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Optimisation of *Botryococcus* sp. Growth Using Synthetic Media (N: P) for Biodiesel Production

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ABSTRACT

Algae biodiesel is undeniably very promising as an energy substitute for fossil fuel. It mass cultivation though requires huge capital investment. The aim of this study was to find a simple, inexpensive and tolerable media for algae growth. The optimal growth conditions for algae growth were studied. *Botryococcus* sp. was isolated from Sembrong Dam in Johor, Malaysia. In this study, two media were used, namely bold's basal medium (BBM) and synthetic media from nitrogen and phosphorus compound. The synthetic media consisted of ammonium chloride and monopotassium phosphate that were blended together and modified into desired ratios. The N: P ratio of 1.5:1 yielded the highest chlorophyll-a concentration and the optimal growth conditions of algae for both media were at 6000 Lux, pH 7 and 30 rpm. The BMM had the highest algae growth, 3.25 x 10^7cell/ml while the synthetic media yielded a maximum cell concentration of up to 1.025 x 10^7 cell/ml which is 68.5% lower compared with BBM. The findings of this study point to the importance of large scale production of algae useful for industrial production of biodiesel.

Keywords: Algae, biodiesel, *Botryococcus* sp., growth media

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INTRODUCTION

In recent years, there has notable development of microalgae biodiesel for fuel in western countries, such as American and Brazil because of the dwindling of fossil fuel stock. Therefore, there is an urgent need to stop dependency on fossil fuels and | find alternative ways for renewable fuel production. Researchers have discovered commercial value of microalgae as it can

produce more oil compared with other feedstock which is 3-35 times higher than terrestrial plants in terms of oil content (Mata et al., 2010). The large scale culturing microalgae for mass production of biodiesel is usually associated with high cost in terms of execution and maintenance. The use of algae growth media is quite expensive for large scale production of microalgae (Lim et al., 2012).

Many algae growth media have been introduced, yet, the high culturing cost impede the development of algal biodiesel production except the mass culturing in ponds where nutrients are supplied by the fish or shrimp through their life cycle (Lim et al., 2012). As the available commercial media growth is made of several chemicals is a complicated process and not a cost-effective one, there is a quest for cheaper yet efficient media growth. In this study, media growth is due to most important nutrients for algae growth, nitrogen and phosphorus (Hu et al., 2004). Ammonium ion (NH_4^+) is easier for microalgae to absorb than ammonia (NH_3), according to Harold (1966). Most algae has high amounts of phosphate as polyphosphate granules important for plant growth and animal tissue as well use to synthesize protein in them while phosphorus is an essential nutrient in converting sunlight into usable energy and vital for cellular growth and reproduction. In this study, two different types of media were compared which are N:P media and Bold's Basal Medium (BBM). The ammonium source (NH_4Cl) and phosphate (KH_2PO_4) were used to produce synthetic media and the ratio between them was adjusted for its effectiveness vis a vis algae growth. Favourable environment parameters such as intensity of light, pH culture and bioreactor speed also need to be controlled for ensuring the culture results in optimal growth.

MATERIALS AND METHODS

The Selection of Synthetic Media Grow

In this study, several ratios of N: P were tested to stimulate nutrient strength (0.6:0.2, 0.8:0.4, 1:0.6, 1.2:0.8, 1.5:0.8, 1.5:0.9, 1.5:1, 2:1, 2:1.5). The ratio selections were based on strength of N: P in Sembrong Dam water in which the average ammonia content was1.14 mg/L of ammonia and total phosphate is 1.05 mg/L. The ammonium chloride (NH_4Cl) and monopotassium phosphate (KH_2PO_4) were diluted according to desired concentration. All samples were prepared in triplicate. 250 ml of Erlenmeyer flasks were used with the initial algae concentration of 1000 cells/ml for all samples. The *Botryococcus* sp. used was isolated from the Sembrong Dam (Wellson et al., 2016). *Botryococcus* sp. was cultured under the light of white fluorescent bulbs of 4000±100 lux (photoperiod of 16:08 h light dark). The cultures were shaken gently manually twice a day for 20 days and exposed to room temperature at 27°C ± 2.

Optimization of *Botryococcus* sp.

Light Optimisation. The light intensity varied based on previous studies (Lavens & Sorgeloos., 1996; Yan Li., 2005; Stuart et al., 2011). The light intensities were 4000, 6000, 8000 and 10000 Lux. 100 ml of synthetic media was used for every sample in 250ml of conical flask. The samples were prepared in triplicate with initial inoculation of 1000 cells/ml for all sample

and shaken gently by hand twice per day and exposed to room temperature at 26°C ± 2 and cultured for 20 days. The initial pH of cultures was set at 7 and manually shaken twice per day.

