Malaysian Journal of Microbiology, Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Efficiency of chitosan and eggshell on harvesting of *Spirulina* sp. in a bioflocculation process

Yuen Hing Lai, Fatin Hamimi Md Azmi, Nur Ain Fatehah, Soopna Puspanadan and Chee Keong Lee*

Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.
Email: cklee1311@yahoo.co.uk

Received 26 June 2018; Received in revised form 19 November 2018; Accepted 22 November 2018

ABSTRACT

Aims: Microalgae were very small in size (a few µm) and have a low concentration in the medium. Due to their size, harvesting of microalgae from their growth medium remain a major obstacle in downstream processing. Efficient harvesting method must be applied to ensure it is cost effective, preserves quality and improves the culture process which is important for commercial algal production. Common harvesting methods use to harvest microalgae from their growth medium are centrifugation, filtration, flotation, sedimentation, and flocculation. Flocculation is a common method use to harvest microalgae due to low cost, save time and highly efficient method for algae biomass recovery. The purpose of this study was to investigate the effects chitosan and eggshell on flocculation of microalga *Spirulina platensis*. Chitosan and eggshell were chosen as flocculant due to their biodegradability, non-toxicity and safe to handle.
Methodology and results: The efficiencies of flocculation process were examined by conducting experiments over a range of culture pH, flocculant concentrations and flocculation time using chitosan and eggshell as flocculant agent. Under optimized flocculation conditions of 50 mg/L chitosan at pH 8 culture media for 90 min of flocculation time and 4 mg/mL eggshell at pH 4 culture media for 8 min of flocculation time, the maximum flocculation efficiency obtained was 79.98±1.65% and 97.17±1.38%, respectively.
Conclusion, significance and impact of study: Therefore, it can be concluded that chitosan and eggshell could be used as flocculants for harvesting large scale microalgal biomass production. Nevertheless, eggshell is more economical and more efficient compared to chitosan in harvesting microalgae biomass.

Keywords: Bioflocculation, *Spirulina platensis,* chitosan, eggshell, flocculation efficiency

INTRODUCTION

Algae were first used by the Chinese to survive a famine that can be traced back to 2000 years ago (Saravanamuthu, 2010). Nevertheless, microalgal biotechnology has started to establish in the middle of the last century. In the early of 1960s, the first commercial pilot plant of *Chlorella* was established in Japan by Nihon Chlorella (Richmond, 2008). It was followed by the establishment of *Spirulina* production facility in the late 1970s in Lake Texcoco, Mexico (Vonshak, 1997; Borowitzka, 1999). Nowadays, microalgal biomass has become a potential feedstock for biofuels production due to its advantageous characteristics. Microalgal can be grown in a broad range of environments such as wastewater, brine and non-arable land. In addition, algae have higher biomass productivity compared to green plant and crops. Hence, microalgae can be used to supersede the food crops such as potato and corn in the production of biodiesel in order to mitigate global food crisis (Demirbas and Demirbas, 2011; Maheswari and Ahilandeswari, 2011; Sani *et al.*, 2013).

Microalgae cultivation is very simple. It can be classified as open system such as open pond or closed system such as photo-bioreactor. Photo-bioreactor is the simplest way to cultivate microalgae. Thus, light is important to increase growth of microalgae and subsequently increase production of microalgae biomass through carbon fixation process (Milano *et al.*, 2016). Production of microalgae has so far been limited to high-value applications. In order to realize large-scale production of microalgae biomass for low-value applications, new low-cost technologies are needed to produce and process microalgae. A major challenge lies in the harvesting of the microalgae.

