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バージョン：

Tubular Photobioreactor: A Preliminary Experiment Using *Synechococcus* sp. (Cyanobacteria) Cultivated in NPK Media for Biomass Production as Biofuel Feedstock

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Photobioreactor (PBr) is a system that usually used for producing high amount of biomass. This system was designed for supplying multiple factor that providing a good biological condition for growth of the microalgae contained in the system. This research used *Synechococcus* sp. HS-9, an indigenous cyanobacteria isolated from Rawa Danau hot spring, Banten. This strain was selected because their ability to adapt and reproduce. *Synechococcus* sp. HS-9 was inoculated into two units of tubular photobioreactor (tPBr) along with NPK medium and then observed using turbidimeter. This strain of cyanobacteria is grown up to 4.44 x 10^6 cells/mL (tPBr-1) and 4.29 x 10^6 cells/mL (tPBr-2) (cell number) or 56.5 NTU (tPBr-1) and 55.9 NTU (turbidity) after eleven days. The results showed that combination of NPK media, tPBr system, and *Synechococcus* sp. HS-9 are producing potential biomass for biofuel feedstock. This research also proved that use of NPK media as growth medium are promising, because of the good efficiency of cost production.

Keywords: Tubular, Photobioreactor, *Synechococcus* sp., NPK, Biofuel.

1. Introduction

The need of energy for life is one of many problems that humankind cannot be avoided. Human are using certain amount of energy sources such as fossil fuel and electricity. Nowadays, crisis of energy is starting to approach human life. In example, Japan has been the third largest energy consumer country in the world with 461 MTOE (Million Tonnes of Oil Equivalent) primary energy consuming per year[1)]. To maintain energy, many researchers had been trying using many components to made alternative fuels[2,3)]. One type of alternative fuel is biofuel[4)]. This kind of fuel can be obtained from many bioresources such as plants and microorganisms[5)]. Cyanobacteria, a member of microalgae, is potential bioresources for producing biofuel[6)]. This happen because their photosynthetic ability and their biomolecules that very similar to plants. In other perspective, use of cyanobacteria are very promising because of their fast growth ability[7)].

To increase the microalgae doubling processes, many researchers and industrial factory are using photobioreactor (PBr) system to support the cyanobacteria growth factor[8)]. There are two main type of PBr system, open pond PBr system and closed PBr system. Closed PBr system can be divided by their shape, i.e. flat panel PBr and tubular PBr. For many years, tubular PBr system are known for producing biomass effectively[9)]. Some research showed that this type of PBr system are good for green algae (chlorophytes) and blue-green algae (cyanobacteria).

Microalgae also needs nutrients to grow, beside the system and another environment factors. The growth media is the critical point for producing cyanobacterial biomass[10)]. There are many types of growth media that can be used for cultivating cyanobacteria, for example Blue-Green 11 (BG-11), Bold's Basal Medium (BBM) and Cyanobacteria-TAPS (CT)[11)]. Nevertheless, these growth mediums are expensive in cost, because of the using of many types of pure inorganic compounds. Those mediums are effectively used for laboratory scale culture, not for the industrial scale[12)]. Many industrial factories are searching and using alternative media for producing more cost-efficient biomass. By using *Synechococcus* sp. HS-9 (indigenous cyanobacteria isolated from Rawa Danau, Banten)[13)] this research aim is to observe the growth *Synechococcus* sp. HS-9 inside the tubular photobioreactor system (tPBr) along with NPK as growth media. This research also conducted to measure cost-efficiency of NPK media compared to other general laboratory growth media.

2. Methods

There are some stages in this research, first is the assembly of tPBr system. There are two units of tPBr system. This system is assembled using custom made acrylic tubes and some aquarium components. All the components are assembled according to the design shown in fig. 1. After the tPBr system are assembled, all units are placed in controlled area to reducing unnecessary factor that can affect the results.

