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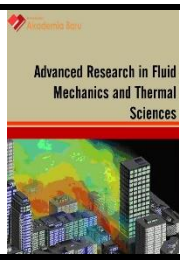

Difference in Time of Audible Sound Exposure to Chlorella DPK-01 in Tubular Photobioreactors: A Strategy to Improve Photobioreactor System

Rubiantin Mesha Nauli Tambunan[1], Yosua Adi Santoso[1], Santoso Soekirno[2], Nasruddin[3], Nining Betawati Prihantini[1,*]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, 16424, Depok, West Java, Indonesia
[2] Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, 16424, Depok, West Java, Indonesia
[3] Department of Mechanical Engineering, Faculty of Engineering, Universitas Indonesia, 16424, Depok, West Java, Indonesia

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ABSTRACT

The cultivation of *Chlorella* DPK-01 in tubular photobioreactors (PBRs) with difference in time of audible sound exposure was done. The study aims to evaluate the effect of difference in time of audible sound exposure in tubular PBRs to the growth and lipid percentage of microalgae *Chlorella* DPK-01. This study was using three groups of *Chlorella* DPK-01 PBRs. One group was control group and not exposed to any sound (Control-PBR), one group was exposed to audible sound in the light (PBR-A), and another group was exposed to audible sound in the dark (PBR-B). Each group consists of three units of PBR. The audible sound (279.9 Hz sine wave) was played in the light for PBR-A and in the dark for PBR-B. The observation period was 14 days. The growth rate of *Chlorella* DPK-01 was 47.6% per day (Control-PBR), 0.44 per day (PBR-A), and 0.55 per day (PBR-B) respectively. Meanwhile, the lipid percentage of *Chlorella* DPK-01 was 16% (Control-PBR), 31% (PBR-A), and 11% (PBR-B) respectively. Therefore, exposing audible sound in the light and in the dark may differently affect the growth and lipid production of *Chlorella* DPK-01.

1. Introduction

People's dependency to fossil fuel in running various sectors in life causes resource depletion of fossil fuel in nature. Alternative fuels from organic materials (biofuel) are needed due to this resource depletion. Previous studies have shown that lipid content from microalgae biomass can be used as biofuel after several processing steps [1-2].

Chlorella is one of the potential organisms from microalgae group whose biomass can be used as raw material for biofuel. It is because *Chlorella* has Palmitate acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:3) in its biomass. These lipid constituents have been proven suitable to be processed into biofuel [3]. Besides, *Chlorella* is cosmopolite microalgae, so it can easily be found, both in aquatic and terrestrial environments [4]. Reproduction stages of *Chlorella* is quite simple. The stages are

* *Corresponding author.*
E-mail address: nining.prihantini@gmail.com / nining@sci.ui.ac.id

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growing, ripening, and cell division. Growing and ripening until *Chlorella* becomes a mature mother cell occurs in the light. Meanwhile, the process of releasing young daughter cells from the mother cell occurs in the dark [5]. Therefore, this study uses *Chlorella* DPK-01, Indonesia's indigenous microalgae that was isolated from soil in Depok, West Java.

Large amount of *Chlorella* biomass with high lipid content is needed to be used as raw material for biofuel. This can be achieved by cultivating *Chlorella* in a photobioreactor (PBR) system. Photobioreactor system can regulate several factors that affect the growth and lipid production during the cultivation of microalgae, including *Chlorella.* Those factors are light intensity, photoperiodicity, temperature, acidity of growth media (pH), and physico-stimulant such as audible sound exposure [6].

Previous study has shown that difference in photoperiodicity can cause difference in the growth of *Chlorella* from Depok, West Java (as known as *Chlorella* DPK-01) [7]. Other study has shown that different sound waves can cause differences in difference in the growth of *Chlorella* [8]. However, there are no studies that show the effect of exposure of the same sound wave at different times (light/dark) to the growth of *Chlorella*. Therefore, this study is conducted to measure and compare the cell size, cell density, and lipid percentage of *Chlorella* DPK-01 that was exposed to audible sound (279,9 Hz sine wave) at different times (light/dark).

2. Methodology

2.1 Photobioreactors (PBRs) and Incubation Cabinet

Nine units of Photobioreactors (PBRs) were used in this study. Three units as control PBR, three units as PBR-A (exposed to audible sound during light), and three units as PBR-B (exposed to audible sound during dark). Photobioreactors in this study were acrylic-based tubular PBRs. The thickness of the acrylic was 3 mm. The lid of the photobioreactor is equipped with a microphone port. Microphone port was used as the entry point of Sound Level Meter's microphone so it could measure sound intensity inside the PBR. Besides, the lid is also equipped with a small hole as an inoculation and sampling port (Figure1).

