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Preface: The 3rd International Seminar on Fundamental and Application of Chemical Engineering 2016 (ISFAChE 2016)

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Cultivation of *Chlorella Vulgaris* Using Different Sources of Carbon and Its Impact on Lipid Production

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ABSTRACT. A cultivation process of *Chlorella vulgaris* has been done in different treatment to investigate the optimum condition for lipid production. Firstly, autotroph and heterotroph condition have been applied to test the significance impact of carbon availability to the growth and lipid production of *Chlorella vulgaris*. And for the same purpose, heterotroph condition using glucose, fructose and sucrose as carbon sources was independently implemented. The growth rate of *Chlorella vulgaris* in autotroph condition was much slower than those in heterotroph. The different sources of carbon gave no significant different in the growth pattern, but in term of lipid production it was presented a considerable result. At lower concentration (3 and 6 gr/L) of carbon sources there was only slight different in lipid production level. At higher concentration (12 gr/L) glucose as a carbon source produced the highest result, 60.18% (w/w) compared to fructose and sucrose that produced 27.34% (w/w) and 18.19% (w/w) respectively.

Keywords : *Chlorella vulgaris*, fructose, glucose, lipid, sucrose

INTRODUCTION

A rapid growth rate of world population since the era of industrial revolution has resulted in serious problems on the human life condition. United Nations (UN) has announced Sustainable Development Goals (SDGs), in which 17 issues reflecting those problems are elaborated in detail. Energy is an essential need to support daily activities and industrial sector, the demand on energy is now much higher in parallel with global population number. Nowadays, supply to fulfill energy demand is still relying on non-renewable energy sources. Unfortunately, the continual usage of fossil fuels (e.g. oil, coal) produces side products that negatively affect the quality of environment. Several environmental problems such as ozone depletion, climate change, acid rain and local air pollution are exist because of gasses from the combustion process of fossil fuels (Khan et al, 2009). Moreover dependency on fossil fuels will create economic problem since these are considered finite materials. The stock of fossil fuels is gradually decreasing and it will significantly increase the price. To anticipate that condition, UN has declared in SDGs that by 2030 the share of renewable energy in the global energy mix should increase substantially. Thus the introduction of alternative energy needs to be fostered. Microalgae is a potential natural resource for substituting fossil fuel because of its high lipids content. Several researches reported that biomass of microalgae is a promising material to fulfill the global energy demand especially in the transportation sector (Christi 2009, Demirbas and Ayhan 2010). Comparing the efficiency in oil production, microalgae has 20 times more productivity than palm oil in the same size of application area (Khan et al., 2009). Unlike the utilization of vegetable oil for biodiesel that creating conflict as it also used for food ingredient, microalgae will not interfere the process supply of food material. In addition, microalgae will positively contribute to prevent eutrophication in the water body by absorbing excess nutrients from domestic wastewater and/or runoff water from farming lands. Among various species, *chlorella vulgaris* has been identified as the most potential microalgae for biodiesel production since the measured lipids content were as high as 58% (Mata et al., 2010).

In biodiesel production from microalgae, there are two significant processes: (1) cultivation of microalgae to get the biomass and lipids content as high as possible and (2) synthesis process in order to

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get the biodiesel itself. Optimum result from both processes will yield a high volume of biodiesel, thus the introduction of this alternative source will be more feasible. For that reason, research to determine the most suitable condition for both cultivation and synthesis process is continually undertaken. The aim of this study is to evaluate the effect of internal carbon source (heterotroph) and external carbon source (autotroph) to the cultivation process of *Chlorella vulgaris*. Fructose, glucose and sucrose in different concentrations have been used to test the most suitable carbon source to the lipids production.

