Effect of LED Wavelengths and Light-Dark Cycle on Photosynthetic Production of *Chlorella Kessleri* for Algae-Based Biosensor Optimization

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Abstract. Observation of cell culture with a micro sensor system for biomedical application in measuring chemical, biological and physical parameters are widely used to evaluate cell metabolism respiration. Cellular respiration depicts the energy formation and molecules in the form of dissolved oxygen (pO2) level needed in the photosynthesis process. This paper presents the observation of pO2 level produced from the algae *Chlorella kessleri* influenced by LED light source at wavelength of 480 nm (blue) and 650 nm (red). It aims to show the relationship between photosynthesis production activities to the signals produced by each condition for biosensor optimization. Artificial light settings consist of light-dark cycle to simulate a day light period in producing chlorophyll and night period as a time of relaxation. Sample of 150 μL *Chlorella kessleri* with density 1.4·10⁶ cell/ml is immobilized in biochip-C and stimulate with two different wavelength light sources for 30 minutes period ON/OFF. The result shows that the red light has 18% pO2 level higher than blue light since the algae is sensitive to the red light and absorbed more energy to produce chlorophyll.

1. Introduction

Micro sensor system is useful for biomedical tools in measuring chemical, biological and physical parameters for evaluating cell metabolism respiration. Cancer cells formed through a process of uncontrolled proliferation such as neoplasm, which consist of benign neoplasms and malignant neoplasms [1]. Specific characteristics of tumor cells indicated in cell metabolism that changes according to the periodic increase in cancer cell density due to metabolic instability [2].

Evaluation of side effects symptoms, mechanisms and treatment of substances in living organisms as parameters of tumor cell metabolism are included in the field of toxicology observed as biosensor substances. Other applications is to evaluate the use of ingredients in medicines, additives in the food industry and chemical components for cosmetics as well as analyzing of tumor tissue growth by identification of phenotypes of extracellular anomalies and oxygen concentrations [3]. Cell response to specific multicellular depends on a number of factors including cell type, duration of specific oxygen concentrations, cell cycle, pH and concentration of nutrients [4].

Observation of the cell is more informative and efficient with a laboratory-integrated platform in the form of a lab-on-chip concept with a relative small size [5]. The substance used in this work is *Chlorella kessleri* green alga as substance analyte that produces an electrical signal. In [6], *Chlorella*


kessleri algae were used as a bio indicator of pollution in waters due to high level of sensitivity to the pollutants around environment. Algae produces pO2 because of photosynthesis mechanism which expressed in the form of biosensor basal potential represents a change in algal activity and vitality, pO2 concentration is inversely proportional to the algal vitality level and vice versa [7].

Loveless [8] defined that sunlight is polychromatic, but the absorption of chlorophyll in the process of photosynthesis requires monochromatic light with red and blue lengths [9]. Blue and red light with wavelengths of 400 nm to 450 nm and 650 nm to 750 nm absorb more chlorophyll pigments, while green light is reflected back by plants so the leaves appear green. Algae plants need blue light for vegetative growth and red lights are needed for algal generative growth such as flowering and algal germination [10]. Various types of light sources with different spectrum selected to control the process of algae photosynthesis in Photo-Bio Reactors (PBR) with light sources Cool White Fluorescent lights, Gro-Lux, Incandescent Light, halogen lamps and LEDs. The results show that the production of algae photosynthesis is most effective at LED wavelengths of 640-700 nm [11]. This artificial light has several drawbacks such as unknown wavelengths, relative large sizes, light produced are polychromatic and high heat generated implies an influence on algae. The measurement process has also been implemented using biochips-G, which connected to the Arduino Uno based biosensor module for the pO2 production using Chlorella vulgaris algae [12]. Light-dark experiments with ON/OFF phase lighting periods of 20 minutes using fluorescent lamps Osram Dulux S11W/840 were realized for the photosynthetic process of Chlorella vulgaris green algae as an indicator of air quality [13,14].

