

Emulsifying Properties of Proteins Extracted from *Jatropha curcas* L. to Harvest *Chlorella vulgaris* sp. Microalgae

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ABSTRACT

A bio-floatation technique was used to test the effectiveness of *Jatropha curcas* seed extract in recovering microalgae from an aqueous medium. This technique used *Jatropha curcas* oil-protein extract emulsion for floatation removal of *Chlorella vulgaris* microalgae. The effects of variables have been investigated in this process, including operating parameters such as protein-oil emulsion dosage, pH, and mixing time. A maximum floatation efficiency of 81% was observed when a 20 ml/l protein-oil emulsion dosage was tested at pH 2 and a mixing time of 4 min. Furthermore, the analysis of the microalgae's zeta potential changes revealed a notable variation in value caused by the protein-oil emulsion. This bio-floatation technique is useful for the high-efficiency harvesting of microalgae to produce cost-effective biofuel.

Key words : Microalgae, Bio-floatation, Emulsion, *Jatropha*

Introduction

Population explosion in the world is a leading cause of the over-exploitation of natural resources, leading to many socioeconomic and global issues like high inflation, global warming, etc. The world population is estimated to be 9.2 billion by 2040 (Global Trends, 2040). One of the many approaches to meet the daily requirement of fuel, biofuel, food, etc. is harvesting the microorganisms from various natural resources used in more economical and upcycling methods. Microalgae are extensively researched for their potential application and replacement for producing nutraceuticals, biofuels, fine chemicals, pharmaceuticals, and cosmetics (Roy and Mohanty, 2019). Microalgae are unicellular photosynthetic microor-

ganisms that proliferate fast and thirst for carbon dioxide. Biomass, rich in biochemical composition, favors the production of a wide range of marketable products (Roy and Mohanty, 2019; Dwivedi and Dwivedi, 2022). They can accumulate proteins, lipids, and other high-value products like EPA, DHA, and pigments (Ummalyma *et al.*, 2017). Microalgae are harvested using many versatile physicals (sedimentation, centrifugation, floatation, filtration), chemical (inorganic flocculation, organic flocculants), and biological (auto-flocculation, bio-flocculation) techniques (Ummalyma *et al.*, 2017; Kandasamy and Shaleh, 2018). One of the major obstacles to the full-scale production of algal biofuel is to lower the energy cost of harvesting algae. Compared to bio-flocculation, other methods such as

physical and chemical harvesting are very expensive (like energy requirements for mechanical machinery), especially for large-scale operations and high yields. Introduction of an innovative bio-flocculation technique significantly reduces the energy consumption for the harvesting of microalgae (Ummalyma *et al.*, 2017). This technique involves flocculation promoted by extracellular polysaccharides (natural polymers) and proteins derived from microorganisms. However, there is still no one best technique for harvesting microalgae. At the very least, biomass recovery from the culturing medium accounts for 20–30% of the total biomass production cost (Barros *et al.*, 2015).

A recent study showed that the bacterial strain *Citrobacter* W4 facilitates bio-flocculation of wastewater-cultured microalgae *Chlorella pyrenoidosa* (He, *et al.*, 2022). Another study showed the effectiveness of activated sludge-derived-extracellular polymeric substance (ASD-EPS) as a bio-flocculant for microalgae harvesting. Microalgae *Chlorella vulgaris* showed high flocculation with ASD-EPS and large lipid production (Choi *et al.*, 2020). Flootation by emulsion is a method usually used in the mining industry to clean raw coal (Kumar *et al.*, 2022).

In the present study, a newly developed simple flootation technique with protein-oil emulsion was developed (Suparmaniam, *et al.*, 2019). A mix of sunflower oil with *Jatropha curcas* (Jc) protein was used, and its effectiveness on *Chlorella vulgaris* sp. (*C. vulgaris*) microalgae recovery was studied. This has been the first of its kind to use *Jatropha* protein in bio-flootation, which will perform both flocculation and flootation effects on microalgae.

