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Growth Kinetic of *Chlorella* sp. Microalgae at Flate Plate Photobioreactor

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Abstract. *Chlorella* sp. microalgae are often cultivated for various purposes such as medicines, cosmetics, and alternative energy such as biodiesel, bio-crude oil. Different concentrations of nutrients in culture medium can give different effect on population growth and essential content of *Chlorella* sp. One of them is the concentration of nitrate and phosphate. In order to increase the biomass produced, it is necessary to enhance its growth with another medium that that are cheaper and easier. Urea and TSP was used as source of nitrogen and phosphate element in various ratio for the cultivation at flat plate photobioreactor. Among the process conditions tried, cultivation using urea and TSP fertilizer medium in ratio 2:1 (C1) was considered as the optimum composition that resulted in highest cell density, 4.28×10^7 cells/mL. Then, medium in ratio 4:1 (C2) which was 3.10×10^7 cells/mL and the lowest cell density found in ratio medium 6:1 (C3) which was 1.65×10^7 cells/mL. Medium with low nitrogen content had the highest cell density. The temperature culture during cultivation was in the range of 25 – 26 ^oC and pH value was in the range of 8.3 - 8.7. Temperature and pH culture during cultivation are still in optimal conditions for the growth of *Chlorella* sp.

INTRODUCTION

One of the renewable energy sources is bioenergy (biofuel) which is can be obtained from biological sources, that is from biomass conversion (plants, animals, and microorganisms). The common biomass used as energy resources comes from crops such as sago, corn, and palm. However, there is a conflict between energy sector used for fuel and food sector [1].

Microalgae is one of the potential biomass that can be developed into biofuels because it comes from non-food raw materials and can be cultivated on relatively small land. Moreover, microalgae is considered to be an efficient biomass alternative to be converted into biofuel because it has fast growing and can produce relatively high biomass [2]. Microalgae are unicellular photosynthetic microorganisms that live in fresh water or saline water environments, and then convert sunlight, water, and carbon dioxide (CO_2) into biomass [3].

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Microalgae need a medium that have appropriate nutritional content during cultivation to produce optimum biomass and essential content. Nutrition in medium is one of the factors that influence the growth and biochemical composition of microalgae [4]. Microalgae can grow in medium that has enough macro and micro nutrients, especially nitrogen (N) and phosphate (P). Nitrogen in nitrate is one of macro nutrient that is very influential in growth and productivity of biomass because it is needed for the formation of protein, fat and chlorophyll [5]. Generally, microalgae cultivation used pro-analyst (PA) fertilizer medium such as Walne, Miquel Allen, and Bold Basal Medium. However, the use of pro-analyst fertilizer was considered less economical for large scale application because the price is relatively expensive. Therefore, it is necessary to find alternative medium that are cheaper and easier by using technical fertilizer such as urea and TSP (*Triple Super Phosphate*). Thus, this study aims to find the optimal composition by using technical fertilizers in *Chlorella* sp. cultivation through varying the ratio of urea and TSP.

Microalgae cultivation system can be done with an open pond or a closed pond (photobioreactor). One of the closed pond cultivation can be used flat plate photobioreactor. This type of photobioreactor can be used on the exterior of a building as a facade to create a green building. By utilizing the nature of microalgae that require CO_2 and light, flat plate photobioreactors will be able to convert CO_2 and utilize sunlight in the cultivation of microalgae so as to produce biomass that has added value.

The microalgae species used in this study is *Chlorella* sp. that live in fresh water. *Chlorella* sp. is one of the microalgae that classified as green algae (*Chlorophyceae*). Characteristic of *Chlorella* cells are generally round or elliptical (ovoid) body shape with diameters range from 2 to 12 microns and its reproduction is done asexually through self-division.

METHODS

Materials used in this study were culture of *Chlorella* sp., Urea, TSP, water, *Calcium Hypochlorite* ($Ca(ClO)_2$), and *Sodium Thiosulfate* ($Na_2S_2O_3$). Firstly, sterile water was made by adding *Calcium Hypochlorite* to the water as much as 60 ppm and left for one night. Then added 30 mg/L of S*odium Thiosulfate*. Fertilizer media was the combination among urea and TSP. Urea was used as a source of nitrogen for growth and TSP as a source of phosphate. The fertilizer medium composition used consists of 3 combinations of urea and TSP, which are 2:1; 4:1; and 6;1. After being weighed, each fertilizer composition was added to sterile water.