Harvesting process in the microalgal biomass production is critical which accounts for about 20-30% of the total production cost. This process is costly and

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

challenging due to their small size (3-30 µm in diameter) and low biomass concentration in growth medium (Gudin and Thepenier,1986; Grima *et al.*, 2003). Therefore, an efficient and economic harvesting technique is necessary to be developed. There are several methods that have been developed for harvesting microalgae such as centrifugation, filtration, gravity sedimentation, flocculation, electro-flocculation and foam fractionation (Chen *et al.*, 2011; Salim *et al*., 2011; Kothari *et al.*, 2017). Among the above methods, flocculation is considered to be a promising method to harvest microalgae because it is relatively cheap and allows rapid treatment of huge quantities of microalgae cultures (Oh *et al.*, 2001). Flocculation of microalgae can be achieved through chemical, biological, and physical methods and by genetic modification. In the flocculation process, the microalgae suspended growth medium are destabilized by the addition of flocculants in which the destabilized particles aggregate to form flocs and effectively removed by sedimentation (Divakaran and Pillai, 2002; Vandamme *et al*., 2010; Salim *et al.*, 2011).

Bioflocculation is a biological method because it is using the organic substances or compounds for harvesting of microalgae. During flocculation, single cells form larger aggregates that can be separated from the medium by simple gravity sedimentation. This method excludes the use of chemical coagulants. However, it involves integration with other type of microorganism to induce flocculation. For example, the hyphae of fungus are positively charged can neutralised the negatively charged microalgae cells and thus induce by resulted formation of flocs (Zhou *et al.*, 2013). The production of these bioflocculants requires different cultivation conditions which acquire additional medium costs and increases the risk of microbial contamination of the medium (Vandamme *et al.*, 2013).

Chitosan is a well-known positively charged biopolymer which is a waste product from shellfish production. It has linear copolymer of d-glucosamine and N-acetyl-d-glucosamine that produced by the deacetylation of chitin (Rinaudo, 2006). Chitosan intrinsically possesses high cationic charge density, long polymer chains, bridging of aggregates and precipitation (in neutral or alkaline pH conditions) that make it an effective flocculants for microalgae harvesting (Bratby, 2006). It also consists of others beneficial properties such as non-toxic, biodegradable, non-corrosive and safe to handle. Nonetheless, chitosan also possesses disadvantages for flocculation in which it is only efficient over a limited pH range and a negative effect on flocculation performance would be happened when it exceeded the optimal dose (Guibal, 2004; Renault *et al.*, 2009). Recently, Choi (2015) suggested that waste eggshell can be used as a flocculants to harvest microalgae from the growth medium. Eggshell consists of several beneficial properties as a flocculant because it is readily available, cheap, biodegradable, biocompatible, non-toxic and non-corrosive as compared to other chemical flocculants. In addition, eggshell possesses enormous cationic charge density in which it can easily attract and destabilize the negative surface charges of microalgae in suspension as a result of flocculation.

Thus, in this research, harvesting of microalgae *Spirulina platensis* using bioflocculant such as eggshell and chitosan was performed. The flocculation efficiency was compared in order to determine its suitability for used in harvesting of *S. platensis*.

MATERIALS AND METHODS

Microalgae cultivation

The microalgal species used in this study was *S. platensis*. The culture (1 L) was grown in Zarrouk's medium using 2 L Erlenmeyer flask, with continued bubbling filtered air (2 L/min), at a room temperature (25±1 °C) under continuous illumination using cool white fluorescence lights at light intensity of 28 µmol photons $m^{-2}s^{-1}$ (2000 Lux). The culture was harvested at early stationary phase and stored in a refrigerator at 4 °C for subsequent used in the flocculation experiments. Each liter of Zarrouk's medium consisted of (g/L): sodium bicarbonate ($NaHCO_3$, 16.89), sodium nitrate ($NaNO_3$, 2.50), sodium chloride (NaCl, 1.00), dipotassium phosphate (K_2HPO_4, 0.25), potassium sulfate (K_2SO_4, 1.00), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, 0.20), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, 0.04), iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 0.01), disodium ethylenediaminetetraacetate dihydrate (Na_2EDTA, 0.08) and 1.0 mL of trace element solution. The trace element solution has the following composition (g/L): boric acid (H_3BO_3, 2.86), ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}$, 0.02), chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$, 1.80), copper (I) sulfate (Cu_2SO_4, 0.08) and zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, 0.22) (Zarrouk, 1966).

Preparation of flocculants

Chitosan was obtained in the form of a pale brown powder which is soluble in dilute acetic and hydrochloric acids. Chitosan powder was dissolved in 10 mL of 0.1 M HCl solution before it was further diluted to a final concentration of 25 mg/mL using distilled water.

