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Biomass Production of Subang Isolated *Scenedesmus* in Tube-shaped Photobioreactor with the Exposure of Audible Sound (Music): Photobioreactor Improvement Strategy

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Abstract: The cultivation of Subang Isolated *Scenedesmus* in tube-shaped photobioreactors (PBRs) with the exposure of audible sound (music) was done. The study was done to know the effect of different music to the growth and culture appearance of Subang Isolated *Scenedesmus*. Subang Isolated *Scenedesmus* itself is Indonesia's indigenous microalgae from Subang, West Java. This study was using three groups of Subang Isolated *Scenedesmus* PBRs. One group was control group and not exposed to any sound (Control-PBR), one group was exposed to music called "Blues for Elle" (PBR-A), and another group was exposed to music called "Bloom" (PBR-B). Based on 8 days of observation, it is known that Subang isolated *Scenedesmus* in PBR-A has the highest average cell density at the peak phase ($1.81 \pm 0.16 \times 10^5$ cells/mL), highest growth rate (26.22 % per day), and most intense green color compared to Subang isolated *Scenedesmus* in PBR-B and Control-PBR. Related-Samples Friedman's Two-Way ANOVA by Ranks test has proven that the average cell density value of Subang isolated *Scenedesmus* in Control-PBR, PBR-A, and PBR-B are not the same (α = 95%, sig = 0.013). Therefore, different audible sound (music) with certain intensity and frequency may affect the growth of Subang Isolated *Scenedesmus* differently.

Keywords: Audible sound; Cell density; Growth; Music; Photobioreactor (PBR); *Scenedesmus*

Introduction

Microalgae is a group of photosynthetic microorganisms (Barsanti et al., 2023) that are on the rise in the research world. It happens because the biomass of microalgae is suitable to be used in various sectors of human life. Biomolecules of microalgae can be processed into food supplements (Draaisma et al., 2013), drugs (Skjånes et al., 2013), cosmetics (Begum et al., 2016), and even biofuels (Milano et al., 2016).

One of the organisms from microalgae group that is potential to be used in various sectors of human life is microalgae from Genus *Scenedesmus*. Taxonomically, *Scenedesmus* belongs to Chlorophyta Division (Barsanti et al., 2023). Most of microalgae from Chlorophyta Division, including *Scenedesmus*, are cosmopolite. As a cosmopolite microalga, *Scenedesmus* can be found in various habitats, including fresh water, brackish water, even in the sea (Bellinger et al., 2015).

Scenedesmus is considered as a potential bioremediation (Santoso et al., 2020) and biofuel agent (Difusa et al., 2015). Indonesia has its very own indigenous *Scenedesmus*. It was isolated from a pond of rubber waste storage in Subang, West Java, Indonesia (Prihantini et al., 2007).

To be able to use in various sector of human life, a large amount of the biomass of microalgae, including *Scenedesmus* should be obtained in a short time. It can be achieved if the *Scenedesmus* is cultivated in a low cost photobioreactor (PBR) system. There are two types of photobioreactors, which are open-pond and closed-system photobioreactors (Huang et al., 2017). This research used closed-system. Regulation of several abiotic factors such as light intensity and temperature in closed-system PBR is providing the optimum condition for microalgae, including *Scenedesmus*, to grow (Slade et al., 2013). Besides, the risk of contamination can be minimized by cultivating microalgae in closed-system PBR (Rastogi et al., 2018). Contamination should be

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avoided in microalgae cultivation process, so that the biomass obtained will have a good quality to be processed further (Zhu et al., 2020).

One physical factor that has been proven as an accelerator of microalgal growth in PBR system is audible sound (music). Cultivation of microalgae with audible sound (music) exposure has been done on *Haematococcus pluvialis* (Christwardana et al., 2017), *Chlorella* DPK-01 (Tambunan et al., 2020) and *Synechococcus* HS-9 (Santoso et al., 2020). Music used in those studies were music from "Microbial Bebop" album. The "Microbial Bebop" itself was created by mapping data of several physical parameters that affect the activity, metabolism, and abundance of several microorganisms, including microalgae, into music (Larsen et al., 2013).