Chlorella sp. was cultivated in flat plate photobioreactor (see Fig. 1 (a), (b)) with dimensions of 40 x 3 x 60 centimeters. The total capacity was 4 liters and also supported by temperature sensor. As many as 2 L culture of *Chlorella* sp. was added to 2 L of fertilizer medium. The cultivation was held for 7 days for each fertilizer ratio, with 24 hours lighting lamps and aeration

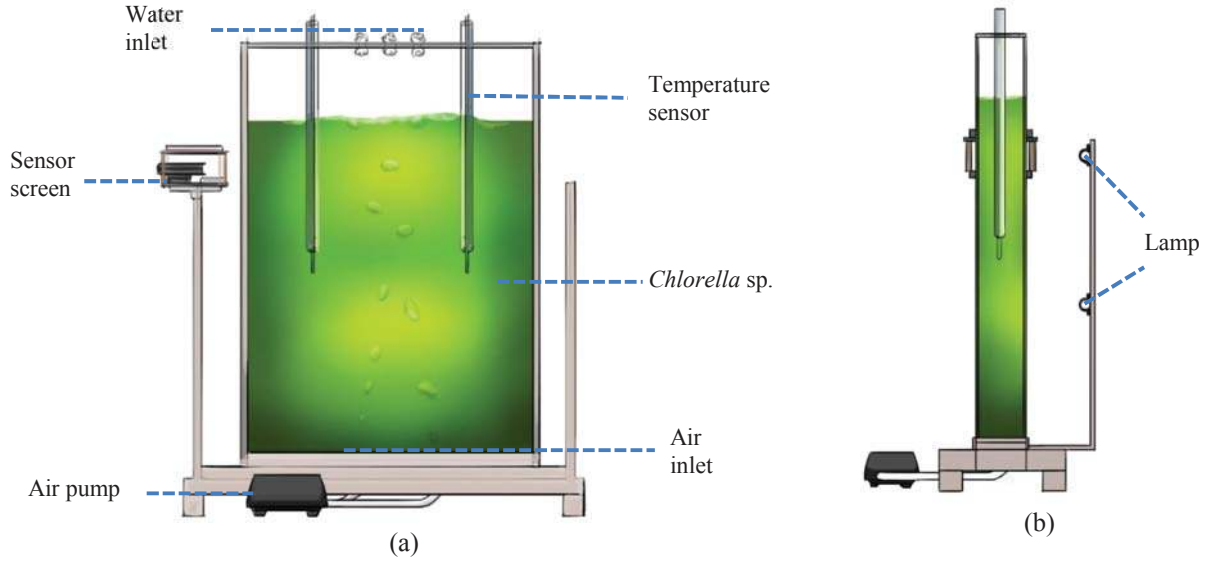

FIGURE 1. Flat plate photobioreactor design (a) Front view; (b) Side view

Cell density calculation of *Chlorella* sp. culture was done by using a Neubauer Improved Haemocytometer and microscope every day for 7 days. Observations were made by sampling 3 mL *Chlorella* sp. culture by using micropipette. Then, it moved to haemacytometer. Haemacytometer was placed under the objective lens of microscope and the number of cell was counted. The number of cell counted on five chambers, four on the corner and one on the center was counted. Afterward, cell density was calculated using Eq.1 [6].

$$A = N \times \frac{25}{5} \times 10^4 \tag{1}$$

Besides, the environmental factors were also measured. Environmental parameters measured were temperature and pH of culture. The measurement was taken every day during cultivation. Temperature and pH was measured by using a digital device. The device was dipped in culture, then on screen the temperature and pH values were known.

