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NEXT GENERATION MICROALGAE BIODIESEL: A STRATEGY TOWARDS CIRCULAR BIOECONOMY

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ABSTRACT

The present study investigates the potential of chicken compost as an alternative nutrient source to grow *Chlorella vulgaris* in batch bubble column photobioreactor system for lipid-based biofuel production. Experimental results showed that microalgae cultivated in chicken compost medium exhibited higher biomass concentration (1.0 g/L) as compared to the bold basal media (BBM). Additionally, it was shown that *Chlorella vulgaris* cultivated in recycled media produced biomass concentration that was similar to that of the fresh medium. Meanwhile, the carbon dioxide (CO_2) fixation efficiency obtained in this study was 20% higher than those reported previously. Microalgae harvesting using biofloculant prepared from shell waste resulted in 99% flocculation efficiency without a negative impact on the lipid content. The fatty acid methyl ester (FAME) acquired comprised of two main alkyl groups namely, C16 and C18, which maintain the suitability of microalgae lipid for quality biodiesel production.

Keyword: *Microalgae; Organic fertilizer; Nutrient recycling; Bioflocculant; Biodiesel; Circular bioeconomy*

1. INTRODUCTION

In the recent millennium, microalgae biomass which is composed of valuable compounds viz., carbohydrate (12–17%), lipid (14–22%), protein (51–58%), carotenoids, and pigments has been time-honoured for the productions of various high-value commodities such as biofuels, nutraceuticals, and pharmaceuticals (Yadav et al., 2015). Among these, microalgae biofuels including hydrocarbons, methane, syngas, kerosene, diesel, and gasoline are of high industrial interest (Gorry et al., 2018). In fact, microalgae-derived biodiesel has the potential to replace non-renewable transport fuels without negatively influencing the supply of food and other crop products (Chisti, 2008). Besides, the lipid-extracted residual biomass contains high amounts of carbohydrates and protein that can be converted into useful biomaterials such as bioethanol (Xiu et al., 2017), animal feed (Raposo et al., 2010), and biogas (Yang et al., 2011), which reduces toxic nutrient leaching and greenhouse gaseous (GHG) emissions, thus greatly improve the environmental and economics of green biorefinery. Among the examined species, *Chlorella vulgaris* shows distinctive commercial values due to its ability to grow under high carbon dioxide (CO_2) concentration (up to 40 %) and possess great photosynthetic efficiency with high tolerance to stress factors (Bui et al., 2018).

Nevertheless, the high cost of chemical nutrients and excessive use of freshwater for microalgae cultivation along with energy-intensive harvesting technologies have limited the commercialization potential of microalgae-based biodiesel. It was reported that, the cost of chemical nutrients or inorganic fertilizers could account for 10–20% of the entire cultivation capital cost, turning it a fundamental factor to be considered for commercial microalgae cultivation processes (Tan et al., 2018). In this regard, utilization of waste-based nutrients coupled with spent media recycling to grow microalgae have been identified as effective ways to improve the economic and environmental sustainability of microalgae industry (Zhang et al., 2016). On the other hand, bioflocculants derived from waste resources such as *Cicer aretinum*, *Moringa oleifera*, and cactus have demonstrated very high turbidity evacuation abilities in wastewater treatment facilities. These waste materials are abundant in nature, eco-friendly and biodegradable that present a lucrative opportunity for potential application in microalgae harvesting (Suparmaniam et al., 2020). Therefore, the current work focuses on the use of cheap chicken waste compost as nutrients source to cultivate microalgae and the feasibility of reusing spent culture medium to further reduce the nutrients and water footprint. Moreover, carbon capture performances of microalgae at lab- and pilot-scale were assessed. Additionally, shell waste such as chicken's eggshell was explored as low-cost and novel biomaterial to prepare bio flocculantion for effective recovery of microalgae biomass from aqueous broth for further processing for lipid extraction and biodiesel synthesis.