METHODOLOGY

PHM media solution

PHM media solution was prepared from the mixture of 0.5 ml Fe, 0.5 ml trace metal solution, 0.5 ml solid extract solution, 0.5 gr KNO_3, 0.5 gr $MgSO_4.7H_2O$ which then diluted to 500 ml with demineralized water. The solution was put into autoclave for 15 minutes in 121^oC temperature prior to the addition of 0.5 ml K_2HPO_4 in the laminar flow.

Cultivation process of microalgae

Stock culture of *Chlorella vulgaris* (LIPI Serpong) was kept aseptically in PHM media solution. Inoculation was done by preparing stock culture and PHM media solution in 1 to 10 comparison (1 ml microalgae : 10 ml PHM media solution). 500 ml of these solutions were put into conical flask that previously has been sterilized in autoclave for 30 minutes at the temperature of 121^oC. For autotroph experiment, these conical flasks were put on shaker plate and stored into a box with an illumination of 4300±300 lux from cool white fluorescent tube and light – dark cycles of 16 – 8 hours. Each flask was equipped with an air tube connected to aerator to provide atmospheric CO_2. As for heterotroph experiment, the same volume of samples was prepared and several carbon sources, e.g. fructose, glucose and sucrose were added independently to the conical flasks. Variable of concentration for each carbon source was 3, 6, 9 and 12 g/L. For both autotroph and heterotroph experiments, sampling was done every 12 hours to monitor the growth process from lag to endogenous phase. Dry weight was measured and used as an indicator of the microalgae growth.

Lipid of microalgae

The harvested biomass was filtered using filter paper and put it in an oven at temperature of 105^oC for 12 hours. Following the oven process, biomass was kept in desicator for the same duration. After the period of storing, the weight of biomass was calculated by subtracting the weight of empty filter paper from the total weight of filter paper and biomass. The dried biomass on the filter paper was put into soxhlet and 70 ml of n-hexane were added. 25 ml of n-hexane were added into the empty round flask with known weight, at the same time condenser could be set up. Putting the soxhlet with dried biomass, round flask with n-hexane and condenser on heating mantle. Temperature was set at 69^oC. By doing so, lipids will release and mix with n-hexane in the round flask. Following that process, distillation apparatus was prepared by filling the liebigh cooler with water and another round flask was located under the cooler. By setting the temperature at 70^oC, distillation process was started and after reaching the setting temperature lipids will completely separated from n-hexane solution. The total weight of lipids could be defined by calculating the difference of lipids filled round flask and empty round flask.

RESULTS AND DISCUSSION

Growth of Chlorella vulgaris

Figure 1. Cultivation process of *Chlorella vulgaris* on plate shaker

During the cultivation process, *Chlorella vulgaris* in conical flask were put on the shaker plate (Figure 1), and sampling was done every 12 hours followed by dry weight analysis to monitor the growth phase. There are 4 (four) phases in the growth process of microalgae, which are (1) lag phase; (2) exponential growth; (3) stationer phase and (4) endogenous phase (3 – castelanos 2013). Lag phase is an adaptation period, in this phase microalgae will adapt to the new substrate or living condition. Exponential phase, is a phase when microalgae utilize substrate optimally and result in a maximum number of new cell. At certain point the available substrate will get limited, in this phase some of microalgae start to decay but the net between reproduction and decay is still equal, this is so called stationary phase. Following to that, endogenous phase is when the substrate is no more sufficient and the number of decay will increase significantly. For the duration of 168 hours observation, *Chlorella vulgaris* in autotroph condition grew very slowly as can be seen in figure 2. The biomass accumulation process was very limited; from the initial to the end of measurement the biomass concentration increased from 2 mg/L to 2.22 mg/L.

