Analysis of Carbon Dioxide Solubility Increasement Caused by Baffle Diameter Variation in Airlift Photobioreactor to Growth Rate of Synechococcus HS-9 Biomass

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Abstract: Photobioreactor (PBR) have been one of the most popular system that used for multiplying microalgal biomass. Many researches have been conducted to develop photobioreactor that suit the most with prominent microalgae strains. Carbon dioxide supply is one of many sectors that play an important role for growing microalgae. Carbon dioxide are used by the microalgal cells to produce many chemical compounds that support growth. In many cases, carbon dioxide must be solved into growth media to be utilized by the cells. This research focused on analyzing the effect of carbon dioxide solubility increasement caused by variation of baffle in airlift photobioreactor (APBR) to growth rate of *Synechococcus* HS-9 biomass. Twenty-one day of experiment resulting that there are growth rate differences occurs. The highest growth rate occurs in APBR-A, APBR-B, and control PBR are 0.515, 0.463, and 0.738 respectively. Despite of the maximum growth rate occurred, there are fluctuation of growth rate happen during experiment. Carbon dioxide solubility test then conducted to see effect of baffle variation. The best carbon dioxide solubility occurred in APBR-B with 23 mg/L, then followed by APBR-A with 22 mg/L, and control PBR with 20 mg/L after 24 hours of treatment. Further analysis resulting that there is no strong correlation between carbon dioxide solubility increasement to growth rate of *Synechococcus* HS-9 biomass. This occurred due degression of acidity value (pH) caused by excess of carbonic acid formed by carbon dioxides who diluted into growth media.

Keywords: Carbon dioxide; Growth Rate; Photobioreactor, Solubility; *Synechococcus* HS-9

1. Introduction and background

Energy is one of the most important things that support human lives. Human nowadays are exploiting non-renewable energy resources to maintain their activities[1)]. In order to maintain energy sustainability, sustainable development goals (SDG) stated that we need to achieve affordable and clean energy for our future[2)]. This goal can be achieved by change our old energy resources to the renewable energy resources[3]) One of many types of renewable energy resources came from utilizing microalgae biomass. Prokaryotic microalgae (cyanobacteria) is a type of microalgae that has the potential to be used as a renewable energy source. This is because they can produce lipids properly. Examples of potential cyanobacteria are genus *Synechococcus*[4)] and the genus *Leptolyngbia*[5)].

Microalgae are photosynthetic microorganism that have contents similarities with plants[6)]. This microorganism can produce biochemical substances that can be utilized for developing biofuels[7)]. The biochemical substances occurred in microalgae are carbohydrates, lipid, and protein[3)]. Carbohydrates and lipid extracted from microalgae bodies, later can be derived into many kinds of biofuels such as biodiesel and bioethanol[7)]. Microalgae utilized photon from light sources and turn it into biochemical substances through photosynthesis. The waste product of this mechanism is oxygen that later can

be accumulated in the atmosphere and utilized by other organisms[8]. Based on that ability, microalgae can be a good chance to be used to achieve affordable and clean energy resources.

Utilization of microalgae as biofuel feedstock need a lot of biomass. To achieve that, many researchers are conducting experiment to develop optimum system for growing microalgae biomass. The system used for multiplying microalgae biomass is photobioreactor (PBR)[9]. One type of photobioreactor that is commonly used to increase microalgae biomass is the bubble column photobioreactor (BCPBR). This type of photobioreactor is commonly used because of its slim shape, so that the use of area for the photobioreactor is more efficient than other types of photobioreactors[10]. Controlled factors in the system include the availability of nutrients, light, temperature, acidity (pH) and air[11]. The combination of all these factors affects the quantity and quality of biomass produced. The determination of the combination of all the factors above is carried out specifically for the microalgae used, so that the development of a photobioreactor system needs to pay attention to the type of microalgae used.