RESULTS AND DISCUSSIONS

Based to the experimental data (see Fig. 2), culture of *Chlorella* sp. was cultivated by using urea and TSP fertilizer medium in the ratio of 2:1 (C1); 4:1 (C2); 6:1 (C3) showed different cell density. The difference occurs because the different of nutritional content. In medium C1 and C2, the lag phase occurs very short, one day on the first day. Whereas, in C3 medium the lag phase occurs longer this is 6 days. The lag phase showed the duration of adaptation of *Chlorella* sp. with a new medium. During the adaptation period the cells restore the enzymes needed for growth and nutrient entry into the *Chlorella* sp. cells through the diffusion process. This happens because of differences in concentration between body fluids in *Chlorella* sp. with new media [7]. In this study, the lag phase was very short because there are differences in body fluid concentrations of *Chlorella* sp. cells with new media are not high. While the lag phase occurs long enough because the new culture medium relatively had a high nutrient element in which the media C3 had the highest element of nitrogen. This result stood in line with the previous work by Wijoseno [8].

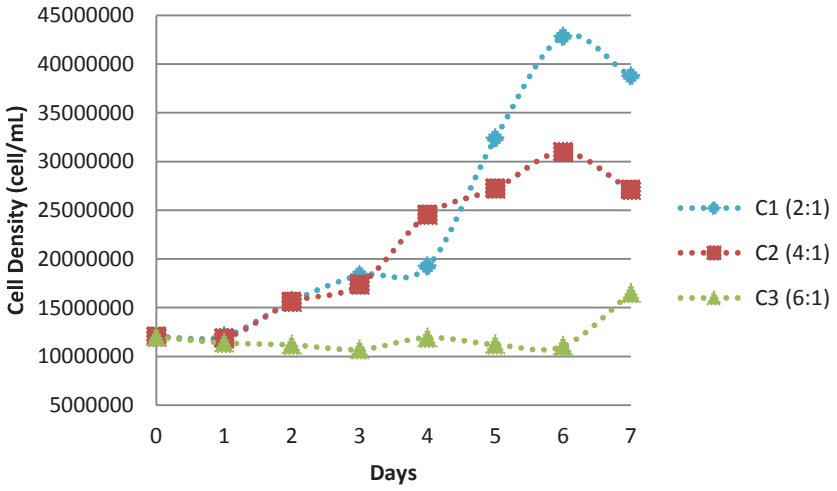

FIGURE 2. Experimental data of cell density

After the lag period was completed, the graph moved up on each medium. This indicated that the cell density increases because the cells already have utilized the nutrients in the medium for rapid growth of cell division. The exponential phase is the peak of growth where cell reaches the highest level. The results showed that C1 and C2 medium reached the highest cell density on day 6. While the C3 medium began to increase on day 7 which means it requires a longer growth period.

The highest cell density was found in culture of *Chlorella* sp. with C1 medium which was 4.28×10^7 cells/mL on day 6. Then the second highest cell density was found in culture with C2 medium which was 3.10×10^7 cells/mL

and the lowest cell density found in the C3 medium which was 1.65×10^7 cells/mL. The treatment of the nitrogen content of each medium was different, C3 > C2 > C1. Study by Nigam [9] showed that high nitrogen levels will result in high cell density too. However, it is different from the results of this study where the cell density of *Chlorella* sp. in C1 medium with low nitrogen content had the highest cell density.

This difference results because the form of nitrogen elements that cannot be absorbed by cells. Nitrogen is an important component as a source of microalgae nutrients for its growth phase. Nitrogen is absorbed in the form of ammonium (NH_4^+) and nitrate (NO_3^-). According to Vincent [10], ammonium is a form of nitrogen compound that is more preferred microalgae. This is because the process of transportation and assimilation of ammonium ions by phytoplankton cells requires less energy than nitrate ions.

Measurement of medium culture condition was done by observing temperature and pH parameters. Temperature measurement of *Chlorella* sp. medium culture was taken based on data that reads on the sensor screen. Then, pH value was taken with a digital pH meter. According to experimental data, the temperature change during the period of cultivation tends to be constant, in the range of 25 – 26 ^oC. The teori by Isnansetyo and Kurniastuty [11], the optimal temperature of *Chlorella* sp. between 25 – 30 ^oC. Temperature changes in this study are still within the optimal temperature range for the growth of *Chlorella* sp.

