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Influence of Artificial Light Color on Cellular Respiration of Green Algae Photosynthesis Activity

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Abstract. Photosynthesis is the process by which plants use the light to produce glucose from carbon dioxide and water and then converted into pyruvate which releases adenosine triphosphate (ATP) by cellular respiration. This paper presents a biosensor using *chlorella kessleri* green algae as living transducer and used for cellular respiration detection in the form of producing dissolved oxygen (DO) in water. Green algae by 150μL with colony of $2.26 \cdot 10^6$ cells/ml is immobilized into biochip chamber purchased from cellasys GmbH and supplied with 50μL Algae Culture Broth (ACB). Above the biochip, an artificial light replacing natural light is mounted to stimulate the photosynthesis mechanism of the algae, which is controlled using a computer. The influence of artificial light color for the DO production was measured using artificial light sources from LED Grow Light (GL), fluorescent tube lamp (TL) 21 watt and red or blue filtered TL lamp. The results show that the TL lamp light produces the highest DO level with an average potential of 1657.64 mV and a red filtered light with a value of 1728.77 mV. The results indicate that the TL light spectrum affects algae cell metabolism better than filtered TL light since it consists of various wavelengths needed by algae cells.

INTRODUCTION

Dissolved oxygen is the number of oxygen molecules dissolved in oxygen water and arises from photosynthesis by chlorophyll organisms, the process of diffusion from free air into water and mechanical aeration. The amount of oxygen in water under normal conditions reaches 12mg/L [1]. This parameter can be used as an indicator to determine the quality of water by observing the amount of dissolved oxygen. The increasing amount of DO shows that the water quality is in a good condition and vice versa. As the DO level decreases, the organic substances produce foul-smelling gas which harmful to other organisms.

The measurement of dissolved oxygen (DO) is previously carried out using the principle of idometry titration using Manganese (II) chloride ($MnCl_2$) and Azide (NaOH-KI) solution [2]. Dissolved oxygen levels were titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$). The advantage of this method is that it has an accurate determination of dissolved oxygen level, but the process of titration is complicated and must pay attention to some iodine evaporation and air oxidation.

A bioindicator is used as an analyte to determine the DO level in water by observing the activity of algae life. Algae are eukaryotic protists that can grow if exposed to sunlight or autotrophically by synthesizing or providing their own food with the help of solar energy and chlorophyll. The cell multiplication process can be stimulated using artificial light sources as an energy source for photosynthesis [3].

The amount of DO produced is determined by the light needed by plants to photosynthesize with short wavelengths, red light (650-750 nm) and blue light (400-450 nm) [4]. Light-emitting diodes (LEDs) are widely used as artificial light sources for photosynthesis due to constant intensity, low heat generation and relatively high efficiency [5-8]. The

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productivity of cultivated *Chlamydomonas Reinhardtii* observed in various light colors [9] while [10] and [11] used LEDs for variations in wavelengths, intensity and exposure time to increase lipid production from microalgae.

In this work, an amperometric DO biosensor integrated into a single mini-sized chip called lab-on-chip is used to measure the dissolved oxygen level production from the algae activity, which has three electrodes. They are working electrode (WE), reference electrode (RE) and an auxiliary electrode (AE) [12]. The biosensor measures biological reactions by generating signals proportional to the concentration of an analyte in the reaction. For instance, pollution is an 'analyte' in a biosensor and the green algae is the bio indicator that specifically recognizes the analyte. The vitality of the algae to produce DO depends on its environment, light for photosynthesis mechanism and also nutrient. An electronic module consists of electronic circuitry that performs signal conditioning is used to evaluate the DO as basal potential [13]. Influence of artificial light color on algae photosynthesize mechanism was measured using light sources from Grow Light Lamp 6 watt (purchased from MW-Hydro), Fluorescent Tube Lamp (purchased from Osram T521W Cool White) and blue or red light color obtained using polypropylene plastic filter sheet. Level of DO produced from the algae photosynthesis is then represented by the basal potential of the sensor.