To develop optimum PBR system for microalgae, several factors must be researched. These factors are light intensity, temperature, nutrition, acidity, and gas supply[12]. One of the most important factors to be researched is gas supply. Microalgae used carbon dioxide (CO_2) to be carbon sources for producing biochemical substances. Microalgae usually utilized dissolved CO_2 and absorb it into their cell[13]. Higher amount of dissolved CO_2 mean that more carbon can be utilized by the cells. In order to increase CO_2 solubility, physical modification of PBR system such as baffle addition can be conducted. Addition of baffle is increasing CO_2 residence time[14]. It means CO_2 injected to the system had more time to react with water and dissolved.

Optimal gas solubility can be obtained by adjusting the current pattern in the photobioreactor system. The optimization process is carried out by adding a baffle component[10]. The addition of these components causes the conventional BCPBR system to be modified into an airlift photobioreactor (APBR).

Synechococcus HS-9 is used in this research as biological agent for microalgae biomass production. This kind of microalgae is indigenous cyanobacteria isolated from Rawa Danau hot spring in Banten, Indonesia[15]. Synechococcus has round (coccoid) with a size ranging from 0.5-2.5 µm[16]. *Synechococcus* HS-9 is known to contain fatty acids which can potentially be used as biodiesel agents[17]. The strain was chosen because it can be multiplied rapidly and their ability in extreme area[18]. The biochemical substances contained inside the cells also abundant to be used as biofuel feedstock [19]. This research aim is to analyze effect of CO_2 solubility increasement impact to growth rate of *Synechococcus* HS-9 biomass.

2. Method and experimental setup

There are several steps in this research. The research scheme can be seen in figure 1. The first step is the PBR system preparation. There are nine units of PBR used in this research (figure 2). These nine units of PBR are consists of three variation with three time of replications. The first system is bubble column photobioreactor (BCPBR) as control. Second and third system is airlift photobioreactor (APBR) with baffle diameter variations. Variations conducted in APBR system are 6 cm and 8 cm. All of the PBR system was assembled according to the design in figure 3, and figure 4, meanwhile figure 5 describing diameter of baffle variations.

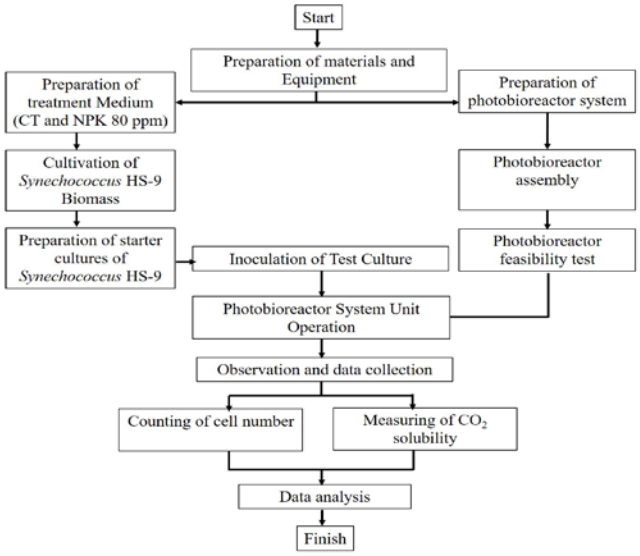

Fig. 1: The research scheme

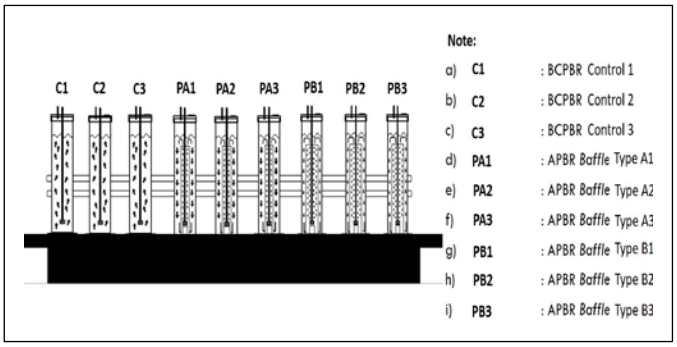

Fig. 2: The nine units of PBR: three BCPBR for control; three APBR type A; three APBR type B