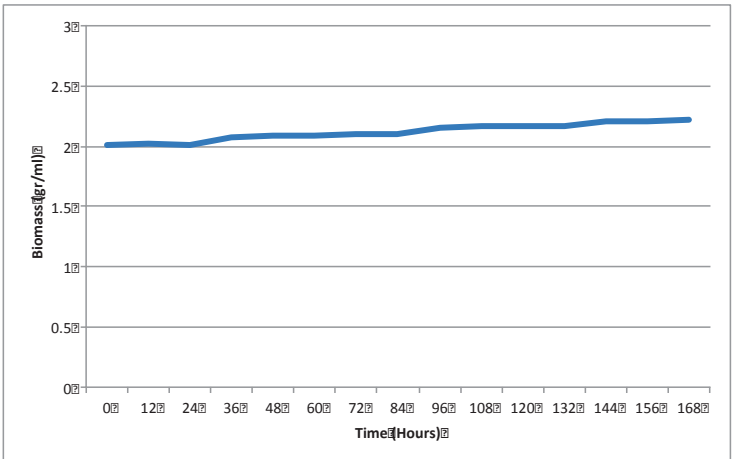
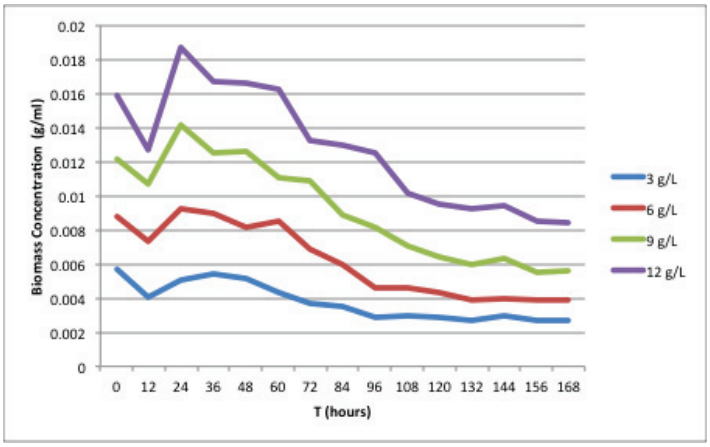


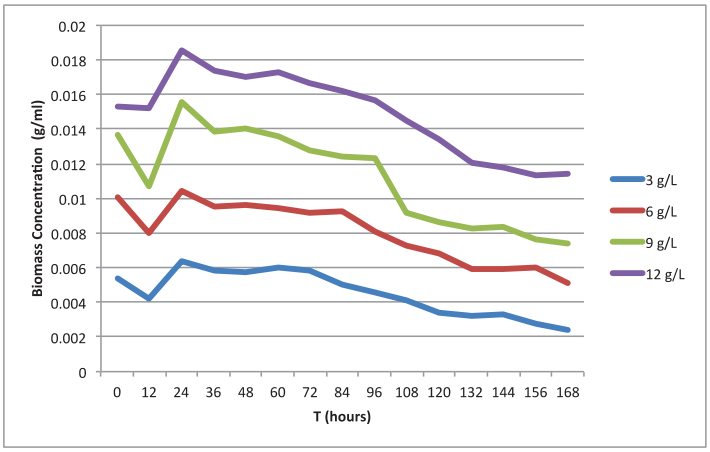
Figure 2. Growth of *Chlorella vulgaris* under Autotroph condition

Unlike the autotroph condition, *Chlorella vulgaris* in heterotroph condition grew more substantially. In the same duration of observation, the growth of *Chlorella vulgaris* in three different carbon sources has the similar pattern as shown in figure 3(a), 3(b) and 3(c). At the first 12 hours, microalgae tended to adapt to the new living condition prior to the growth phase. At different concentration, pattern of biomass growth appear in the same range, exponential growth at 12 to 24 hours, stationer at 24 to 48 hours and

start to decay after 48 hours. At the end of monitoring (168 hours), the collected biomass produced was 0.0027 g/mL 0.0039 g/mL, 0.0056 g/mL and 0.0084 g/mL for glucose; 0.0024 g/mL, 0.0051 g/mL, 0.0074 g/mL and 0.0114 g/mL for sucrose; 0.0023 g/mL, 0.004 g/mL, 0.0057 g/mL and 0.0074 g/mL for fructose, following the variation concentrations for each carbon source, which were 3 g/L, 6 g/L, 9 g/L and 12 g/L respectively. This means that *Chlorella vulgaris* is able to grow in all carbon sources.