2. METHODOLOGY

2.1 Microalgae Cultivation in Lab- and Pilot-Scale Using Organic Fertilizer.

Chlorella vulgaris was cultivated in a 5L lab-scale photobioreactor (PBR) (Figure 7a) containing organic fertilizer medium that composed of various essential nutrients to support microalgae growth. The pH value in the cultivation medium was adjusted by using 1 M of sulphuric acid (H_2SO_4) and sodium hydroxide (NaOH). Throughout the experiment, photobioreactors were continuously illuminated with cool-white, fluorescent light (Philip TL-D 36 W/865) and aerated with compressed air at a temperature of 25 ± 5 °C. Furthermore, the influence of spent medium recycles on the growth performance of *Chlorella vulgaris* was also investigated to reduce the nutrients and water footprint for microalgae biodiesel production (Suparmaniam et al., 2020). The feasibility of an upscaling of microalgae cultivation was investigated using a pilot-scale PBR. The upscaled bubble column PBR was made with acrylic material and has a working volume of 60 L as illustrated in Figure 7b. Microalgae cultivation in this PBR is conditioned according to the previously studied lab-scale PBR and the growth was monitored using a spectrophotometer (Shimadzu UV-1280) (Dasan et al., 2021; Dasan et al., 2020; Suparmaniam et al., 2020).

2.2 Preparation of Bioflocculant from Chicken's Eggshell for Microalgae Harvesting

Chickens' eggshells waste was collected from nearby cafeteria at Universiti Teknologi PETRONAS due to ease of availability. These eggshells waste was first washed using distilled water and dried in an oven at 102 °C (Memmert 100–800). The dried shells were then ground to fine powder by using a pastel and mortar. Then, a 100 mg of the grounded shell powder were suspended in 10 mL of 0.5 mol/L hydrochloric acid solution and continuously stirred for 30 min using a magnetic stirrer on a hot plate magnetic stirrer (Fisher Scientific Isotemp) at room temperature to facilitate the extraction of bioflocculants. The resulting solution was filtered through filter paper (Double Rings 101) and the filtrate was then marked up to 100 mL with deionized water to a final bioflocculant concentration of 1000 mg/L. The bioflocculant extract was later tested for flocculating *Chlorella vulgaris* using Jar test experiment and compared with gravitational sedimentation in terms of harvesting efficiency and contact time (Suparmaniam et al., 2020).

2.3 Microalgae Lipid Extraction and Biodiesel Conversion

A modified Bligh and Dyer method was applied to extract lipid from 0.2 g of microalgae biomass, as described in previous. The crude lipid (3 mg) extracted from *Chlorella vulgaris* were dissolved in 3 mL of methanol containing 10 μL of concentrated sulfuric acid (H_2SO_4) and agitated in an incubator shaker (200 rpm) at 60 °C for 6 h. Upon purification, the top layer containing hexane and fatty acid methyl esters (FAME) was transferred into another vial for fatty acid profile analysis by gas chromatography (Shimadzu GC-2010, Japan) (Dasan et al., 2021; Suparmaniam et al., 2020).

3. FINDINGS

3.1 Effect of Chicken Compost as Nutrients Source and Culture Medium Recycling on Chlorella Vulgaris Growth

Chlorella vulgaris was found to grow faster using chicken waste compost than that of BBM medium up to 1.0 g/L of biomass concentration at 12th day of cultivation (Figure 1). During the early stage of growth, a shorter lag phase was observed when organic fertilizer was used as nutrients source, which clearly indicated that the microalgae cells could adapt well and propagate rapidly under the supplied nutrients. Interestingly, there were no stationary phases observed for both cultures using different nutrients source, further proving the efficiency of organic fertilizer as compared to the well-established chemical-based medium. As for culture medium recycling, the results revealed that the growth of *Chlorella vulgaris* was not significantly affected with spent water and nutrients looping system, regardless of cultivation cycles (Figure 2). The biomass concentration of the *Chlorella vulgaris* was maintained in the range of 0.8 to 1.0 g/L within 3 cycles of cultivation. Furthermore, the slight reductions of microalgae biomass concentration in recycled medium could be due to the contamination of culture medium after multiple cycles of batch cultivation and the *Chlorella vulgaris* was unable to re-grow under the same optimum conditions (Suparmaniam et al., 2020). Hence, the use of chicken waste compost as a source of nutrients with spent medium recycling is highly encouraged for mass microalgae cultivation as it can tackle the high cost associated with microalgae biodiesel production and at the same time avoid chemical waste that can harm the environment.