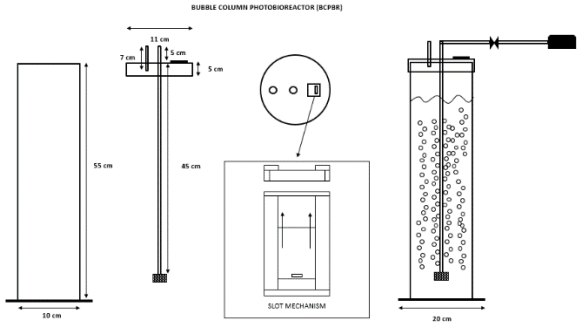

Fig. 3: The design of bubble column photobioreactor (BCPBR)

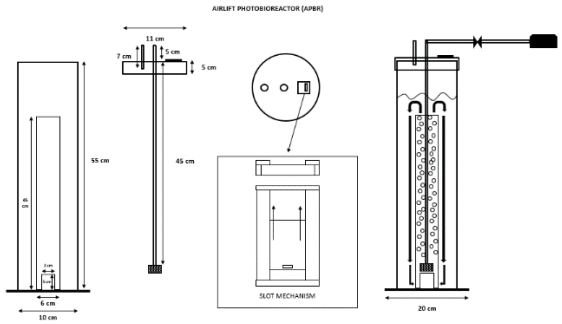

Fig. 4: The design of airlift photobioreactor (APBR)

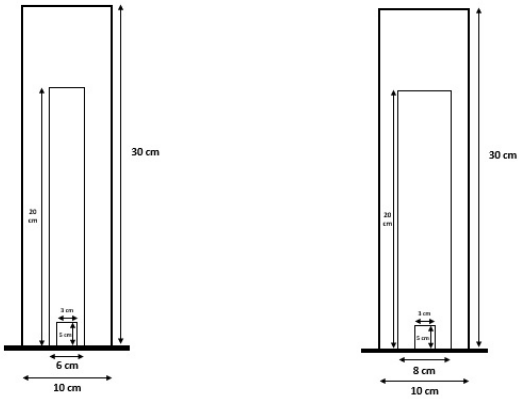

Fig. 5: Diameter (Ø) variations of baffle (APBR A (left): 6 cm; APBR B (right): 8 cm)

The *Synechococcus* HS-9 biomass later was cultivated into NPK 80 ppm growth media with acidity number (pH) 6.5 and then incubated inside the system[20]. The observation was conducted for 21 days. Data collections including *Synechococcus* HS-9 cell number and CO_2 solubility. Cell number acquired in this research then processed into growth rate through this formula 1[11].

$$r = \frac{lnNt - lnN0}{\Delta t} \qquad (1)$$

Carbon dioxide (CO_2) solubility was measured using titration methods. NPK 80 ppm media was treated inside the PBR system for 4 hours and 24 hours. Later the growth media was sampled and measured by using Na_2CO_3 0.045 N and phenolphthalein (PP). Data acquired then processed into this formula 2[21].

$$mg.L^{-1}\ CO_2 = \frac{1000}{v} \times p \times 0{,}5 \qquad (2)$$

Later, all of the acquired data will be accumulated and processed into table and graph.

3. Results and Discussion

3.1. Analysis of carbon dioxide (CO_2) solubility

Carbon dioxide (CO_2) solubility was measured by using titration methods. Data acquired in this step include CO_2 solubility in distilled water and NPK 80 ppm growth media after 0 hours, 4 hours, 24 hours, and NPK 80 ppm with *Synechococcus* HS-9 inside it. All of the parameters measured from all three types of PBR systems. Results of this step are described in table 1.

