JURNAL IPTEK
MEDIA KOMUNIKASI TEKNOLOGI
homepage URL : ejurnal.itats.ac.id/index.php/iptek



Development of Renewable Photobioreactor (FBR) Technology with Fluid Hydrodynamics System-Online Monitoring Microcontroller as SNI Standardized Pure Oxygen Producer

Mahendra Satria Hadiningrat[1], *Mayang Sari*[2], *Ninik Nigusti Ayu Sunardi*[3]
Institut Teknologi dan Sains Nahdlatul Ulama Pasuruan, Pasuruan, 67171

ARTICLE INFORMATION

Jurnal IPTEK – Volume 26 Number 2, December 2022

Page:
151–160
Date of issue :
December 30, 2022

DOI:
10.31284/j.iptek.2022.v26i2.3373

ABSTRACT

This photobioreactor research was carried out using Chlorella vulgaris algae as an O_2-producing reactor and optimizing light energy as its energy source, with dimensions of 40x50x60 cm with control of pH, temperature, and chemical visibility factors. Variations are given by providing a supply of CO_2 in both types of photobioreactors. Then it can be seen the concentration of O_2 produced from the photobioreactor and its ability to overcome CO_2 gas emissions. The use of glass as a reactor-making material is because glass is able to absorb visible light wavelengths in the range of 400–750 nm where at that wavelength microalgae can live and reproduce well. Before selecting the lamp used for the photobioreactor system. Measurements were carried out on two photobioreactors, namely, photobioreactors supplied and not supplied with CO_2, and using three types of light sources, namely halogen lamps, LEDs, and sunlight. The maximum oxygen concentration value was produced by the photobioreactor supplied with CO_2. The average percent error of the designed tool is 1.383% which is obtained by comparing the value of the designed tool with the reference measuring instrument.

Keywords: *Arduino Uno R3; Chlorella vulgaris microalgae; KE50 Sensor; Non-inverting amplifier; Photobioreactor.*

E-MAIL

[1]mahendra@itsnupasuruan.ac.id
[2]mayangsari@itsnupasuruan.ac.id
[3]niniksunardi@gmail.com

PUBLISHER

LPPM-Institut Teknologi Adhi Tama Surabaya
Address:
Jl. Arief Rachman Hakim No. 100, Surabaya 60117,
Tel/Fax: 031-5997244

Jurnal IPTEK by LPPM-ITATS is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License.

ABSTRAK

Penelitian ini menggunakan mikroalga Chlorella vulgaris sebagai penghasil oksigen murni melalui reaktor dan optimalisasi dari sumber cahaya tertentu, dengan dimensi 40x50x60 cm (dalam kondisi pH, suhu dan faktor visibilitas normal). Variasi yang diberikan ada dua jenis yaitu menggunakan CO_2 dan tanpa menggunakan CO_2 yang akan memproduksi persentase gas oksigen murni melalui reaktor. Penggunaan reaktor kaca untuk menyerap energi sumber cahaya tampak berkisar dari 400–750 nm agar produksi mikroalga menjadi maksimal. Dengan pengukuran visibilitas, melalui lampu halogen. LED dan bantuan cahaya matahari akan terjadi penyerapan yang sangat optimum. Hasil dari penyerapan cahaya ke mikroalga meningkat searah dengan persentase gas oksigen yang dihasilkan.

Kata kunci: *Arduino Uno R3; Fotobioreaktor; Mikroalga Chlorella vulgaris; Penguat non-inverting; Sensor KE50.*

INTRODUCTION

Lately, we often hear about Global Warming which is increasing day by day along with the emergence of new industrial factories in the world, where each factory always produces liquid, gas, or solid waste which causes problems in handling it. Especially waste gas in the form of greenhouse gases which includes CO_2 (carbon dioxide) gas emissions from burning fossil fuels that have accumulated in the atmosphere which has an impact on warming and climate change on earth. In dealing with this problem, there have been many ways to reduce Global Warming, one of which is the idea of making bioreactors using algae that are easy to breed and have greater potential to reduce greenhouse gases than by handling forest reforestation [1][2].

