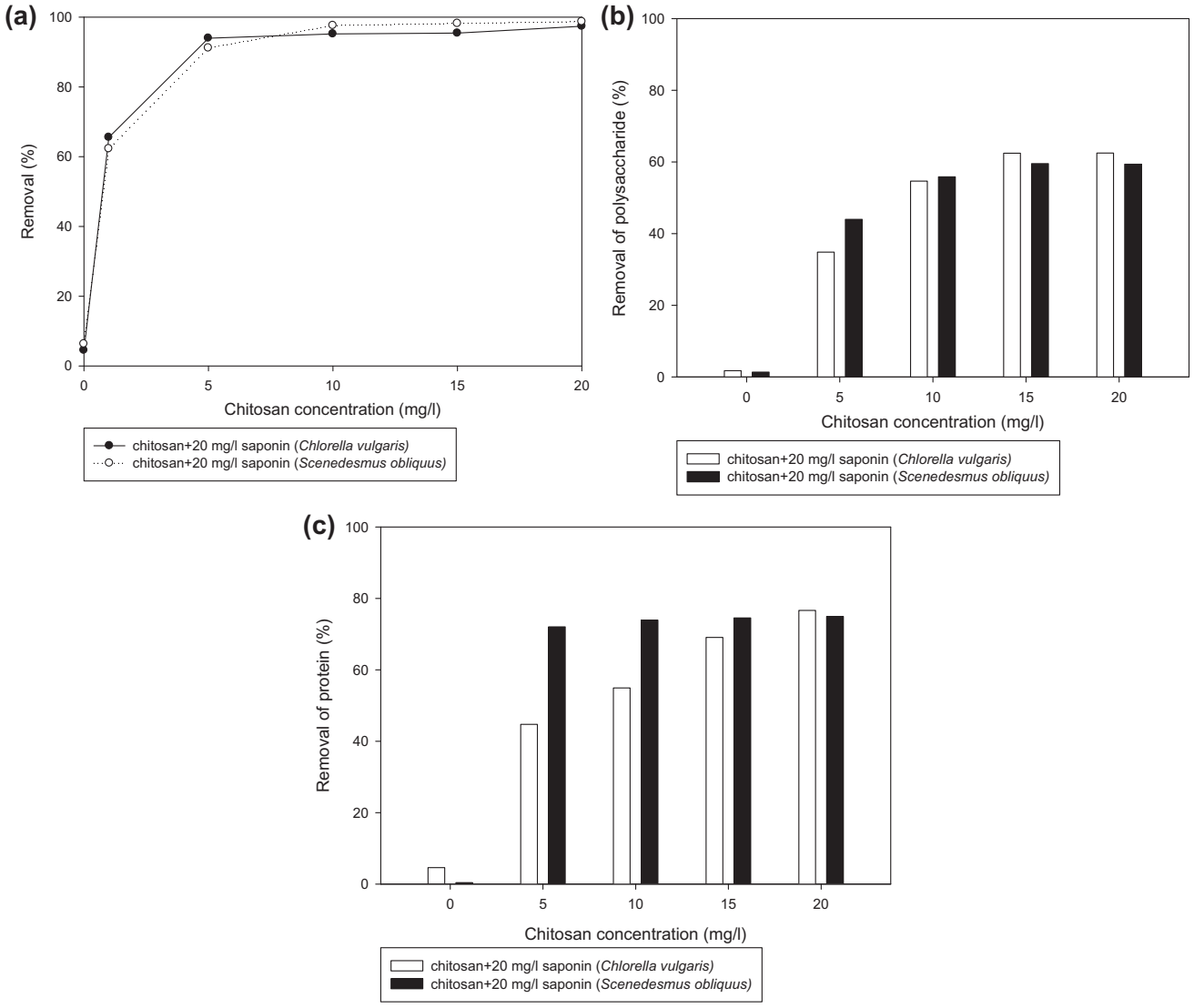

Fig. 2. Effect of chitosan dose (at 20 mg/L of saponin) on the separation of (a) microalgae cells (b) polysaccharide; (c) protein.

addition of chitosan induced effective flocculation and the mechanisms involved are adsorption and charge neutralization (Tran et al., 2013). It is noted that the presence of the AOM enhances flocculation of microalgae and very large flocs are formed (Pranowo et al., 2013; Vandamme et al., 2014). Consequently, flotation separation efficiency improved as a result of the flocculation by chitosan, which is beneficial for flotation. Fig. 2b and c show polysaccharide and protein removal when using saponin and chitosan. Removal of polysaccharide remarkably increased at 5 mg/L of chitosan, and increased with increasing chitosan dose. Total of >52.9% was removed at chitosan dose of 20 mg/L. Similarly for protein, its removal increased with increasing chitosan dose and reached >73.09% at 20 mg/L of chitosan. These results again indicated the AOM interacted with chitosan and resulted in enhanced separation efficiency from water. It has been reported that chitosan is able to form complexes with polyanions such as alginate for the enhancement of protein and water recovery efficiency (Widjaja et al., 2009).

