Composition of fatty acids from cultivated *Botryococcus braunii* algae in dome-shaped photobioreactor for biofuel production

Dusit Buagate[a], Pisit Maneechot[a], Wattanapong Rakwichian[b], Anusorn Vorasingha[c] and Prapita Thanarak[a]

[a]School of Renewable Energy Technology,
Naresuan University, Muang, Phitsanulok 65000, Thailand
Tel. +66-5596-3193 Fax. +66-5596-3182
E-mail: dusitbuagate@gmail.com, pisitm@nu.ac.th, prapitat@nu.ac.th
[b]School of Energy and Environment, University of Phayao, Muang, Phayao 56000, Thailand
[c]Department of Chemistry, Faculty of Science, Naresuan University, Muang, Phitsanulok 65000, Thailand
Tel. +66-5596-3461 Fax. +66-5596-3401
E-mail: anusornv@nu.ac.th

Abstract

Dome-shaped photobioreactor (PBR) was characteristically designed to cultivate for *Botryococcus braunii* (Bb) algae for investigating oil production. Its volumetric capacity was 30 L in each culture unit. Experimental treatments were designed to circulate a Bb culture solution in 4 different flow rates as 1, 5, 10 and 15 L/min. In these experiments, 12 culture units were structured to demonstrate comparison in various different conditions which each unit was arranged with a completely randomize design (CRD). The experiments were performed in 3 replicates. Experimental site was established at Rim Si Muang Sub district, Khao Kho District, Phetchabun Province, Thailand (16° 33′ 29.8″ N and 101° 01′ 19.6″ E). Elevation of the location is 815 m. above mean sea level where a Bb strain was found and collected from a fish pond. Then Bb algae were cultured from February 23 - March 14, 2012 with modified Chu 13 media for 20 days as previously done by Velichkova et al. (2012) under an ambient condition without adding CO_2 for photosynthesis. Mean daily ambient temperature was in range of 16.6 - 35.9 ºC. Bb algae were harvested after 20 days of cultivation. Next, the water was staved off from Bb algae using a centrifuge (FALCON 6/300) at 3,000 rpm for 15 min. In addition, algal broth was preliminarily dried using a hot air oven (Memmert, Model 100-800) at temperature of 90 ºC for 48 hrs. And was powdered using a cylindrical ball mill for 4 hrs. Algae oil extraction was done in a hexane and using sonication for 60 min. Finally, hexane was evaporated from the algae oil using a rotary evaporator. The results showed that dry weights of biomass production were 0.009±0.02, 0.137.0±0.06, 0.696±0.06, and 0.006±0.06 g dry weight/L with flow rates of 1, 5, 10 and 15 L/min, respectively. Algae oil production was 0.0753 ±0.02 and 0.383 ±0.01 g/ with flow rates of 5 and 10 L/min, respectively, whereas it was regardless for flow rates 1 and 15 L/min because of insufficient quantity of biomass. Highest dry weight of biomass production was 0.696± 0.06 g/L with flow rates 10 L/min. Conclusion; the suitable flow rate of 10 L/min had significantly a great effect in an increase of biomass concentration of Bb algae when cultured in a prototype of dome-shaped PBR. Fatty acid composition was analyzed by a gas chromatography – mass spectrometry (GC-MS) that was Docosanoic acid (Behenic acid: C22:0), Heptadecanoic acid (Margaric acid: C17:0) and Octadecanoic acid (Stearic acid: C18:0). The quantity of fatty acids was approximately 6.96 % v/v (all contain) were which can utilize to compound for biofuel production.

Keywords: *Botryococcus braunii cultivation, Dome-shaped photobioreactor, Biofuel production, Fatty acid, Algae oil extraction*

1. Introduction

New energy consumption is continuously increasing because of industrialization and world population. The major sources of this energy are petroleum, natural gas, coal and nuclear [1]. The disadvantage of using petroleum based fuels is atmospheric pollution created by refining process of petroleum; along with the burning of coal and petroleum combustion which is the largest contributor to increase CO_2 in atmosphere as a source of greenhouse gas. Petroleum diesel is considered as a

major source of other air contaminants including NOx, SOx, CO, particulate matter and volatile organic compounds [2]. Furthermore**,** it is predicted that worldwide petroleum oil discoveries have been less than annual production since 1980 and worldwide production is near its maximum [3], [4]. The main cause of global warming is the burning of an enormous amount of fossil fuel that increased the CO_2 level in the atmosphere. Of the major problems mentioned above, renewable sources of energy are being highly sought.