Algae are often better known as algae, including phytoplankton, which is included in the world of Tallophyta (thallus plants) because they do not have true roots, stems, and leaves. Algae are chloroplast organisms that produce oxygen through the process of photosynthesis. Their abundance and easy propagation methods allow algae to become a renewable energy source. So it is considered effective in reducing CO_2 emissions because of its ability to reduce CO_2 in the photosynthesis process. In this study, research was conducted on photobioreactors using Chlorella vulgaris algae as an O_2-producing reactor and optimizing light energy as an energy source, which used the Flat-plate type with dimensions of 40x50x60 cm with control of pH, temperature and chemical visibility factors. Variations are given by providing a supply of CO_2 in both types of photobioreactors. Then it can be seen the concentration of O_2 produced from the photobioreactor and its ability to overcome CO_2 gas emissions [3][4].

The problems studied in this study are: (1) how to design a closed photobioreactor so that optimal lighting is obtained from a given light source in the form of a 20 watt 220 Volt Halogen lamp, (2) how to determine the concentration of O_2 produced by the photobioreactor Chlorella vulgaris with CO_2 supply and without CO_2 supply, (3) how to find out the appropriate wavelength for photosynthesis Chlorella vulgaris in a closed photobioreactor, and (4) how to produce pure oxygen gas for mass development according to SNI standards.

This research aims to: (1) know the appropriate design for optimizing closed photobioreactors from a given light source, (2) know the concentration of O_2 produced by the photobioreactor Chlorella vulgaris with CO_2 supply and without CO_2 supply, (3) know the appropriate wavelength for the photosynthesis process of Chlorella vulgaris in a closed photobioreactor, and (4) produce pure oxygen gas production for mass development according to SNI standard.

To achieve the desired planning, the urgency of research is needed to facilitate the analysis in this study, which includes: (1) the use of Chlorella vulgaris as a reactor to mitigate CO_2 emissions, (2) the use of a flat-plate type as a closed photobioreactor design and a light chamber as a light source optimization, (3) providing a light source with a 20-watt 220-volt Halogen lamp with an intensity of 1000 lux, and (4) measurement of pure O_2 concentration produced for mass marketing with SNI standards.

LITERATURE REVIEW

Photobioreactor

A photobioreactor is a bioreactor that is combined with a certain light source for the intake of light energy into the reactor. Bioreactor itself is a closed system of a biological system for a biotechnological process. Bioreactors provide a stable environment for the optimization of organism growth and metabolic activity. The photobioreactor is a closed system that is easier to control and adapt to the installation location, is more able to prevent contamination, prevent evaporation of water and CO_2, and does not require a large area. With photobioreactors, high biomass productivity can be achieved and contamination is easier avoided. Several photobioreactor models have been studied, beginning in the 1950s by Davis and colleagues (1953) at the Carnegie Institution in Washington. The photobioreactor has a capacity of one litre, sixty-five percent of which is in the form of glass or plastic tubes and the rest is in the form of a settling chamber [5].

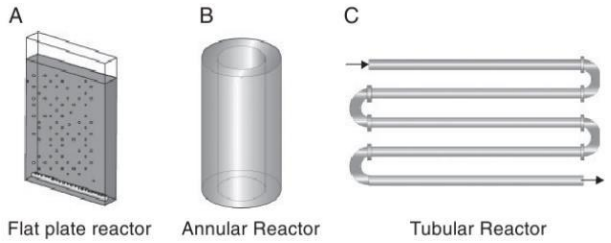


Figure 1. Photobioreactor model.

The disadvantage of using plastic tubes as photobioreactor vessels is the instability of plastic tubes against heat and sunlight. Plastic tubes are easily damaged due to photodegradation, so a circulation process is needed to cool the culture. As a result, production costs for the closed system are higher than for conventional culture methods. The culture cooling system is a very expensive process. Complete removal of this cooling system will reduce investment by up to 50%, so algae production costs will be the same as for an open system [5][6].