(a)



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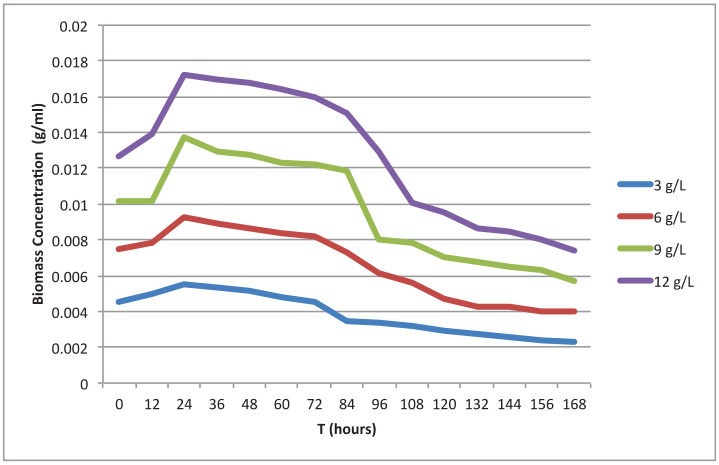

(c)

Figure 3. Growth of *Chlorella vulgaris* under Heterotroph condition using (a) glucose; (b) sucrose; (c) fructose as source of carbon

The Impact of Different Carbon Sources on Lipid Production

Lipid production from *Chlorella vulgaris* in autotroph condition is much lower (2.5%) compared to the one in heterotroph condition. In autotroph condition the metabolism of microalgae tends to form protein rather than lipid (Miao et al., 2006). Meanwhile in heterotroph condition, microalgae will convert most of carbon to the lipid. Process conversion of carbon to the selected material in microalgae growth can be indicated from the color of biomass. The color *Chlorella vulgaris* in autotroph condition is green, and yellow in heterotroph condition. Yellow color indicates the existence of lipid content in the system. Figure 4 shows the color difference of Chlorella vulgaris in heterotroph (yellow, in the upper part) and in autotroph condition (green, bottom part – right side).

Figure 4. Color of *Chlorella vulgaris* in heterotroph condition (upper part) and in autotroph condition (bottom – right side)

Results of distillation process describe that the different source of carbon gives impact on the lipids production. Among of three, *Chlorella vulgaris* with glucose as the carbon source produces the highest amount of lipid (in weight lipid/weight biomass). Glucose is a monosaccharide type; a very simple structure of carbohydrates thus is easier to be digested by living organism. Most of carbon in glucose has been converted into lipid. Sucrose belongs to the group of disaccharides, which is more complex and more difficult to be decomposed. In this experiment, *Chlorella vulgaris* with sucrose as the source of carbon provides the least amount of lipid. Meanwhile, the use of fructose another type of monosaccharide result in a number of lipid that bigger than sucrose but less than glucose. Lipid production for every type of carbon source in different concentration compared to the autotroph condition can be seen in figure 5.