Table 1. CO_2 solubility inside PBR systems (ØAPBR A: 6 cm; ØAPBR B: 8 cm)

Parameters	CO_2 solubility (mg/L)
Distilled Water	8
NPK 80 ppm	10
NPK 80 ppm BCPBR (4 hours)	13
NPK 80 ppm BCPBR (24 hours)	20
NPK 80 ppm BCPBR + *Synechococcus*	15
NPK 80 ppm APBR A (4 hours)	20
NPK 80 ppm APBR A (24 hours)	22
NPK 80 ppm APBR A + *Synechococcus*	17
NPK 80 ppm APBR B (4 hours)	21
NPK 80 ppm APBR B (24 hours)	23
NPK 80 ppm APBR B + *Synechococcus*	19

Based on the results, data shown that CO_2 solubility inside distilled water and normal NPK 80 ppm are 8 mg/L and 10 mg/L respectively. Meanwhile, the highest CO_2 solubility in BCPBR 20 mg/L after 24 hours, APBR A was 22 mg/L after 24 hours and APBR B was 23 mg/L after 24 hours. As we see in the results, CO_2 solubility increase progressively from the normal CO_2 solubility to maximum CO_2 solubility. This result was influenced by addition of baffle inside APBR system. Baffle inside APBR system create two zones with different pressure[22]. The zone was down comer and upriser. Zone created inside the system created flow pattern as seen in figure 6.

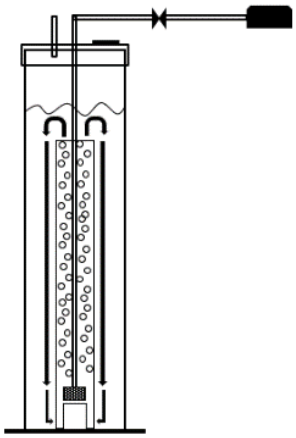

Fig. 6: Illustration of gas flow pattern inside APBR system

Pressure in down comer area was low meanwhile in upriser was high. This different pressure influencing the CO_2 residence time inside APBR system[23]. Air bubble injected from inlet will have more time to react with water and the CO_2 will turn into carbonic acid (HCO_3^-) (soluble form). The HCO_3^- later will be utilized by *Synechococcus* HS-9 cells for photosynthesis and used for many metabolism pathways[24].

3.2. Analysis of *Synechococcus* HS-9 growth rate

Increasement of CO_2 solubility affect several things inside the system. These changes were affecting the *Synechococcus* HS-9 biomass growth. Based on 21 days of observations, the growth curve was obtained. After analyzing the growth curve, log phase of *Synechococcus* HS-9 was determined and analyzed. The log phase was occurred for 5 days with four intervals. Later, the data acquired from growth curve was processed using formula (1). Result of analysis was shown in figure 7.

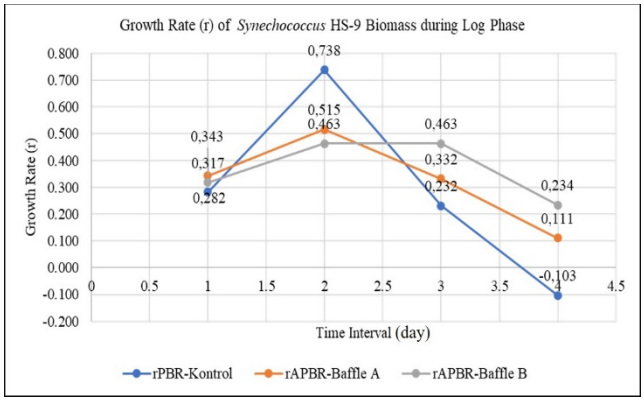

Fig. 7: Growth rate (*r*) of *Synechococcus* HS-9 during Log Phase

Based on the result shown, the growth rate of *Synechococcus* HS-9 in 4 four days of log phase was fluctuated. First interval (T0-T1) and second interval (T1-T2) shown increasement of growth rate. Meanwhile, third interval (T2-T3) and fourth interval (T3-T4) shown decreasing of growth rate. Data also shown the highest growth rate for BCPBR and APBR A occurred in the second interval (T1-T2) with 0.738 per day and 0.515 per day respectively. Meanwhile the highest growth rate of APBR B occurred in third interval (T2-T3) with number of 0.463 per day.