METHOD

Biochip-C and Algae Cultivation Preparation

The biochip-C consists of four different sensors: pH, DO, temperature and impedance sensor [14]. For algae photosynthesis mechanism, the amperometric based DO sensor was applied to measure the soluble oxygen. Fig. 1 depicts the biochip-C with specification as shown in Table 1.

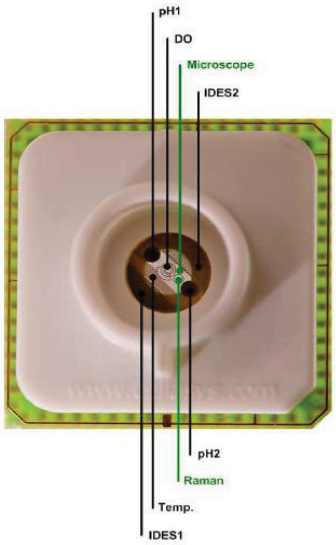

TABLE 1. Biochip C specification

Parameter	pH	Diss. Oxygen
Dimension	~ 3 mm²	~ 3 mm²
Linear range	pH 5,0 to pH 11,0	0 to 120 %DO
Sensitivity	~ - 40 mV/pH	1 nA/pDO +/- 10 %

Parameter	Impedance	Temperature
Dimension	~ 10 mm²	~ 4 mm²
Linear range	10 Ω to 5 kΩ	0° to +80°C

FIGURE 1. Biochip-C for cell metabolism observation

Biochip C was attached in an adapter connecting the sensor terminals with a biosensor module so it can be used for process measurements of living cells directly using the wireless principle. The electronic of biosensor has two parts; remote device that is directly connected to biochip sensor with immobilized algae and receiver module connected to the computer. Measured data were acquired and saved in computer for further analysis using Sigma Plot software. Above the biochip with a distance of 10 mm, a light holder was mounted, which is used in varied light sources; GL Lamp consists of a LEDs combination with a mixture of 44 red lights (625-630nm) and 16 blue light (445-470nm), white light T521W TL (72.63 µmol photons m^{-2} s^{-1}) and also filtered red or blue light.

A strain code 211-11h of green algae species *Chlorella kessleri* obtained from the collection of Sammlung von Algenkulturen Göttingen (SAG) Germany was used as a bioindicator in this research. Alga nutrient was prepared by mixing 1,87gr Algae Culture Broth (ACB) powder (purchased from Sigma Aldrich, Code: 17124) in 1 liter of distilled water in an Erlenmeyer flask and was stirred using an incubator shaking automatic stirrer for 30 minutes at 150 rpm. The ACB medium was then sterilized using an autoclave of 15 minutes with a temperature 121ºC.

The algae seeds were cultivated in the ACB medium and placed in the algae cultivation room, while for the photosynthesis, the seeds were stimulated by an artificial daylight using a light source which its wavelength is in accordance with the amount of photosynthesis active radiation, with a 12 hours ON and 12 hours OFF lighting cycle. A CO_2 aerator produces carbon dioxide gas into alga medium. After 10 days cultivation, the population of algae was calculated using a haemocytometer observed under a microscope to determine the increase in the number of algae cells. Algae cells can be sampled in the process of measuring dissolved oxygen levels if the number of algal cells has experienced a significant increase.

Measurement Set Up

The scheme of measuring dissolved oxygen from algae can be seen in Fig. 2.