The *C. Vulgaris* cultivation and protein extraction of Jc were both done continually, along with the flootation experiment on a lab scale. The performance of this method was evaluated under different operating conditions including pH, mixing time, and dosage. This technique is thought to lower the cost of microalgae biomass recovery.

Methodology

C. vulgaris culture and growth condition

The freshwater microalgae *C. vulgaris* sp. was acquired from the School of Fisheries and Aquaculture Science, Universiti Malaysia Terengganu (UMT). Several 15 ml centrifuge tubes filled with regular water were used to hibernate the algae strain. Later

inoculated into 1 L Erlenmeyer flasks containing Bold's Basal Medium and scaled up repeatedly for a harvesting study. The cultures were grown for at least 15 days under controlled environmental conditions with proper aeration at $24.5 \pm 1^\circ\text{C}$ with the illumination of 750lux. A UV-VIS spectrophotometer measured microalgae growth to determine the absorbance of microalgae cultures. When the absorbance value remained constant for three consecutive days at this stage, samples were collected for the flootation experiment. Additionally, biomass concentration was calculated by the TSS test (He *et al.*, 2022).

Microalgae growth monitoring

C. vulgaris growth was determined by measuring the absorbance using Double Beam UV-VIS Spectrophotometer (Shimadzu UV-1800, Japan). UV-VIS was used to determine the wavelength of *C. vulgaris* and the cell density was calculated. *C. vulgaris* samples were filtered using a glass fiber filter (ADVANTEC type GC-50, 47 mm diameter, 0.45 μm). The dry mass of *C. vulgaris* was measured by drying the filter paper at 105°C for 24 hours. By lab analytical balance, the initial and final weights of the filter paper were measured. *C. vulgaris* concentration was calculated from microalgae dry weight produced per liter (mg/l) (Pandey *et al.*, 2019).

The specific growth rate (μ) was calculated according to the following formula (Wong *et al.*, 2017):

$$\mu = \ln (A_2/A_1) / (T_2 - T_1) \quad \dots (1)$$

where A_2 and A_1 are the absorbance (A_{650}) at the times T_2 and T_1 , respectively.

Biomass productivity (B) was calculated by the formula (Wong *et al.*, 2017).

$$B = (B_2 - B_1) / (T_2 - T_1) \quad \dots (2)$$

where B_1 and B_2 are the mean dry biomass concentration at the times T_1 and T_2 , respectively.

Preparation of *Jatropha curcas* seed powder

100g of Jc seed was dried by using an oven within 2 days at 60°C . The dried seed was manually crushed and deshelled. The weight after the seed has been deshelled is recorded at $60 \pm 2\text{g}$. The kernels were manually crushed by using pastel and mortar, and then the powder was passed through a mesh sieve to obtain fine powder and stored at room temperature for further analysis (Khodapanah *et al.*, 2018).

Oil removal of *Jatropha curcas*

Oil was extracted by using 350 mL absolute ethanol

as a solvent in a Soxhlet apparatus. A 10 g of fine powder of Jc was put into the thimble inside the extraction chamber connected to the condenser. The process was performed for almost 16 h in a fume hood. The extracted press cake was collected and dried for 30 min at 50-60 °C to remove the moisture and the mass of the dried press cake was recorded. The solvent was removed by using a rotary evaporator for 5 hours and the *Jatropha* oil was collected (Khodapanah, *et al.*, 2018).

Preparation of *Jatropha curcas* protein extraction

A 2g of press cake was mixed with 100 ml of distilled water at 60 °C in the 200 ml beaker. This process was performed at room temperature. A magnetic stirrer continuously stirred the solution, and the pH of the solution was kept at 10 for 1 h by adding a drop of 1 or 0.1M NaOH. Then the extract solution was centrifuged at 3000 g for 20 min at room temperature, and the insoluble residue was removed. The supernatant was collected into the beaker, and was continuously stirred for 10 min using a magnetic stirrer, and pH was adjusted to 5 by dosing 1 or 0.1M HCl. The solid residue was collected, and mixed with 100 ml distilled water, and stirred for 4 h. The solid was discharged, and the liquid part collected and stored at 4°C for further experiments (Khodapanah *et al.*, 2018).