Research Update

Kim and Lee [7] observed that the lamp increased the productivity of microalgae biomass and overall photosynthetic efficiency. Algal growth kinetics and oxygen production rates under flashing light with various flashing frequencies (5 Hz–37 kHz) were compared with those under equivalent continuous light in photobioreactors. Flashing may be a reasonable solution to overcoming reciprocal shadows, especially in high-density algal cultures. Detrell [8] examines how water chemistry changes, what controls this variability, and where in the critical zone this occurs. For his research, he developed a new sample bottle design for water samples to maintain sample integrity for reactive elements such as Fe and Mn. Kazbar et al [9] hypothesizes that the critical point of photosynthesis occurs more frequently in cases of higher sedimentation. Abiusi et al [10] predict the potential for bioavailability in the water column and sediments and their relationship with enzymatic hydrolysis, estimate the impact of land use, anthropogenic activities on bioavailability and water quality.

Referring to the several studies above, that research is still on a laboratory scale with an increase in the effectiveness of algae culture. So that in this research it is developed to a large scale and priority is to produce pure oxygen with SNI standards with an Online Microcontroller.

Phase of Growth and Development of Algae

Delayed Phase (lag phase)

After the administration of vitamins into the culture medium, a delayed phase occurs due to the adjustment of the new environment before starting culturing (cell division). Adjustment in this case means a time when cells are deficient in metabolites and enzymes due to unfavorable conditions in previous cultures.

Logarithmic Growth Phase (log phase)

During this phase, the cells divide rapidly. The cells are in a stable state and the number of cells increases at a constant rate. New cell materials are formed at a steady rate. Nevertheless, they are catalytic and increase in mass exponentially. This depends on one of two things that happen, namely if not one or more nutrients in the hatchery are depleted, then of course the toxic metabolites will accumulate and inhibit growth.

Growth Rate Decline Phase

In this phase, the rate of cell growth decreases due to high competition in the living medium, and the nutrients available in the medium are not sufficient to meet the needs of the rapidly growing population in the exponential phase. As a result, only a portion of the population gets enough nutrients to grow and divide.

Stationary Phase

During this phase, the number of cells tends to be constant. This is caused by the depletion of nutrients in the medium or by the accumulation of toxic metabolites resulting in growth arrest. In most cases, cell turnover occurs in the stationary phase, where there is slow cell loss due to death that is offset by the formation of new cells through division. When this happens, the number of cells will increase slowly even though the number of living cells remains.

Variables Affecting Growth

Algae in the photobioreactor, in order to obtain high biomass, the selection of the type of photobioreactor is an important thing to do. Microalgae are usually cultivated in open (open photobioreactors) and closed (closed photobioreactors) systems by being illuminated either by artificial light or by sunlight at a temperature of 270–300°C and a pH of 6.5–8.

Light

Light as an energy source for photoautotrophic life is a fundamental limiting factor in photobiotechnology. In most microalgae, photosynthesis is optimal at around 1,700–2,000 E/(m^2) radiation. In flat-plate photobioreactors, with a surface-to-volume ratio (SVR) of 20–80 m^2/m^3 and luminance up to 1.15 E/(m^2s), with a layer thickness of up to 5 mm, productivity can reach up to 2–5 g dry weight per day.

CO_2/O_2 Balance

Adjustment of the CO_2/O_2 balance at high photosynthetic rates occurs by using CO_2 in the reaction with the main enzyme RUBISCO for carboxylation in the Calvin cycle, instead of using O_2 for respiration (catabolism in the light; photorespiration). Most species of Chlorella can adapt to conditions of CO_2 content in the air up to 12%. By using the perfect gas equation, the weight of CO_2 gas introduced into the culture can be calculated by converting the volume into CO_2 weight. The calculation is according to the formula:

$$pV = nRT \quad .... (1)$$

with P = pressure (1 atm); V = volume of gas intake (litre); n = number of gas molecules (molecular weight CO_2 [g/mol]); R = gas constant (0.082 L.atm/ K.mol); T = temperature (°K) [11][12].