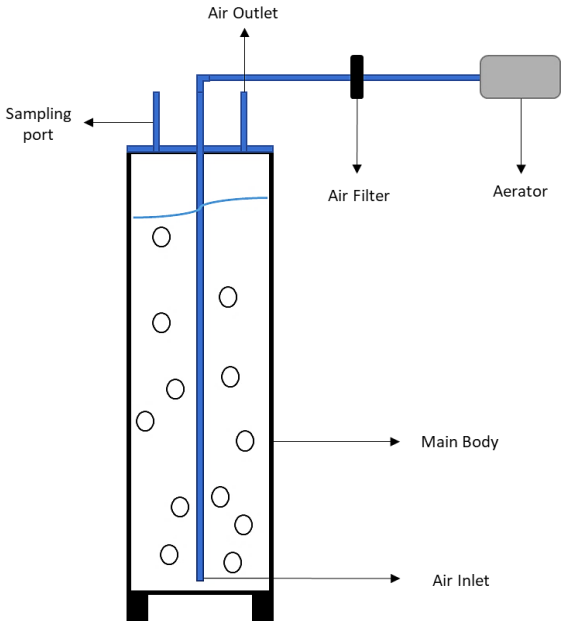

Fig. 1: Schematic of Tubular Photobioreactor System (tPBr)

Optical density methods were chosen, to calculate the biomass growth[14]. Standard curve of biomass optical density was acquired by using serial dilution methods. This stage resulting a standard curve and an equation. This curve and equation later used as a standard to daily measurement and data collecting. The curve and equation are shown in fig. 2.

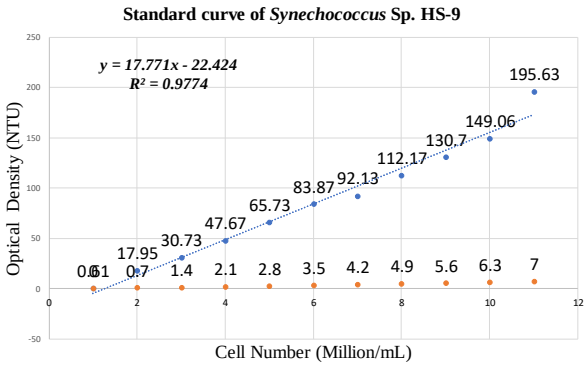

Fig. 2: Standard Curve of *Synechococcus* sp. HS-9

The next step is inoculation process, which some amounts of inoculums are pour into growth medium. Media that used for this experiment is made by dissolving 320 mg of Grow More™ NPK fertilizer into four (4) liters of distilled water and then sterilized by using autoclave, which have 80 ppm of final concentration. *Synechococcus* sp. HS-9 inoculum is inoculated into tPBr system with 1:10 ratio (200 mL biomass of *Synechococcus* sp. HS-9 into 1800 mL medium)[15]. The tPBr that now contain microalgae culture was placed in controlled room which have 15000 lux light intensity and constant 27°C temperature. Data observations are done by collecting turbidity data using turbidimeter[16]. Results from turbidimeter (NTU/Nephelometric Turbidity Unit) are transformed into cell number using the equation from standard curve.

3. Experimental Results

3.1 Growth Measurement of *Synechococcus* sp. HS-9 Biomass

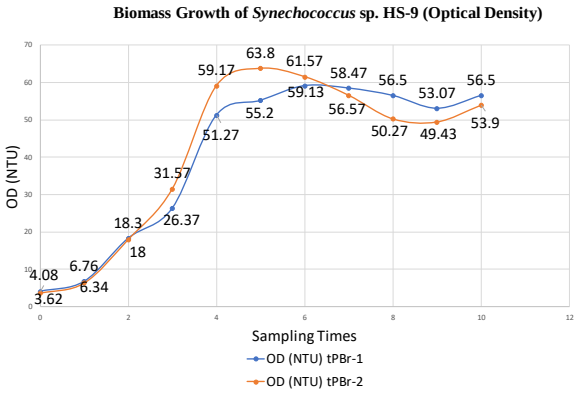

Fig. 3: Biomass Growth of *Synechococcus* sp. HS-9 (Optical Density)