3.2. Zeta potential and size distribution

Henderson et al. (2010) demonstrated that zeta potential can be used to assess flocculation and flotation of microalgae. Fig. 3a shows the profile of zeta potential for both species of microalgae as affected by CTAB and saponin dose. The zeta potential of *C. vulgaris* became significantly less negative due to the addition of CTAB. However, only slight change was found for *S. obliquus*. It can be reasoned that the adsorption of cationic CTAB caused charge neutralization of algae cells. On the other hand, saponin dose had little effect on zeta potential of algal suspensions, probably because saponin is hardly adsorbed for lack of electrostatic interaction. The slight increase of zeta potential as influenced by saponin to *C. vulgaris* hinted limited adsorption of saponin via hydrogen bonding or van der Waals force. It is in agreement with aforementioned results that saponin was a poor collector when used alone. Fig. 3b shows the profile of zeta potential for both species of microalgae when saponin and chitosan were used. The adsorption of chitosan onto the microalgae cells made the shift in the zeta potential as expected. Although no charge reversal was found, it has been widely accepted that patch flocculation is good for effective flocculation of microalgae cells and full charge neutralization may not be necessary (Rashid et al., 2013). The positively charged patches on flocs could serve as active sites for saponin adsorption. Chitosan acts as an activator in the flotation process that facilitates effective flotation separation using saponin.

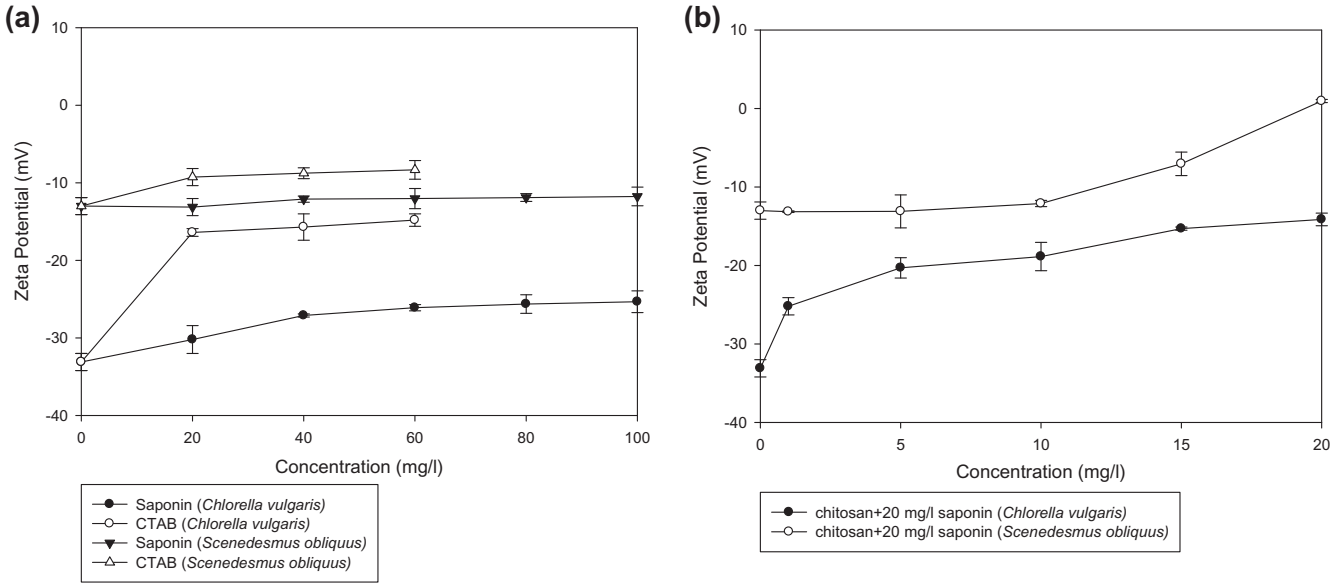

Fig. 3. Zeta potential of *C. vulgaris* (pH = 6.41 ± 0.2) and *S. obliquus* (pH = 7.29 ± 0.2) (a) as affected by collector dose; (b) as affected by chitosan dose (at 20 mg/L of saponin).

Table 2
Median diameter (volume-based) of microalgae cells as affected by collector dose.

Collector dose (mg/L)	*C. vulgaris* (μm)	*S. obliquus* (μm)
0	3.8	7.8
Saponin (20 mg/L)	3.8	7.4
Saponin (60 mg/L)	3.7	8.6
Saponin (100 mg/L)	4.0	7.1
CTAB (20 mg/L)	14.4	19.0
CTAB (60 mg/L)	984.3	23.0

Particle size distribution was assessed in this study to correlate the relationship between floc size and flocculation efficiency. Table 2 shows the size distribution of microalgae cells as affected by collector dose. The median diameter of *C. vulgaris* was 3.8 μm, while that of *S. obliquus* was 7.8 μm. It is noted that saponin had little effect on microalgae size at dose from 20 to 100 mg/L. However, the median diameter of *C. vulgaris* increased to 14.4 μm and 984.3 μm, and that of *S. obliquus* to 19.0 μm and 23.0 μm at CTAB dose of 20 and 60 mg/L, respectively. Due to its long-chain character, CTAB could also act as a flocculant as well (Lien and Liu, 2006).