"Blues for Elle", the music that was created based on chlorophyll A concentrations and nutrient uptake ability of photosynthetic microorganism (Larsen et al., 2013) has been used in studies above. Meanwhile, "Bloom", the music that was created based on chlorophyll A concentrations and salinity of marine photosynthetic microalgae (Larsen et al., 2013) has not been used in any previous study of microalgal cultivation. Therefore, this study was done to determine the effect of "Blues for Elle" and "Bloom" in PBR system to the growth and culture appearance of Subang Isolated *Scenedesmus* based on 8 days of observation period. Data obtained from this research can be used as initial information for Indonesian indigenous microalgae bioprospecting activities, specifically *Scenedesmus* bioprospection.

Method

Research Scheme

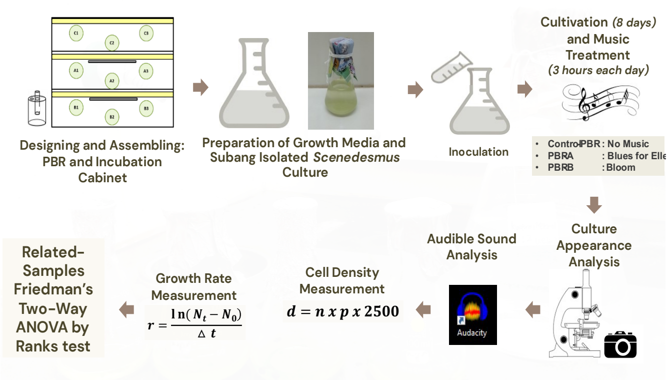

Figure 1. Research scheme

This research consists of several steps. Those steps are designing and assembling photobioreactors (PBRs) and incubation cabinet, preparation of growth media, preparation of Subang Isolated *Scenedesmus* culture, inoculation, cultivation, also data collection and analysis. General schematic view of this research can be seen in Figure 1.

Incubation Cabinet and Photobioreactors (PBRs) Design

Design of the incubation cabinet and photobioreactor in this study was similar to the design that was used by Tambunan et al. (2021) in their study. The incubation cabinet were metal-based, while the photobioreactors were acrylic-based. The design of the incubation cabinet and photobioreactor can be seen in Figure 2 and Figure 3 respectively.

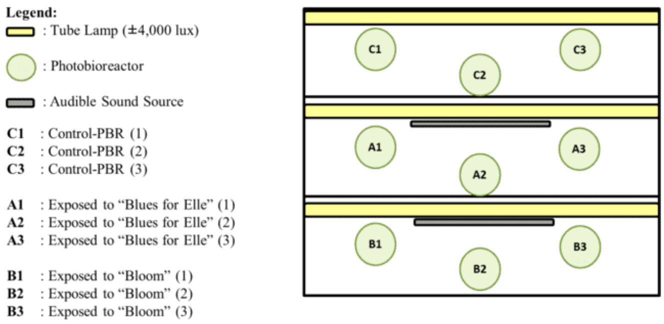

Figure 2. Incubation cabinet design

Figure 3. Photobioreactor (PBR) design

The metal-based incubation cabinet was coated with egg foam and glass wool carpet to avoid noise from the surroundings. The thickness of the egg foam and glass wool carpet were 30 mm and 7 mm respectively. The incubation cabinet was partitioned into three sections. In each section, one tube lamp (±4,000 lux) was provided as a light source, so that the *Scenedesmus* can undergo photosynthesis. Two speakers and two music players were placed inside the incubation cabinet. One speaker and one music player were provided on the second row, while another speaker and music player were provided on the third row of the incubation cabinet.

Photobioreactors in this study were acrylic-based tube-shaped PBRs. The thickness of the acrylic was 3 mm, while the total volume of each PBR was 1,177 mL. The lid of the photobioreactor is equipped with a microphone port and a small hole. Microphone port was the entry point of Sound Level Meter's microphone, so that sound intensity inside each PBRs could be measured. Meanwhile, the small hole was used as inoculation port at the beginning of the study, then used as the sampling port during the observation period.

Preparation of Growth Media

The NPK medium was used as microalgal growth media in this study. That medium was chosen because it contains essential macronutrients, such as Nitrogen (N), Phosphorus (P), and Potassium (K), which are needed by microalgae, including *Scenedesmus,* to grow optimally (Prihantini et al., 2020). The NPK medium was made by dissolving 120 mg NPK inorganic fertilizer (Grow More, 2013) to 1,000 mL of distilled water, so that the final concentration would be 120 ppm.