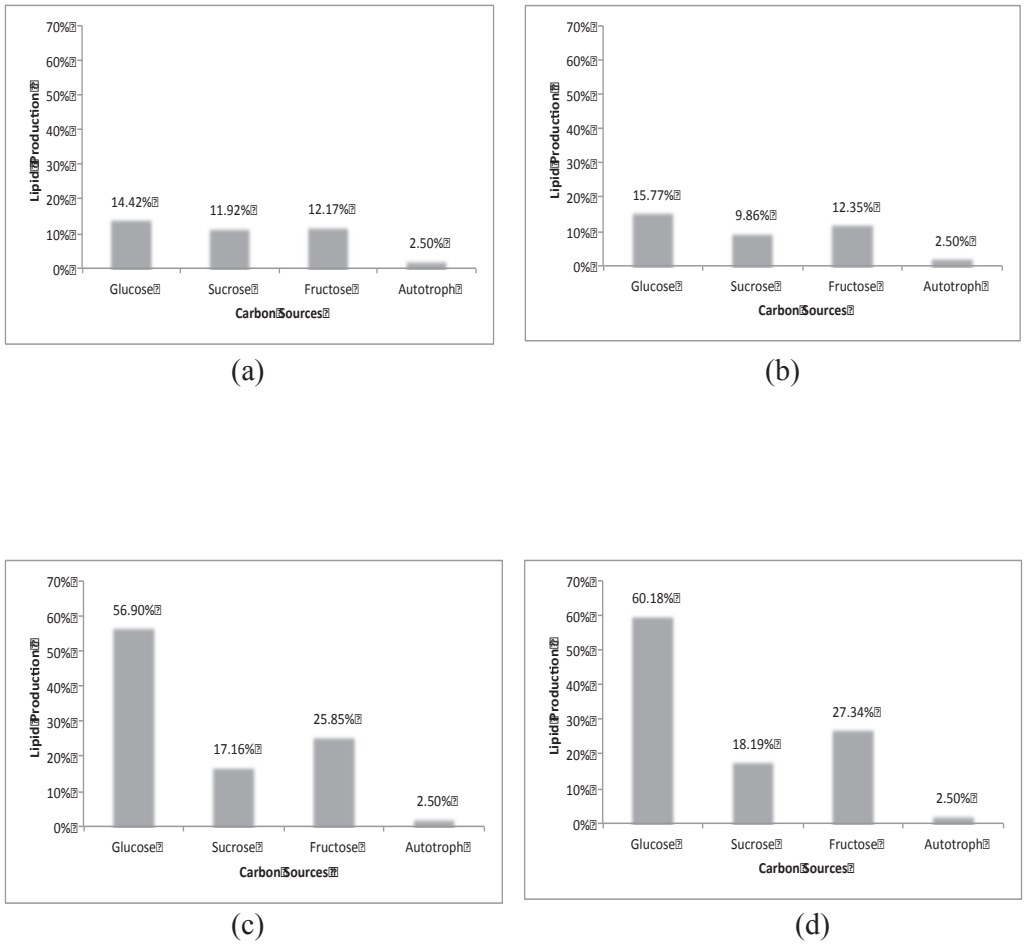

Figure 5. Lipid production of *Chlorella vulgaris* with various source of carbon in different concentration (a) 3 g/L; (b) 6 g/L; (c) 9 g/L; (d) 12 g/L

The Effect of Carbon Concentration on Lipid Production

Concentration of carbon source seems to be an important factor in determining the amount of lipid production as displayed in figure 6. The similar pattern has been found in all sources of carbon that at concentration of 3 g/L and 6 g/L the increase of lipid production is not significant. At concentration of 9 gr/L there is a significant increase of lipid production, which are 56.90%, 17.16%, 25.85% for glucose, sucrose and fructose respectively. And at the highest concentration that has been applied (12 gr/L), the lipid production was measured as high as 60.18%, 18.19%, and 27.34% for glucose, sucrose and fructose respectively. Correlation between the availability of carbon source and lipid production is determined by several factors such as temperature, pH, metabolism of microalgae and harvesting time (Kong et al., 2011 and Widjaja et al., 2008). The composition of those factors will result in optimum concentration that can be converted into lipid.