The median diameter of *C. vulgaris* doubled after flocculation by 1 mg/L of chitosan. It then increased remarkably with chitosan dose and median diameter of 419.8 μm was found at 20 mg/L of chitosan (Fig. 4a). Similarly for *S. obliquus*, it increased slightly from 7.8 μm to 9.1 μm at chitosan dose of 1 mg/L, and increased as chitosan dose became higher (Fig. 4b). Combining results from zeta potential and size analysis, it could be concluded that saponin worked effectively in combination with chitosan for flotation separation of algal cells. Although saponin was not effective for

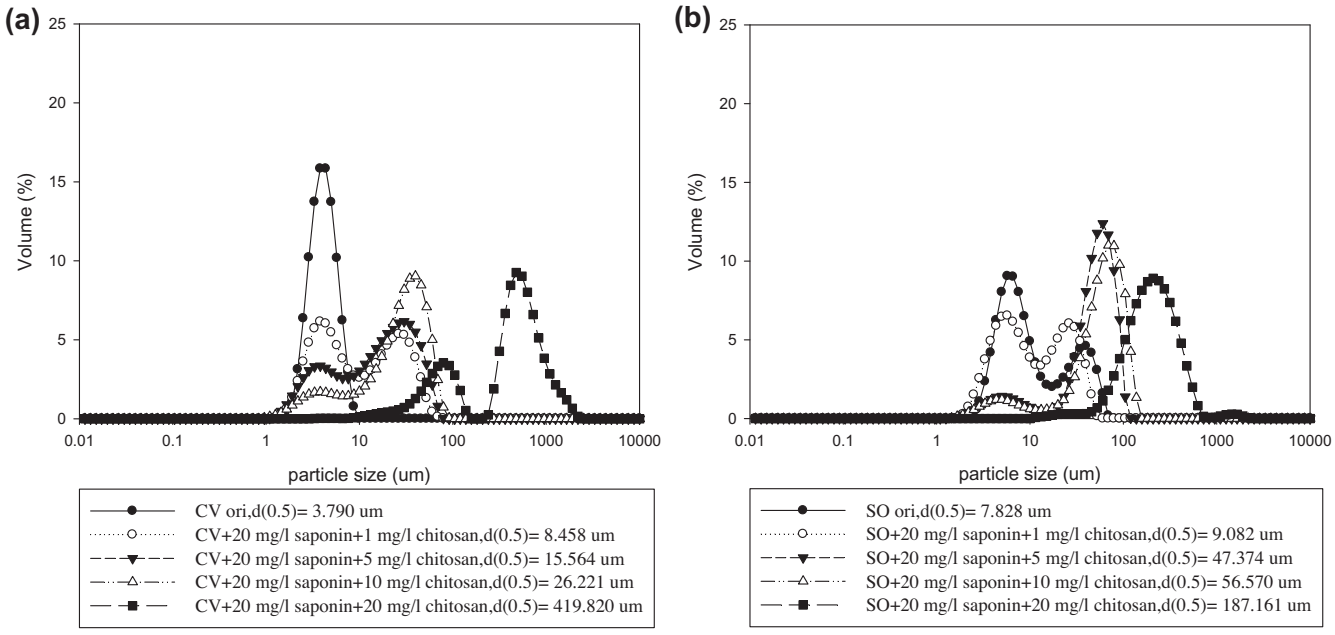

Fig. 4. Volume-based size distribution as affected by chitosan dose (at 20 mg/L of saponin) for (a) *C. vulgaris*; (b) *S. obliquus*.

cells separation when used as collector in DiAF, and very high separation efficiency was observed for algal cells as well as polysaccharide and protein.

Compared with synthetic cationic surfactants such as CTAB or dodecyl amine (DAC), chitosan-saponin system showed distinct advantages since both are natural chemicals. In reviewing potential for biodiesel production from microalgae, the importance of microalgae-based biorefinery has been emphasized (Lam and Lee, 2012; Yen et al., 2013). In addition to biodiesel production, algae biomass has other valuable components, including carbohydrates, long chain fatty acids, pigments and proteins. For example, algal polysaccharides can be regarded as new bioactive materials that find many downstream applications in food, cosmetics, textiles, and etc. (Yen et al., 2013). Researchers have shown that biosurfactants are generally more biocompatible and digestible (Rahman and Gakpe, 2008). It is apparent that combined use of two natural chemicals, chitosan and saponin, in DiAF possesses special advantage in case biorefinery is considered as an integral part of microalgae-based biorefinery. When applied in flotation separation of algae, the system demonstrated effective separation of algae cells and AOM. Thus, it is full of potential to further develop highly efficient flotation separation process using biosurfactant and biopolymer.

4. Conclusions

Dispersed air flotation using saponin and chitosan for separation of microalgae was investigated. Combined use of saponin and chitosan yielded high separation efficiency of microalgae cells, polysaccharide, and protein. Saponin played the role of frother and collector, while chitosan induced flocculation for the enhanced flotation separation. The study demonstrated that the use of saponin-chitosan, both natural chemicals, is full of potential to be applied in the integrated microalgae-based biorefinery.

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