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# Effect of Bubble Size on the Growth of *Synechococcus* HS-9 Microalgae with Double Loop Fluid Oscillator

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**Abstract:** The capacity of fossil fuel production is decreasing through the years. Energy diversification is needed to solve this problem. Microalgae is one type of microorganism that has the potential as an alternative fuel. Large scale microalgae cultivation utilizes photobioreactors with aeration of microbubbles. One method to produce smaller bubbles uses a fluid oscillator. This study aims to analyze if there is any effect of the bubble size difference on *Synechococcus* HS-9 growth. Bubble formation utilizes a double loop fluid oscillator and continuous flow. Bubble size data is taken using a high-speed camera at 6 lpm and 10  $\mu$ m micro porous shafts and processed through image processing using imageJ application. *Synechococcus* HS-9 growth data is taken daily optical density by using spectrophotometer. The result showed double loop fluid oscillator produces smaller bubbles than the conventional bubble aeration, thus affects a higher growth of *Synechococcus* HS-9. *Synechococcus* HS-9 growth is higher in oscillating flow conditions than in continuous flow.

Keywords: microalgae, *Synechococcus*, bubbles, microbubbles, fluid oscillator, sparger

## 1. Introduction and background

Indonesia's energy consumption is projected to increase in the coming decades <sup>1</sup>. Indonesia can import energy resources from abroad, but this can have an economic impact in the long term. There needs to be a solution for Indonesia to become an energy independent country. Renewable energy sources are a solution to the increasingly limited fossil fuels. The use of renewable energy sources, the highest amount is biomass (18.55%), hydropower (2.84%), and geothermal (1.23%). The use of petroleum (35.21%), coal (24.69%), and gas (17.37%) are still relatively high compared to the use of renewable energy resources <sup>2</sup>. Indonesia government has set objective for the proportion of new and renewable energy (NRE) in the national energy sector up to 23 percent by the year 2025<sup>3</sup>.

Indonesia is a country full of biodiversity. Indonesia, located in tropical area, has the potential for biomass resources. There are various types of the biomass that have the potential to be a source of energy. Microalgae can produce large amounts of oil with a narrower area of breeding grounds <sup>4</sup>.

Microalgae is a group of microscopic organisms, both

single cells, and colonies that live in all areas of freshwater and marine waters <sup>5</sup>. Microalgae have various types of benefits. These microorganisms can be used as food, medicine, and also biofuel. *Synechococcus* HS-9 has the potential to be used as biofuel. The content of *Synechococcus* that can be used as biofuel is lipids and fatty acids<sup>6</sup>. Biofuel requires large quantities of microalgae biomass. Cultivation of microalgae for large amounts using a device called a photobioreactor. A recent study showed that *Synechococcus* had been harvested in a tubular photobioreactor showing potential for biomass product because of it relatively rapid growth <sup>7</sup>. Moreover in 2020, Arif has found a high saturated fatty acids content in *Synechococcus* HS-9<sup>8</sup>.

Inside microalgae photobioreactors, microalgae will do photosynthesis as in the microalgae habitat. In the process, photosynthesis requires light and carbon dioxide as an energy source for microalgae growth. Carbon dioxide has a significant influence on the process of photosynthesis. Carbon is the primary constituent of microalgae cells and is estimated at 50% of the elements of dry biomass microalgae<sup>9</sup>. During the photosynthesis process in photobioreactors, microalgae absorb the

content of water-soluble carbon dioxide, a suitable medium for the transfer process between carbon dioxide and microalgae requires microbubbles. Microbubbles have a higher surface area to volume than conventional bubbles<sup>10)</sup>. Another characteristic of microbubble is its slow rising velocity. Thus the mass transfer of CO<sub>2</sub> from the bubbles to medium have more time, and bubbles can dissolve in the microalgae medium completely<sup>11)12)</sup>. Tiny microbubbles have shown beneficial extraction properties called aprons. Lye and Stuckey, for example, report high mass transfer coefficients using colloidal liquid aprons in the extraction of erythromycin<sup>13)</sup>. Furthermore, recently, a study indicates the slower bubble rising velocity due to the baffle on photobioreactor resulting in the higher growth rate of microalgae<sup>14)</sup>.