Eggshell waste was collected from the cafeteria at Universiti Sains Malaysia. The eggshells were washed with tap water to remove the unwanted substances attached on its surface. The eggshells were then rinsed with distilled water and dried in an oven at 65 °C for overnight (Binder hot air oven, USA). The dried eggshells were ground into fine powder by mortar and pestle. Then, the eggshell powder was sieved mechanically using a 325-mesh size sieve. The eggshell powder was dissolved in 10 mL of 1M HCl with continuous stirring for 30 min. The final eggshell solution (1.0 mg/mL) was obtained by diluting the acid solution using deionized water (Elga PURELAB Prima 7-15-30, United Kingdom).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

Determination of flocculation efficiency

A lab-scale Jar Test (VELP Scientifica, model JLT4, Europe) was used to determine the flocculation performance of eggshell and chitosan as bioflocculant on harvesting of microalgae *S. platensis* under various conditions (pH, flocculants concentration and flocculation time). Before the flocculation process begin, the optical density (OD) of the test samples were recorded at wavelength of 680 nm by UV-Vis spectrophotometer (Hitachi Model U-1900). Then, the samples (500 mL) were stirred at 100 rpm for 5 min prior to the addition of flocculant (chitosan or eggshell separately). The samples were continuous mixed at 100 rpm for 1 min prior to reduce the agitation speed to 40 rpm and stirred for another 15 min. Thereafter, the samples were allowed to settle (10 min) and an aliquot of the samples were withdrawn and measured the optical density at wavelength 680 nm using UV-Vis spectrophotometer at (Hitachi Model U-1900). The flocculation efficiency was calculated using the following equation (Huo *et al.*, 2014). Experimental conditions which contributed to the highest flocculation efficiency was chosen for used in next experiment unless otherwise stated.

$$\text{Flocculating efficiency (\%)} = \left(1 - \frac{A}{B}\right) \times 100$$

Where,
A= is the absorbance reading of the sample at specified time interval and
B= is the initial absorbance reading of the sample.

Factors affect flocculation of *S. platensis*

For the effects of culture pH, the experiments were conducted using various culture pH ranging from 4 to 8 (1 pH unit interval). Other the other, for the effects of flocculants concentration, the experiments were conducted using different concentration of eggshell (2 mg/L to 10 mg/L, 2 mg/L interval) and chitosan (50 mg/L to 250 mg/L, 50 mg/L interval). Finally, for the effect of flocculation time, the experiments were conducted for 2 to 16 min (2 min interval) and 15 to 120 min (15 min interval) using chitosan and eggshells, respectively. Thereafter, the samples were allowed to settle (10 min) and an aliquot of the samples were withdrawn and measured the OD at wavelength 680 nm using UV-Vis spectrophotometer at (Hitachi Model U-1900). The flocculation efficiency was calculated according to the equation described above.

Statistical analysis

All data reading was in triplicates. Data was analysed statistically by single factor analysis of variance (ANOVA) using IBM SPSS Inc. software 20.0 (SPSS Inc, Illinois, USA). Data presented are from the mean values of triplicate readings. The comparisons of means were made using Duncan's test.

RESULTS AND DISCUSSION

Effect of culture pH on flocculation of *S. platensis*

The pH is critical in the harvesting of microalgae by flocculation because of the zeta potential of charged particles in the suspension changes with pH and eventually it may influences the flocculation (Clasen *et al.*, 2000; Divakaran and Pillai, 2002; Chaiwong and Nuntiya, 2008; Vandamme *et al.*, 2010). The flocculation efficiencies of chitosan and eggshell were tested by adding 250 mg/mL chitosan and 8 mg/mL eggshell accordingly into 500 mL of test cultures under various pH conditions as shown in Figure 1. The flocculation efficiencies of chitosan were relatively low under acidic conditions (pH 4 to 6) which ranged from 57.12±1.76% to 76.17±6.15%. This probably due to the change of the molecular structure of chitosan in which the net positive charges (cationic) of chitosan is minimum under acidic condition (Huei and Hwa, 1996). The positive charges of chitosan are required to interact with the net negative charge carried out by the microalgae cell surface (acidic condition) and thus formation of flocs and precipitation. This consequently increased the flocculation efficiency at higher pH condition.

