Assessing the effects of operating parameters on flocculation of *Chlorella vulgaris* using bioflocculants extracted from miscellaneous waste biomass

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Abstract. Harvesting of microalgae is one of the main challenges in the production of biodiesel due to the small cell size of microalgae cells. Chemical flocculants have been generally used in the harvesting of microalgae, but they are harmful to the environment and relatively costly. Therefore, the utilization of waste biomass in producing bioflocculants is the current research niche to introduce environmental-friendly harvesting method and to minimize the cost of biodiesel production. Thus, in the current work, flocculation *Chlorella vulgaris* using mild acid-extracted bioflocculants from miscellaneous waste biomass (cockle shell, peanut shell and banana peel) were conducted by varying the pH values, the dosage of bioflocculants and temperatures. Cockle shell bioflocculant demonstrated the best flocculation performance, with highest flocculation efficiency of 85.2% compared to the peanut shell bioflocculant with flocculation efficiency of 37% and banana peel bioflocculant with flocculation efficiency of 16.3%. The optimum flocculation conditions for cockle shell bioflocculant were determined as follow: pH 9, bioflocculant dosage of 140mg/L and temperature of 30°C. The findings herein presented practical applicability of bioflocculants extracted from cockle shell for safe, rapid and inexpensive microalgae harvesting.

1 Introduction

With the rapid development of science and technology, the global energy demand is expanding while the energy sources are depleting at the same time. Fossil fuels such as coal, petroleum and natural gas as the non-renewable energy that remains as the largest source of energy and they are expected to fulfil the 80% of the world's energy demand continuously [1]. As these energy sources are very limited and will not be replenished, the world will be placed at edge of energy crisis if there is no alternative renewable energy to substitute fossil fuels [1]. In addition, air contaminants can be released from the combustion of fossil fuels leading to air pollution and global warming. Therefore, these critical global issues have led to the exploration of eco-friendly and alternative renewable energy sources, such as solar energy, geothermal energy, biomass energy and biofuels. As the energy consumption of the transportation sector is almost 30% of the world's energy consumption, much attention is given to biofuels for further development [2].

Among all the green fuels, biodiesel is one of the promising candidates to overcome the world energy crisis and environmental issues. Most of the biodiesel produced in the industry are derived from vegetable oils (e.g. soybean, rapeseed and canola), animal fats and waste cooking oil [3]. Other than that, biodiesel is toxic-free and highly biodegradable as well as able to maintain ecological balance in a more effective way if compared with petroleum diesel [4]. However, there are some limitations in the production of conventional biodiesel. As some biodiesel is produced from plants, arable lands will be required for biodiesel production causing food scarcity. The massive use of vegetable oils may lead to the occurrence of starvation in developing countries. When there is excessive demand for land for biodiesel production, it will lead to deforestation. Water resources also can be an issue when the water demand for some biodiesel crops increases [5]. Moreover, the production cost of biodiesel is generally high due to the cost of raw materials and the cost of processing. The cost of raw

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materials (vegetable oil) is contributing 60-75% of the overall total cost of biodiesel [6].

To overcome the limitation, biodiesel produced from microalgae becomes a sustainable solution for diversifying the raw materials issues. This is due to the fact that the oil productivity of microalgae is higher than that of conventional crops and microalgae has the capability of producing oil throughout the year [7]. Besides, microalgae can produce biomass very rapidly, with some even performing two doublings per day [8]. In addition, cultivation of microalgae requires less land and water in comparison to traditional oil-bearing crops [5]. However, recovery of microalgae biomass from culture remain as one of the main issues that hampering industrial scale production of microalgae biodiesel as microalgae cell appear as a dilute suspension that consists of 0.1-2.0g/L of dried biomass which needs further concentration before drying [9]. There are two common types of harvesting methods of microalgae biomass, namely physical and chemical methods. As for the physical method, they are further classified into sedimentation, centrifugation, filtration, and flotation. Nevertheless, the chemical method like flocculation is the most ubiquitous technique to recover microalgae cells from culture broth as it is more effective and inexpensive [9]. Through the flocculation process, microalgae cells will be concentrated followed by the settlement to the bottom of cultivating apparatus as the density of the concentrate increases [10]. The flocculants can be categorized into inorganic chemicals (e.g. aluminium sulfate), organic polymers (e.g. polyethylenimine) that secreted by microorganisms or bioflocculants which can be extracted from waste biomass (e.g. plant and shell waste) [11]. Organic flocculants such as aluminium salts (alum) are one of the superior flocculating agents that have been proved to have high flocculation efficiency to aggregate microalgae cells in terms of dosage and quality of biodiesel produced [9].