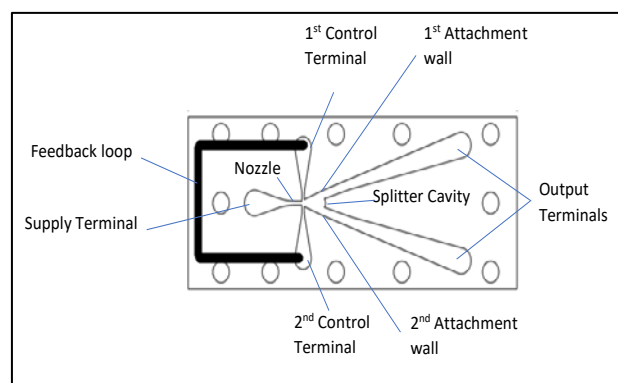
Several methods can obtain microbubbles. These methods include pressurized gas, ultrasound, and fluid oscillation<sup>15)</sup>. Pressurized gas supplied a high pressure on the air to dissolve in water. Pressurized air will then pass through a special nozzle to produce bubbles of small size. The Ultrasound method provides ultrasonic sound waves in water, thereby inducing local cavitation to create bubbles. Fluid oscillation makes the air flow unstable so that the bubbles that are formed are still close to the diameter of the output<sup>16)</sup>. Fluid Oscillation is the more considered cause of its low power, operational and the manufacturing costs are affordable<sup>17)</sup>. Fluid oscillation is considered as a potential method for aerating microalgae photobioreactors. Fluid oscillation is induced using a device called a fluid oscillator.

Recent research has been done using fluid oscillators as aerators for cultivating microalgae. Microalgae harvest analyses showed that the best overall cost-benefit relationship was achieved by the ceramic flat plate sparger with an oscillating airflow, using a model of single actuating fluid oscillator<sup>18)19)</sup>. This development of microbubbles is assisted by nozzle using a microporous sparger. Some common sparger types have been employed to generate and disperse gases efficiently, including pipe spargers<sup>20)</sup>, multiple ring spargers<sup>21)</sup>, wheel type spargers<sup>22)</sup>. The results showed that bubble size using a single loop fluid oscillator with a microporous sparger averaged size around 300  $\mu\text{m}$ <sup>23)</sup>. However, this method hasn't been reported using the *Synechococcus* HS-9 strain.

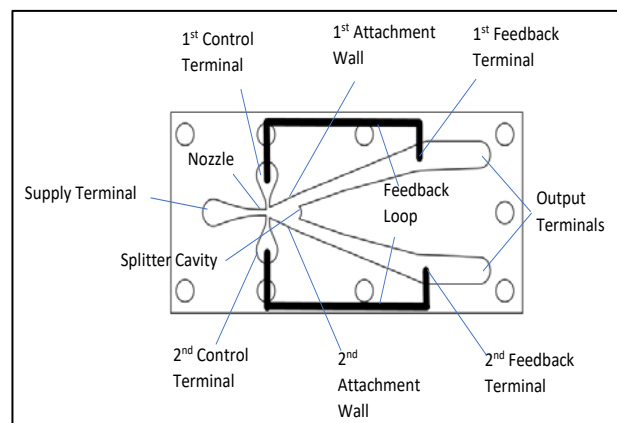
This research will be using a double loop fluid oscillator originated by Tesar<sup>24)</sup>. The supplied flow produces a jet in the single loop fluid oscillator (Figure 1) due to the nozzle's existence. Subsequently, the splitter cavity will direct the flow to one of the attachment wall surfaces because of the Coanda effect and keeps the jet flow attached. Because this flow is only connected to one side of the attachment, pressures on the flow side will be lower than on the other side. This phenomenon will induce a feedback flow in the control terminal from the high-pressure side to the low-pressure

side. Eventually, this feedback flow will deflect the main supply flow hence changing the flow direction into the other attachment wall, and the cycle will go back to the first step. On the other hand, in a double loop fluid oscillator, the feedback flow is more specific, as shown in Figure 2. A portion of the flow is withdrawn from the output channel and returned to the control nozzle on the same side via the feedback channel, thus destabilizing and deflecting the main flow to attached to the other attachment wall.