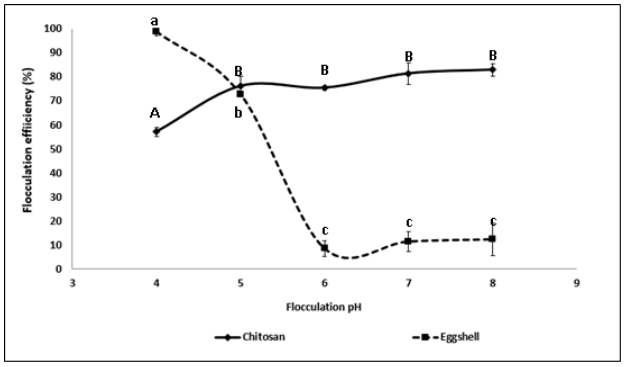

Figure 1: Effect of pH on flocculation of *S. platensis* using chitosan and eggshell. Error bars indicate means with standard error of three replicates. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan's test.

Nevertheless, the flocculation efficiencies of chitosan were higher under neutral (pH 7) and alkaline (pH 8) conditions which were more than 80%. Moreover, the addition of chitosan caused the formation of large and dense flocs under alkaline conditions meanwhile relatively small flocs with dispersed texture were formed under acidic conditions.

In this study, the maximum flocculation efficiency of chitosan was 82.97±2.60% at pH 8 and followed by 81.43±4.50% at pH 7. Although the flocculation efficiencies of chitosan have no significant change ($P>0.05$) between pH 7 and pH 8, pH 8 should be chosen as optimum pH for chitosan to flocculate the algae as it was relatively close to the condition of original algae

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

culture medium. Divakaran and Pillai (2002) reported that the maximum flocculation efficiency of chitosan on flocculation of freshwater microalgae was obtained at pH 7 with about 90% removal meanwhile a higher optimal pH was obtained by Cheng *et al.* (2011) which was pH 8.5. According to Roussy *et al.* (2005) and Guibal *et al.* (2006), the ionic flocculant like chitosan has shown significant impact on flocculation process over the changes of pH. Moreover, Qian *et al.* (2004) reported that the structure of flocculants in suspension has a significant impact on the flocculation performances. The change of pH influences the molecular structure of chitosan in which the positive charges of chitosan disappeared and precipitated with coil structure under alkaline conditions (Huei and Hwa, 1996).

On the other hand, the flocculation efficiencies of eggshell were high under acidic conditions. The flocculation efficiencies decreased with increasing of pH reaching minimum at pH 8. The highest flocculation efficiency of eggshell was 98.92±1.60% which was obtained at pH 4 and followed by 72.88±1.07% at pH 5. On top of that, the flocculation activities of eggshell rapidly decreased at higher pH (pH 6 to 8) which ranged from 8.53±3.13% to 12.32±6.80%.This indicated that eggshell was more effective in removal of microalgae under acidic condition (pH 4, $P<0.05$) where the net negative charge carried by the microalgae cell surface most likely favors the electrostatic attraction between the eggshells and microalgae. In fact, the change of pH influences the physicochemical interactions between eggshells and microalgae as well as the structure of eggshell in suspension. Moreover, eggshells have a high cationic charge density and attract the negatively charged microalgae as a result of charge neutralization and precipitation Choi (2015). However, Choi (2015) reported that the optimal pH was pH 6 which was higher compared to the present study. Henderson *et al.* (2008) suggested that the difference in culture media, growth conditions and unique strain properties, such as cell morphology, extracellular organic matter and cell surface charge would also influence on the flocculation performance.