Based on results, fluctuation of *Synechococcus* HS-9 biomass growth rate was influenced directly by CO_2 reaction inside PBR system. Carbonic acid (H_2CO_3) formed inside system are higher than normal system. This phenomenon causing the cells can absorb adequate carbon for their metabolism. Cyanobacteria utilize CO_2 gas as a raw material for glucose anabolism to produce energy. To use CO_2, cyanobacteria cells need to move CO_2 from the environment into the body[25]. The transfer process is carried out through various types of different Ci transporters. CO2 gas in liquid media dissolves in the form of HCO_3^- according to the existing equilibrium reaction. Cyanobacteria cells have ability to absorb CO_2 gas both in gaseous and dissolved form (HCO_3^-)[23]. The ability of cells to absorb these two forms of CO_2 differs depending on the Ci transporter and the rubisco formation they have.

Synechococcus is a β-cyanobacteria with 5 Ci transporters and 1B rubisco[24]. The cyanobacteria group tends to utilize carbon dioxide in the form of HCO_3^- compared to CO_2 in the form of gas. This is due to the Ci transporter for the removal of HCO_3^- does not require energy (passive), while the Ci transporter CO_2 requires energy and CO_2 that has been absorbed needs to be converted into HCO_3^- before it can be utilized. Carbon dioxide that has entered the cell body is then transferred to the carboxisome and converted back into CO_2 by the enzyme carbonyx anhydrase (CA) before it is converted to phosphoglyceric acid (PGA) by rubisco 1B[25].

For short amount of time, increasement of CO_2 solubility can uplift growth rate of *Synechococcus* HS-9 biomass. Meanwhile after that, the growth rate started to fluctuate and even drop. This is happened because process of dissolving carbon dioxide (CO2) gas into carbonate compounds (HCO_3^-) and carbonic acid (H_2CO_3) causes a decrease in the acidity (pH) of the PBR system[26]. The phenomenon then affects many things one of them, namely photosynthetic disorders due to disruption of the nutrient transport process and inhibited metabolic activity[27]. In addition, the phenomenon of photodamage also influences the process of cell photosynthesis in the PBR system. In summary, the process of photosynthesis has the role of producing intermediate compounds in the form of acetyl-CoA. These compounds then play a role in forming derivative compounds such as nucleotide acids, amino acids, lipids, energy (ATP), and several other components[28]. All of these metabolites play a role in the life and reproduction process of *Synechococcus* HS-9 cells, so photosynthetic disorders can cause a gradual decrease in the quality and quantity of cells.

4. Conclusions

The increasement of carbon dioxide (CO_2) solubility is affecting growth rate of *Synechococcus* HS-9 biomass specially during log phase. During 4 days of log phase, the growth rate was fluctuated from rising to fall of growth rate. This is happened because addition of baffle inside APBR system are increasing residence time of CO_2 to react with the growth media. The highest CO_2 solubility occurred in BCPBR, APBR A, and APBR B after 24 hours with 20 mg/L, 22 mg/L, and 23 mg/L respectively. Dissolving process of CO_2 into HCO_3^- causing decrease of acidity and affecting the metabolism process. So, in further research, acidities buffer research must be conducted in order to maintain stable acidity value (pH).

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Nomenclature

pH	Power of hydrogen
PBR	Photobioreactor
HS-9	Hot spring number 9
SDG	Sustainable development goals
BCPBR	Bubble column photobioreactor
APBR	Airlift photobioreactor
r	Specific growth rate
mg. L^{-1}	Milligram per liter
Ø	Diameter of baffle
v	Volume of sample
p	Titrant coefficient
NPK	Nitrogen phosphorous potassium
HCO_3^-	Soluble carbonic acid
H_2CO_3	Carbonic acid
Ci	Carbon inorganic
CA	Carbonyx anhydrase
PGA	Phospoglyseric acid
ATP	Adenosine triphosphate
Acetyl-CoA	Acetyl coenzyme A
ln	Natural logarithm
Nt	Cell numbers in certain time
N0	Cell numbers in first time
Δt	Time interval

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