However, in recent years, the awareness of potential harms brought by inorganic flocculants such as toxicity, instability and non-biodegradability have been increasing, which lead to the research interest in the development of highly efficient and eco-friendly bioflocculants to replace conventional flocculants for microalgae harvesting. On the other hand, organic flocculants make the production of microalgae biodiesel economically unviable due to their high cost and dosage requirement [12]. Therefore, utilization of miscellaneous waste biomass that is abundantly available, cheap in cost and biodegradable for the production of bioflocculants for the wide array of industrial application is the current research niche in developing sustainable environmental technology [13]. Apart from that, the abandoned waste generated from various industries is at a perilous state in which reutilization of these waste materials need to be expanded and diversified [14]. The utilization of *Moreinga oleifera*, a plant waste as one of the promising biomaterial for wastewater treatment and microalgae harvesting have been introduced and studied in the past few years [15]. More recently, calcium-rich waste eggshell was proved as an ideal bioflocculant in the harvesting of *C. vulgaris* as nearly 99% of flocculation efficiency was achieved and charge neutralization by calcium ions was reported as major flocculation mechanism [16].

Similarly, cockle shell that rich in calcium carbonate was reported as an effective adsorbent in providing high removal rate for phosphorous, lead and chromium from polluted river water [14]. On the other note, peanut shells are ample in nature and often used as animal feed [17]. Interestingly, researchers have found a new potential of peanut shell as activated carbon for wastewater treatment due to its strong adsorption effects after chemical modification [17]. Banana peels were also demonstrated to remove chromium, cadmium and copper ions from aqueous solution through biosorption with regards to their high metal binding capabilities and specific heavy metal selectivity [18]. It was reported that, polysaccharide-based biomaterials such as peanut shell and banana peel posse chemical groups such as free hydroxyl and carboxyl groups that provide more adsorption sites for colloidal particles, whereby the bridging between these biomaterials and particles are strengthen and extended [1]. However, to the best of our knowledge, there is no microalgae harvesting attempt has been made using bioflocculants extracted from cockle shell, peanut shell and banana peel. It could be hypothesized that cockle shell, peanut shell and banana peel bioflocculants might promote flocculation of microalgae cells by mechanisms similarly to that proved in the aforementioned literature studies. Therefore, this work was aimed to test and compare the flocculation ability of bioflocculants derived from different types of waste biomass, namely cockle shell (shell waste), peanut shell (plant waste), and banana peel (fruit waste) to harvest *C. vulgaris*, in effort to transform these biological wastes into value-added biomaterials. The effects of different pH values, bioflocculant dosages and temperatures towards the flocculation efficiency of microalgae will be experimentally assessed to find the optimum flocculation condition as well as to select the potential bioflocculant for effective microalgae harvesting.