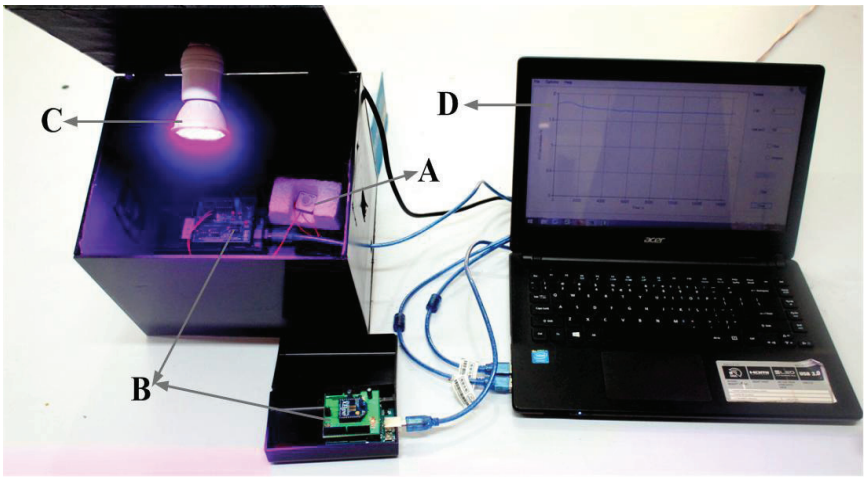

FIGURE 2. Set up of DO measurement with different light sources: A. biochip-C, B. electronic modules, C. light sources and D. computer

The initial measurement of DO level was carried out by running the electronic biosensor module connected with Biochip-C without solution for 15 minutes. This process must be done to ensure that the biochip was connected to the biosensor module. Amounts of 150µL algae with a density of 2.26×10^6 cells/ml and 50µL of ACB nutrient were then immobilized into Biochip-C gently uses an Eppendorf pipette.

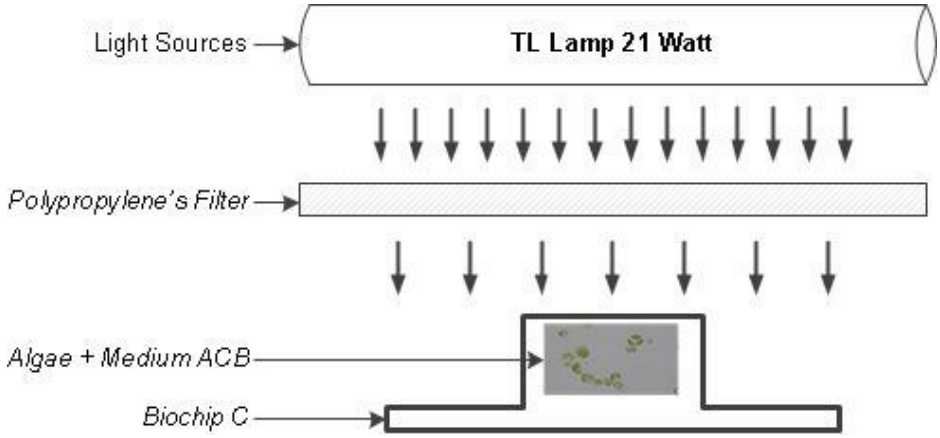

FIGURE 3. The polypropylene material sheet is used to filter the TL light spectrum for the blue and red light sources

The artificial daylight source was toggled ON/OFF to stimulate the algae photosynthesis mechanism using four different light sources: GL lamp, TL lights and red and blue lights obtained by using colored polypropylene plastic

filter with a thickness of 0.56mm. This filter was used to select the appropriate light spectrum of the TL light that can enhance the absorbance of algae chlorophyll in absorbing the spectrum of light. A black acrylic box was designed to avoid external light source affects the photosynthesis reaction inside the biochip, as shown in Fig. 3. Based on Fig. 3, influence of algae vitality to produce DO was compared in various light spectrums.