The double loop fluid oscillator has never been studied until the microbubble generation state. There is a need for a high frequency fluid oscillator to produce smaller microbubbles<sup>25)</sup>. Thus double loop fluid oscillator, which has a higher frequency, is used in this research. A microporous pipe sparger was used for this experiment. This study aims to analyze if there is any effect of bubble size difference on *Synechococcus* HS-9 growth.



**Fig. 1** Single loop fluid oscillator

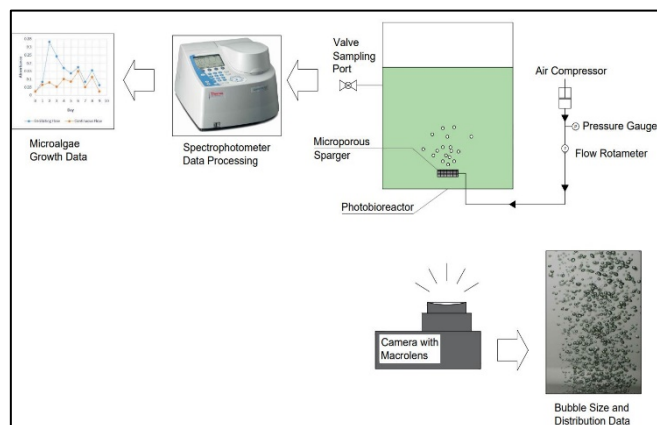


**Fig. 2** Double loop fluid oscillator

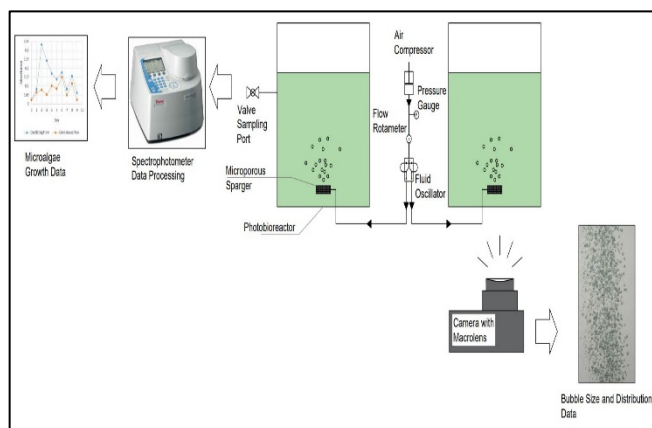
## 2. Method and experimental setup

The experimental set up shown in Figure 3 and, the flow diagram of the experiment and image processing technique is shown in Figure 4<sup>14)</sup>. The test section used two photobioreactors filled with culture volume 20 liters with 15 cm long, 40 cm wide, and 40 cm high. The airflow was compressed air with 1 bar gauge pressure, and this pressure was set to the minimum gauge pressure so the bubble could break off from the

diffuser. Airflow was controlled using a flow meter with flow variance tested 6 Lpm. This volumetric airflow was chosen because of the minimum flow so air can oscillate through a fluid oscillator. After flowing through the oscillator, air will pass the diffuser. The diffuser was a microporous sparger manufactured by SHENZHEN HENGKO TECHNOLOGY with pore diameter size 10  $\mu\text{m}$ .