Effect of flocculant concentrations on flocculation of *S. platensis*

Flocculant concentration is another important factor that influences the extent of flocculation efficiency. A range of flocculant concentrations was used to study the effect of flocculant concentration on flocculation activity of chitosan and eggshell which has shown in Figure 2. The pH of test culture was adjusted to pH 8 (Chitosan) since it is the optimum pH with maximum flocculation efficiency (Figure 2A). The overall flocculation efficiency of chitosan was high in the ranged of 81.60±0.606% to 84.38±1.872% with different tested chitosan concentrations. The highest flocculation efficiency of 84.38% was obtained using 150 mg/L of chitosan. However, the flocculation efficiency was decreased to 81.60±0.606% with an increasing concentration of chitosan to 250 mg/L. In this study, 50 mg/L chitosan has been chosen as the optimum flocculant concentration because it achieved high flocculation efficiency (82.08±0.92%) with a minimum amount of chitosan usage. In addition, statistical analysis also indicated no significant difference ($P>0.05$) in flocculation efficiency obtained among tested chitosan concentrations. One of the flocculation mechanisms of chitosan-based flocculant is the simple charge neutralization in which the electrostatic repulsions decrease to a minimum value when the given dose of flocculant stabilizes the dispersed particles in the suspension by completely neutralized its surface charges. The dispersed particles aggregate to form large flocs and eventually settle. As the flocculant dose in excess, the suspended particles are surrounded by flocculant and the zeta potential deviates from zero. The recharged particles are then stabilized again by the electrostatic repulsion effects which cause reduction in flocculation efficiency. As referred to this mechanism, the optimal dose of chitosan should be determined to avoid re-stabilization in order to achieve maximum flocculation efficiency (Guibal *et al.*, 2006; Yang *et al.*, 2011).

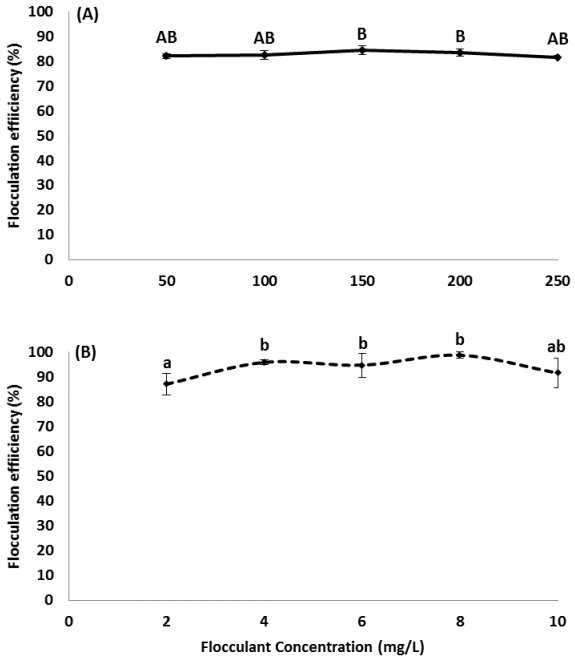

Figure 2: Effect of flocculant concentration on flocculation of *S. platensis* using: (A) chitosan and (B) eggshell. Error bars indicate means with standard error of three replicates. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan's test.

On the other hand, the flocculation efficiencies of eggshell over a range of tested concentrations were higher than 85% (higher than flocculation efficiency using chitosan). The highest flocculation efficiency (98.71±1.32%) was achieved using 8 mg/L of eggshell at

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

pH 4. While, the lowest flocculation efficiency of 81.74±4.33% was detected using 2 mg/L of eggshell. According to Rashid *et al.* (2013), the zeta-potential of microalgae culture generally increased with flocculants dose which then reduced the surface charges of microalgae as a result of neutralization by eggshells. The zeta potential was considered as a key indicator of the dispersion stability of microalgae and eggshells in suspension where high (negative or positive) zeta potentials tend to stabilize the microalgae and remain dispersed in suspension whereas low zeta potentials allow coagulation or flocculation to occur (Choi, 2015). In this study, the selected optimal eggshell concentration for flocculation was 4 mg/L with flocculation efficiency of 95.86±1.00% since it achieved high flocculation efficiency using relatively low concentration of flocculant. On top of that, statistical analysis showed no significant different in flocculation efficiency obtained using 4 mg/L compared with 8 mg/L (showing the highest flocculation efficiency). Therefore, 4 mg/L eggshell was considered favorable to achieve over 95% separation efficiency of *S. platensis*.