3.2 Harvesting of Chlorella Vulgaris Using Bioflocculant Prepared from Chicken's Eggshell.

This section addresses the comparative evaluations of *Chlorella vulgaris* harvesting using gravitational sedimentation and natural bioflocculant (Figure 3 and Figure 4). A maximum flocculation efficiency of $99 \pm 0.5\%$ could be attained within 5 minutes of contact time when *Chlorella vulgaris* was treated with chicken's eggshell bioflocculant. Meanwhile, lower than 10% of harvesting efficiency was achieved by natural sedimentation using gravitational force, which imposed inefficiency of the method in rapid recovery of microalgae biomass. Characterization analysis proved that co-precipitation of calcium ions that are present abundantly in these eggshell-derived bioflocculant are the major contributor towards effective flocculation *Chlorella vulgaris* cells. In comparison to chemical flocculants (alum, iron chloride, etc.) that have been widely used to harvest microalgae cells, bioflocculants extracted from chicken's eggshell waste is low-cost, toxic-free and involves minimal preparation steps (Suparmaniam et al., 2020).

3.3 Carbon Capture Performance of Chlorella Vulgaris

One of the interesting features in microalgae biomass production is the potential to trap CO_2 gas generated from atmosphere in the pond as bicarbonate. Figure 5 illustrates the performance of *Chlorella vulgaris* for carbon dioxide fixation for la- and pilot-scale systems. It was found that the lab scale system can capture up to 158 g of carbon in a year. Meanwhile, the pilot-scale system can capture 12 times more carbon than the lab-scale system. It has been confirmed through this study that the changes in the CO_2 fixation efficiency were closely related to the variation of biomass productivity and carbon content of *Chlorella vulgaris* cell (Dasan et al., 2021). Notably, the carbon fixation rate of *Chlorella vulgaris* obtained in this study was among the highest as compared to the literature (Lam & Lee, 2013; Mohsenpour & Willoughby, 2016; Zheng et al., 2020).

3.4 Lipid Yield and Fatty Acid Profiling of Chlorella Vulgaris

In the current study, the lipid yield of both raw *Chlorella vulgaris* (harvested using gravitational sedimentation) and the one treated with bioflocculant were in the range of 18–20%. The percentage of lipid yield obtained in the current work was aligned with previous findings that reported 6.3–25.8 wt% for *Chlorella vulgaris* biomass (Tan et al., 2018). More interestingly, harvesting microalgae with bioflocculant derived from chicken's eggshell did not significantly alter the fatty acid composition of microalgae lipid. As shown in Figure 6, the FAME derived from *raw Chlorella vulgaris* contained 27.5% of saturated fatty acids and 72.5% of unsaturated fatty acids. On the other hand, 36.42% of saturated fatty acids while 63.58% of unsaturated fatty acids were obtained for *Chlorella vulgaris* treated with chicken's eggshell bioflocculant. The FAME in the current work had displayed high in unsaturation degree which is always preferable in biodiesel production with low kinetic viscosity and high engine performance (Suparmaniam et al., 2020).

4. CONCLUSION

The present study has successfully demonstrated the feasibility of growing *Chlorella vulgaris* using chicken waste compost as nutrient medium in which a higher amount of microalgae biomass was obtained than that of the BBM medium. Moreover, the growth performances of *Chlorella vulgaris* in recycling media were found to be similar to those obtained in fresh medium, indicating that recycled media were able to support healthy proliferation of microalgae cells even without any pre-treatment or purification process. Additionally, utilization of chicken's eggshell waste as bioflocculant served as a green and cost-cutting protocol in replacing conventional chemical flocculants for microalgae harvesting to produce quality biodiesel in a full-scale. All in all, current work introduced a low-cost microalgae cultivation system that can be potentially incorporated in existing microalgae biorefinery schemes which will benefit the economy of microalgae biodiesel production and for better environment protection.