In this research, a light source from Light-Emitting Diodes (LEDs) was used that has advantages high efficiency, long service life, emitting monochromatic light, constant intensity, lower heat produced and relatively small size [14]. Chlorella kessleri is photosynthetic production was detected using biochip-C purchased from cellasys GmbH, Germany, integrated in a chip called lab-on-chip, consist of a collection of electrodes such as DO sensor, pH, impedance and temperature. Experiments with biochip-C can be done repeatedly and the electrical response is fast, sensitive, and small size.

2. Methodology

2.1. Mechanism of photosynthesis

The process of algae photosynthesis conveys in a photosynthetic rate curve against light intensity and artificial lambda-PAR lights to optimize the detection capability of the biochip.

![Figure 1. Photosynthetic response curve to light intensity](image)

The pO2 level is proportional to the increase in the intensity of light or photons received by the algae to produce color chlorophyll that performance a role in the process of photosynthesis. If the light intensity increases sharply, the rate of photosynthesis becomes constant due to damage to chlorophyll
and saturation of the enzyme catalysis that helps photosynthesis reaction [15], in accordance with figure 1.

2.2. Algae Cultivation and Measurement of dissolve oxygen using Biochip-C

Bioreceptor substance was green algae *chorella kessleri* purchased from the Sammlung Algen Göttingen (SAG) Germany, while algae nutrition sources used are Algae Culture Broth (ACB) from Sigma Aldrich 17124 500G. The ACB medium was prepared by adding 1000 ml of distilled water and 1.87 grams ACB, and stirring until homogeneous using an automatic orbital shaking incubator with a speed of 150 rpm and an interval of 30 minutes, then sterilized at 121°C using an autoclave with an interval of 15 minutes. The algae samples are then cultivated in ACB medium, stored in cultivation room illuminated with an artificial lights from fluorescence lamp TL 21W with cycle intervals of 12 hours ON and 12 hours OFF and aerated with CO₂ generator for for photosynthetic process. It takes 14 up to 22 days for harvesting the algae. Algae samples were counting using hemocytometer with XSP-12 Monocular Microscope to determine alga density prior experiment.

![Biochip-C](image)

**Figure 2.** Biochip-C for algae vitality observation [16].

| Parameter     | Diss. Oxygen |
|---------------|--------------|
| Dimension     | ~ 3 mm²      |
| Linear range  | 0 to 120 % DO |
|               | 0°C to +80°C |
| Sensitivity   | 1 nA/pDO +/- 10 % |
| Dimension     | ~ 4 mm²      |

Biochip-C has dimensions of 24 x 24 x 10 mm³, weighing 4.5 g and operating temperatures of 0°C to +80°C. Biochip-C is an integrated sensor in a lab-on-chip concept that can detect many parameters with several electrodes such as amperometric sensors to measure cell respiration (pO2), potentiometric sensors (MeOx) to measure pH changes, impedance (IDES) to measure impedance morphology (Z) and pt1000 sensor to measure temperature changes (T) simultaneously [6]. The electrodes activated in this study are amperometric sensors because algae can produce a pO2 as a result of photosynthesis. The detected signal from biochip-C is evaluated using wireless biosensor module integrated with the data transfer for the measurement of pO2. This tool consists of two parts, the transducer module which consists of a transmitter and biochip-C and the receiver module as the receiver of the measurement signal directly in graphical form. The working principle of the
amperometric sensor uses a fixed potential derived from the reduction potential of the compound by measuring the current generated due to changes in resistance to the analyte concentration. The amount of pO2 in the solution is proportional to the electrons involved, according to the oxygen reaction that occurs at the following electrodes [17].

\[
O_2 + 4e^- + 4H^+ \rightarrow 2H_2O
\]

At initial, an amount of 150 μL algae is immobilized into Biochip-C chamber using an eppendorf pipette, while the light source for stimulating the algae photosynthetic mechanism varied using two 5-mm standard LEDs type C503B-BCS/BCN/GCS/GCN with wavelengths (\(\lambda\)); blue 480 nm and red light of 650 nm, purchased from Vishay Semiconductor.