Initial pH Optimization. The pH values adapted were 6, 7, 8 and 9 based on previous study (Lavens & Sorgeloos, 1996: Yan Li., 2005: Stuart et al., 2011). The hydrochloric acid (HCI) and sodium hydroxide (NaOH) were used to adjust the pH to meet the desired pH values. The initial inoculation cell was 1000 cells/ml under the white compact fluorescent bulb of 23 watts of 6000±100 lux with 16:08 photoperiod. The other conditions were set the same for optimal light.

Shaking Speed Optimization. The rotational speed for culturing *Botryococcus* sp. is essential in order to provide an adequate air mixture and uniformity of nutrients in the cultures. Three different speeds were adapted, 30, 40 and 50 rpm by using laboratory orbital shaker. The inoculation cells, lux, temperature were set at same pH optimisation and the initial pH was set at 7 pH.

RESULTS AND DISCUSSION

Synthetic Media

Based on the result of chlorophyll-a for 9 different of N:P ratios, the highest concentration of chlorophyll-a recorded was at ratio 1.5:1 with 98 µg/L. The second highest is 1.5:09 with 74 µg/L and the third highest with ratio 2:1.5 yielding 68 µg/L. Therefore, the best ratio of N:P is of 1.5:1 and ensures good synthetic media optimisations. The culture with N:P nutrients ratio were cultured for 20 days. Based on observation, the algae was able to live and tolerate the synthetic media even though its biomass was not as high compared with using BBM media growth (as in Figure 1).

Figure 1. Cultures of 1.5:1 NP ratio at 20 days

Optimisation of Light

Over a period of 20 days, the cell concentration of all cultures was counted everyday using haemocytometer in the unit cell/ml (Paran, 2014). Figure 2 shows that growth of algae increased with time and reached optimum level between 16th and 18th day within the stationary phase. The lag phase occurred from 1st to 11t^{h} day and the exponential growth was between 11th and

16th day. Generally, the death phase occurred within 17th to 20th day. The result showed that the growth of *Botryococcus* sp. is optimal under the illumination of 6000 lux. For both synthetic and BBM media at 6000 lux the optimal algae concentration was 1.025 x 10^7 cell/ml and 3.25 x 10^7cell/ml respectively. Using synthetic media results in algae concentration being 68.5% lower than commercial media BBM. This shows that the use of simple and cheap synthetic will not able to give abundant *Botryococcus* sp. as much as using BBM but the former can still be used as a cheap media for experimental purposes. The synthetic media also represent the nutrient strength in actual water source with pollutant loading. The second highest peak cell concentration is at illumination 8000 lux yielding 925 x 10^4 cell/ml equivalent to 30.8% of the peak concentration using BBM which is 3000 x 10^4 cell/ml. Additionally, under the 10000 lux, the peak concentration is 900 x 10^4 cell/ml equivalent to 32.7% of the highest cell concentration using BBM which is 2750 x 10^4 cell/ml. The light intensity of 4000 lux produces the least cell concentration compared with the rest; light intensity is only reached at the peak of 800 x10^4 cell/ml or 40% of the Bbm (2000 x 10^4 cell/ml). This is consistent with the findings of Sharma (2011) who pointed out the optimum range for light is between 5000-7500 lux, and 6000 lux is the optimal for growth of *Botryococcus* sp. Other researchers cultured the botryococcus with light intensity in the range of 6000-7000 Lux (Stuart et al., 2011: Nagaraja et al., 2014).

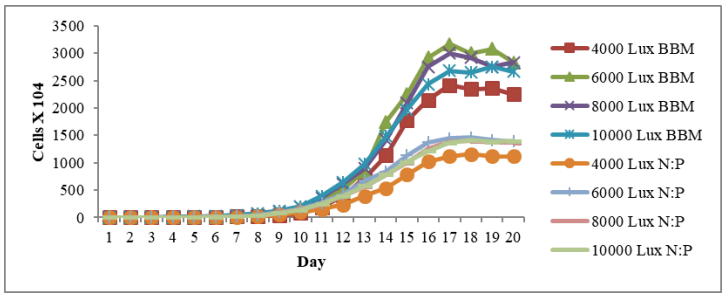

Figure 2. Graph of optimal light intensities

pH Optimisation

The growth of algae is also influenced by the pH level of the media (Mata et al., 2010). Based on Figure 3, the trend of graph is similar to light optimisation. *Botryococcus* sp. grows under optimal conditions of pH 7 with maximum cell concentration of 1100 x 10^4 cell/ml or 56% less than the peak cell concentration using BBM (2500 x 10^4 cell/ml). Furthermore, the graph for pH 7 and pH 8 is almost the same at 15th day, in which the cell concentration at pH 8 is higher than pH 7 until the 14th day before it declines. The peak cell concentration for pH 8 was 1050 x 10^4 cell/ml or 45.5% at the maximum cell concentration using BBM (2250 x 10^4 cell/ml). The peak cell concentration of algae cultured in N:P media with pH 9 yields 900 x 10^4 cell/ml equivalent to 45% of the peak cell concentration of using BBM (2000 x10^4 cell/ml). The