Flow Hydrodynamics in a Bubble Column Photobioreactor

Hydrodynamics (mixing characteristics) is a function of reactor geometry and operating conditions (gas and liquid flow rates). Hydrodynamics affects photosynthetic efficiency, productivity, and cell composition. Several hydrodynamic parameters that are measured in a liquid include volume, shape, the velocity of bubbles, gas hold-up, liquid velocity, slip velocity, axial dispersion, Reynolds number, mixing time, and mass transfer coefficient. Stirring is also related to viscosity, both at low and high viscosity. Viscosity will affect the formation of bubble diameter, while the area of air bubbles is influenced by airflow velocity and bubble diameter.

$V_h=3/4\ r^3\ n_b$ .... (2)

with r = radius of air bubble (cm) dan n_b = number of bubbles per minute. Viscosity is highly dependent and influenced by temperature, concentration, and shear stress which affects fluid motion [13][14][15][16].

METHOD

Photobioreactor Design

The design of this photobioreactor consists of two glass pools in the form of a flat plate with the same dimensions with an effective volume of 2,500 ml of media. This reactor belongs to the type of flat plate Closed-photobioreactor where this design is relatively inexpensive and easier to clean besides the distribution of light as an energy source can be more evenly distributed. The use of glass as a reactor-making material is because glass is able to absorb visible light wavelengths in the range of 400–750 nm where at that wavelength microalgae can live and reproduce well. Before selecting the lamp used for the photobioreactor system, the sample of Chlorella vulgaris was tested for absorbance using a UV-Vis spectrometer. From the results obtained, it is concluded that Chlorella vulgaris absorbs almost all visible wavelengths as well as in the infrared region so halogen lamps are used as system lighting.

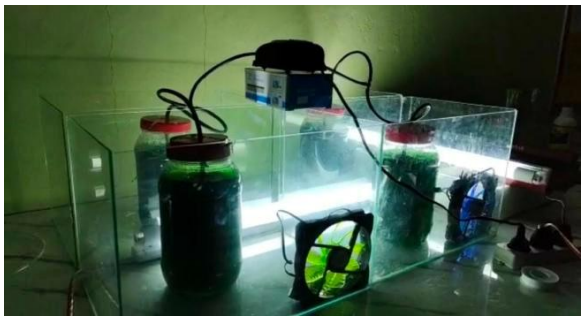


Figure 2. Reactor design.

Based on Figure 2, sanitized water has then given nutrition and aeration for 30 minutes after being given Chlorella vulgaris culture. Giving Chlorella vulgaris culture gives as much 250 ml into the culture medium as much as 2,250 ml so that the effective volume of the medium becomes 2,500 ml. The pH of this media was measured with current indicator paper at the beginning of the process and the end of the photobioreactor process. Then operating conditions of the photobioreactor are measured and maintained the temperature ranges from 26–30°C to the tube thermometer.

Photobioreactor Research Design Flowchart

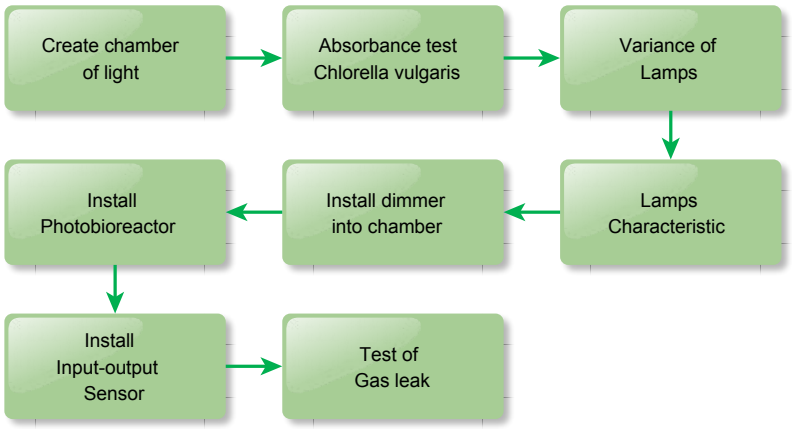


Figure 3. Reactor process.

