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Development of Bioflocculant from Chicken's Eggshell Membrane to Harvest *Chlorella vulgaris*

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Abstract. As microalgae biomass is considered as the most assuring source of biodiesel, flocculation has become a potential technology that could be able to alleviate microalgae dewatering cost which is the cornerstone hindrance of their full-scale application. However, large scale harvesting of microalgae biomass using commercial flocculating agents is obstructed by economic and environmental drawbacks upon downstream discharge. Thus, in the present work, a novel introduction of natural flocculant extracted from waste biomass, which is, chicken's eggshell membrane was made to harvest *Chlorella vulgaris*. Flocculation tests were carried out to test the effectiveness of the natural flocculant to recover microalgae biomass. Chicken's eggshell membrane was proven to be one of the effective bioflocculant as it achieved above 60 % of flocculation efficiency after 1 hour of sedimentation with optimum flocculation parameters of pH 11.8 with 80 mg/L of flocculant dosage at 40 °C.

1. Introduction

The fossil fuels fulfil the energy needed by the world about 90 % and 45 % out of it is concurred by petroleum [1]. As domestic product per capita is the main target of improvement for many countries, statistic showed that the fossil fuel demand will increase, and subsequently placing these limited resources at competition pace [2]. Additionally, lavish utilization of fossil fuel causes acceleration of atmospheric CO₂ concentration and climate change [3]. Besides, petroleum that is being derived from ancient algae deposits, is a non-renewable commodity which will run out or become too expensive to reacquire [2]. Therefore, development of renewable energy that can substitute these depleting conventional fuels is a critical need. Few renewable energy sources are being sought and the most promising sustainable alternatives to fossil fuel are the biofuels, including biodiesel and aviation fuels.

Commercial biodiesel is mainly produced from different type of feedstock such as pure vegetable oil, waste cooking oil and animal fat. However, it is impractical to use these feedstocks due to high cost and limited supply of these sources to meet the massive demand of biodiesel. Apparently, microalgae biomass is being regarded as a promising raw material for generation of biodiesel and other biochemical products [3]. This is owing to the fact that, microalgae cells posse positive traits such as high growth rate, short doubling time and less requirement for water as well as land in comparison to traditional crops [4].



Nevertheless, recovery of microalgae biomass from the dilute suspension remains a major drawback in biodiesel production due to micro-sized cells (3-20 μm) of microalgae, diluted concentration in water and colloidal stability [4]. By estimation, the capital investment of biomass recovery was above 30 % of the total production of microalgae biodiesel. Among the common harvesting methods (e.g. filtration, ultra-filtration, flocculation, sedimentation and centrifugation), flocculation is the primary option to separate the solid biomass from the culture medium due to low cost [5]. Through flocculation process, the microalgae cells are aggregated and thus, easily settled through gravitational sedimentation or flotation [6]. Flocculants can be divided into two categories which are inorganic flocculants (e.g. poly-aluminium chloride and aluminium sulfate) and also organic polymeric flocculants (e.g. polyethyleneimine) through secretion by microorganisms [7–10] or bioflocculants that can be extracted from waste biomass. The use of inorganic flocculants can result in serious health and environmental problems such as toxicity, instability and water pollution [11].

Thus, bioflocculants have received considerable amount of attention from researchers as they are regarded as non-toxic and biodegradable substitutes for harmful synthetic flocculants in many industrial fields such as sludge treatment plants and microalgae harvest [11]. Till date, *Moreinga oleifera*, a plant-based waste have been discovered as effective bioflocculant to treat wastewater and harvest microalgae species [12]. However, there is very less research studies have been conducted on utilization of shell-based wastes for bioflocculation purposes. On the other hand, chickens' eggshells are major waste products of the food industry and produced in large quantities [13]. In effort to transform these wastes into useful biomaterials, chickens' eggshells have been tested to flocculate *Chlorella vulgaris* and bioflocculants extracted from chickens' eggshell was proved as ideal bioflocculants as it achieved 99% of harvesting efficiency [14]. But, to our best knowledge, there is no flocculation trials have been made using chicken's eggshell membrane. Apart from presence of amines and amides, chicken's eggshell membrane are targeted to contain positively charged functional groups that expected able to neutralize negatively charged microalgae cells to form flocs through flocculation process [15]. Thus, this work was aimed to test flocculability of chicken's eggshell membrane and to evaluate the flocculation efficiency of *C. vulgaris* using the extracted bioflocculant. The effect of various flocculation parameters such as pH values of medium, bioflocculant dosage and temperature towards flocculation efficiency of microalgae were also methodically investigated.