Fig. 1. Tubular Photobioreactor Design

The incubation cabinet was a metal cabinet that was partitioned into three sections. It was coated with egg foam (3 cm thick) and glass wool carpet (0.7 cm thick), to avoid noise from outside the system. It is also equipped with tube lamps (4,000 lux) as the light source for *Chlorella* DPK-01 to undergo photosynthesis. Two speakers and two music players were placed inside the incubation cabinet. One speaker and one music player was put at the second row while the others were put at the third row (Figure 2).

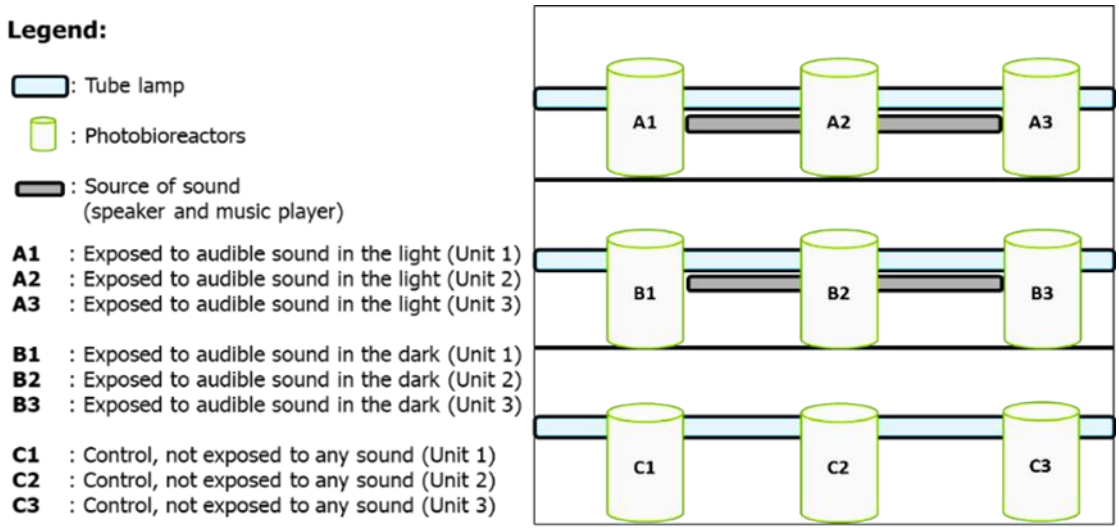


Fig. 2. Incubation Cabinet Design

2.2 Preparation of Growth Media and Cultivation of The Microalgae

About 8,000 mL of microalgal growth media was made in this study. The microalgal growth media in this study was Bold's Basal Medium (BBM). Bold's Basal Medium contained nutrients, both macronutrients and micronutrients, which are needed by *Chlorella* DPK-01 to grow and to synthesize chemical compounds, such as lipid [9]. About 5,700 mL BBM was used to fill each PBR, 630 mL each. An additional 2,000 mL BBM will also be made to make the starter culture. The initial acidity value (pH) of BBM in this study was 7.

Starter culture was made so that the *Chlorella* DPK-01 can adapt before inoculation to photobioreactors. After four days of incubation, about 30 mL *Chlorella* DPK-01 from starter culture were inoculated into 630 mL BBM in each PBR. The initial cell density was $6.3 \pm 0.1 \times 10^5$ cells/ml. There were nine units of PBR used in this study. Those PBRs were incubated in the incubation cabinet for 14 days.

2.3 Audible Sound Treatment

The audible sound in this study was 279.9 Hz sine wave with 51 dB sound level. Frequency generator was used to create the sound, while the music player and speaker were used to expose the sound and adjust the sound level. The audible sound was played from 11 AM to 2 PM (in the light) on the first row of the incubation cabinet and from 11 PM to 2 AM (in the dark) on the second row of the incubation cabinet. Meanwhile, the control in the third row of the incubation cabinet will not be exposed to any sound.

2.4 Lipid Extraction and Measurement

Lipid extraction was done by the Bligh & Dyer (1959) [10] modified method. The extraction used methanol and chloroform (2:1 v/v) as solvents. *Chlorella* DPK-01 biomass was centrifuged then separated from the supernatant. After that, it was mixed with solvents, sonicated, mixed with distilled water, sonicated again, and centrifuged again. The organic layer was separated from the biomass by 0,45 mm filter paper. Those separated layers were collected in two separated flasks. The flasks then dried in 105 °C oven for about 4 hours. Lipid percentage then counted by dividing the dry lipid weight with dry cell weight. Dry lipid weight was the difference between the dried flask with

organic layer and the dried empty flask while the dry biomass weight was the difference between the dried flask with biomass and the dried empty flask.