Effect of flocculation time on flocculation of *S. platensis*

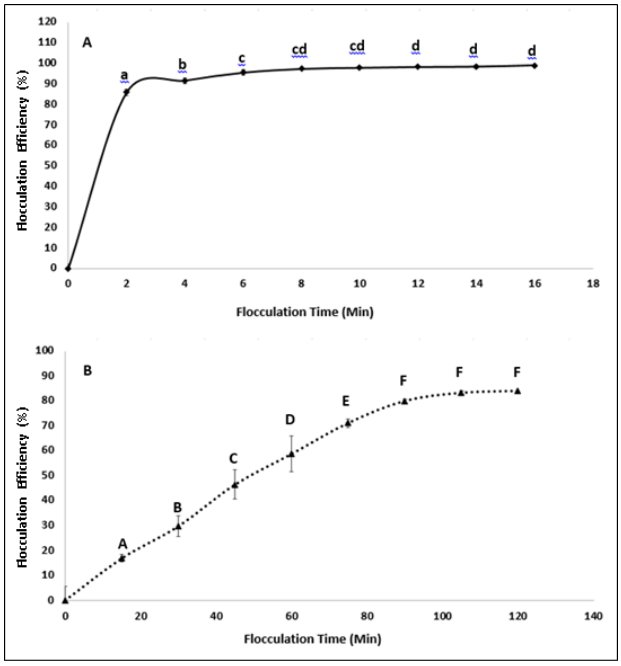

Figure 3: Effect of flocculation time on flocculation of *S. platensis* using (A) chitosan and (B) eggshell. Error bars indicate means with standard error of three replicates. Means with the same letter indicated no significantly difference at 5% level of probability by Duncan's test.

The time taken for the microalgae cell to settle down after flocculation process also play a big role in the harvesting of *S. platensis* biomass. As shown in Figure 3, rapid flocs of microalgae cell were detected using eggshell as flocculant compared with chitosan. About 85.81±2.29% of flocculation efficiency was detected after 2 min of flocculation process when eggshell was used as flocculant compared to chitosan which only achieved about 16.83±5.52% of flocculation efficiency after 15 min. This indicated that formation of flocs occurred more efficient and rapidly using eggshell compared with chitosan. This can be seen from Figure 4 in which the microalgae culture has separate into 2 layers with biomass sediment at the bottom after 2 min of flocculation process using eggshell. On top of that, the flocculation efficiency achieved > 90% after 4 min of flocculation time (eggshells) and reaching >98% after 12 min. In addition, statistical analysis indicated no significant different (P>0.05) in flocculation efficiency obtained between 8 min to 16 min of flocculation time.

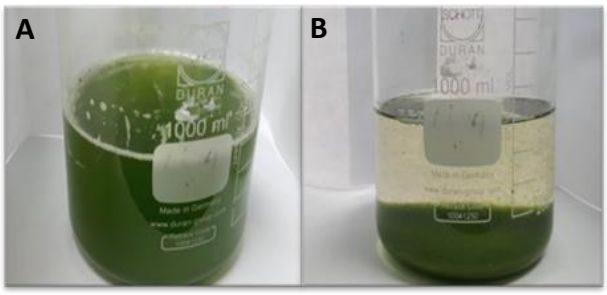

Figure 4: Comparison of the flocculation efficiency of *S. platensis* (A) before and (B) after adding eggshells flocculant at 40 mg/L at pH 4 for 2 min.

On the other hand, flocculation efficiency constantly increased with time when chitosan was used as flocculant and reaching maximum (84.04±0.72%) after 120 min of flocculation process. No flocculation process was performed beyond 14 min (eggshell) and 120 min (chitosan) because only minor changes in flocculation efficiency (P>0.05) was recorded. Based on previous studies, most of the findings demonstrated that the flocculation efficiency achieved 97% after 10 min (Divakaran and Pillai, 2002; Farid *et al.*, 2013) which was in agreement with present study using eggshell as flocculant.