2. Experimental

2.1. Pure microalgae strain and culture conditions

A wild-type *Chlorella vulgaris* was supplied by Prof. Dr. Lee Keat Teong, Universiti Sains Malaysia. The microalgae was preserved and grown in Bold's Basal Medium (BBM), consisting of: (1) 10 mL per liter of culture medium using the following chemicals: NaNO_3 (25 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.5 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (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) 1 mL per liter of culture medium using the following chemicals: EDTA anhydrous (50 g/L), KOH (31 g/L), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (8.82 g/L), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (1.44), MoO_3 (0.71 g/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (1.57 g/L), $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.49 g/L). The initial pH of the medium was adjusted to 6.8. The culture was grown in a 100 mL Erlenmeyer flask containing 50 mL of medium, aerated with compressed air, surrounding temperature of 25–28 $^\circ\text{C}$ and illuminated continuously with cool-white fluorescent light (Philip TL-D 36 W/865, light intensity of 60–70 $\mu\text{mol m}^{-2}\text{s}^{-1}$) [16].

2.2. Cultivation of microalgae with compost

Chicken compost with granular shape was purchased from a local Tesco supermarket. 10 g of the fertilizer was immersed in 600 mL tap water and stirred for 24 hours using a magnetic stirrer. The fertilizer solution was filtered using filter paper (Double Rings 101). Subsequently, 200 mL of the fertilizer medium was introduced into a photobioreactor with 5 liter of tap water (without sterilization) and the pH of the medium was adjusted according to 3 to 3.5. Then, 500 mL of *C. vulgaris* from the seed culture was introduced into the photobioreactor. The photobioreactor was aerated with compressed

air continuously and illuminated with cool-white fluorescent light (Philip TL-D36W/865, light intensity of 60–70 l mol m⁻²s⁻¹) [16].

2.3. Preparation of bioflocculant

Collected chickens' eggshells were washed using distilled water and the membranes were peeled off to be dried at 102 °C in an oven. 100 mg of the membrane peels were dissolved in 10 mL of 0.5 mol/L hydrochloric acid solution and continuously stirred for 30 minutes using a magnetic stirrer. The resulting solution was filtered through filter paper and the filtrate was then marked up to 100 mL with deionized water to a final bioflocculant concentration of 1000 mg/L [14].

2.4. Flocculation experiment for parameter studies

The bioflocculants extracted from chicken's eggshell membrane was tested preliminarily for their ability to flocculate *C. vulgaris* in a beaker at room temperature. The microalgae culture medium was diluted with tap water to maintain the initial absorbance at 2.0 ± 0.05 Abs. 0.8 mL of bioflocculants was introduced into 10 mL of microalgae cells in a 50 mL beaker and stirred for 1 minute. After mixing, the pH of the mixture was slightly increased until visible flocs were formed. The steps were replicated at higher working volume, 400 mL of *C. vulgaris* in 800 mL beaker using 32 mL of and the settling time was further increased to 60 minutes. For the beginning, the dosage of bioflocculant added was fixed at 80 mg/L and the flocculation experiment was conducted at room temperature. In the present work, the effects of pH values of medium, flocculant dosage and temperature on the flocculation efficiency on *C. vulgaris* were analysed.

2.5. Measurement of flocculation efficiency

Flocculation efficiency of *C. vulgaris* was evaluated by the relation:

$$\begin{aligned} &\text{Flocculation efficiency (\%)} \\ &= \frac{OD_{688}(t_0) - OD_{688}(t)}{OD_{688}(t_0)} \times 100\% \end{aligned} \quad (1)$$

Where $OD_{688}(t_0)$ is the turbidity of the sample recorded at time zero and $OD_{688}(t_1)$ is the turbidity of the sample recorded at time t. This sample was taken at the midst height of beaker for control and microalgal suspension containing bioflocculant. A graph of optical density was plotted against different time interval.