About 1,620 mL NPK medium was used to fill nine PBRs in this study, 180 mL each. Meanwhile, about 500 mL additional NPK medium (120 ppm) was also made to make the starter culture. Initial acidity value (pH) of NPK medium in this study was adjusted to 7.

Preparation of Starter Culture and Inoculation

Starter culture was made so that the Subang Isolated Scenedesmus can adapt before the inoculation to PBR system. Starter culture and microphotograph of Subang Isolated *Scenedesmus* can be seen in Figure 4.

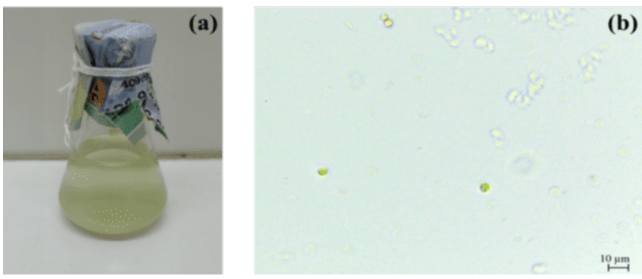

Figure 4. (a) Starter culture and (b) Microphotograph of Subang Isolated *Scenedesmus*

The starter culture was made by incubating Subang Isolated *Scenedesmus* that was cultivated in NPK medium (120 ppm) for 14 days. After 14 days of incubation, about 20 mL of Subang Isolated *Scenedesmus* from starter culture were inoculated into 180 mL NPK medium in each PBR. The initial cell density was $1.29 \pm 0.01 \times 10^5$ cells/ml. There were nine units of PBR used in this study. Those PBRs were incubated in the incubation cabinet for 8 days.

Audible Sound (Music) Analysis and Exposure

The PBR-A group on the second row of the incubation cabinet was exposed to "Blues for Elle", while the PBR-B group on the third row of the incubation cabinet was exposed to "Bloom" (See Figure 1). All music were played from 11 AM to 2 PM (3 hours), during the observation period (8 days). Before being exposed to all PBRs, audible sounds ("Blues for Elle" and "Bloom") were analyzed with a software called Audacity 2.3.0. The audible sound analysis was done to know all frequencies and sound level in certain music. Sound level from certain frequency then converted into sound intensity using mathematical equation below.

$$\boldsymbol{\beta} = (\mathbf{10})\boldsymbol{log}\frac{\boldsymbol{I}}{\boldsymbol{I_0}} \tag{1}$$

where $\boldsymbol{\beta}$ is the sound level (dB), I is the sound intensity (W/m^2), and I_0 is reference intensity level of audible sound, to which all intensities of audible sound are compared (10^{-12} W/m^2) (Serway et al., 2012).

Data Collection and Statistical Analysis

Data obtained from this study were cell density (cells/mL) and growth rate (% per day) of Subang Isolated *Scenedesmus.* Besides, the culture appearance (color) was also examined once a day during the observation period. Cell density data were obtained by counting the cell number with Hemacytometer (Improved Neubauer counting chamber) under the light microscope, then the cell density was measured according to this mathematical equation.

$$\boldsymbol{d} = \boldsymbol{n} \times \boldsymbol{p} \times \mathbf{2500} \tag{2}$$

where d is cell density (cells/mL), n is the number of cells that was seen in counting chamber under the microscope, and p is the dilution factor (Lee et al., 2013). After the cell density was measured, growth rate was measured according to this mathematical equation.

$$\boldsymbol{r} = \frac{\boldsymbol{ln}(\boldsymbol{N_t} - \boldsymbol{N_0})}{\Delta\, \boldsymbol{t}} \tag{3}$$

where r is the growth rate value, (N_t - N_0) is the difference between population size at the end of the exponential phase (N_t) and population size at the beginning of the exponential phase (N_0) and $\Delta\, \boldsymbol{t}$ is time interval (Lee et al., 2013).

All collected data were described by descriptive statistics, such as pictures and graphs. Besides, Related-Samples Friedman's Two-Way ANOVA by Ranks test (Walpole et al., 2012) was also done to examine the average cell density difference of Subang Isolated *Scenedesmus* in Control-PBR, PBR-A, and PBR-B (α = 95%).

