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Sound Wave Exposure as a Strategy for Improving the Tubular Photobioreactor for Cultivating *Synechococcus* HS-9 as Biofuel Feedstock under Different Photoperiods

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Abstract. This study aimed to evaluate the effect of sound wave exposure in different photoperiods on *Synechococcus* HS-9 cell density and lipid content using tubular photobioreactors (PBRs). In this study, nine PBRs were used: three PBRs were exposed to a sine wave of 279.9 Hz for three hours during the day (A), three PBRs were exposed to a sine wave of 279.9 Hz for three hours during the night (B), and three PBRs remained unexposed to any sound wave to serve as a control (K). All PBRs were studied for 18 days. The results showed that the highest average cell densities of *Synechococcus* HS-9 in PBR A, B, and K respectively were 8.883×10^5 cells/mL, 7.242×10^5 cells/mL, and 6.175×10^5 cells/mL. The highest lipid percentage, which was 17%, was observed in PBR A; the percentage in PBR B was 16%, and in PBR K, 7%. However, *Synechococcus* HS-9 in PBR B showed a higher growth rate compared to PBR A and PBR K. Sound waves could have increased cell activity and metabolism which led to the increase in cell densities and lipid percentages in *Synechococcus* HS-9. The photoperiodic differences might have resulted in a lower photosynthetic rate and cell metabolism, but the sound wave could have helped promote the growth of *Synechococcus* HS-9 despite the lower photosynthetic rate.

Keywords: Audible sound; Biomass; Photobioreactor; Photoperiodism; *Synechococcus*

1. Introduction

The dependency on fossil fuels as the main energy source has caused a depletion of fossil fuel reserves (Sukarni et al., 2019) and severe environmental pollution that affects many ecosystems. The development of sustainable and environmentally friendly fuels is needed in order to maintain the balance of the ecosystem and preserve fossil fuels (Machado and Atsumi, 2012). There are some sources of biofuel feedstock, such as food crops (e.g., corn, jatropha, and coconut) and microalgae (Chisti, 2007). Microalgae fixate CO_2 from the environment directly through photosynthesis, during which the CO_2 is converted to several biomolecules such as lipids. The aforementioned features of microalgae show that microalgae have the potential to serve as biofuel feedstocks and

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bioremediation agents.

Cyanobacteria are one group of microalgae that have been considered to be biofuel feedstocks. Cyanobacteria have high growth rates, can easily be genetically manipulated, and do not need to be grown on large and arable plots of land, thus reducing the competition with food crops for growth area (Nozzi et al., 2013; Sarsekeyeva et al., 2015; Farrokh et al., 2019). *Synechococcus* is one genus of cyanobacteria that has been researched for its capability to produce several bioethanol and lipid products that could be synthesized as biofuel (Mashayekhi et al., 2017). *Synechococcus* could be found in various habitats including hot springs. In this study, we use *Synechococcus* (labeled as *Synechococcus* HS-9) that was isolated from the Rawa Danau hot spring in Banten, Indonesia (Prihantini, 2015). *Synechococcus* HS-9 has been studied for its biofuel compounds, such as fatty acids (Prihantini et al., 2018).

PBRs are systems that could be used to increase the biomass of microalgae, including cyanobacteria. PBRs combine several abiotic factors such as growth media, light, photoperiods, and temperature in order to maximize the growth of cyanobacteria (Johnson et al., 2018). Previous research has shown that varying photoperiods could affect the growth of *Synechococcus* PCC 6715 (Klepacz-Smólka et al., 2020). Besides the aforementioned factors, there are also physico-stimulants that could affect the growth of cyanobacteria, such as sound waves. A sound wave is a mechanical wave caused by the movement of energy in a medium (Serway and Jewett, 2014). There are several studies which show that sound waves could promote microalgae growth (Jiang et al., 2012; Christwardana and Hadiyanto, 2017). Furthermore, there is an indication that sound waves could enhance the aeration in PBRs (Rizaldi et al., 2019) and the transesterification process of microalgae lipids (Cercado et al., 2018). Audible sound in the form of music can increase the cell density of *Synechococcus* HS-9 (Santoso et al., 2020). Nevertheless, to the best of our knowledge, there is no study about the effect of sound wave exposure under different photoperiods (light and dark times) on *Synechococcus* HS-9. Therefore, this study was conducted in order to measure and compare the growth and lipid production of *Synechococcus* HS-9 when exposed to sound waves during periods of daylight and night.