APPENDIX

A. LIST OF FIGURES

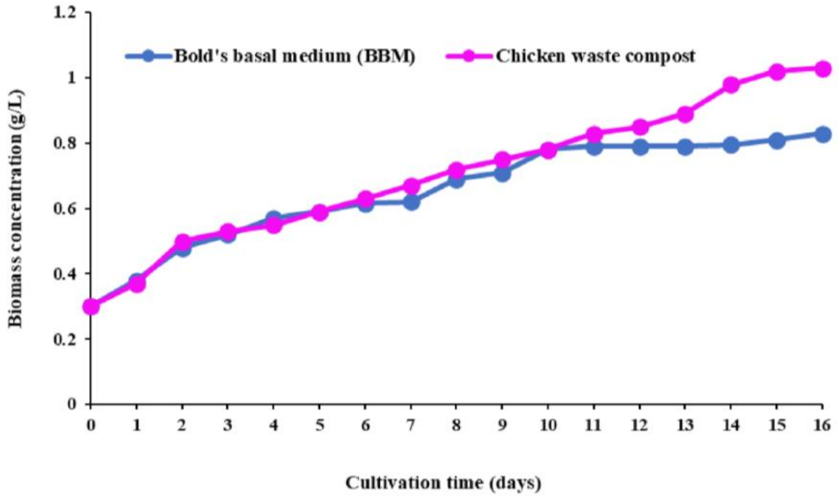

Figure 1 *Chlorella Vulgaris* Growth in Bold Basal and Chicken Waste Compost Media.

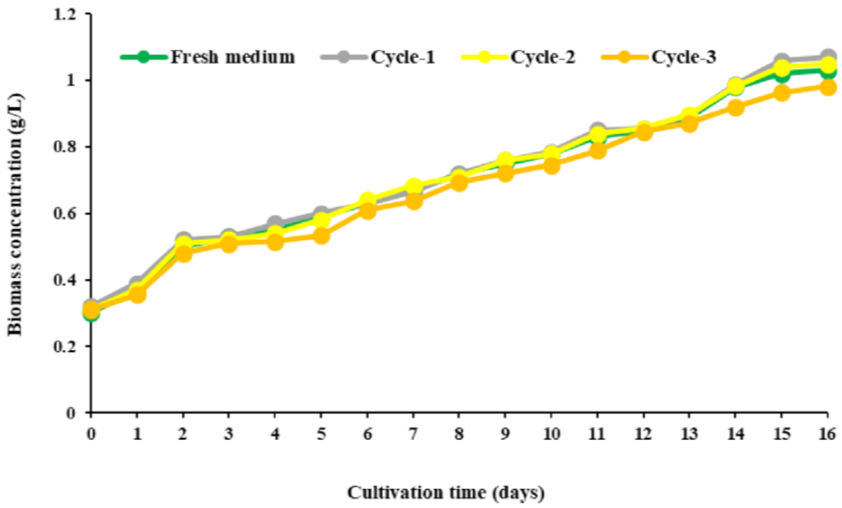

Figure 2 *Chlorella Vulgaris* Growth in Fresh and Recycled Cultivation Media.

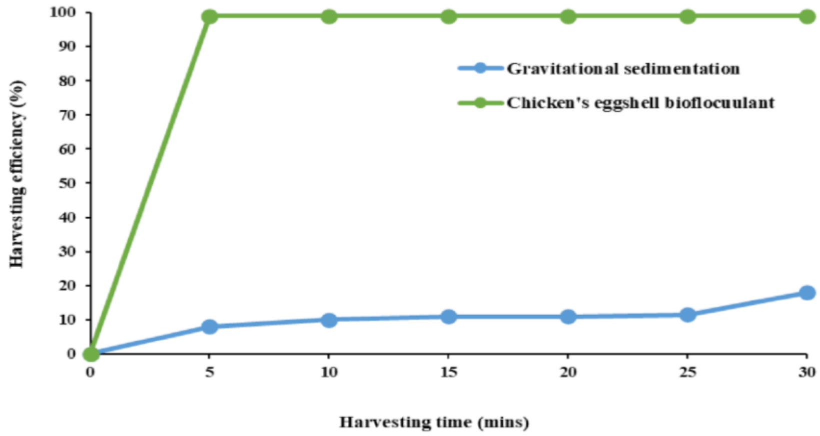

Figure 3 Comparative Harvesting Performances of Gravitational Sedimentation and Chicken's Eggshell Bioflocculant on *Chlorella Vulgaris*.

a)

b)

Figure 4 *Chlorella Vulgaris* Suspension during a) Gravitational Sedimentation and b) Chicken's Eggshell Bioflocculation After 5 Minutes of Contact Time.