Culture Media Flowchart

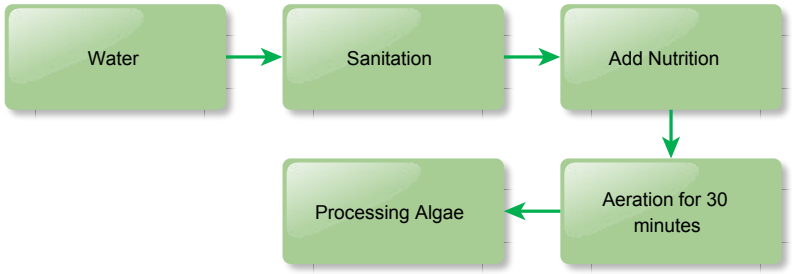


Figure 4. Reactor process.

Photobioreactor Operation

In this study, CO_2 is given periodically by measuring using a flowmeter attached to the CO_2 regulator. Then CO_2 gas is injected into the batch scale to determine the concentration of O_2 produced by microalgae photosynthesis during a phytoplankton life cycle of about 12 days. Photobioreactor with a capacity of 3 litres filled with 2,500 ml of culture media. CO_2 gas is flowed into the reactor in a closed system from the bottom of the reactor using fine porous distributor water (aeration stone). Before the process on the photobioreactor takes place, a gas leak test from the reactor output is carried out using an air pump that is inserted into the reactor, and then the output hose connected to the reactor is inserted into the distilled water and seen whether the output is the same as the output in the reactor. If the resulting output is the same as the air pump output, the reactor is ready for use.

The volume of CO_2 gas injected was 15% and was measured continuously during the experiment with a flowmeter. The lighting is carried out in 9 hours intervals from 08.00 to 17.00. With an Intensity of 1,000 lux measured with a lux meter and set with a dimmer. The pH value was measured using pH indicator paper at the beginning of the process and the end of the photobioreactor process. Meanwhile, the operational temperature of the photobioreactor is monitored daily using a tube thermometer.

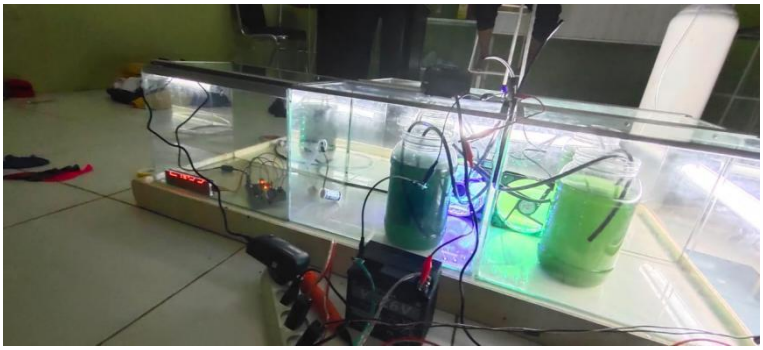


Figure 5. Photobioreactor operation.

Green Algae Cell Density Test

Algae cell density testing using a haemocytometer, was carried out at the Physics Laboratory of the Nahdlatul Ulama Institute of Technology and Science (ITSNU) Pasuruan, within Figure 6, stated as the growth of algae in the presence of particles can be assessed by quantifying algal biomass, usually by measuring chlorophyll fluorescence in vitro or in vivo. However, the particles may interfere with in vivo measurement and therefore an in vitro method is often preferred. For in vitro analysis, chlorophyll is extracted using agents such as acetone or ethanol. In some of these approaches, cells and particles are separated by filtration or flocculation and sedimentation. Fluorescence can be determined rapidly if aliquots of the chlorophyll-containing samples are transferred to microplates and measured using a microplate reader. For the in vivo

method, representative samples of the test dispersion containing algae and particles are transferred directly to microplates.