3. Results and Discussion

3.1. Effect of pH values of medium

Physical characteristics of chicken's eggshell membrane and physicochemical reactions between the membrane particles and microalgae cells could be the fundamental of pH effects on flocculation efficiency of *C. vulgaris* [14]. PH changes can affect the surface charges of microalgae cells, making them to be protonated when the pH values are lowered and deprotonated with increase in pH [14]. In fact, unstability in the surface charge of *C. vulgaris* cells affect the interaction between flocculant particles and the performances of flocculation as well [17]. The efficiency of flocculation of *C. vulgaris* cells at varied pH values was investigated as shown in Figure 1. In the presence of fixed dosage of 80 mg/L of flocculants in a working volume of 400 mL of microalgae culture, the optimum pH was observed to be pH 11.8, reaching about 53.8 ± 0.5 % of flocculation efficiency. At pH 9.8, the flocculation was considered to be effective as it achieved 40.5 ± 0.5 % of flocculation efficiency after 1 hour of settling time. There were no flocs formed at acidic medium of pH 6 with minimal flocculation efficiency of 12.1 ± 0.2 % which nearly the same to control. Nevertheless, pH 11.8 was selected to proceed with other parameters as more than 50 % of flocculation efficiency was achieved and also,

further increase in pH requires high amount of pH buffers which in turn make the condition too alkaline and cause changes in volume of system.

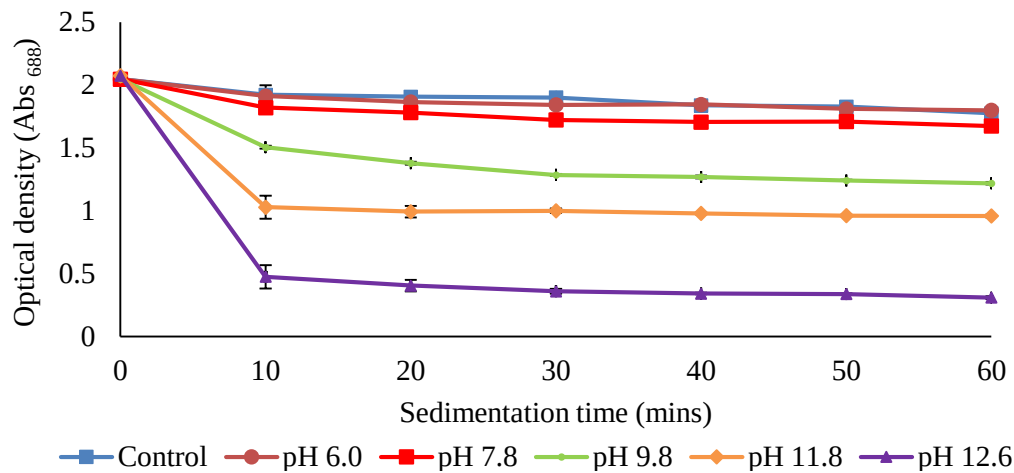


Figure 1. Effects of different pH on turbidity of *C. vulgaris* using chicken's eggshell membrane as bioflocculant.

3.2. Effect of bioflocculant dosage

The flocculation efficiency of *C. vulgaris* was observed by applying different dosage of bioflocculant ranging from 0 to 100 mg/L with different time intervals (minutes) at selected pH 11.8. The sedimentation efficiency increased with increase in dosage of added bioflocculant and contact time. A maximum harvesting efficiency of 54.2 ± 0.4 % was obtained at dosage of 100 mg/L after 60 minutes whereas 14.7 ± 0.4 % was recorded with 20 mg/L of bioflocculant at same contact time. Flocculation efficiency of control, which was without addition of bioflocculants, was found comparatively lower (13.3 ± 0.7 %) than those obtained with added bioflocculant. Change in the turbidity of *C. vulgaris* with different flocculant dosage and time interval is depicted in Figure 2. The flocculating ability of chicken's eggshell membrane was found to be apparently higher than aluminium and iron salts that have been used as coagulants in wastewater treatment. Maximum flocculation efficiency of 98 % was reported by the author in the case of *Chlorella sp.* MJ 11/11 at 400 mg/L of ferric chloride. In the case of potassium aluminium sulfate, a maximum efficiency (98.6 %) for *Chlorella sp.* MJ 11/11 was observed at a concentration of 500 mg/L [18]. However, present bioflocculation process that based on zero-cost and easily available waste chicken's eggshell membrane able to flocculate *C. vulgaris* more than half of the flocculation efficiency as discussed previously with ferric oxide and potassium aluminium sulfate at only 100 mg/L. This could be further applied in commercial scale of microalgae cultivation with the aim to reduce the overall harvesting cost and toxicity in the microalgae biomass.