Preparation of *Jatropha* protein-oil emulsion

The *Jatropha* protein-oil (JPO) emulsion was prepared at room temperature by adding 7 ml of sunflower oil and 1 ml of Polysorbate 20 to 7 ml of Jc protein extract liquid and mixed using a magnetic stirrer for 3 min continuously. The JPO emulsion mixture prepared was used for the floatation harvesting experiment.

Floatation experiment

A floatation experiment was done with a sample of 100 ml microalgae prepared in a fixed concentration of 710 mg/l. The initial pH value of microalgae suspension was identified using a pH meter. Before biofloatation, a sample of *C. vulgaris* was taken to measure the initial absorbance and TSS. The experiment started by stirring the microalgae suspension with a magnetic stirrer for 3 min at the lowest speed of the magnetic stirrers to reduce the sedimentation of the microalgae. To evaluate the optimum dosage of JPO emulsion, several tests were conducted with different JPO emulsion amounts of 10, 20, 30, 40, and 50

ml/l. The process of mixing continued for another 2 min. A series of tests with 2, 4, 6, and 8 min of mixing time with a constant amount of JPO emulsion of 20 ml/l were done. The experiment was repeated to identify the effective pH value by adjusting the initial pH of *C. vulgaris* sample into 4 different values, 2, 4, 6, and 8 (original pH of *C. vulgaris* culture) by adding 20 ml/l of JPO emulsion with 4 min of mixing time. After each biofloatation process, the algae cell was carefully removed from the solution. The remaining liquid was taken to the UV-VIS Spectrophotometer analysis and TSS experiment to identify the floatation efficiency. Floatation efficiency by measuring the absorbance of *C. vulgaris* sp. was calculated as follows:

$$\text{Efficiency (\%)} = (A_1 - A_2) / (A_1) \times 100\% \quad \dots (3)$$

where A_1 is initial absorbance and A_2 is final absorbance.

For the TSS method, the efficiency was calculated as in the following equation:

$$\text{TSS (mg/l)} = ((A-B) \times 1000) / (\text{sample volume}) \quad \dots (4)$$

where A is the weight of filter + dried residue (mg), and B is the weight of filter (mg).

$$\text{Efficiency (\%)} = (T_1 - T_2) / (T_1) \times 100\% \quad \dots (5)$$

where T_1 is the initial TSS value and T_2 is the final TSS value.

Zeta Potential analysis

Zetapotential (mV) was measured for the microalgae at different pHs. A dual automated analyzer Delsa Max PRO (MO, USA), was used for the measurement. 0.1 ml of algae was mixed in 1.9 ml of deionized water. Zeta potential analysis was done before and after the addition of JPO emulsion in the microalgae suspension. Every data was analyzed in triplicate (mean \pm S.D).

Statistical analysis

All the values were expressed as mean \pm S.D. or SEM. Statistical analysis was performed by one-way ANOVA (SPSS-IBM) followed by student's t-test. A value of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Characterization of *Chlorella vulgaris* sp.

The absorbance of *C. vulgaris* medium on day 12 was 0.50 ± 0.08 . The overall Specific Growth Rate was

$0.20 \pm 0.04 \text{ day}^{-1}$ and $0.21 \pm 0.02 \text{ day}^{-1}$, calculated through absorbance and dry biomass, respectively. Biomass productivity was $58.2 \pm 0.03 \text{ mg/l day}^{-1}$.

In the floatation experiment, zeta potential is an important factor. Zeta potential shows a measurement of the apparent surface charge (Gomes, *et al.*, 2022). With a zeta potential close to zero, the particles can move toward each other to the point where they will be aggregated by Van der Waals forces and flocculation or coagulation will occur (Zhang, *et al.*, 2023).