CONCLUSION

The present study reveals that both flocculants (chitosan and eggshell) could be considered as a promising approach to harvest the *S. platensis* with maximum harvesting efficiency of 79.98±1.65% and 97.17±1.38% under the optimal conditions using 50 mg/L chitosan at pH 8 culture media for 90 min and 4 mg/L eggshell at pH 4 culture media for 8 min, respectively. Nevertheless, the harvesting of *S. platensis* using eggshell is more economical and more efficient compared to chitosan as eggshell has achieved higher flocculation efficiency and required lesser quantities. Additionally, it is also much cheaper and abundantly available.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(3) 2019, pp. 188-194
DOI: http://dx.doi.org/10.21161/mjm.180156

ACKNOWLEDGEMENTS

The author (Lee, C.K.) would like to express his greatest appreciation and gratitude to USM for given him USM Research Universiti (RUI) grant (1001/PTEKIND/811273). We would like to acknowledge the contribution from the School of Industrial Technology staffs, especially the laboratory assistant namely Mr. Azmaizan Yaakob and Mrs. Najmah Hamid. They have been very helpful in giving equipment assistance and providing for all my laboratory needs.

REFERENCES

Borowitzka, M. A. (1999). Commercial production of microalgae: ponds, tanks, tubes and fermenters. *Journal of Biotechnology* **70(1), 313-321.**

Bratby, J. (2006). Coagulation and flocculation in water and wastewater treatment. IWA publishing, London, United Kingdom. **pp. 60-75**.

Chaiwong, N. and Nuntiya, A. (2008). Influence of pH, electrolytes and polymers on flocculation of kaolin particle. *Chiang Mai Jounal of Science* **35, 11-16.**

Chen, C.-Y., Yeh, K.-L., Aisyah, R., Lee, D.-J. and Chang, J.-S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology* **102(1), 71-81.**

Cheng, Y.-S., Zheng, Y., Labavitch, J. M. and VanderGheynst, J. S. (2011). The impact of cell wall carbohydrate composition on the chitosan flocculation of *Chlorella*. *Process Biochemistry* **46(10), 1927-1933.**

Choi, H. J. (2015). Effect of eggshells for the harvesting of microalgae species. *Biotechnology and Biotechnological Equipment* **29(4), 666-672.**

Clasen, J., Mischke, U., Drikas, M. and Chow, C. (2000). An improved method for detecting electrophoretic mobility of algae during the destabilisation process of flocculation: Flocculant demand of different species and the impact of DOC. *Journal of Water Supply: Research and Technology-Aqua* **49(2), 89-101.**

Demirbas, A. and Demirbas, M. F. (2011). Importance of algae oil as a source of biodiesel. *Energy Conversion and Management* **52(1), 163-170.**

Divakaran, R. and Pillai, V. S. (2002). Flocculation of algae using chitosan. *Journal of Applied Phycology* **14(5), 419-422.**

Farid, M. S., Shariati, A., Badakhshan, A. and Anvaripour, B. (2013). Using nano-chitosan for harvesting microalga *Nannochloropsis* sp. *Bioresource Technology* **131, 555-559.**

Grima, E. M., Belarbi, E.-H., Fernández, F. A., Medina, A. R. and Chisti, Y. (2003). Recovery of microalgal biomass and metabolites: Process options and economics. *Biotechnology Advances* **20(7), 491-515.**

Gudin, C. and Thepenier, C. (1986). Bioconversion of solar energy into organic chemicals by microalgae. Advances in biotechnological processes (USA). New York, USA. **pp. 73-110.**

Guibal, E. (2004). Interactions of metal ions with chitosan-based sorbents: A review. *Separation and Purification Technology* **38(1), 43-74.**

Guibal, E., Van Vooren, M., Dempsey, B. A. and Roussy, J. (2006). A review of the use of chitosan for the removal of particulate and dissolved contaminants. *Separation Science and Technology* **41(11), 2487-2514.**

Henderson, R., Parsons, S. A. and Jefferson, B. (2008). The impact of algal properties and pre-oxidation on solid–liquid separation of algae. *Water Research* **42(8), 1827-1845.**

Huei, C. R. and Hwa, H.-D. (1996). Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane. *Carbohydrate Polymers* **29(4), 353-358.**