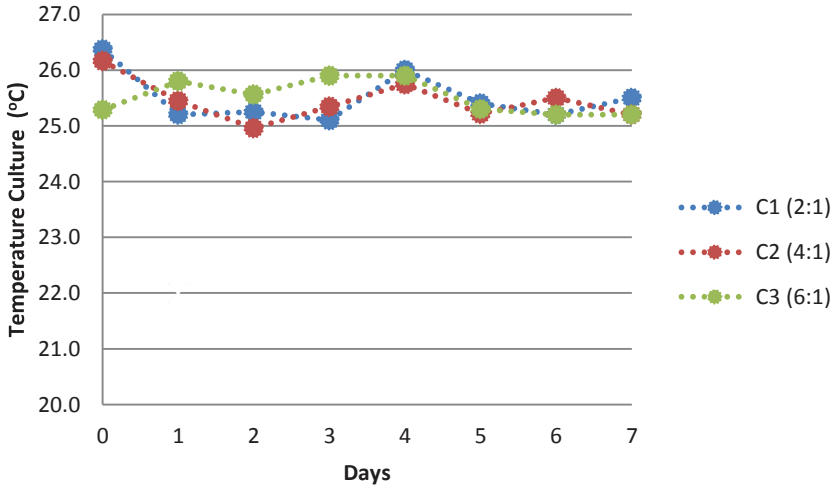

FIGURE 3. Experimental data of temperature culture

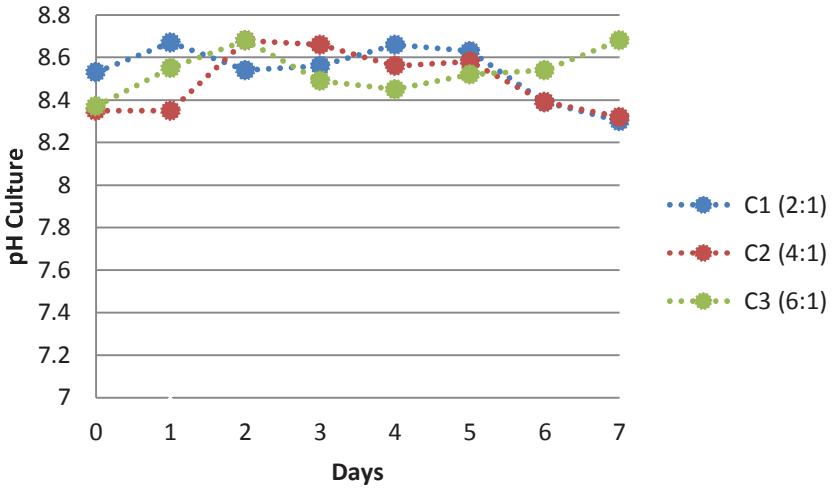

FIGURE 4. Experimental data of pH culture

The results of pH measurements of culture media tend to be constant, in the range of 8.3 - 8.7. This pH range is still in a good range for the culture of *Chlorella* sp. Study by Wigajatri [12], the appropriate pH value for the growth of *Chlorella* sp. ranges from 4 - 9. The pH values that exceed the optimum limit or below the optimum limit will cause a decrease in the growth rate of microalgae [13]. Changes in pH values may occur due to photosynthesis and other processes [14].

In conditions where there is no acid addition, increased phytoplankton growth is followed by an increased pH value. This can be seen in the results of research that at the beginning of the cultivation period the pH value tends to increase. That is because when growth increases the rate of photosynthesis also increases. Thus, the main ingredient for photosynthesis is CO_2 levels decreasing. Decreased CO_2 levels cause H^+ ions to also decrease, so the pH has increased. The increase in pH also occurs due to the process of utilization of nitrogen by *Chlorella* sp. from fertilizers in the culture medium [15].

CONCLUSION

The use of urea and TSP fertilizer media as a source of nitrogen and phosphate affects the population of *Chlorella* sp. Based on experiments, medium with low nitrogen content has the highest cell density. C1 medium in ratio of urea:TSP = 2:1 is the most optimal medium with the highest growth rate and has the highest cell density which was 4.28×10^7 cells/mL. Temperature and pH changes are still within the optimal temperature range for the growth of *Chlorella* sp.

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