RESULT AND DISCUSSION

This experiment is aimed to determine the Biochip-C response in the presence of simulated daylight (lightly ON) and night (OFF), and also the ACB. A red LED with 650 nm wavelength was used as artificial daylight to stimulate the biochip and ON-OFF cycle was controlled using a computer. Figure 4 shows that the sensor output in A (OFF conditions) has a higher potential of 1995.15 mV, since the DO production was minimized. An amount of 150µl ACB nutrient solution was immobilized into the Biochip-C with artificial light conditions "ON". The potential decreases to a minimum and it increases to reach the saturation point at 1860.97 mV. This study has previously been carried out for biochip-G characterization that shows a potential decrease against the PBS solution [15].

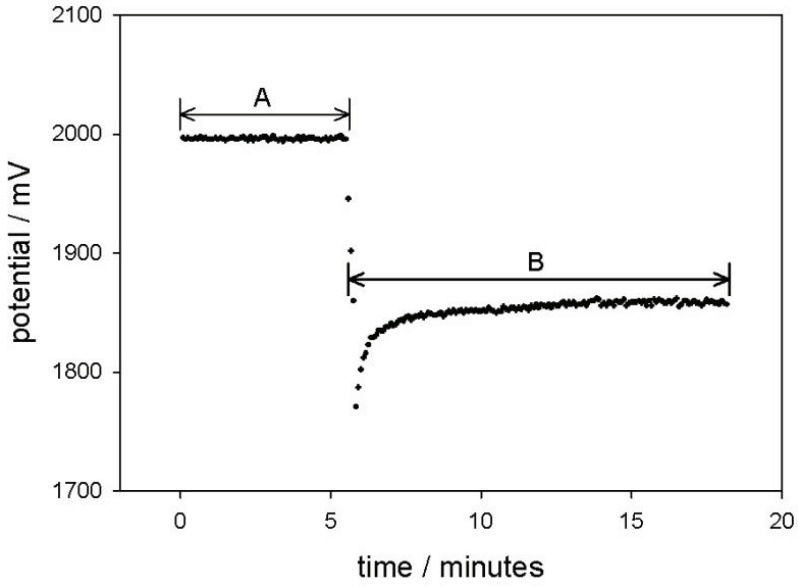

FIGURE 4. Sensor output at specified LED status, A: LED off and B: LED ON+ACB.

There was a large fluctuation in this experiment, as shown in Fig. 4. The sensor output was not affected by ACB solution nor the light spectrum, as long as the biochip is empty. Alteration of sensor output was only influenced by the presence of ACB nutrients in Biochip-C.

Figure 5 shows measurements of DO levels obtained from the photosynthesis process of *Chlorella* algae using GL LED and TL lamp. A strain code 211-11h of green algae species *Chlorella kessleri* obtained from SAG Germany was used as a bio indicator, which is immobilized in the biochip C chamber and fed with algae nutrient.

In this experiment, the green algae was immobilized into the biochip, which has given a reaction as shown in Fig. 5. For the range A, the artificial light was OFF and no photosynthesis observed. In range B, when the artificial light started to stimulate the algae, the measured potential is markedly decreased, which is characterized by an increased amount of dissolved oxygen detected by the sensor. At the initial, the potential of each light source shows a slight variance due to differences in the number of algae colonies used, since the DO level depends on the number of algae colonies. Both measured potentials were decreased in the same tendency for about 20 minutes and then split into two levels of potentials. They reached a saturation point in C, that the TL lamp affects the algae to produce more dissolved oxygen than the GL lamp with a potential of 1643.44± 1.802mV and 1699.82± 2.364mV respectively. The dissolved oxygen level is proportional inversely to the measured potential using the amperometric biosensor circuit, more oxygen molecules produced by algae, smaller the potential released by the amperometric sensor. Fluorescent tube lamp lights have more influence the algae to produce DO since the TL lamp released wide spectrum needed by the

algae for metabolism activity than the grow light LED. According to [16] large power will produce a large intensity of light as well, so that algae obtains optimum light to carry out photosynthesis.