Result and Discussion

Audible Sound (Music) Analysis

Sound intensity is a measure of magnitude and direction of sound energy flow. The greater the intensity of the sound, the greater the energy that carried by it (Serway et al., 2012). Based on the analysis with Audacity 2.3.0 and Equation (1), it is known that "Blues for Elle" has 279.9 Hz dominant frequency and 1.62 x 10-

^{11} W/m^2 intensity. Meanwhile, "Bloom" has 258.4 Hz dominant frequency and 1.19 x 10^{-11} W/m^2 intensity. The dominant frequency and intensity of "Bloom" is slightly lower than "Blues for Elle". Hence, the energy carried by "Bloom" is also slightly lower than "Blues for Elle".

Cell Density and Growth Rate Measurement of Subang Isolated Scenedesmus

The graph of Subang Isolated *Scenedesmus* average cell density during observation period (8 days) can be seen in Figure 5. It is shown that Subang Isolated *Scenedesmus* in Control-PBR, PBR-A, and PBR-B reached the highest cell density or peak phase on the fourth day of the observation period (t_4). It is also shown that Subang Isolated *Scenedesmus* in PBR-A has the highest cell density during the observation period compared to Subang Isolated *Scenedesmus* in another PBR groups. Besides, it is shown that Subang Isolated *Scenedesmus* has the lowest average cell density during first three days ($t_0 - t_3$) and last two days ($t_7 - t_8$) of the observation period, yet it has slightly higher cell density on the fourth until sixth day ($t_4 - t_6$) of the observation period.

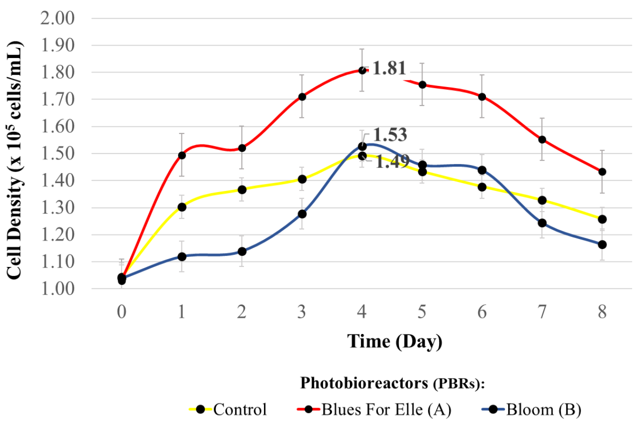

Figure 5. Graph of Subang Isolated *Scenedesmus* average cell density in function of time

Growth rate was measured using Equation (3). It is known that the growth rate value of Subang Isolated *Scenedesmus* in Control-PBR, PBR-A, and PBR-B is quite similar, which are 25.77 % per day, 26.22% per day, and 25.83% per day respectively. Based on that result, Subang Isolated *Scenedesmus* in PBR-A has the highest growth rate value compared to Subang Isolated *Scenedesmus* in another PBR groups. This result is similar with the study of Tambunan et al. (2020), where *Chlorella* DPK-01 that was exposed to "Blues for Elle" also has the highest growth rate compared to *Chlorella* DPK-01 in another PBR groups.

Since the growth rate value of Subang Isolated Scenedesmus in all PBR groups are quite similar, Two-Way ANOVA by Ranks test was also done. The test was done to determine the significance of the difference in the average density of Subang isolated *Scenedesmus* cells in all PBR groups. Based on the test, it is known that the average cell density value of Subang isolated *Scenedesmus* in Control-PBR, PBR-A, and PBR-B are not the same (α = 95%, sig = 0.013). It confirms that audible sound (music) with different dominant frequency and intensity affects the growth of Subang Isolated *Scenedesmus* differently.

Effect of Different Audible Sound (Music) Exposure to Scenedesmus Physiological Mechanisms

Different audible sound (music) may affect physiological mechanisms of microalgae, including *Scenedesmus,* differently. Besides the difference in average cell densities (cells/mL) and growth rate value (% per day), culture appearance (color) of Subang Isolated *Scenedesmus* shows slight difference. It can be seen in Figure 6.