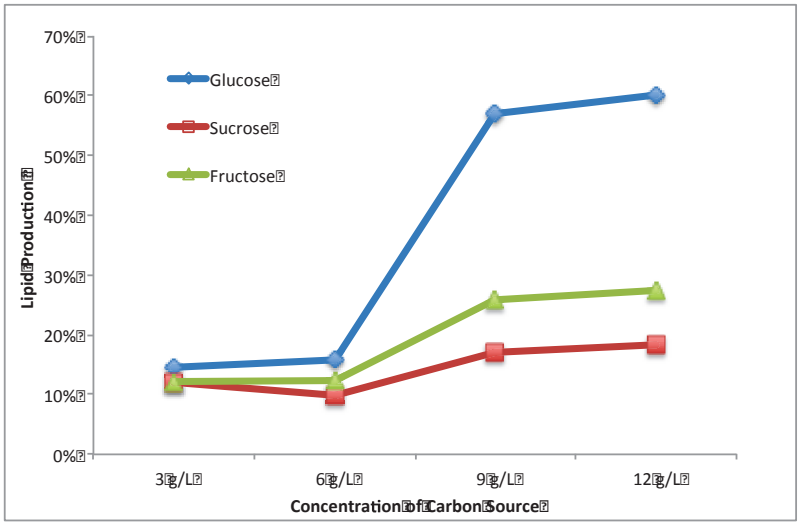

Figure 6. The effect of carbon source in different concentration on lipid production

CONCLUSION

This study demonstrates that the growth of *Chlorella vulgaris* in heterotroph condition is more effective in producing lipid compare to autotroph condition. Moreover, the source of carbon plays significant role on increasing the lipid content, in this case glucose has been indicated as the best carbon source for *Chlorella vulgaris*. Substrate concentration also is a critical factor in determining the lipid production. The lipid production is 60.18% at concentration of 12 g/L glucose. This is the highest value among other variation concentrations of carbon sources.

REFERENCES

1. Christi, Y 2007 ,'Biodiesel from microalgae', *Journal of Biotechnology Advances* , vol. 25, pp.294-306
2. Christi, Y 2009 ,'Biodiesel from microalgae', *Biotechnology advances*', vol. 25, no. 3, pp. 294-306
3. Demirbas and Ayhan 2010 ,'Importance of Algae Oil as a source of biodiesel", *Journal of Energy Conversion and Management*,' vol. 52, pp. 163-170, 2010
4. Hadiyanto, H, Azimatun Nur, MM, Hartanto, GD 2012),' Cultivation of *Chlorella sp.* as Biofuel Sources in Palm Oil Mill Effluent (POME)', Int. Journal of Renewable Energy Development vol. , no. 2, pp. 45-49.
5. Khan, SA, Rashmi, Hussain, MZ, Prasad, S & Banerjee, UC 2009 ,'Prospect of biodiesel production from microalgae in India', *Renewable and Sustainable Energy Reviews,* vol. 13, no. 9, pp. 2361-2372
6. Kong, Weibo, Song, Hao, Cao, Yuntao, Yang, Hong, Hua, Shaofeng &Xia, Chung 2011 ,'The characteristics of biomass production, lipid accumulation and chlorophyll biosynthesis of *Chlorella*

vulgaris and chlorophyll biosynthesis of *Chlorella vulgaris* under mixotrophic cultivation', African Journal of Biotechnology, vol. 10, no 55, pp. 11620-11630

7. Mata, TM, Martins, AA & Caetano, NS 2010 ,'Microalgae for biodiesel production and other applications: A review', *Renewable and Sustainable Energy Reviews,* vol. 14, no. 1, pp. 217-232.
8. Miao, X., Wu, Q 2006 ,'Biodiesel production from heterotrophic microalga oil.Bioresource Technology', vol. 97, pp. 841–846.
9. Radaelly G, Fleischer, D, Vick, B, Caspari, M, Weissman, J, & Rice, D 2009, *Methods for concentrating microalgae*, United States Patent Application 12/339521
10. Widjaja A, Chien CC, Ju YH 2008 ,'Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris'*, *Journal of the Taiwan Institute of Chemical Engineers,* vol. 40, pp. 13 – 20.