Bioenergy is one of the most important sources to mitigate greenhouse gas emissions and substitute for fossil fuels [5]. Biomass is part of the bioenergy and considered as better sources of energy [1]. Biomass has been focused on as an alternative energy source because it is a renewable energy resource and fixes CO_2 in the atmosphere by photosynthesis. If biomass is grown in a sustained way, its combustion has no impact on the CO_2 balance in the atmosphere [6]. Furthermore, an introduction of biomass energy could contribute to sustainable development for environment, sociality and economy. [7]. Biodiesel is the one of the alternative fuels-obtained by transesterification of triglyceride oil with monohydric alcohols [8]. It can be obtained from many crops such as canola and soybean, palm, sunflower oil, or algal oil as a diesel fuel substitute [9]. and is also a nontoxic and biodegradable alternative fuel that is obtained from renewable sources. Among biomass, algae are targeted as they usually have higher photosynthetic efficiency than other biomass and considered to be one of the best sources of biodiesel [10]. Algae are the highest yielding feedstock for biodiesel as they can produce up to 250 times the amount of oil per acre as compared to that of soybeans and 7 to 31 times greater oil than palm oil [10]. Microalgae require 140-200 kg of water per kilogram of CO_2 fixed compared with more than 550 kg of water per kg of CO_2 fixed by trees [11]. Unlike for trees, microalgae can utilize water of low quality (waste water) or even high salinity water where both are unsuitable for agriculture use or human consumption [11]. In addition, microalgae would be the best algae for biodiesel because they are an organism capable of photosynthesis, are much faster and easier to grow and produce more oil other than macro algae [10].

Botryococcus braunii (Bb) is a pyramid shaped planktonic microalga, generally live in freshwater [12] , [13]. It is a unicellular species which forms colonies that are held together by lipid biofilms [14]. It contains high lipid content in the form of hydrocarbon ranging from 25 to 76% which can be converted into biofuels [15], [16], [17]. In this organism, up to 40% of dry cell weight consists of fatty acids. It has received great attention in the last two decades, because the hydrocarbon has the potential for use as an alternative fuel [18]. It can also be used as feedstock for hydrocracking in oil refinery to produce gasoline, kerosene, and diesel [19]. With characteristics of Bb that can grow rapidly in nature [20] and uses sunlight and CO_2 for growth so that it can reduce CO_2 in the atmosphere which is the main cause of global warming [21].

An interesting in Bb has historically focused upon its role in the formation of oil deposits [22]. It has been suggested that Bb might be a large renewable source of liquid hydrocarbon because it forms massive floating blooms depend on the potential for large scale cultivation and efficient harvesting [23].

Cultivation of Bb in photobioreactors offers a sustainable method for CO_2 capture and storage. It is suitable in semi-arid or arid lands without competing with human habitat or agriculture production [11]. In addition, CO_2 fixation using microalgae grown in photobioreactors does not require CO_2 capture, concentration, and scrubbing of SO_x and NO_x prior to using the flue gas from fossil fuel refinery.

Recently, present reviews on PBR for mass cultivation of microalgae, they are found that include following PBR systems; open ponds, tubular PBR, vertical-column PBR and flat-plate PBR [24].

Firstly, open ponds are widely used because of low cost and ease of operation. However, they are sensitive to contamination leading to introduction of unwanted fast growing organisms in the ponds. These organisms will feed on the autotrophic biomass and lead to loss of productivity [17]. Secondly, tubular PBR system is better in contamination control, but photosynthesis will generate high oxygen

level in the system. Algae cells can be damaged by photo oxidative induced by a high concentration of dissolved oxygen in combination with intense sunlight. Thirdly, a vertical-column PBR consumes low energy and the system is easy to sterilize. This system has a low illumination surface area. The construction requires complicated materials and creates shear stress to algal culture. Lastly, flat-plate PBR system has large illumination surface area with low oxygen build up. However, this system requires many compartments and support materials for scale-up design. Possibility of hydrodynamic stress to some algal strains is taken into account.