Figure 3. Setup the measurement of pO2 with a variation of defined Lambda-PAR

The measurement of biosensor with biochip-C modules are turned on for 10 minutes without algae samples, graphical responses are then displayed and recorded on a PC which used as a reference calibration shows the conditions ready for the measurement process, this aims to ensure detection is not affected by light. 150μL algae samples were immobilized in biochip-C and the measurement for duration 10 minutes the curve stationary using the eppendorf pipette and counted with a Hemocytometer. The measurement of photosynthesis process with an artificial LEDs condition for 15 minutes to reach the stationary point and continued with the LED OFF condition for 15 minutes to reach the stationary for LED wavelength blue and red. The obtained result shows the changes of pO2 represented in the basal potential against time for each condition. Since the biochip detects ions in the algae through the process of current-voltage conversion, so the potential value generated states of the pO2 in the algae.

3. Result and Discussion
Evaluation of the performance of the biosensor module in detecting pO2 produced from the algae with LEDs as an artificial light aims to show the relationship between photosynthetic production activity and the signal produced by each condition, as shown in figure 4.
Figure 4. Measurement of pO2 curve with ON/OFF cycle

Artificial LED sources produce chlorophyll for light period and dark cycle for the time of rest. Under illumination, biosensor response is characterized by potential decrease, which represents an increase for pO2 produced in the photosynthesis process. An increase in potential value occurs when the LED in dark condition which states the result of respiration which consumes oxygen and produces CO2 so that the amount of pO2 decreases.

Figure 5. Result of pO2 measurement depicted in basal potential with blue and red LED source. Alga density is $1.4 \times 10^6$ cell/mL in 30 minutes/cycle and pH 7.

The results show, that the biochip condition without light produces an average output potential value of 1968 mV as illustrated in Figure 5. Signal fluctuation occurs in the measurements are due to noise in the biosensor circuit. The basal potential dropped to the 1932 mV from the initial value due to interaction between immobilized algae and sensor electrode. As the LED ON, the algae absorbs photon energy from the artificial light and uses for photosynthesis mechanism, cell metabolism produces oxygen molecules in the medium and detected by the pO2 sensor. The dissolved oxygen is proportional inversely to the basal potential detected by biosensor, and to the algae population. Red
light source results a higher potential value than the blue LED for 18% than blue light, since the algae more sensitive and absorb more energy to produce chlorophyll. Decreasing of basal potential depicts more oxygen molecules in the medium and the results are agree after [10, 14].

Table 2. Maximum and minimum algal potential yield

| λ (nm) | LED | Rate potential (mV) |
|--------|-----|---------------------|
| Biru 480 | ON | 1845                |
| Biru 480 | OFF | 1892                |
| Merah 650 | ON | 1808                |
| Merah 650 | OFF | 1866                |

From figure 6, the potential value does not return to the initial potential as the light OFF for blue and red light, blue light has potential ΔU₁ for 40 mV and red light ΔU₂ for 60 mV. This is due to slowly algae response from ON/OFF state and changing the wavelength of light sources implies delay of remains photosynthesis process, so that the algae still in the photosynthesis mechanism.

4. Conclusion
The wavelength light source influences the algae vitality in producing dissolved oxygen by cell metabolism. Results show the algae photosynthesis process is more efficient using red light of 650 nm than blue light of 480 nm for 18%, since the algae absorb more photon energy from the red light. Algae responses at difference of LED wavelength show the basal potentials for every light sources slightly lower than initial potential value, blue light for 87 mV and red light for 124 mV. This is due photon energy is remain in the chlorophyll algae cell to produce oxygen molecules.

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