2 Methodology

2.1 Microalgae seed cultivation

A wild-type *C. vulgaris* was obtained from Prof. Dr Lee Keat Teong from School of Chemical Engineering, Universiti Sains Malaysia. The microalgae is preserved and grown in Bold's Basal Medium (BBM), consisting of: (1) 10mL/L of culture medium using the following chemicals: $NaNO_3$ (25g/L), $CaCl_2{\cdot}2H_2O$ (2.5g/L), $MgSO_4{\cdot}7H_2O$ (7.5g/L), K_2HPO_4 (7.5g/L), KH_2PO_4 (17.5g/L), NaCl (2.5g/L) and (2) 1mL/L of culture medium using the following chemicals: EDTA anhydrous (50g/L), KOH (31g/L), $FeSO_4{\cdot}7H_2O$ (8.82 g/L), $MnCl_2{\cdot}4H_2O$ (1.44 g/L), MoO_3 (0.71g/L), $CuSO_4{\cdot}5H_2O$ (1.57g/L), $Co(NO_3)_2{\cdot}6H_2O$ (0.49g/L). The initial pH of the medium was adjusted to 6.8. Then, the

culture was grown in a 100mL Erlenmeyer flask containing 50mL of medium, aerated with compressed air and illuminated continuously with cool-white fluorescent light (Philip TL-D 36 W/865, light intensity of 60-70μmol m^{-2} s^{-1}) under surrounding temperature of 25-28°C [11].

2.2 Microalgae cultivation with organic fertilizer

For subsequent microalgae cultivation, organic fertilizer was used as the main nutrient source for growth. Chicken compost was used in the present project and was purchased from a local supermarket. 10g of the compost was immersed in 600mL tap water and stirred thoroughly for 24 hours using a magnetic stirrer. Non-soluble particulate solids were observed and filtered using filter paper (Double Rings 101). 200mL of the produced compost medium was introduced into a 5L photobioreactor that was filled with 4300mL of unsterilized tap water and the pH of the cultivation medium was adjusted to 3 to 3.5. Following the step, 500mL of microalgae suspension from the seed culture with an initial cell concentration of 0.3×106 cells was introduced into the photobioreactor. Then, compressed air with a flow rate of 0.4L/min was aerated into the photobioreactor to impart carbon source for microalgae growth and was illuminated continuously with a cool-white fluorescent light (Philip TL-D36W/865, light intensity of 60-70mol m^{-2} s^{-1}) [11]. The well-growth of *C. vulgaris* was monitored by measuring the optical density of the culture at 688nm by using UV visible spectrophotometer (Shimadzu UV-2600) and was maintained by adding a substantial amount of chicken compost necessary for the microalgae cell doubling.

2.3 Extraction of bioflocculants

Waste cockle shells, peanut shells, and banana peels were collected from local markets and smallholder in Perak. They were washed thoroughly with distilled water after separated from peanuts, cockles and bananas. Then, they were dried at approximately 40°C in an oven to remove the moisture. The dried shells and peels were grinded into a fine powder and sieved manually using a micro sieve. After obtaining the sieved fine powder, 100mg of dry powder was dissolved in 10mL of 0.5mol/L hydrochloric acid solution with continuous stirring for 30 mins using a magnetic stirrer. The solution was diluted to 100mL using deionized water to the final bioflocculants' concentration of 1000mg/L [11].

2.4 Flocculation jar test experiment

The flocculation of *C. vulgaris* by using bioflocculants extracted from cockle shells, peanut shells and banana peels were investigated by jar test apparatus as shown in Fig.1. Microalgae suspension was first diluted with tap water to maintain the initial absorbance at 2.3±0.05 Abs. Then, bioflocculants prepared from different waste biomass were introduced into 800mL beaker containing 500mL of microalgae suspension at designated dosages and stirred at 150rpm for 15 mins. After intensive mixing, the pH values of the mixtures were slightly increased until visible flocs were observed and allowed to settle for 60 mins. As for constant flocculation condition, the bioflocculants' dosages were fixed at 100mg/L and the experiments were conducted at room temperature. In the current work, the effects of operating parameters such as pH values (6 to 10 with 1 unit interval), bioflocculant dosages (80 to 200mg/L with 20mg/L interval) and temperatures (20 to 60°C with 10°C interval) on the flocculation efficiency of *C. vulgaris* were methodically analysed.