We tested inorganic and organic particles (including alloys and polymers), ion-releasing and non-releasing materials, and particle sizes in the nanometer to micrometer range with a variety of shapes (spherical, flaky, and fibrous). Some of the materials were nontoxic, whereas others showed varying degrees of toxicity (ErC50 = 0.2–100 mg/L in both methods). There were only minor differences between the methods in ErC50 values and the percent inhibition at various test concentrations, but the confidence intervals for the ErC50 values in vivo were narrower and were covered by the range observed in vitro.

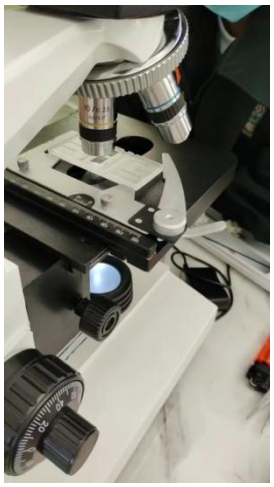


Figure 6. Density test using Haemocytometer

Overall Hardware Design

A schematic design of the overall physical form of the tool. The general design consists of a KE50 oxygen sensor, a signal conditioning circuit (non-inverting amplifier), a 12 V power supply, a microcontroller (on the Arduino Uno board), and an LCD display in Figure 7.

The sensor senses oxygen, then the oxygen detected by the sensor is converted into electrical quantities. The sensor output voltage is amplified by a non-inverting amplifier circuit, to activate the op-amp in a non-inverting amplifier circuit, a 12 V power supply is used. The output voltage from the non-inverting circuit which is still an analog voltage is processed using ADC to be processed by the microcontroller. The oxygen concentration value measured by the device is displayed on the LCD.

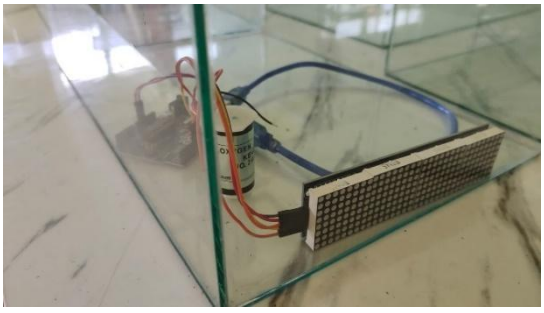


Figure 7. Sensor install design.

In Figure 7, the oxygen sensor KE-50 has a steady output voltage on the mV scale. The sensor output is still on the mV scale cause the sensor output cannot be read by the microcontroller, so we need a signal conditioning element in the form of an amplifier noninverting. A non-inverting amplifier is selected to amplify the voltage sensor output to 0–5 V so that it can be read by the microcontroller. The way the circuit works is the output of the sensor is connected directly to the op-amp's non-inverting input. Enter starting from the feet (+) KE-50 sensor goes to the (+) op-amp

and then connects to port A2 Arduino Uno. The IC used is LM324N with R1 = 1 kΩ, R2 = 200 kΩ, R3 = 10k Ω and R4 = 20kΩ. The voltage for the supply is the power supply 12V.

RESULTS AND DISCUSSION

Data Discussion I

KE50 Sensor Characterization Results

Initial testing of the KE50 sensor was carried out by looking at the comparison between the output voltage on the sensor and the oxygen concentration value on the comparator measuring instrument. The test is carried out by providing several variations of oxygen concentration from oxygen gas cylinders and from free air. The output voltage data from the sensor is taken for each variation of oxygen concentration using a digital multimeter. The increase in the KE50 oxygen sensor output voltage occurs as the percent oxygen concentration increases. This test is carried out to see the linearity between the sensor output voltages. Data characterization of sensor output voltage using oxygen concentration measuring instrument.

Table 1. Shows voltage value.

Oxygen Concentration (%)	Output Voltage of sensor (mV)
20.00	3.30
20.20	3.41
20.80	3.51
21.84	3.62
21.96	3.81

Linear sensor output with oxygen concentration value. Each increase in the value of oxygen concentration represents an increase in the value of the sensor output voltage. This shows that the sensor output voltage value is linear with the oxygen concentration.