The growth of living things such as plants and algae that needs photosynthesis. Consequently, light is essential in the photosynthesis. If there has more radiation receptive area enough, the growth of algae is better. Many species of algae could be naturally occurred in local area while Bb algae are more interesting that easy to find. Bb algae are naturally able to move by the movement of other living things such as fish etc. in the water.

Nowadays, Biodiesel has become very attractive as a biofuel because of its environmental benefits. It has less air pollutants per net energy than another commercial fuel and is nontoxic and biodegradable and because it produced from renewable sources with high energetic efficiency than the energy invested in producing its self.

Most biodiesel is produced today by the transesterification of triglycerides of refined/edible type oils using methanol and together with alkaline catalyst (sodium hydroxide; NaOH, potassium hydroxide: KOH, sodium methoxide: $NaOCH_3$)

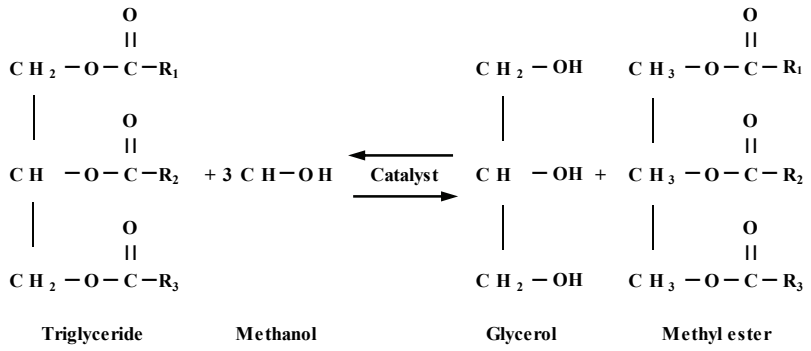

Figure 1. Transesterification of triglyceride

The reaction was normally performed at 70-80ºC. The glycerol and Fatty Acid Methyl Ester (FAME) were separated by setting after catalyst neutralization. The crude glycerol and biodiesel obtained are the purified. The process of transesterification as show in Fig.1

Therefore, researcher focused on dome-shaped type since there has more radiation receptive area than other types. Moreover, it could steady control in flow rate as well. This research was mainly aimed to mitigate some disadvantages occurred in the systems of photobioreactor including investigates the quantitative change of biomass in various flow rates, finds out growth rates of Bb algae under ambient condition and reveals fatty acid composition of Bb algae for biofuel production.

2. Materials and methods

2.1 Construction of dome-shaped PBR as a prototype for Bb culture

Experimental site was established at Rim Si Muang Sub district, Khao Kho District, Phetchabun Province, Thailand (16° 33′ 29.8″ N and 101° 01′ 19.6″ E) and elevation of the location is 815 m. above mean sea level.

Dome-shaped PBR was constructed that was of 1 m. in diameter and 0.5 m. in height. Its surface area was covered with PVC transparent tube of 1 inch inside diameter and 1/8 inch thickness. Chimney was placed on top of transparent acrylic tube which was 2 inch in diameter and 40 inch in length (Thai Pipe Co., Ltd.). Total volumetric capacity of 30 L in each dome-shaped PBR was then fitted Fig.2.