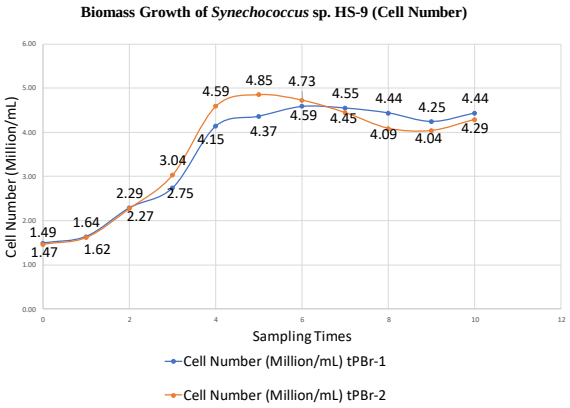

Fig. 4: Biomass Growth of *Synechococcus* sp. HS-9 (Cell Number)

The growth of *Synechococcus* sp. HS-9 were observed for 11 days, data shown an increasing trend for *Synechococcus* sp. HS-9 biomass. The cell numbers on the last days of observations are 56.5 NTU or 4.44 x 10^6 cells/mL for tPBr-1 and 55.9 NTU or 4.29 x 10^6 cells/mL for tPBr-2. The graphic shown that biomass of *Synechococcus* sp. HS-9 are significantly increased, start from day 2 (T_1) until day 5 (T_4) in both tPBr system. After showing an increase trend, the graphic enters stationery phase. This phenomenon showed from day 6 (T_5) until the end of data collecting period. This is caused by decreased nutrients level inside the medium.

The nutrients inside the medium are used by the cell for metabolism and other living processes. After the cell reach the carrying capacity of medium, the cell will enter the stationery phases and will not increase further[17].

According to the equation.1, the rate of growth for *Synechococcus* sp. HS-9 can be calculated by using the formula. Letter *r* is symbol for rate of growth. The equation can be solved by knowing cell number in the beginning (*No*), cell number at the end of period (*Nt*), and interval of the period (Δt)[11]. The equations are showed in equation 1.

$$r = \frac{ln\frac{(Nt)}{(No)}}{\Delta t} = \frac{\ln Nt - \ln No}{\Delta t}$$

Equation. 1: Equation for Measuring Rate of Growth

$$r_{tPBr-1} = \frac{\ln(\frac{4.44 \times 10^6}{1.49 \times 10^6})}{10} = 0.108\ /day$$

$$r_{tPBr-2} = \frac{\ln(\frac{4.29 \times 10^6}{1,47 \times 10^6})}{10} = 0.106\ /day$$

Equation. 2: Rate of Growth Equation for *Synechococcus* sp. HS-9 in Tubular Photobioreactor System

From the equations.2, the rate of *Synechococcus* sp. HS-9 biomass growth is 0.108/ day for tPBr-1 and 0.106/day for tPBr-2. This result is not significantly different from each unit. Even though the growth rate of *Synechococcus* sp. HS-9 in NPK media are showing increase trend. The rate of growth still can be enhanced by increasing the media concentration.

Other factor that contributed in *Synechococcus* sp. HS-9 biomass growth are air flow and nutrient distribution inside the tPBr system. Since it has tubular shape, the flow of air and medium inside the system are patterned like fig. 5. This pattern of flow made every single cell inside the system get adequate nutrients and light for its metabolism[18].