2.5 Measurement of flocculation efficiency

The flocculation efficiency of microalgae was calculated by using the following equation:

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

Where $OD_{688}(t_0)$ is the optical density of microalgae suspension recorded at time zero and $OD_{688}(t)$ is the optical density of microalgae suspension recorded at time t [11]. The samples were taken at the middle levels of beakers for control and microalgae suspensions containing bioflocculants to measure the absorbance after 60 mins. Graphs of flocculation efficiency (%) against operating parameter values were plotted to evaluate the flocculation process of *C. vulgaris*.

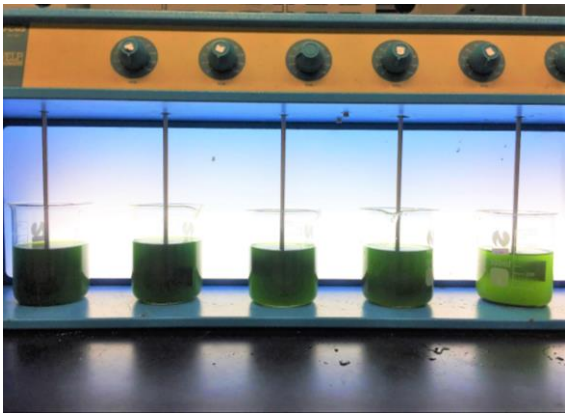

Fig.1. Flocculation jar test apparatus.

3 Results and Discussion

3.1 Effect of pH value on flocculation efficiency

The pH value of medium is one of the important factors that affect the flocculation process of microalgae cells. Any variation in pH values change the charge density of microalgae cells and rearrange the molecular fragments as well as functional groups of bioflocculants, thus alter the physicochemical interaction between the reacting

particles [19]. It is widely known that the active sites on the microalgae cell wall were likely to be protonated at low pH and deprotonated when the pH increased [20]. Therefore, calcium-rich cockle shell extract together with extracts of peanut shell and banana peel is targeted to contain various functional groups that can strongly absorb and destabilize the negative charges on microalgae cells which leads to effective flocculation [11]. The flocculation efficiencies of *C. vulgaris* at varied pH by using bioflocculants extracted from cockle shell, peanut shell and banana peel were examined as shown in Fig.2.

With the addition of 100mg/L of cockle shell extract as bioflocculant, the highest flocculation efficiency was 69.7±0.6% which occurred at pH 9. At pH 8, the flocculation process was observed to be effective, reaching about 63.70±0.4% of flocculation efficiency after 60 mins of settling time. However, the flocculation efficiency decreased to 64.1±0.3% when the pH of medium further increased to 10. As for microalgae suspension added with bioflocculant extracted from peanut shell, the highest flocculation efficiency recorded was 8.7±0.1% at pH 10. Similarly, 10 was the optimum pH for flocculation of *C. vulgaris* aided by bioflocculant extracted from banana peel, reaching about 12.4±0.1%. Acidic medium seemed to yield minimal flocculation efficiencies for both bioflocculants. The optimal flocculation efficiencies observed for both bioflocculants at alkaline medium with pH as high as 10 might due to the opposite charges carried by bioflocculant particles and microalgae cells at pH 10 that resulted in attraction between each other and subsequently higher flocculation performances [16].

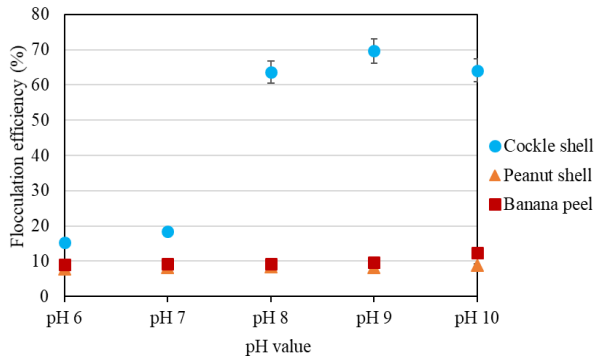

Fig.2. Effect of different pH values on the flocculation efficiency of *C. vulgaris* using cockle shell, peanut shell and banana peel extract as bioflocculants.