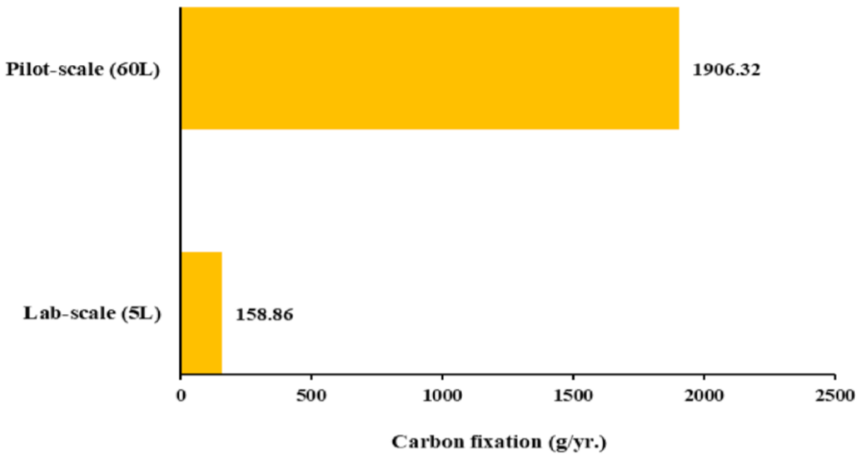

Figure 5 Carbon Dioxide Fixation by Microalgae at Lab- and Pilot- Scale Photobioreactors.

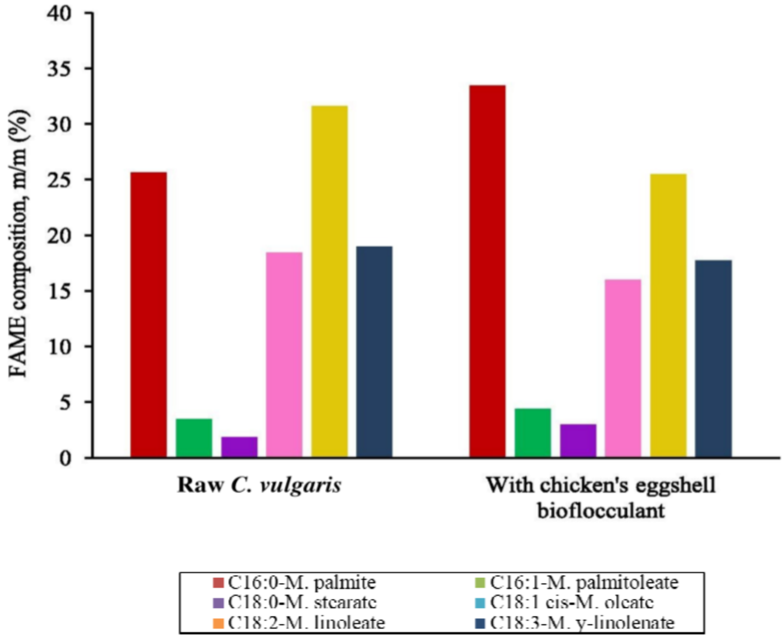

Figure 6 FAME Composition of Raw *Chlorella Vulgaris* Biomass and Treated with Bioflocculant.

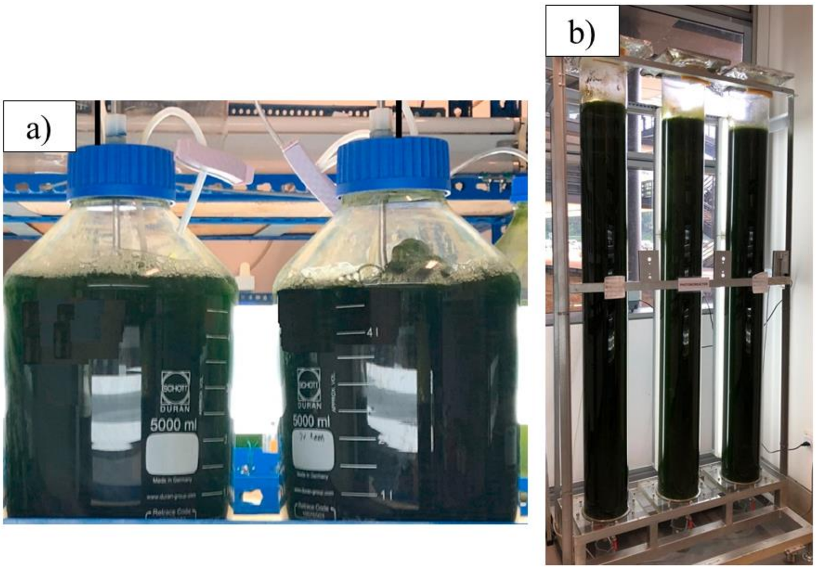

Figure 7 Microalgae Cultivation at a) Laboratory and b) Pilot-scale.

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