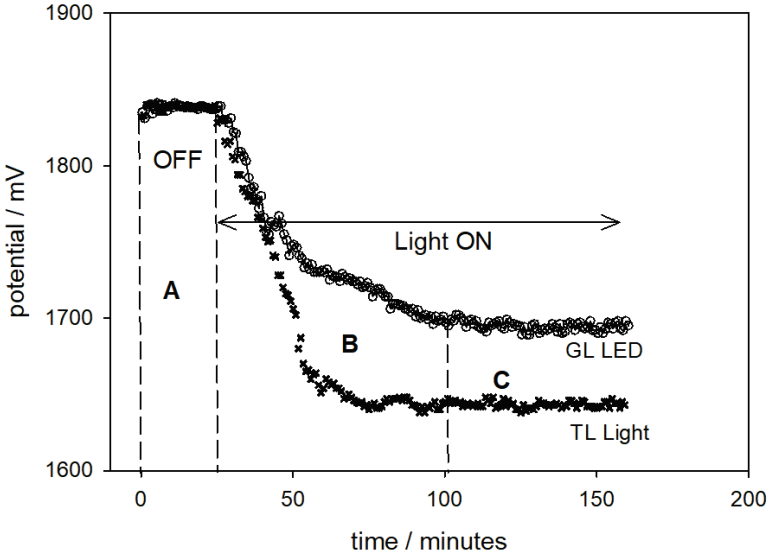

FIGURE 5. Measurement of DO level with GL and TL light sources

Based on this result, the TL lamp white spectrum was filtered to obtain the appropriate selected light spectrum using a red and blue filter, which produces red and blue light. The same procedure experiment has been made to obtain the influence of the light color on the DO production and the results can be seen in Fig. 6.

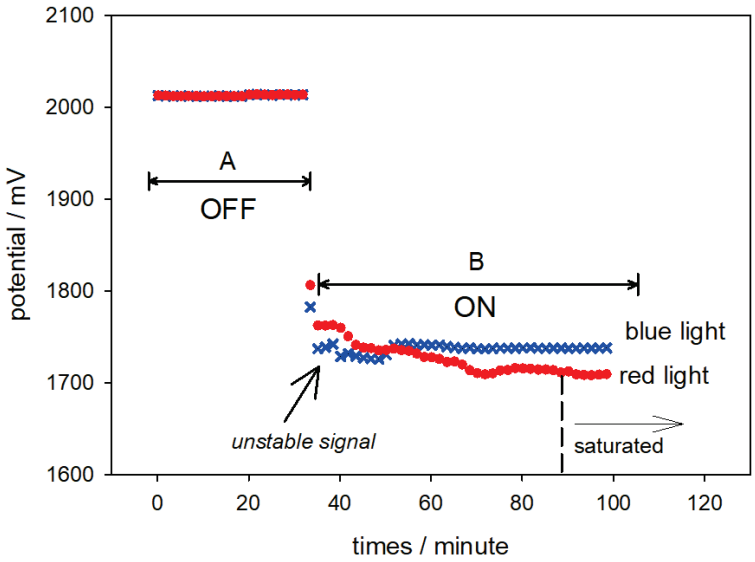

FIGURE 6. Dissolved oxygen concentration measured using a blue and red filter

Based on Fig. 6, the range a shows the algae without light, while B was measured using filtered lights. In B, there were unstable potential at the initial due to the increase of DO in the biochip chamber detected by the sensor electrodes. Moreover, the algae need time to adapt the incoming photon energy from the filtered light sources [15]. Red light produces oxygen with a potential of 1707.58 ± 1.414 mV higher than the blue light with a potential of 1737.27 ± 0.171 mV. Based on [17], the blue light is absorbed by algae in the process of reforestation, chlorophyll synthesis, and chloroplast formation, while the red light is needed by algae to process growth, to increase the cell size and

photosynthesis mechanism. From this result, it is shown that the algae can absorb more energy for photosynthesis illuminated using red light, which is more sensitive to affects the algae to produce DO. But the red light has slightly less output voltage than the TL Lamp, since the photon energy is reduced by the filter sheet.