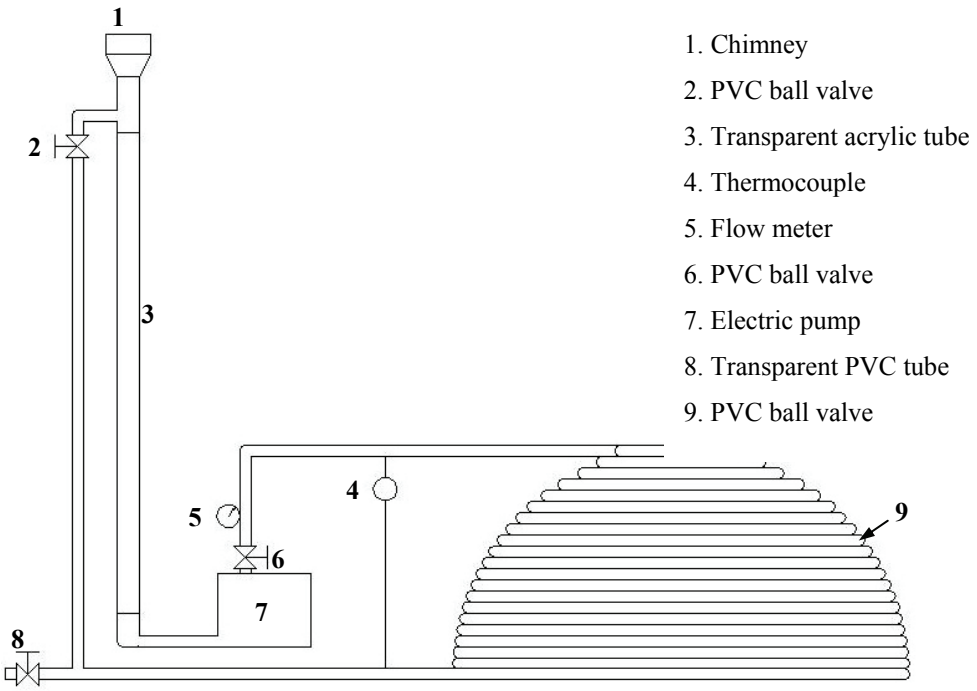

Figure 2. Simplified diagram of dome-shape photobioreactor

The system operation is described as follows;

Firstly, Bb algae solution was filled into a chimney (1) in each unit (30L). Then, the solution was circulated by an electronic pump of 0.37 kW (7) to shift in dome-shaped PBR system. The solution flow rates were specifically controlled by valve (2) and valve (6), were 1, 5, 10 and 15 L/min while a valve (8) was installed to harvest Bb algae production for investigating the growth rate of Bb algae in every 5 days interval. Moreover, a monitoring point was made from a transparent acrylic tube (3) to observe the solution color change with the eyes throughout cultivation time. The solution temperature was measured at around a point of entrance way of dome-shaped PBR by a thermocouple also (4). Then, they were transferred and recorded at every 1 hr. interval during daytime and nighttime throughout cultivation time with a data logger. The system operation time was daily begun at 5:00 a.m. until 5:00 p.m.

The experiment was arranged incompletely randomized design (CRD) with 3 replicates therefore tested with 12 culture units. The Dome-shaped PBR was designed similar to a canopy of trees. It was better than other types due to it has a bigger radiation receptive area (RRA), produces more productivity. Moreover, when the scaled up underneath area of dome could use for other beneficial purposes. Comparison on calculated RRA between dome-shaped PBR with other types as shown in Table 1.

Table 1: Calculated RRA of various types of PBR

Types	RRA* (m^2)	Ratio
Flat plate PBR	2.20	7
Open pond	0.33	1
Tubular PBR	1.30	4
Vertical-column PBR	1.30	4
Dome-Shaped PBR	4.90	15

Note: * Calculation at solution capacity of any types of PBR 100 liters.

Photobioreactors for mass cultivation of microalgae, it is found that they include following PBR systems; open ponds, tubular PBR, vertical-column PBR and flat-plate PBR.

From Table 1 indicated that dome-shaped PBR has a biggest RRA equals to 4.90 m^2 which could receive solar radiation more than open pond that was 15 times at the same solution capacity (100 liters).

2.2 Sampling of Bb algae

Bb algae strain was selected from a natural pond where was around near the experimental area.