**Fig. 3a** Double Loop Fluid Oscillator Experiment Setup

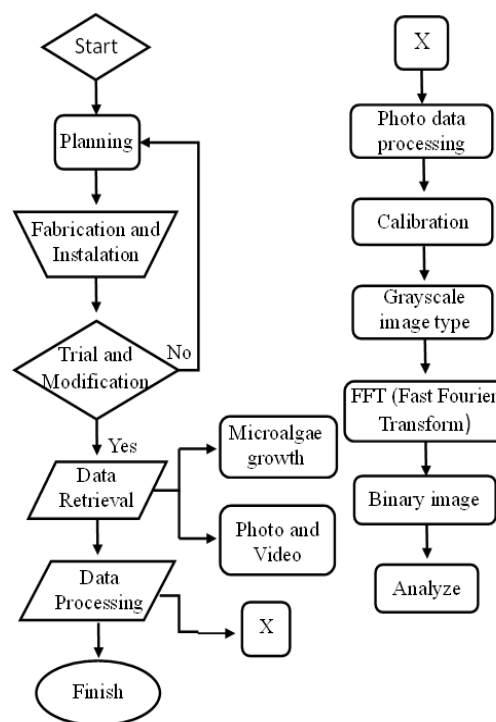


**Fig. 3b** Continuous flow Experiment Setup

Data acquisition was acquired by digital image analysis. The configuration of the experimental apparatus used camera Nikon D5200 with settings 1/4000 shutter speed, ISO 6400 attached with Nikon AF-S FX Micro-NIKKOR Standard Macro Lens set with a focal length and aperture of 85 mm and 2.5 mm was used to capture the microbubbles. A diffused layer was placed between the test section and a 50 W LED lamp at the back of the test section. This shadow photographic technique was used to get a sharper image of the bubbles. Before the experiment was started, a ruler was placed inside the test section and on the sparger to calibrate the dimension of the object to the pixel dimension.

Image captured the microbubbles was then processed using ImageJ software. The image captured was transformed using a bandpass filter to sharpen the edge of the bubbles. After a series of processing, the picture

finally transformed into a binary image. This binary, therefore, would be analyzed, the area of the bubble would be known and further processed; thus the diameter of the bubbles would be known. Bubbles were analyzed through a 20 mm x 20 mm rectangular sampling. The sampling was 20 times for each image for more accurate results.



**Fig. 4** Flow diagram of the experiment and image processing technique<sup>14)</sup>.

The microalgae *Synechococcus* HS-9 strain was obtained from Danau Rawa in Banten, Indonesia<sup>26)</sup>. NPK was used as the growth medium because it has shown promising results in fertilizing *Cyanobacteria*, giving better economic benefits<sup>27)</sup>. The alga was grown in 20 liters flat plate photobioreactor for nine days. Two aeration methods are used in this experiment, one with fluid oscillator and the other using continuous flow. Growth conditions are presented in table 1.

Table 1. Growth condition of microalgae

Variables	Value
Temperature	39 – 42 °C
pH	7 – 9
Medium	NPK
Light Intensity	1100 Lux
Type of aeration	Air

Each day a 10 ml samples are obtained from both photobioreactors. The sample then went into Thermo Scientific GENESYS10S UV-Vis Spectrophotometer for optical density measurement. The spectrophotometer was set to wavelength 663.0 nm to count the total of *Chlorophyll a* inside the microalgae cells<sup>28)</sup>.

### 3. Results and Discussions

The processed picture provided a distribution of the size of bubbles in each of the experiment variables. It has been discovered that oscillating bubbles have a narrower bubble. Figure 5 shows the results of bubbles generated by a double loop fluid oscillator and continuous flow. From the photo captured, visually, it can be seen that the oscillating flow bubble has a more dense microbubble population compared to the continuous flow bubble. These photos were further processed to clarify the visual assumption and bubble distribution. The results of the distribution of the bubbles are shown in figure 6. The bubble distribution showed that oscillating flow has a higher the number of bubbles under 200 microns (about 39%) than continuous flow (about 28%). The bubble distribution in the oscillating flow has equal size rather than the continuous flow. The phenomenon happened due to a rise in the frequency that led the air stream to oscillate quicker so that the bubble size formed closer to the sparger pore size. According to Vaclav Tesar's studies that the greater the oscillator frequency, the lower the bubble size.