3.2 Effect of flocculant dosage on flocculation efficiency

The flocculation efficiencies of *C. vulgaris* suspension was observed by applying different bioflocculants' dosages at selected optimum pH as depicted in Fig.3. The dosages of bioflocculant extracted from cockle shell were varied from 80mg/L to 160mg/L and a maximum flocculation efficiency of 79.3±0.5% was recorded at 140mg/L of bioflocculant at pH 9 and room temperature while the lowest flocculation efficiency obtained using 80mg/L of bioflocculant, reaching about 59.2±0.2%. Nonetheless, when the bioflocculant dosage was further increased to 160mg/L, the flocculation efficiency was dropped to 76.0±0.5%. As for flocculation assisted by bioflocculants prepared from peanut shell, the dosages were varied from 80mg/L to 200mg/L at pH 10. It can be observed that the highest flocculation efficiency occurred when 180mg/L of bioflocculant was used, reaching approximately 37.0±0.2% but any further rise in bioflocculant dosage up to 200mg/L caused the flocculation efficiency to drop to 12.6±0.1%. 160mg/L of bioflocculant extracted from banana peel able to harvest *C. vulgaris* cells with the highest flocculation efficiency of 14.2±0.1% while the lowest obtained at a dosage of 80 mg/L, recorded about 8.1±0.1%.

It was documented that, greater interaction between flocculants and suspended particles can be induced by a higher dosage of flocculants, which will further enhance the separation of suspended particles from the dilute medium. When the flocculant dosage is higher, the chances for more flocculants to bind on the active sites of the microalgae cell will be higher too. Nevertheless, a very extreme dosage could lead to poor flocculation performance due to saturated polymer bridging sites, causing re-stabilization of the destabilized particles due to an insufficient number of suspended particles to form more inter-particle bridges. On the contrary side, inadequate dosage causes inefficient charge neutralization of negative charges on microalgae cells, resulting in low flocculation efficiency [21]. Present findings on potential of bioflocculants extracted from cockle shell in flocculating *C. vulgaris* cells was comparable to other common flocculants that applied in wastewater treatment and microalgae harvesting. Maximum flocculation efficiency of 98% was documented by the author in flocculating *Chlorella* sp. MJ 11/11 at 400mg/L of ferric chloride [22]. In the case of *Moreinga oleifera* seeds as bioflocculant, a maximum dosage of 600mg/L was needed to harvest *C. vulgaris* up to 80% of flocculation efficiency [1]. Requirement for a lower dosage of bioflocculants in the current work to flocculate *C. vulgaris* more than half of flocculation efficiencies as reported in the aforementioned literature findings, with respect to flocculant dosage: flocculation efficiency ratio, proved that cockle shell bioflocculant can be effectively and feasibly used to harvest microalgae.

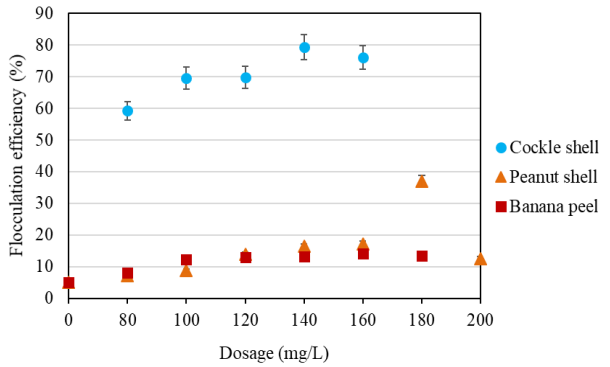

Fig.3. Effect of different bioflocculant dosages on the flocculation efficiency of *C. vulgaris* using cockle shell, peanut shell and banana peel extract as bioflocculants.