Huo, S., Wang, Z., Zhu, S., Cui, F., Zou, B., You, W., Yuan, Z. and Dong, R. (2014). Optimization of alkaline flocculation for harvesting of *Scenedesmus quadricauda* 507 and *Chaetoceros muelleri* 862. *Energies* **7(9), 6186-6195.**

Kothari, R., Pathak, V. V., Pandey, A., Ahmad, S., Srivastava, C. and Tyagi, V. (2017). A novel method to harvest *Chlorella* sp. *via* low cost bioflocculant: Influence of temperature with kinetic and thermodynamic functions. *Bioresource Technology* **225, 84-89.**

Maheswari, N. U. and Ahilandeswari, K. (2011). Production of bioplastic using *Spirulina platensis* and comparison with commercial plastic. *Resource Environment and Life Science* **4(3),133-136**.

Milano, J., Ong, H. C., Masjuki, H., Chong, W., Lam, M. K., Loh, P. K. and Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews* **58, 180-197.**

Oh, H.-M., Lee, S. J., Park, M.-H., Kim, H.-S., Kim, H.-C., Yoon, J.-H., Kwon, G.-S. and Yoon, B.-D. (2001). Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. *Biotechnology Letters* **23(15), 1229-1234.**

Qian, J., Xiang, X., Yang, W., Wang, M. and Zheng, B. (2004). Flocculation performance of different polyacrylamide and the relation between optimal dose and critical concentration. *European Polymer Journal* **40(8), 1699-1704.**

Rashid, N., Rehman, M. S. U. and Han, J.-I. (2013). Use of chitosan acid solutions to improve separation efficiency for harvesting of the microalga *Chlorella vulgaris*. *Chemical Engineering Journal* **226, 238-242.**

Renault, F., Sancey, B., Badot, P.-M. and Crini, G. (2009). Chitosan for coagulation/flocculation processes–an eco-friendly approach. *European Polymer Journal* **45(5), 1337-1348.**

Richmond, A. (2008). Handbook of microalgal culture: Biotechnology and applied phycology. John Wiley and Sons. Oxword, United Kingdom. **pp. 3-20.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science* **31(7), 603-632.**

Roussy, J., Van Vooren, M. and Guibal, E. (2005). Influence of chitosan characteristics on coagulation and flocculation of organic suspensions. *Journal of Applied Polymer Science* **98(5), 2070-2079.**

Salim, S., Bosma, R., Vermuë, M. H. and Wijffels, R. H. (2011). Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology* **23(5), 849-855.**

Sani, Y., Daud, W. and Abdul Aziz, A. (2013). Biodesel feedstock and production technologies: Successes, challenges and prospects. *Intech, Open Access Journal* **77-94**.

Saravanamuthu, R. (2010). Industrial Exploitation of Microorganisms. IK International Pvt Ltd. New Delhi, India. **pp. 70-85**.

Vandamme, D., Foubert, I., Meesschaert, B. and Muylaert, K. (2010). Flocculation of microalgae using cationic starch. *Journal of Applied Phycology* **22(4), 525-530.**

Vandamme, D., Foubert, I. and Muylaert, K. (2013). Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in Biotechnology* **31(4), 233-239.**

Vonshak, A. (1997). *Spirulina platensis* arthrospira: Physiology, cell-biology and biotechnology. CRC Press, London. **pp. 79-100.**

Yang, Z., Shang, Y., Lu, Y., Chen, Y., Huang, X., Chen, A., Jiang, Y., Gu, W., Qian, X. and Yang, H. (2011). Flocculation properties of biodegradable amphoteric chitosan-based flocculants. *Chemical Engineering Journal* **172(1), 287-295.**

Zarrouk, C. (1966). Contribution a l'etude d'une cyanobacterie: Influence de divers facteurs physiques et chimiques et la photosynthese de Spirulina maxima (Setchell et Gardener) Geitler. PhD. Thesis, University of paris, France.

Zhou, W., Min, M., Hu, B., Ma, X., Liu, Y., Wang, Q., Shi, J., Chen, P. and Ruan, R. (2013). Filamentous fungi assisted bio-flocculation: A novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Separation and Purification Technology* **107, 158-165.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538