Data Discussion II

Preparing the Sample for Hemocytometer Count

A total of 1 ml of the medium in the 500 mL flat bottom flask was drawn with a pipette containing the cells of the freshwater microalgae and was appropriately prepared before applying it to the hemocytometer. The hemocytometer was cleaned using 70% ethanol. The shoulders of the hemocytometer were moisturized and the coverslip was affixed using mild pressure and tiny circular motions. The phenomenon of Newton's rings was seen when the coverslip was correctly affixed, hence the depth of the chamber was confirmed.

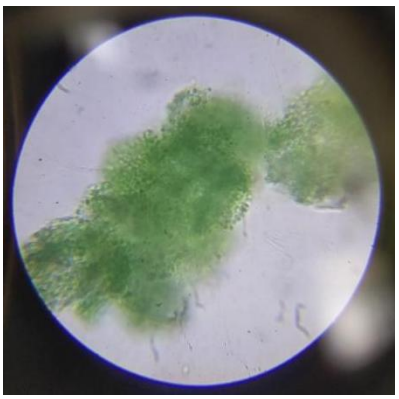


Figure 8. Cells suspension of Algae using microscope.

Based on Figure 8, free-floating plants include large true plants like duckweed that float on the surface and microscopic algae that live suspended in the water itself. These algae may exist as single cells, or in long filaments. Most of these algae are suspended in the water. A green or brownish tinge to water may be the only evidence that algae are present, but under a microscope, an amazing and diverse world is revealed. In moving water, microscopic algae are generally found attached to rocks or other substrates.

As light, nutrients, and other environmental conditions of attached microalgae were different from the suspended microalgae, the physiological properties of attached microalgae also varied from the suspended ones. Besides the relatively lower biomass accumulation rate, attached microalgae also had a lower oxygen-evolving activity (65% on average) compared to suspended microalgae. The composition of microalgae changed towards accumulating more protein when suspended microalgae turned to attached status. The relative protein content of attached microalgae (50.1 ± 10.1%) was approximately 30% higher than the suspended algae (36.0 ± 16.1%) on average. The discovery of physiological properties of attached microalgae in this paper could help the production of high-protein microalgae-related products and explain some phenomenon during the production of microalgae-related products.

CONCLUSION

From the measurement results obtained, it appears that the concentration of oxygen produced by the photobioreactor supplied with CO_2 is higher than that which is not supplied, this indicates that CO_2 optimizes the photosynthetic process in the photobioreactor. However, in the photobioreactor at 0 lux the oxygen concentration produced by the photobioreactor supplied with CO_2 is lower, this is because photosynthesis barely occurs in dark conditions so the addition of CO_2 causes a reduction in the oxygen concentration produced by the photobioreactor.

ACKNOWLEDGMENT

Many thanks to funded grants from Direktorat Riset, Penelitian dan Pengabdian Masyarakat (DRPM)-Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi (Kemendikbudristek) 2022.

REFERENCES

[1] B. Ak, E. Atak, M. D. Köse, and O. Bayraktar, "Production of Chlorella sp. in a Designed Photobioreactor," *Celal Bayar Üniversitesi Fen Bilim. Derg.*, vol. 15, no. 4, pp. 377–383, Dec. 2019, doi: 10.18466/cbayarfbe.523332.

[2] A. S. Afifah, I. W. K. Suryawan, and A. Sarwono, "Microalgae production using photo-bioreactor with intermittent aeration for municipal wastewater substrate and nutrient removal," *Commun. Sci. Technol.*, vol. 5, no. 2, pp. 107–111, Dec. 2020, doi: 10.21924/CST.5.2.2020.200.

[3] J. K. B. Bishop and T. J. Wood, "Year-round observations of carbon biomass and flux variability in the Southern Ocean," *Global Biogeochem. Cycles*, vol. 23, no. 2, p. n/a-n/a, Jun. 2009, doi: 10.1029/2008GB003206.