2. Methods

2.1. Growth Media Preparation and Cultivation

Eight (8) liters of growth media were made for this study. Cyanobacteria TAPS (CT) media is used for this study because CT is the basal media with complete nutrients for most freshwater cyanobacteria. CT consists of C media buffered with TAPS (N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid) (NIES, 2001). The chemical used for C media were imported from Merck, Darmstadt, Germany. The pH of CT media was adjusted by adding 1N solution of NaOH until the pH value reaches 8. *Synechococcus* HS-9 culture was cultivated in CT media for seven days to make a starter culture for inoculation. After the starter culture is ready, 50 mL of *Synechococcus* HS-9 culture was inoculated to PBR filled with 450 mL of CT. There are three PBR for every treatment, which acts as the replication for every treatment. The PBR then were placed in the incubation cabinet.

2.2. Sound Treatment

The treatment in this study consists of sound wave exposure at different photoperiodism time. The first treatment was PBR with sound exposure during the day (9 AM to 12 PM, labeled as PBR A), while the second one was PBR with sound wave exposure during the night (9 PM to 12 AM, labeled as PBR B), and the third one was PBR without any sound exposure as control (labeled as PBR K). The sound wave used for this study was a

sine sound wave, with a frequency set at 279.9 Hz. The sound level was set at 51 dB, with an intensity of 1.3×10^{-6} W/m^2. The sound was played every day for 18 days.

2.3. Design of PBR and Incubation Cabinet

The PBR used for this study was tubular in shape and made from acrylic with a 3 mm thickness. The diameter of the PBR was 10 cm, while the height of the PBR was 15 cm. The PBR tube was then closed with an acrylic lid, with one small hole as a port for *Synechococcus* HS-9 inoculation and sampling. The lid was equipped with a small tube in which to place the microphone to measure sound intensity. Figure 1 below shows the design of the PBR.

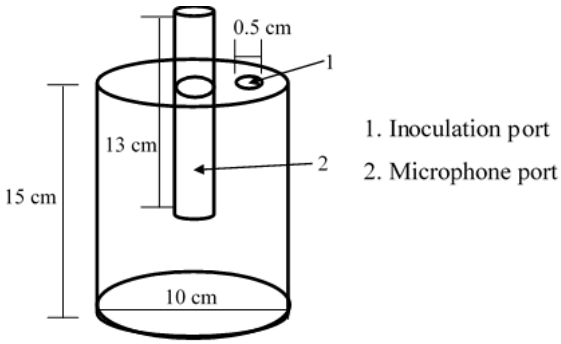

Figure 1 Design of the PBR tube

The incubation cabinet was made from iron. The inside of the cabinet was partitioned into three sections. The top and the middle sections (first and second) were used to place the PBRs exposed to sound waves. The bottom section (third) was used to place the control PBRs. The inside of the cabinet was coated with sound insulation foam and glasswool carpet to prevent sound from escaping from each section of the cabinet, thus creating a soundproof system. The incubation cabinet was equipped with an LED lamp (4500 lux) as a light source, an electric socket as a power supply for the speaker (NUBWO NSB-16) and MP3 player (Ruizu X02), and a timer to set the lighting time. The lamp was set to turn on during the day (9 AM to 9 PM) and turn off during the night (9 PM to 9 AM). Figure 2 below shows the design of the incubation cabinet and the PBR placement scheme.

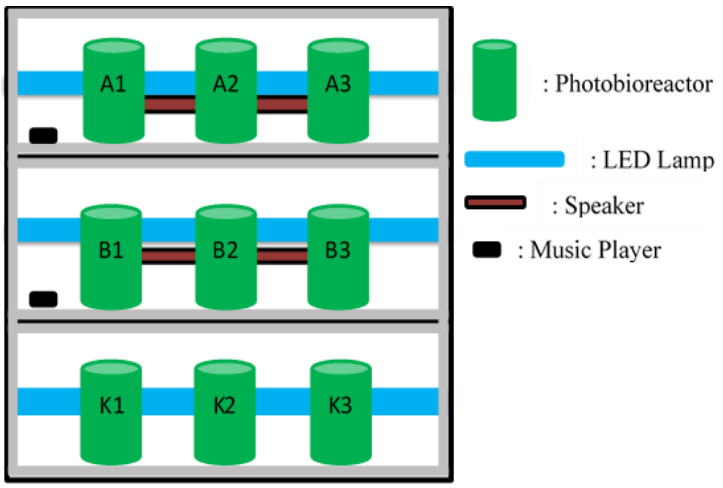

Figure 2 Design of incubation cabinet and scheme of PBR placement in incubation cabinet (front view, open door). A) PBRs exposed to sine wave during the day; B) PBRs exposed to sine wave during the night; K) PBRs not exposed to any sound wave (control)

2.4. Data Collection and Analysis

Data collected from this study consisted of cell density, growth rate, and lipid percentage from *Synechococcus* HS-9 biomass. For the cell density measurement, about 2 mL of *Synechococcus* HS-9 samples were taken from each PBR with a syringe so that the cell density could be calculated using a direct count method. The growth rate is defined as the increase in cell numbers in the population per unit time, compared to the number of cells in any sampling periods (Andersen, 2005). The growth rate could be calculated with Equation 1 (Andersen, 2005).

$$r = \frac{ln\frac{Nt}{N0}}{\Delta t} = \frac{ln\ Nt - \ln N0}{\Delta t} \quad (1)$$

where r is the growth rate, Nt is the number of cells at the end of the log phase, $N0$ is the number of cells at the beginning of the log phase, and Δt is the time interval.