2.3 Culture conditions

The algae solution was mixed with water of 22.5 L, Bb algae strains of 7.5 L and modified CHU 13 medium of 30.722 g. Firstly, the solution of 1 L was randomly taken to measure dry weight of Bb algae sample which happened that was of 0.083 g of dry weight/L. Solution pH for algal culture was adjusted to 6.5-8.5 by using KOH or H_2SO_4. The mixed Bb algae solution was put into each culture unit (30 L in volume) for cultivation. The culture unit was placed outdoors in order to expose to solar radiation in natural condition.

2.4 Data collection

1. Daily minimum, maximum, and average temperature of either solution or ambient air
2. Daily minimum, maximum, and average solar radiation
3. Dry weight of Bb algae was calculated after 20 days of cultivation
4. Oil production from dry weight
5. Types and amount of fatty acid for biofuel production

Percentage of Bb algae is calculated by the following formula.

$$Y = \frac{w_2 - w_1}{w_1} x100 \quad (1)$$

When Y = percentage of Bb
w_1 = dry weight of Bb algae as a starter
w_2 = dry weight of Bb algae at specific time of cultivation

Percentage of oil is calculated by the following formula.

$$P = \frac{v}{w} x100 \quad (2)$$

When P = percentage of oil
w = dry weight of Bb
v = volume of oil (by weight)

2.5 Harvest and oil extraction

After 20 days of cultivation, Bb algae cells were harvested and processed by using a centrifuge to produce algal broth. The broth was directly dried in sunlight for 1 day and then heated at 90°C in a hot air oven until obtaining dried cells. Dry weight of Bb cells was then calculated. The ground algae cells were extracted for oil in hexane. The mixture for extraction was left for 24 h for settling. Extracted oil was purified by a rotary evaporator to evaporate and release hexane. Triglyceride produced from the extraction was reacted with methanol through the process of transesterification.

2.6 Transesterification

The transesterification was carried cut in a batch reactor. The algae oil (50 cm^3) in a 500 cm^3 round-bottom flask equipped with a reflux condenser was stirred at 70º C. A mixture of methanol (MeOH) and catalyst preheated at 60º C was added to the oil. Then the transesterification reaction was conducted under conditions of various MeOH to oil ratios (9:1, 12:1, 15:1 and 18:1) and catalyst amount of 1.0 wt % for the required reaction time. To finish the reaction, the catalyst was separated from biodiesel product by centrifugation, and then excessive amount of methanol was evaporated before analysis of % FAME.

3. Results and Discussion

The outlook of Bb colony and Bb cell used for this study were shown in Figure 4.

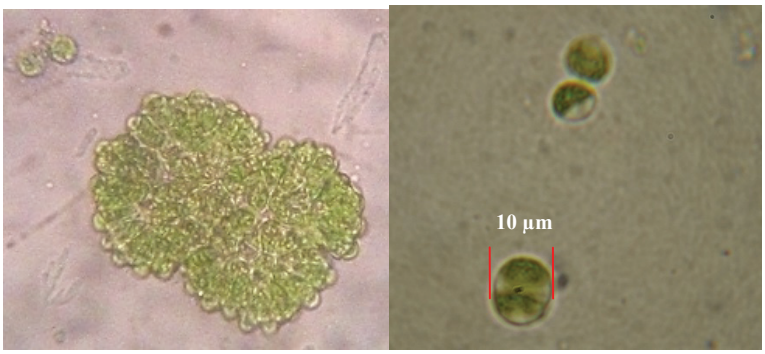

Figure 4. Collected Bb algae colony and Bb algae cell (During cultivation) observed under microscope

During 20 days of cultivation (February 23 - March 14, 2012), temperature and solar radiation were measured at 5 days interval at the site of the experiment. It was found that mean temperature of culture solution at daytime and nighttime was 27.3 and 22.6º C, respectively while maximum and minimum temperature during cultivation were 34.2 and 12.6 º C, respectively. As for mean and maximum solar radiation were 369.5 w/m^2 and 842.5 w/m^2, respectively (Fig 5).

