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Cultivation of *Chlorella vulgaris* in a pilot-scale sequential-baffled column photobioreactor for biomass and biodiesel production



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ABSTRACT

Pilot-scale cultivation of *Chlorella vulgaris* in a 100 L sequential baffled photobioreactor was carried out in the present study. The highest biomass yield attained under indoor and outdoor environment was 0.52 g/L and 0.28 g/L, respectively. Although low microalgae biomass yield was attained under outdoor cultivation, however, the overall life cycle energy efficiency ratio was 3.3 times higher than the indoor cultivation. In addition, negative energy balance was observed in producing microalgae biodiesel under both indoor and outdoor cultivation. The minimum production cost of microalgae biodiesel was about RM 237/L (or USD 73.5/L), which was exceptionally high compared to the current petrol diesel price in Malaysia (RM 3.6/L or USD 1.1/L). On the other hand, the estimated production cost of dried microalgae biomass cultivated under outdoor environment was RM 46/kg (or USD 14.3/kg), which was lower than cultivation using chemical fertilizer (RM 111/kg or USD 34.4/kg) and current market price of *Chlorella* biomass (RM 145/kg or USD 45/kg).

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1. Introduction

Cultivation of microalgae biomass for biodiesel production has gained wide attention in these recent years [1]. Microalgae are fast growing microorganism (100 times faster than terrestrial plants) and they can double their biomass in less than one day [2]. It is estimated that a realistic value of microalgae biomass production rate could lies between 15 and 25 tonne/ha/year. By assuming the microalgae cells consisting of 30% of lipid content, the average crude microalgae lipid production rate could reach 4.5–7.5 tonne/ha/year, which is higher than oil production from soybean (0.4 tonne/ha/year), rapeseed (0.68 tonne/ha/year), oil palm (3.62 tonne/ha/year) and jatropha (4.14 tonne/ha/year) [3–5]. Thus, cultivating microalgae for biodiesel production requires only a minimum land area and holding an important key for sustainable land utilization [6].

There are several advantages of producing microalgae-based biodiesel, such as microalgae can be cultivated on marginal non-agricultural land, has high lipid productivity, can be coupled with wastewater treatment process, and able to bio-mitigate CO_2 from atmosphere and flue gases [4,5,7–9]. In addition, researchers across the world have successfully demonstrated the potential of converting microalgae lipid to biodiesel through transesterification reaction [10–13]. Some of the microalgae strains are capable to accumulate significant amount of lipid within their cells, such as *Botryococcus braunii* (lipid content of 25–75%), *Chlorella* sp. (28–32%), *Scenedemus* sp. (20–21%) and *Nannochloropsis* sp. (31–68%). Furthermore, an added advantage of microalgae biodiesel is that it does not compete with food production and thus, promotes a more sustainable energy development for the future. Through some intensive researches in the last few years, microalgae have positioned itself as one of the third generation biofuel feedstock which successfully open up a new dimension in renewable energy industry [14–16].

Nevertheless, most of the research findings on microalgae cultivation are lab-scale whereas studies related to scale-up process are still scatted in literature. High biomass productivity by microalgae is always observed under lab-scale cultivation due to easily control of the cultivation environment, such as temperature, pH and illumination. In addition, most of the life cycle assessment (LCA) on microalgae biofuel production are calculated based on lab-scale data, in which positive energy balance are always observed [5,17,18]. However, when microalgae cultivation is scaled-up for mass production and aiming for commercialization purpose, a lot of unforeseen circumstances could be surfaced. These include the inconsistent changes of outdoor temperature and weather, contamination by bacteria or fungus (especially when nutrients source is wastewater), reusability of cultivation medium and etc. All these

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factors could inevitably affect the biomass productivity by microalgae and subsequently leads to negative impact on the sustainability of microalgae biofuels production.

Thus, in the present study, a pilot-scale sequential baffled column photobioreactor was designed to evaluate the feasibility of mass cultivation of *Chlorella vulgaris* with organic fertilizer as nutrients source (contaminated environment). Potential of semibatch cultivation under indoor and outdoor environment in the pilot-scale photobioreactor was also carried out to accelerate the biomass productivity. In addition, life cycle energy balance on producing microalgae biodiesel was performed based on the experimental data obtained in the present study. Energy efficiency ratio (EER) was used as an indicator to determine the sustainability of microalgae biodiesel production from the energy perspective. Apart from that, economic assessment on producing microalgae biomass and biodiesel was also carried out to estimate the production cost of this renewable feedstock.

2. Materials and methods

2.1. Pure microalgae strain and cultivation conditions

A wild-type *Chlorella vulgaris* was isolated from local freshwater located at Penang, Malaysia. The microalgae was preserved and grown in Bold's Basal Medium (BBM), consisting of: (1) 10 mL per liter of culture medium with the following chemicals: NaNO₃ (25 g/L), CaCl₂·2H₂O (2.5 g/L), MgSO₄·7H₂O (7.5 g/L), K₂HPO₄ (7.5 g/L), KH₂PO₄ (17.5 g/L), NaCl (2.5 g/L) and (2) 1 mL per liter of culture medium with the following chemicals: EDTA anhydrous (50 g/L), KOH (31 g/L), FeSO₄·7H₂O (4.98 g/L), H₂SO₄ (1 mL), H₃BO₃ (11.4 g/L), ZnSO₄·7H₂O (8.82 g/L), MnCl₂·4H₂O (1.44 g/L), MoO₃ (0.71 g/L), CuSO₄·5H₂O (1.57 g/L), Co(NO₃)₂·6H₂O (0.49 g/L). The initial pH of the medium was adjusted to 6.8. The seed culture was grown in a 100 mL Erlenmeyer flask containing 50 mL of medium, aerated with compressed air with surrounding temperature of 25–28 °C and illuminated with cool-white fluorescent light (Philip TL-D 36 W/865, light intensity of 60–70 µmol m⁻² s⁻¹).