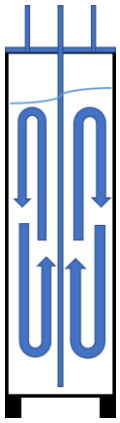

Fig. 5: Flow Pattern in tPBr System

3.2 Macroscopic and Microscopic Observation of *Synechococcus* sp. HS-9 Biomass

Macroscopic observation was done by taking a photo of PBr on first day of observation (t0), four (t5), and seven (t8) to acquire colour change. The colours of *Synechococcus* sp. HS-9 biomass are changing from grey green (t0), moss green (t5) and light green (t8). Change in colour of microalgae culture are indicating the growth of microalgae[19] which cultured on PBr. With the changing of the colour in every sampling day, the number of cells on the PBr system also increased, until it hits a peak and the colour will decrease and become fader.

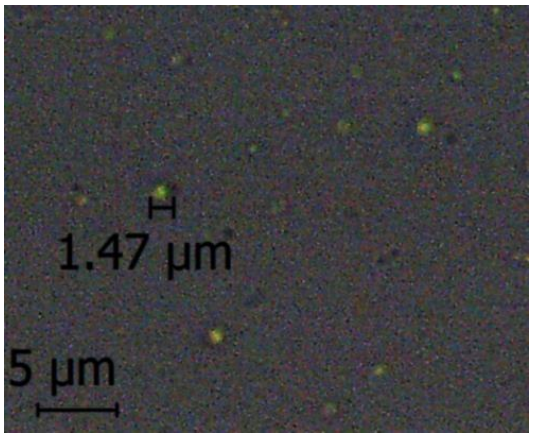

Fig. 6: Photomicrograph of *Synechococcus* sp. HS-9

On the last day, the microalgae under the microscope to see and calculate the size of the microalgae that had been cultured on the PBr system and collect microscopic data. *Synechococcus* sp. are 0.8 to 1.5 microns (µm) in size[20]. From fig.6, *Synechococcus* sp. HS-9 seems to have more

rounded-shape cell. This could happen because *Synechococcus* as a microorganism, responds to its surrounding environment[21]. Shape of microorganism are designed based on how they regulate their metabolism and how effective it was[22].

3.3 NPK Fertilizer as Photobioreactor Media

Based on previous research, NPK media can be used as growth media in PBr system[23]. Even though the results are lower from Bold's Basal Media, use of this media can be more profitable than other growth media. From chemical perspective, NPK fertilizer that were used for making NPK growth media are consisted from several nutrients such as Nitrogen (N), Phosphoric Acid (P_2O_5), Soluble Potassium (K_2O), and other micronutrients.

Meanwhile, Bold's Basal Media (BBM) is consisted of 16 nutrients consist of macronutrients and micronutrients[24]. Nutrient composition in BBM are more promising for growing *Synechococcus* sp. greatly. It happens because, this media is providing all nutrients that needed for the metabolism. Although, not all components in it are utilized by related algae[25].

Meanwhile on the economical perspective, use of NPK fertilizers as growth media is relatively cheap. One kilogram of Grow More™ NPK fertilizer cost around $5 USD, and only used about 100 mg to make one-liter media (100 ppm), which only cost for $0.0005 USD/liter media. This fertilizer already contains all the required Nitrogen, Phosphor and Potassium. This is a very good cost for producing high amount of *Synechococcus* sp. biomass compared to cultivation using standard media that can be cost around $1-10 USD/liter. Use of NPK media also more practical than standard growing media.

Conclusion

Biomass growth of *Synechococcus* sp. HS-9 in tubular photobioreactor are very potential to be used as biofuel feedstock. The biomasses are grown from 56.5 NTU or 4.44 x 10^6 cells/mL for tPBr-1 and 55.9 NTU or 4.29 x 10^6 cells/mL for tPBr-2. This is happened because the cell got adequate nutrient flow inside the tPBr system. This research also proven that NPK fertilizer can be used for *Synechococcus* sp. growing media. Use of NPK media also more economical and more practical for large scale cultivation. Even that good result, optimal concentration for long-lasting and high producing biomass growth media must be conducted in the recent future.

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