[4] E. S. Sofiyah, A. Sarwono, I. Yenis Septiariva, D. I. Wayan, and K. Suryawan, "The Opportunity of Developing Microalgae Cultivation Techniques in Indonesia," *Ber. Biol.*, vol. 20, no. 2, pp. 221–233, Oct. 2021, doi: 10.14203/BERITABIOLOGI.V20I2.4000.

[5] A. U. Farahdiba, O. Cahyonugroho, S. N. Nindhita, and E. N. Hidayah, "Photoinhibition of Algal Photobioreactor by Intense Light," in *Journal of Physics: Conference Series*, Jul. 2020, vol. 1569, no. 4, doi: 10.1088/1742-6596/1569/4/042095.

[6] H. Kim, J. K. B. Bishop, T. J. Wood, and I. Y. Fung, "Autonomous water sampling for long-term monitoring of trace metals in remote environments," *Environ. Sci. Technol.*, vol. 46, no. 20, pp. 11220–11226, Oct. 2012, doi: 10.1021/es3006404.

[7] N. J. Kim and C. G. Lee, "A theoretical consideration on oxygen production rate in microalgal cultures," *Biotechnol. Bioprocess Eng.*, vol. 6, no. 5, pp. 352–358, 2001, doi: 10.1007/BF02933005.
[8] G. Detrell, "Chlorella Vulgaris Photobioreactor for Oxygen and Food Production on a Moon Base—Potential and Challenges," *Front. Astron. Sp. Sci.*, vol. 8, p. 124, Jul. 2021, doi: 10.3389/FSPAS.2021.700579/BIBTEX.
[9] A. Kazbar *et al.*, "Effect of dissolved oxygen concentration on microalgal culture in photobioreactors," *Algal Res.*, vol. 39, p. 101432, May 2019, doi: 10.1016/J.ALGAL.2019.101432.
[10] F. Abiusi, R. H. Wijffels, and M. Janssen, "Doubling of Microalgae Productivity by Oxygen Balanced Mixotrophy," *ACS Sustain. Chem. Eng.*, vol. 8, no. 15, pp. 6065–6074, Apr. 2020, doi: 10.1021/ACSSUSCHEMENG.0C00990/ASSET/IMAGES/LARGE/SC0C00990_0004.jpeg.
[11] P. C. Oostlander, J. van Houcke, R. H. Wijffels, and M. J. Barbosa, "Growth and fatty acid content of Rhodomonas sp. under day:night cycles of light and temperature," *Algal Res.*, vol. 51, Oct. 2020, doi: 10.1016/j.algal.2020.102034.
[12] P. C. Oostlander, J. van Houcke, R. H. Wijffels, and M. J. Barbosa, "Optimization of Rhodomonas sp. under continuous cultivation for industrial applications in aquaculture," *Algal Res.*, vol. 47, May 2020, doi: 10.1016/j.algal.2020.101889.
[13] P. C. Oostlander, J. van Houcke, R. H. Wijffels, and M. J. Barbosa, "Microalgae production cost in aquaculture hatcheries," *Aquaculture*, vol. 525, Aug. 2020, doi: 10.1016/j.aquaculture.2020.735310.
[14] K. H. Park and C. G. Lee, "Optimization of algal photobioreactors using flashing lights," *Biotechnol. Bioprocess Eng.*, vol. 5, no. 3, pp. 186–190, 2000, doi: 10.1007/BF02936592.
[15] J. Wang, "Estimation of Phosphorus Bioavailability in the Water Column of the Bronx River, New York," *J. Environ. Prot. (Irvine,. Calif).*, vol. 03, no. 04, pp. 316–323, 2012, doi: 10.4236/JEP.2012.34040.
[16] R. Thunyaporn, I. Doh, and D. W. Lee, "Multi-volume hemacytometer," *Sci. Reports 2021 111*, vol. 11, no. 1, pp. 1–9, Jul. 2021, doi: 10.1038/s41598-021-93477-1.