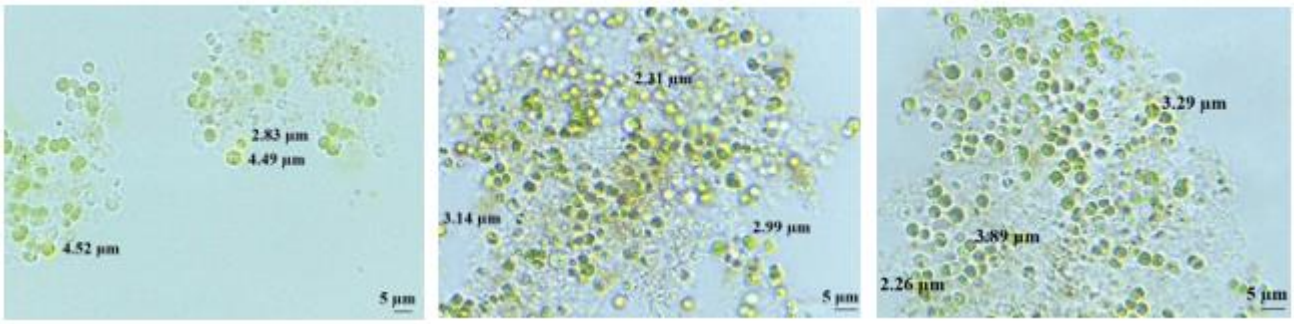
2.5 Data Collection and Analysis

Data obtained from this study are cell size (µm) and appearance, cell density (cells/mL), and lipid percentage (%). Cell size and appearance were examined on the last day of observation. Cell number was counted by direct counting method with Improved Neubauer counting chamber, then the cell density was counted. Cell density was counted every day during the observation period (14 days). Lipid percentage was measured at the end of the observation period. About 1 mL of sample from each PBR was taken with a syringe and observed under the microscope to measure the cell size and count the cell number. Lipid percentage was measured by dividing dry lipid weight with dry cell weight. All collected data are described by descriptive statistics.

3. Results

3.1 Cell Size and Appearance of Chlorella DPK-01

Cell size and appearance of *Chlorella* DPK-01 in each PBRs can be seen in Figure 3. *Chlorella* DPK-01 in each PBRs at the end of the observation period have similar sizes (2—5 µm), still in the range of normal cell (2—10 µm) [11]. Meanwhile, Figure 3 shown that *Chlorella* DPK-01 in each PBRs have different appearance. *Chlorella* DPK-01 in Control-PBR formed a tenuous colony without transparent layers, while *Chlorella* DPK-01 in PBR-and PBR-B formed solid colonies with transparent layer. The transparent layer presumed to be carbohydrate layer.



(a) (b) (c)

Fig. 3. Microphotograph of *Chlorella* DPK-01 in (a) Control-PBR, (b) PBR-A, and (c) PBR-B

Chlorella DPK-01 in PBR-A has thicker carbohydrate layer than *Chlorella* DPK-01 in PBR-B. This phenomenon might happen due to the difference in time of audible sound exposure. It is known that light is an important factor in carbohydrate synthesis [5]. Exposing sound in the light might give *Chlorella* some additional energy to undergo carbohydrate synthesis, causing thicker carbohydrate layer around cells.

3.2 Cell Density, Cell Growth, and Lipid Measurement of Chlorella DPK-01

The graph of *Chlorella* DPK-01 cell density during observation period can be seen in Figure 4. It is shown that *Chlorella* DPK-01 in all PBRs reached the highest cell density on t_4. It is also shown that *Chlorella* DPK-01 in PBR-B has slightly higher cell density than *Chlorella* DPK-01 in another PBRs.

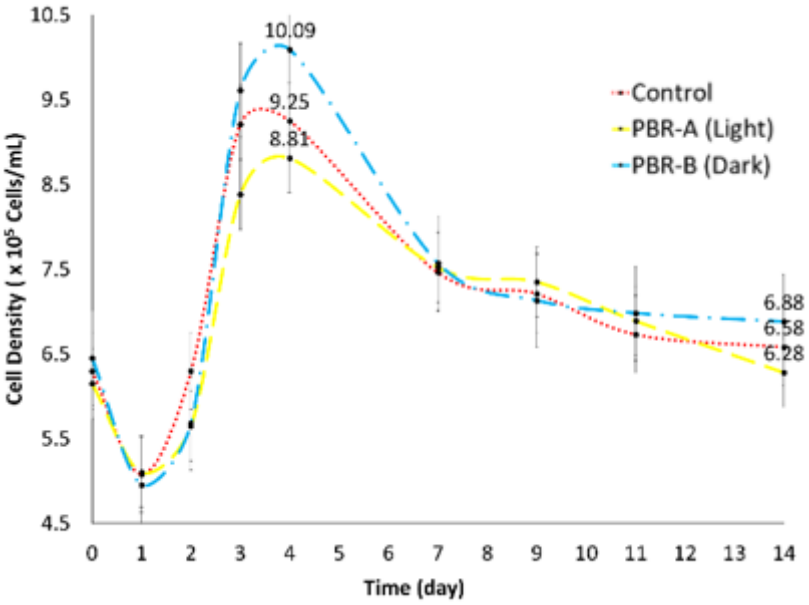


Fig. 4. Graph of *Chlorella* DPK-01 Average Cell Density Value in Function of Time