In this study, zeta potential had the nearest value to zero which was $-18.33 \pm 0.18 \text{ mV}$ at pH 2. At cultivation pH of *C. vulgaris* (pH = 8), the zeta potential value had a high negative value at $-34.64 \pm 0.14 \text{ mV}$. The negative value of zeta potential shows that the *C. vulgaris* suspensions were stabilized by the surface charge of the cells. The surface charge of *C. vulgaris* sp. originates predominantly from the presence of carboxylic (-COOH) and amine (-NH₂) groups on the cell surface (Vandamme *et al.*, 2013). The difference between pH 2 and pH 4, 6, and 8 was statistically significant ($P < 0.05$).

Effect of dosage and mixing time on floatation efficiency

As a result of the optimum dosage, the maximum efficiency of $72 \pm 0.8\%$ was observed by 20ml/l of JPO emulsion with 2 min of mixing time.

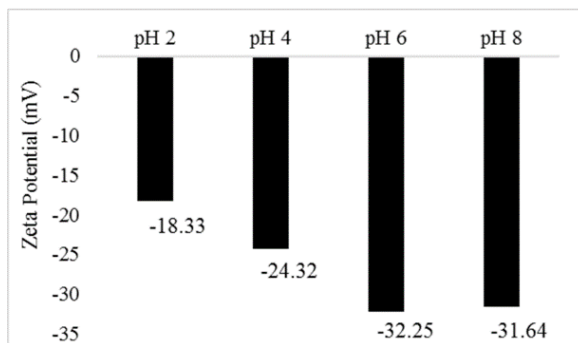


Fig. 1. Zeta potential value of microalga, *C. vulgaris* at different pH.

Additionally, an optimum mixing time of 4 min was obtained by a 20ml/l dosage of JPO emulsion with an efficiency of $78 \pm 0.4\%$.

However, a poor result of $60 \pm 0.7\%$ floatation efficiency was achieved at a mixing time of 8 min. A blank sample was relative with no addition of JPO emulsion have a small amount of reduction in absor-

bance due to the sedimentation of flocs over time.

Bio-floatation efficiency of microalgae using JPO emulsion with pH modification

The maximum absorbance efficiency of floatation harvesting was calculated, and $81 \pm 0.7\%$ of removal was found from the addition of 20ml/l of JPO emulsion to the culture medium at pH 2 with 4 min of mixing time. Similar results were calculated from the TSS method; $82 \pm 0.5\%$. Therefore, more than 80% of microalgae was successfully removed from its culturing medium. Additionally, the zeta potential measurement of the culture medium after the bio-floatation process at pH value 2 showed stabilization at $-0.78 \pm 0.08 \text{ mV}$. Therefore, the interaction between the negatively charged functional groups in the microalgae and the emulsion may have been one of the main driving forces behind the adsorption onto the algal surface and floatation reaction.

This study showed a comparable efficiency with the previous study, where Potocar *et al.* (2020) showed effective harvesting of *C. vulgaris* via floatation using a cooking oil-CTAB emulsion. Over 90% floatation efficiency was observed at pH 10 and 12. In another study, Kandasamy and Shaleh (2018), achieved a satisfactory floatation efficiency of 86% at 50 ml/l within the range of pH 8.

Conclusion

This study proposed a new technique for harvesting *Chlorella vulgaris* sp. The Bio-floatation technique is believed to be effective, biodegradable, non-toxic, and cost-effective. It is important to note that *Jatropha* protein is advantageous since the press cake is a waste by-product containing a significant amount of protein. The isoelectric precipitation principle applied to extract the protein is low-cost and environmentally friendly. As a third-generation feedstock for biodiesel, microalgae have significantly higher yields than other crops. Thus, successful bio-floatation will bring the development of harvesting technology which will increase the feasibility of microalgae as the source of biofuels in the future.

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