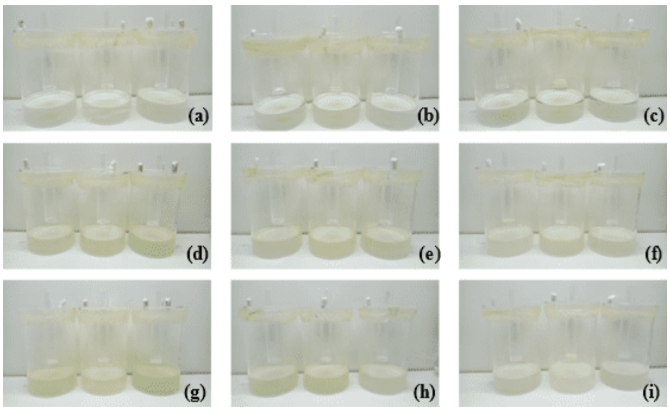

Figure 6. Culture appearance of Subang Isolated *Scenedesmus* on t_0 in (a) Control-PBR, (b) PBR-A, and (c) PBR-B; on t_4 in (d) Control-PBR, (e) PBR-A, and (f) PBR-B; on t_8 in (g) Control-PBR, (h) PBR-A, and (i) PBR-B

Based on Figure 6 (a), (d), and (g), the change of color intensity of Subang Isolated *Scenedesmus* in Control-PBR can be seen. As the observation period goes by, the culture color gets darker and more intense. Similar phenomenon can be seen in Figure 6 (b), (e), and (h), where the change of color intensity of Subang Isolated *Scenedesmus* in PBR-A happened. Meanwhile, based on Figure 6 (c), (f), and (i), it can be seen that the culture color in PBR-B also get more intense, yet the color did not get darker as (a) to (g). This phenomenon might happen because the average cell density of microalgae, including *Scenedesmus,* is not always linear to the turbidity of its culture. It might happen because cell shape, size, and pigment production are interrelated in influencing turbidity of cell culture (Francois et al., 2005).

Sound waves, including audible sound (music) affect the activity of microorganism by transmitting energy to the cell through vibration. This vibration was responded by mechanosensitive channels contained in cell membrane (Reguera, 2011). If the vibrations carry the right amount of energy needed by cells to grow or synthesize pigments, then the biological signal that regulates growth or pigment synthesis will be amplified. In contrast, if the vibrations do not carry the right amount of energy needed by cells to grow or synthesize pigments, biological signal that regulates growth or pigment synthesis will be amplified (Mishra et al., 2016).

This study showed that the energy carried by the dominant frequency of "Blues for Elle" (279.9 Hz) and "Bloom" (258.4 Hz) affect physiological mechanisms of Subang Isolated *Scenedesmus* differently. Energy from "Blues for Elle" might be suitable with the need of Subang Isolated *Scenedesmus* to grow and produce pigment, such as chlorophyll. It can be seen from the growth rate value (26.22 % per day) and greener culture color of Subang Isolated *Scenedesmus* in PBR-A on t_4 (peak phase), compared to Subang Isolated *Scenedesmus* in another PBR groups. Meanwhile, energy from "Bloom" might be suitable with the need of Subang Isolated *Scenedesmus* to grow, yet might not be suitable with its need to produce pigment, such as chlorophyll. It can be seen from the growth rate value of Subang Isolated *Scenedesmus* in PBR-B (25.83% per day), which is slightly higher than the growth rate value of Subang Isolated *Scenedesmus* in Control-PBR (25.77 % per day). Nevertheless, the culture color of Subang Isolated *Scenedesmus* in PBR-B on t_4 is less green than the culture color of Subang Isolated *Scenedesmus* in Control-PBR on the same observation day. This confirms the notion that audible sound (music) with different dominant frequency and intensity affects the physiological mechanism of Subang Isolated *Scenedesmus* differently.

Conclusion

Audible sound (music) exposure affects the growth of microalgae, including Subang Isolated *Scenedesmus*. It confirms the notion that music can be used to improve PBR system. Subang Isolated *Scenedesmus* that was exposed to "Blues for Elle" for 3 hours every day in 8 days has a higher growth rate (26.22% per day) and more intense green culture color, compared to Subang Isolated *Scenedesmus* that was exposed to "Bloom" and not exposed to music. Further study is needed to find out how music quantitatively affects Subang Isolated *Scenedesmus* biomolecules (e.g., carbohydrate, lipid, and protein) and pigments (e.g., chlorophyll and carotene) production. Another study is needed to find out whether these higher cell densities and growth rate values also applies when music exposure is done to Subang Isolated *Scenedesmus* in larger PBR volume and longer cultivation period. The results from those kinds of studies can be used to determine the economic and practical viability of the audible sound exposure in PBR system for mass production.

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