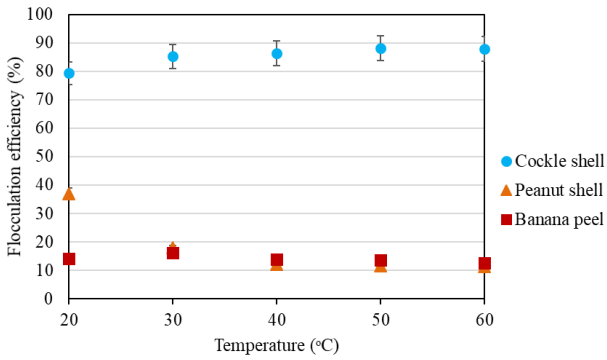

Fig.4. Effect of different temperatures on the flocculation efficiency of *C. vulgaris* using cockle shell, peanut shell and banana peel extract as bioflocculants.

3.3 Effect of temperature on flocculation efficiency

In order to determine the optimum temperature for effective flocculation of *C. vulgaris*, the temperatures were varied from 20°C to 60°C at optimized pH values and bioflocculant dosages for bioflocculants prepared from cockle shell, peanut shell and banana peel. The effects of temperature on the flocculation efficiency of *C. vulgaris* is displayed in Fig.4. After 60 mins of settling time, the highest flocculation efficiency that can be obtained using cockle shell bioflocculant was 88.0±0.4% at 50°C while the lowest was 81.5±0.1% at 20°C. Any further rise in the temperature above 50°C caused a slight drop in flocculation efficiency, reaching about 87.93±0.4%. Even though the best flocculation performance observed at 50°C, the optimum temperature was determined as 30°C, achieving 85.2±0.4% of efficiency. This is owing to the reason that the efficiency difference between 30°C and 50°C is insignificant, with percentage of only 2.8%. Therefore, setting 30°C as the optimum temperature for flocculation of *C. vulgaris* by cockle shell bioflocculant can minimize the energy use as well as help to reduce the harvesting cost.

The correlation between temperature and flocculation efficiency of *C. vulgaris* flocculated using peanut shell extract showed that a maximum of 37.0±0.2% could be attained at the lowest temperature of 20°C. Nevertheless, increasing the temperature beyond 20°C resulted in reduced flocculation efficiency. Similarly, in the case of flocculation assisted by bioflocculant extracted from banana peel, the highest flocculation efficiency was recorded at fairly a low temperature of 30°C, reaching about 16.3±0.1% while the lowest efficiency obtained at an extreme temperature of 60°C with 12.65±0.1%. Therefore, it was clear that increase in temperature increases the rate of floc formations and hence the rate of flocculation only in the case of cockle shell bioflocculants. As for peanut shell and banana peel bioflocculants, a very high temperature could lead to deterioration of active sites on bioflocculants and structural deformation of microalgae cells, thus causing poor flocculation activities [11].

Conclusion

The search for cheap and biodegradable flocculants to harvest microalgae cells has barely started. Current attempt to explore different types of waste biomass as zero-cost material to synthesize flocculants have made some practical contribution and could serve as a reference point to ascertain if future works are compromise goals. Present work provides comparative experimental data on the influence of major process parameters to achieve optimized flocculation conditions. In the present study, the potential of low-cost flocculants extracted from waste cockle shell, peanut shell and banana peel to recover unicellular microalgae cells, *C. vulgaris* was investigated. Among all, flocculation ability of bioflocculants extracted from cockle shell are quite promising, with highest flocculation efficiency of 85.2% under the optimized conditions of pH 9, bioflocculant dosage of 140mg/L and temperature of 30°C followed by peanut shell bioflocculant, achieving flocculation efficiency of about 37% at pH 10, dosage of 180mg/L and room temperature. To further enhance the research in waste biomass-derived natural flocculants for microalgae harvesting, it is recommended to characterize the bioflocculants extracted from these miscellaneous waste biomass to allow better understandings on the content or components which exhibit flocculation properties, degradation pathway and mechanism related to the ability to aggregate microalgae cells.

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