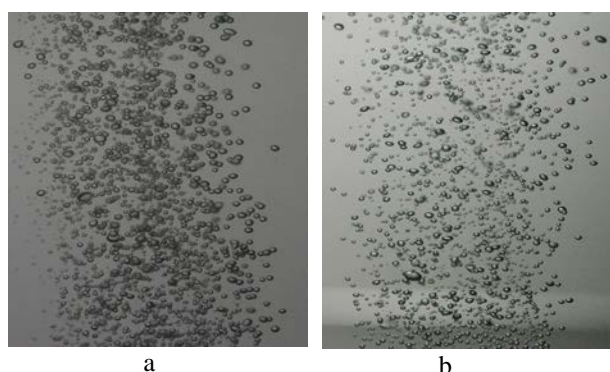


Fig. 5 (a) Oscillating flow bubble; (b) Continuous flow bubble

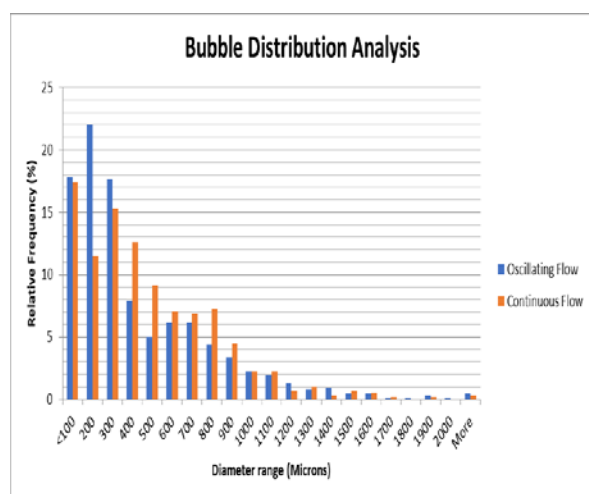


Fig. 6 Bubble Distribution Analysis

From the data obtained, It could be found that the growth of *Synechococcus* HS-9 had varied from time to

time (see Fig. 7). In Oscillating flow conditions, microalgae absorbance (optical density measurement) reached its peak at day 2, while the continuous flow condition reached its peak at day 6. On average, *Synechococcus* HS-9 growth is higher in oscillating flow conditions than in continuous flow. This phenomenon indicates that *Synechococcus* HS-9 would grow better if its bubble size were smaller.

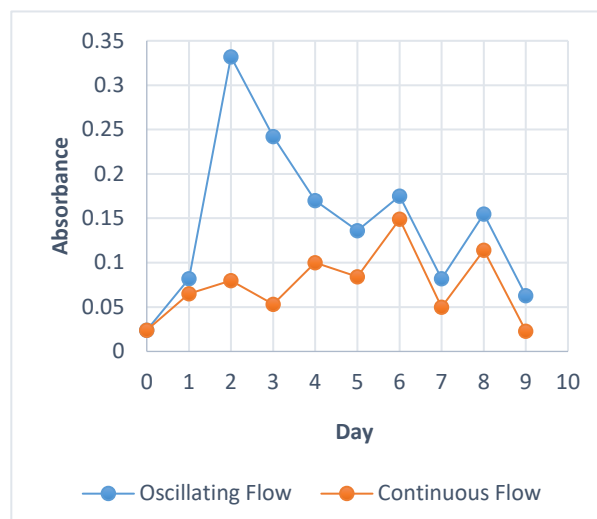


Fig. 7 *Synechococcus* HS-9 growth rate

### 4. Conclusions

In this study, bubble distribution phenomena between oscillating condition and continuous flow condition were analyzed. It could be found that double loop fluid oscillator could make the size of the bubble smaller than the continuous flow bubble, thus will affect the growth of *Synechococcus* HS-9. *Synechococcus* HS-9 growth is higher in oscillating flow condition than in continuous flow. The smaller size of bubbles will affect to higher *Synechococcus* HS-9 growth rate. To produce microbubbles, a fluid oscillator is capable of generating high frequencies with low airflow.

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