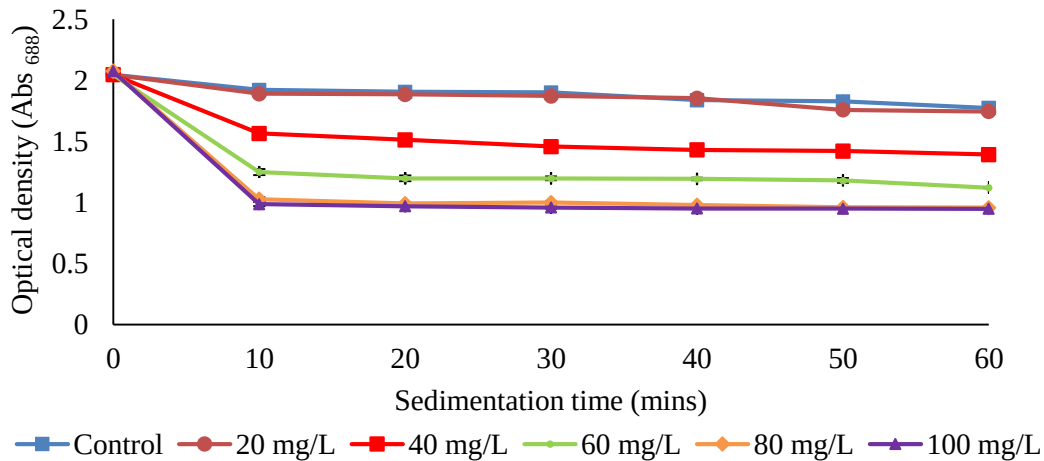


Figure 2. Effects of different bioflocculant dosage on turbidity of *C. vulgaris* using chicken's eggshell membrane as bioflocculant.

3.3. Effect of temperature

The efficiency of chicken's eggshell membrane (80 mg/L) to flocculate *C. vulgaris* over a range of temperatures (20 °C, 30 °C, 40 °C, 50 °C and 60 °C) was also studied to get more insight of its flocculating ability as showed in Figure 3. The correlation between temperature and flocculation efficiency of *C. vulgaris* showed that a maximum of 66.8 ± 0.9 % could be attained with maximum temperature of 40 °C, whereas the lowest flocculation efficiency was 30.1 ± 0.3 % at 50 °C with minor difference with those obtained at 60 °C. Increase in temperature increased the rate of flocculation as temperature changes induce floc formations [17]. However, a very high temperature, which was 50 °C in our case, caused drop in the flocculation efficiency of *C. vulgaris* might due to the effect of high temperature on the binding of bioflocculant with algal cell surfaces.

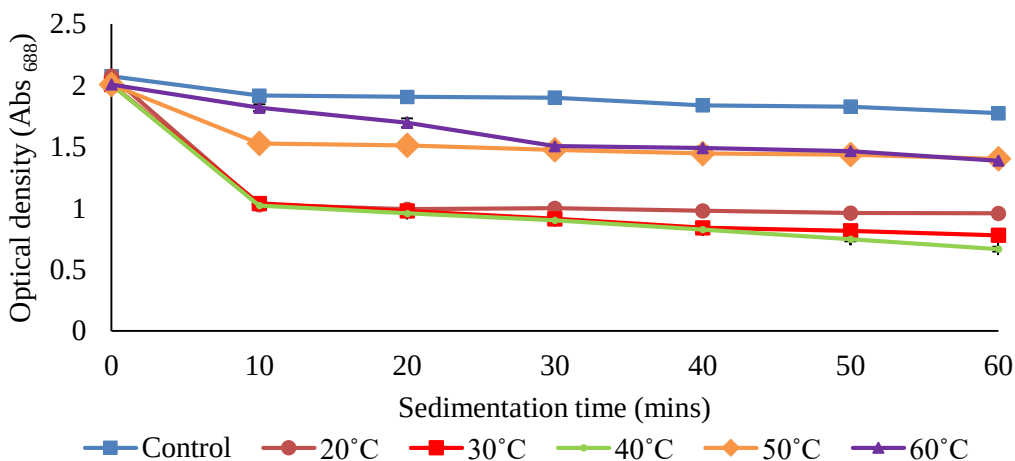


Figure 3. Effects of different temperature on turbidity of *C. vulgaris* using chicken's eggshell membrane as bioflocculant.

4. Conclusions

Through addition of flocculants, finely suspended or dispersed particles aggregated together to form bigger flocs for speedy sedimentation and clarification. The results from the present study proved the potential of chicken's eggshell membrane as a promising bioflocculant to harvest *C. vulgaris* with

separating efficiency of more than 60 % at the optimal conditions: pH 11.8, minimal flocculant dosage of 80 mg/L at 40 °C. The use of waste chicken's eggshell membrane as bioflocculant is recommended for non-toxic, easy and safe harvesting of microalgae at zero-cost.

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