CONCLUSION

It can be concluded that the artificial light from TL lamp could generate the DO level higher than Grow Light since the spectrum of TL lamp is wider than the Grow Light. The algae needs appropriate light sources to stimulate the photosynthesis mechanism. The filtered red light show that red light produces more DO than the blue one because red spectrum is needed by the algae for generative growth. The filtered light source produces sensor output voltages slightly below the white spectrum of TL lamp due to absorbance of polypropylene plastic filter sheet that does not allow for the light source to illuminate the algae.

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REFERENCES

1. R.G. Wetzel and G.E. Likens, *Dissolved Oxygen. Limnological Analyses*, pp. 73–84 (2013).
2. Y. Zhao, T. Ye, H. Chen, D. Huang, T. Zhou, C. He and X. Chena, *Luminescence* ***26(1),*** pp. 29–34(2011).
3. O.K. Agwa, S.N. Ibe, and G.O. Abu, *International Research Journal of Microbiology (IRJM)* ***3(9)***, pp. 288-295(2012).
4. L.A. Lewis and R. McCourt, *American Journal of Botany* ***91(10)***, pp. 1535-1556(2004).
5. W. Blanken, R.H. Wijffels, M. Cuaresma, M. Janssen, *Cultivation of microalgae on artificial light comes at a cost. Algal Res.* ***2***, pp. 333–340(2013).
6. C.Y. Wang, C.C. Fu, Y.C. Liu, *Biochemical Engineering Journal* ***37(1)***, pp. 21-25, 15 October(2007),
7. C.Y. Chen, Y.C. Chen, H.C. Huang, C.C. Huang, W.L. Lee, J.S. Chang, *Engineering strategies for enhancing the production of eicosapentaenoic acid (EPA) from an isolated microalga Nannochloropsis oceanica CY2. Bioresour. Technol.* ***147***, pp. 160–167(2013).
8. C.H. Shu, C.H. Tsai, W.H. Liao, K.Y. Chen, H.C. Huang, *J. Chem. Technol. Biotechnol* ***87***, pp. 601–607(2012).
9. T. Mooij, G. Vries, C. Latsos, R.H. Wijffels, and M. Janssen. *Algal Research* ***15***, pp. 32-42, April (2016).
10. C.H. Ra, C.H. Kang, J.H. Jung, G.T. Jeong and S.K. Kim, *Bioresource Technology* ***212***, pp. 254–261(2016).
11. C.L. Teo, M. Atta, A. Bukhari, M. Taisir, A.M. Yusuf and AniIdris, *Bioresource Technology* ***162***, pp 38-44. June (2014).
12. J. Wiest, T. Stadthagen., M. Schmidhuber, M. Brischwein, J. Ressler, U. Raeder and H. Grothe, *Analytical Letters* ***39***, pp. 1759–1771(2006).
13. L.Umar, Y. Hamzah, and R.N. Setiadi, *Measurement Science and Technology* ***30(6)***: 65102.(2019).
14. P. Staudacher, B. Wolf, J. Wiest and M. Schmidhuber. Mobile biosensor for water quality monitoring detection of oil in biotope water with a whole-cell biosensor sistem, IEEE Africon 2011 - The Falls Resort and Conference Centre, Livingstone, Zambia, 13 15 September (2011).
15. U. Lazuardi, A.A. Frank, W. Joachim, Application of algae-biosensor for environmental monitoring, 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). Milan Italia (2015).
16. A.P. Carvalho, S.O. Silva, J.M. Baptista, F.X. Malcata, *Applied Microbiology and Biotechnology* ***89***, pp. 1275-1288(2011).
17. Y. Yang, Effects of Temperature, Light Intensity and Quality, Carbon Dioxide, and Culture Medium Nutrients on Growth and Lipid Production of Ettlia oleoabundans. Dissertation. Worcester Polytechnic Institute. (2013).