The lipid was extracted from *Synechococcus* HS-9 using a modified version of the method proposed by Bligh and Dyer (1959). About 45 mL of *Synechococcus* HS-9 samples from all treatments were used for lipid extraction. The lipid percentage was measured with Equation 2 (Andersen, 2005).

$$L = \frac{DLP}{DCW} \times 100\% \quad (2)$$

where L stands for lipid percentage, DLP for dry lipid weight, and DCW for dry biomass weight. The lipids were extracted on day 18.

3. Results and Discussion

3.1. Measurement of Synechococcus HS-9 Cell Density, Growth Rate, and Lipid Percentage

Synechococcus HS-9 in all treatments exhibited different growth curves. The highest average cell density of *Synechococcus* HS-9 in all treatments was achieved on day 14. The highest average cell density of *Synechococcus* HS-9 in PBR A was 8.883×10^5 cells/mL, while in PBR B, it was 7.242×10^5 cells/mL, and in PBR K, 6.175×10^5 cells/mL. *Synechococcus* HS-9 that were exposed to sound waves had higher average cell densities compared to the controls. The highest average cell density was observed in PBR A. Figure 3 below shows the growth curve of *Synechococcus* HS-9.

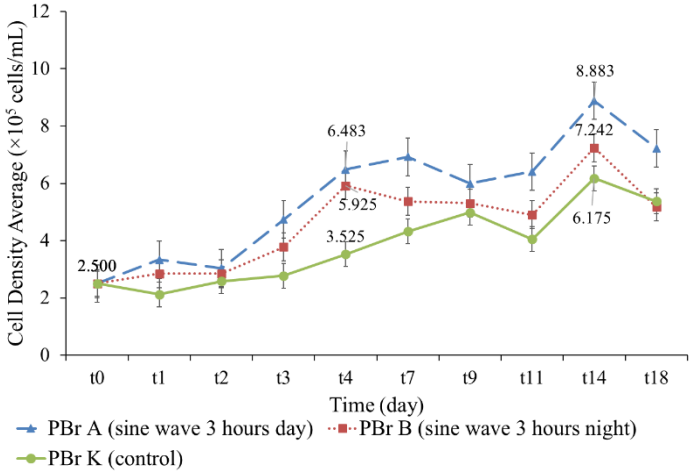

Figure 3 Average cell densities of *Synechococcus* HS-9 in the presence and absence of sound waves at different photoperiods

The growth rate of *Synechococcus* HS-9 was measured from day 1 (t1) to day 4 (t4). *Synechococcus* HS-9 in PBRs that were exposed to audible sounds had higher growth rates compared to the controls, with the highest growth rate seen in PBR B at 0.244 per day, followed by PBR A at 0.222 per day, and PBR K at 0.169 per day. *Synechococcus* HS-9 that were exposed to audible sounds had higher lipid percentages compared to the controls. The highest lipid percentage was observed in PBR A at 17%, then PBR B at 16%, and PBR K at 7%. The growth rates and lipid percentages are shown below in Table 1, Figure 4, and Figure 5.

Table 1 Growth rates and lipid Ppercentages of *Synechococcus* HS-9

Photobioreactor	Cell Density in day 1 (1×10^5 cells/mL)	Cell Density in day 4 (1×10^5 cells/mL)	Growth Rate (r) (per day)	Dry Lipid Weight in day 18 (gr)	Dry Biomass Weight in day 18 (gr)	Lipid Percentage in day 18 (%)
A	3.333	6.483	0.222	0.0091	0.0544	17
B	2.850	5.925	0.244	0.0080	0.0560	16
K	2.125	3.525	0.169	0.0040	0.0592	7