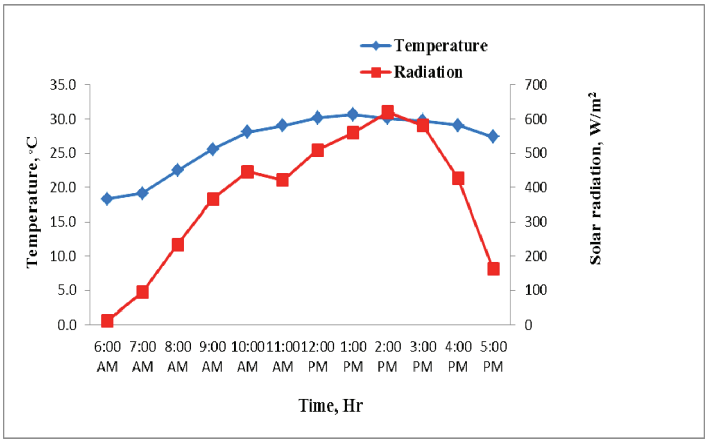

Figure 5. Mean temperature and solar radiation average in cultivated time

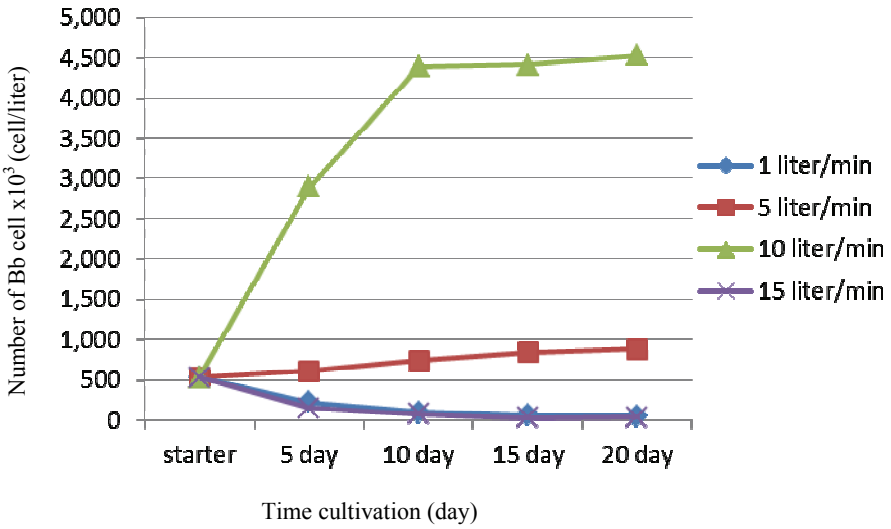

Figure 6. The growth of Bb in cultivated time

From the figure 6.A number of different algal cells, which are results of the flow rate of the solution used for cultured. And the number of algal cells increased the most was 10 liter / min when fed through to 10 day and a little more until harvest. The second is that the algae increase the solution flow rate 5 liter / min, the flow rate of solution of 1 liter / min and 15 liter / min number of cells is decreased rapidly until dead.

Dry weight of Bb algae was greatly affected by flow rate and the algae production was a greatest value that equals to 0.696 g/L when cultured at the flow rate of 10 L/min compared with the flow rates of 5 L/min that equals to 0.137 g/L while the lowest- and highest flow rates were 1 and 15 L/min, respectively, showed that have adverse effect to dry weight of Bb algae and negative values in percentage of change as show in Table 2.

Table 2: Comparison in dry weight between starter and harvest in various flow rates.

Flow rate (L/min)	Dry weight (g /L)		% change
	starter[x]	Harvest	
1	0.083	0.009 ± 0.02 a	-89
5	0.083	0.137 ± 0.06 b	65
10	0.083	0.696 ± 0.06 c	739
15	0.083	0.006 ± 0.06 a	-93

x = dry weight of the sample taken from culture solution mixed with Bb algae stock before cultivation for each flow rate.

Note: same letters within the same column indicates there is no significant difference at p 0.05.

Bb algae growth in a natural pond could be moved with a fish or an aquatic animal because the Bb algae cell has no tail (flagellum) for dislocating to receive solar radiation therefore circulation process was important for Bb cultivation. Suitable flow rate for Bb production was showed in Table 3.

Table 3: Oil production in any flow rates.