The growth rate and lipid percentage of *Chlorella* DPK-01 in each PBRs were measured to support the data in Figure 4. The growth rate of *Chlorella* can be measured by using this equation.

$$r = \frac{\ln (N_t - N_0)}{\triangle t} \tag{1}$$

where (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 $\triangle t$ is time interval. In this study, the time interval chosen was day 1 to day 4 (t_1—t_4). Meanwhile, the lipid percentage can be measured by this equation.

$$\text{Lipid Percentage} = \frac{\text{DLP}}{\text{DCW}}\ x\ 100\% \tag{2}$$

where DLP is dry lipid weight and DCW is dry cell weight [12].

Results of Eq. (1) and Eq. (2) can be seen in Table 1. Table 1 shown that the growth rate value and lipid percentage of *Chlorella* DPK-01 in each PBRs were inversely related. *Chlorella* DPK-01 in PBR-B has the highest growth rate value, yet it has the lowest lipid percentage. Meanwhile, *Chlorella* DPK-01 in PBR-A has the lowest growth rate value, yet it has the highest lipid percentage. This phenomenon might happen because of the difference in time of audible sound exposure to *Chlorella* DPK-01. Cells that were exposed to audible sound in the dark receive additional energy from the sound to undergo cell division, causing higher growth rate value. Meanwhile, cells that were exposed to audible sound in the dark receive additional energy from the sound to undergo biochemical synthesis, such as lipid synthesis.

Table 1
Chlorella DPK-01 Growth Rate Value and Lipid Percentage

Photobioreactor	Growth Rate Value (per day)	Lipid Percentage (%)
Control-PBR	0.48	16%
PBR-A (Light)	0.44	31%
PBR-B (Dark)	0.55	11%

3.3 Effect of the Difference in Time of Audible Sound Exposure to Physiological Mechanisms of Chlorella

Audible sound carries some amount of energy, according to its frequency and intensity [13]. It transmits energy through vibration to the cell. The vibration is responded by mechano-sensitive channels in the cell membrane. Mechano-sensitive channels then amplified several biological signals, so that the energy can be used to undergo intracellular activities of the cell [14], such as biochemical synthesis and the cell growth. Besides, vibrations from sound waves, like vibrations in any other forms such as mechanical agitation, are able to maximize the contact between cells and nutrients in the growth media. It helps cells to absorb nutrients efficiently, so that the growth and biosynthesis process can be accelerated [15].

Since *Chlorella* has different intracellular activities in the dark and in the light [5], difference in time of audible sound exposure causes different effects on the growth and lipid synthesis of *Chlorella* DPK-01. Exposing audible sound in the dark promotes the cell growth of *Chlorella* DPK-01. It might happen because the energy from audible sound carries additional energy for cell to undergo the process of releasing young daughter cells from the mother cell, that occurs in the dark. Besides, the contact between cells and nutrient in growth media was maximum during audible sound exposure in the dark. Audible sound exposure in the dark might increase the cell division rate so that the *Chlorella* have more cell numbers. Meanwhile, since the ripening of *Chlorella* cells occur in the light, exposing audible sound to *Chlorella* DPK-01 in the light might increase lipid percentage from cell biomass. It might happen because the energy from audible sound carries additional energy for cell to undergo biochemical synthesis, such as lipid, that occurs in the light. Besides, in this case, the contact between cells and nutrient in growth media was maximum during audible sound exposure in the light. Audible sound exposure in the light might increase the lipid synthesis rate so that the *Chlorella* have more lipid percentage.

4. Conclusions

Difference in time of audible sound exposure has an effect to the growth and lipid production of *Chlorella* DPK-01 in tubular photobioreactor. Exposing *Chlorella* DPK-01 to audible sound at different time may be used to improve PBR system. *Chlorella* DPK-01 in PBR-B (exposed to audible sound in the dark), has higher growth rate (0.55 per day), yet it has lower lipid percentage (11%) compared to *Chlorella* DPK-01 in another PBRs. Meanwhile, *Chlorella* DPK-01 in PBR-A (exposed to audible sound in the light), has higher lipid percentage (31%) yet it has lower growth rate (0.44 per day) compared to *Chlorella* DPK-01 in another PBRs. Different in time of audible sound exposure can be adjusted to the growth phase of *Chlorella* DPK-01, so that the cell can reach large amounts of cell number, then produce large amount of lipid as well. Besides, further study should be done to determine the economic viability of the difference in time of audible sound exposure for mass production.

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