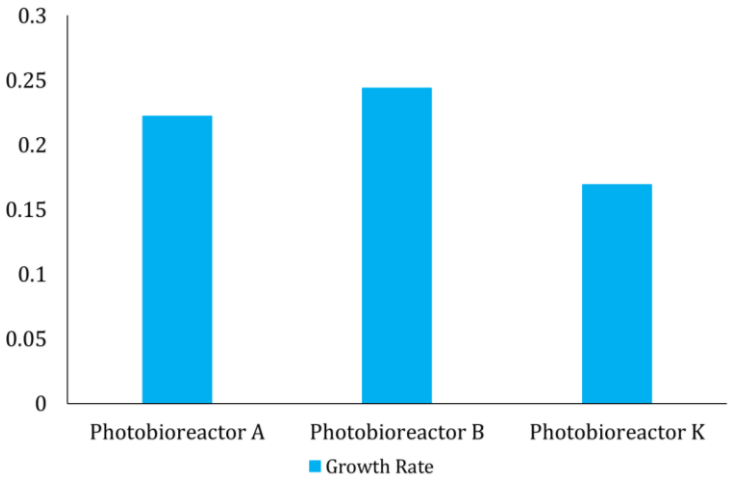

Figure 4 The growth rates of *Synechococcus* HS-9 in the presence and absence of sound waves at different photoperiods

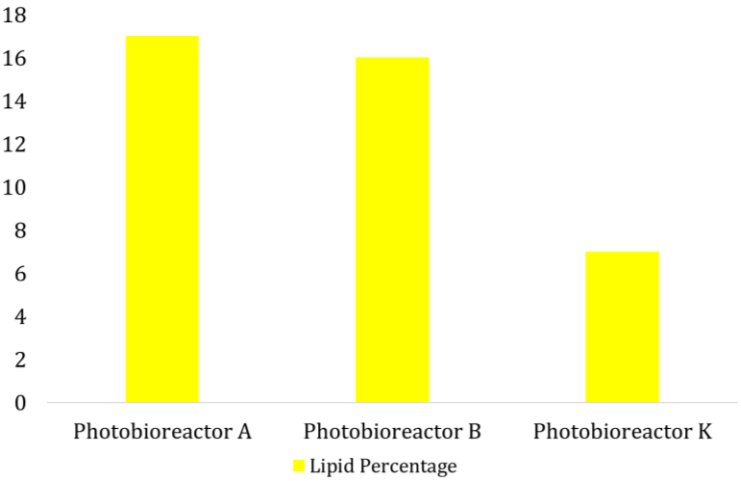

Figure 5 Lipid Percentages of *Synechococcus* HS-9 in the presence and absence of sound waves at different photoperiods

3.2. The Effect of Sound Wave Exposure and Varying Photoperiods on the Physiological Activity of Synechococcus HS-9

Energy from a sound wave is transmitted when the sound wave propagates through a medium or an object (Serway and Jewett, 2014). The energy from sound waves could act as an external force that stimulates the cell by increasing the activity of mechano-sensitive channels in the cell membrane. The increase in activity of mechano-sensitive channels leads to the increase of the ion and molecule transportation rates, thus causing an increase in cell replication and metabolism (Mishra et al., 2016), as seen in Figure 3. The increase in transportations of ions such as Ca^{2+} leads to an increase in the rate of photosynthesis, which can increase the lipid production of cyanobacteria (Checchetto et al., 2012; Wang et al., 2019).

The results of this study demonstrated that different photoperiods have different effects on the growth and lipid production of *Synechococcus* HS-9 that are exposed to sound waves. *Synechococcus* HS-9 that were exposed to sound waves in periods of light (PBR A) had higher cell counts and lipid yields, but *Synechococcus* HS-9 that were exposed to sound waves in darkness (PBR B) had higher growth rates. In darkness, cyanobacteria tend to switch their metabolism mode from aerobic to anaerobic, due to the lack of light as an energy source. This alteration in the mode of metabolism leads to a decrease in the photosynthetic rate of cyanobacteria (Hood et al., 2015). Nevertheless, the energy from sound waves could increase cell replication and metabolism (Mishra et al., 2016), which might increase the growth rate despite the decrease in photosynthetic rate.

4. Conclusions

Sound wave exposure could increase the cell density and lipid production of *Synechococcus* HS-9 in a tubular PBR. The results of this study demonstrated that the average cell densities of *Synechococcus* HS-9 in PBR A (8.883×10^5 cells/mL) and PBR B (7.242×10^5 cells/mL) were higher compared to that in PBR K (6.175×10^5 cells/mL). The different photoperiods had different effects on *Synechococcus* HS-9 that were exposed to the sound wave. *Synechococcus* HS-9 that were exposed to sound waves in the night (dark period) had lower cell densities and lipid yields (16%) compared to *Synechococcus* HS-9 that were exposed to sound waves during the day; however, *Synechococcus* HS-9 that were exposed to sound waves during the day had higher growth rates (0.244/day). Further studies could be done to examine the economic viability of this system for mass production. These results indicate that sound wave exposure could be used as a strategy for improving the PBR system in conditions of low light.

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