Botryococcus sp. grew slowly at pH 6 as the maximum yielding concentration was 775 x10^4 cell/ml or 59% than the peak cell concentration using BBM (1600 x10^4 cell/ml). The optimal pH range for the cultivation of microalgae of all species is between 7 and 9 (Huo et al., 2011; Sharma, 2011). It will be easier for microalgae to capture carbon dioxide in the atmosphere when the growing condition is alkaline, which can produce greater biomass (Zang et al., 2011). The *Botryococcus* sp. is not suitable to be cultured under the pH below 7. Based on the study by Nagaraja et al. (2014), the *Botryococcus* sp. optimal growth was at pH of 6.5 using BBM.

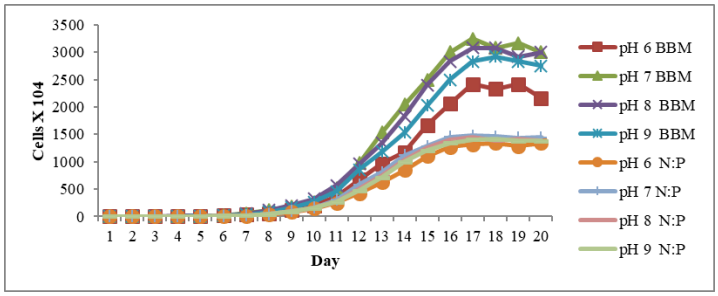

Figure 3. Graph of pH optimisation

Shaking Speed Optimisation

Mixing is necessary for microalgae cultivation to prevent sedimentation and to ensure all cells receive equal amount of light and nutrients. Figure 4 shows a less cell concentration is yielded when cultured under continuous rotating orbital shaker and the growth curve of algae has the same trend of light and pH optimisation. As the rpm speed declined the cell concentration increased. The speed at 30 rpm yields the highest cells concentration, 157.5 x 10^4 cell/mL or 52.5% of the peak concentration using BBM (300 x104 cell/ml) while speed at 40 rpm yields 150 x 10^4 cell/ml equivalent to 54% of the highest concentration using BBM (275 x 10^4 cell/m). The greater the speed of rpm the less concentration of cells is observed. At 50 rpm, the cultures were only able to reach a peak with 145 x 10^4 cell/ml or 58% of the peak cell concentration using BBM (250 x10^4 cell/ml). The rotation at 60 rpm gives the lowest peak concentration, which is 137.5 x10^4 cell/ml or 61%, than the peak cell concentration using BBM (225 x 10^4 cell/ml). Under continuous rotation, *Botryococcus* sp. was seen accumulated at the centre of the flask as a result of centrifugal forces. Eroglu, Okada and Melis (2011) reported the same scenario with *Botryococcus braunii* when the orbital shaker were set continuously at a greater rpm.

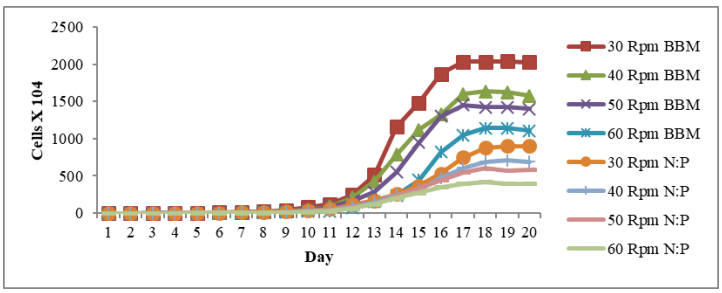

Figure 4. Graph of optimal shaking rate

CONCLUSION

In conclusion, BBM to grow *Botryococcus* sp. is more promising compared with synthetic media which provides 68.5% lower yield than the cell concentration using BBM. *Botryococcus* sp. can be cultured in synthetic media by adjusting N: P ratio of 1.5:1 to produce moderate cells concentration and stimulate the actual condition of *Botryococcus* sp. growth. The optimal growth is observed under the exposure of 6000 lux of light, pH 7 and shaking rate of 30 rpm. The N:P can be further studied, improved and blended with some other nutrients for better performance.

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