2.2. Preparation of organic fertilizer

Baja Serbajadi Humus 27 (organic fertilizer or compost) with granular shape was purchased from a local market. 10 g of the fertilizer was immersed in 600 mL tap water and stirred for 24 h using magnetic stirrer. Non-soluble particulate solids were observed after the stirring process and were filtered using filter paper (Double Rings 101). The resulting organic fertilizer medium was dark-brown in color with typical characteristics as shown in Table 1.

| Table 1 | |
|---|--|
| Characteristics of organic fertilizer medium. | |

| Parameter | Unit | Concentration | Method |
|----------------------------|------|---------------|----------------------|
| COD | ppm | 1729.9 | APHA 5220 B (2005) |
| BOD – 5 days test at 20 °C | ppm | 576.0 | APHA 5210 B (2005) |
| Nitrogen | ppm | 1323.2 | APHA 4500-NH3 F |
| Phosphorus | ppm | 620.1 | APHA 4500-P E (2005) |
| Potassium | ppm | 634.4 | APHA 3111 B (2005) |
| Calcium | ppm | 269.9 | APHA 3111 B (2005) |
| Magnesium | ppm | 54.5 | APHA 3111 B (2005) |
| Manganese | ppm | 1.0 | APHA 3111 B (2005) |
| Boron | ppm | 4.1 | APHA 4500-B C (2005) |
| Iron | ppm | 1.3 | APHA 3111 B (2005) |

2.3. Lab-scale (5 L) cultivation

In 5 L lab-scale cultivation, optimized conditions in previous study was applied [19]. Briefly, 100 mL organic fertilizer medium was introduced into a photobioreactor with 5 L of tap water (without sterilization) and the cultivation was adjusted to pH 5. Then, *Chlorella vulgaris* with initial cell concentration of 0.3×10^6 cells (around 10 mL from the seed culture) was introduced into the photobioreactor. The photobioreactor was aerated with compressed air with flow rate of 0.4 L/min and illuminated continuously with cool-white fluorescent light (Philip TL-D 36 W/865, light intensity of 60–70 µmol m⁻² s⁻¹).

2.4. Pilot-scale (100 L) cultivation

A pilot-scale photobioreactor was developed to cultivate *Chlorella vulgaris*. This newly designed sequential baffled column photobioreactor was made with acrylic material (highly transparent) and has a working volume of 100 L as illustrated in Fig. 1. The developed baffle system is aimed to prolong the retention time of CO_2 in water and to provide homogenize mixing to the microal-gae instead of solely depending on bubbling aeration.

2.4.1. Cultivation under indoor environment

In 100 L pilot-scale study, batch and semi-batch cultivation were conducted. For batch cultivation, optimized conditions in previous study was applied [19]. Briefly, the cultivation was supplied with 2 L of organic fertilizer medium (or corresponded to 26.7 mg/L nitrate), pH of 5 and continuously illuminated with fluorescent light. The initial seed culture was 5 L (Section 2.3) or corresponded to about 2.3–2.5 g/L dry cell weight of *Chlorella vulgaris*. Then, the cultivation was aerated with compressed air (0.03%) with total air flow rate of 6 L/min in the presence and absence of sequential baffled device. Further study with 5% of CO₂ was also conducted to determine the CO₂ removal efficiency by the microalgae.

For semi-batch cultivation, once the microalgae had reached stationary phase, 20 L of cultivation medium was withdrawn from the photobioreactor and replenish with 20 L of fresh tap water into the photobioreactor. Then, the microalgae were allowed to grow for the next 5 days. For the subsequent cycles of semi-batch cultivation, previously withdrawn cultivation medium was recycled instead of using fresh tap water. Nitrate content in cultivation medium was checked to ensure sufficient nutrients are available for microalgae to utilize. All measurements were measured triplicate with relative standard deviation less than or equal to 8%.

2.4.2. Cultivation under outdoor environment

The cultivation conditions for outdoor environment (batch and semi-batch) were the same as under indoor environment (Section 2.4.1), except illumination was provided by sunlight. Sunlight intensity and water temperature was checked daily with light intensity meter and thermometer, respectively. Five measurements of light intensity and water temperature were taken at different interval daytime (7 a.m. to 7 p.m.) and an average value was recorded. All measurements were measured triplicate with relative standard deviation less than or equal to 8%.

2.4.3. Microalgae biomass harvesting

When *Chlorella vulgaris* had grown to stationary phase, air aeration to the 100 L photobioreactor was stopped. The microalgae were let to settle naturally to the bottom of photobioreactor for two days. Two distinguished layers were observed, in which the upper layer consist of water with suspended microalgae cells and the bottom layer was microalgae biomass. The upper layer water was slowly decanted, leaving behind the microalgae biomass Download English Version:

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