Flow rate (L/min)	Oil (g/g dw)
1	N
5	0.753 ±0.02
10	0.383 ±0.01
15	N

Note: Data recorded after culture for 20 days. Values are mean ± SD, N = Not specified as insufficient quantity of the samples

Table 3 showed that oil productions of Bb algae were 0.0753 and 0.383 g/g dw the flow rate of 5 and 10 L/min., respectively, while which of the flow rate of 1and 15 L/min were insignificantly lowest value. As for the oil production with the flow rate of 1 and 15 L/min, they could not be investigated since the obtained quantities of the biomass samples were inconsiderably very small.

The influence of the flow rate of algal cultivation in the system demonstrated that was an important factor for the overall production. Among the flow rates used, there was apparent that the flow rate of 10 L/min could produce best oil production for Bb algae cultivation in dome-shaped bioreactor under ambient conditions and with no CO_2 supply. As for the flow rate of 10 L/min, the increased algal biomass, the highest biomass production and the highest volume of oil (by weight) were 739%, 0.696± 0.06 g/L and 0.383 g/L, respectively.

Using a GC-MC, fatty acids obtained that were compounded of docosanoic acid (behenic acid: C22:0), heptadecanoic acid (margaric acid: C17:0) and octadecanoic acid (stearic acid: C: 18:0) which were the amount of 6.96 % v/v (all contain). Nevertheless, the occurred productivity of algae biomass was considered that was quite low when compared with a previous research was carried out by Ranga Rao, A. et al., (2012) which the results showed that 1.5 g/L of algae biomass received from Bb cultured with the accompany of supplemented of 0.1% $NaHCO_3$. As for the low Bb algae oil

production in this study, it may cause from influence of $NaHCO_3$ and CO_2 control condition because $NaHCO_3$ and CO_2 was not supplemented in order to reduce production costs.

4. Conclusion

Temperature is crucial for growth of algae and the optimum is 23 ºC (27). This experiment was carried out at the site where its altitude is 815 m above mean sea level with mean temperature is 23.4 ºC. Thus the site is considered to be a suitable commercial oil production site for Bb culture, especially when the cost of monitoring of temperature is taken into account for. The designed dome shaped PBR for Bb culture in this study also could be an effective bioreactor for Bb culture as its light receptive area is greater by a simple calculation for surface area between dome-shape PBR and other existing models of PBR. The results showed that the dry weight of biomass was the highest with a flow rate of 10 L/min; more or less such a flow rate was much more ineffective. Naturally, Bb algae are immobile by themselves, so they relied on some movable organism to contribute for water movement in order to circulate them in water. Bb algae therefore will displace in order to get light for photosynthesis. Bb algae cell number. In addition, during the first 0-10 days of Bb algae cultivation, there was an increase in the amount of Bb algae cells very rapidly because Bb algae might be budged to gain an appropriate amount of light for their growth. After that until the end of the algae cultivation time (10-20 days), the number of cells Bb algae gradually increased slowly until eventually rather constant maybe because the amount of Bb algae might be at maximum in the solution which could directly affect to light radiation that is insufficient to the inner layer of the Bb algae cells for photosynthesis.

Therefore the obtained results showed that the researched Bb algae cultivation in dome-shaped PBR with a flow rate of 10L/min was effectively in the highest production of dry weight of Bb algae, oil production and biofuel content. As mentioned above, biofuel extracted from Bb algae, therefore, is technically feasible. Although, some constraints should be minimized in order to obtain the higher productivity of biofuel.

5. Recommendations

- Bb algae cultivation in dome-shaped should be considerably cultured in 10 L/min flow rate and measured the algal growth rate every 1 day.

- Extra experiments should be supplemented to compare the algal production between the CO_2 and the no CO_2 additional conditions (ambient condition).

- Materials of dome-shaped PBR should be constructed with a UV resistant material as an acyclic tube for protecting the obscure vision because solar light will decreasingly radiate thru the PBR into algae.

To determine more comfortable method without using chemical extraction process such as extraction by using mechanical compression.

6. Acknowledgement

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