IMPROVED OPTICAL PROBE FOR MEASURING PHYTOPLANKTON SUSPENSION CONCENTRATIONS BASED ON OPTICAL FLUORESENSCE AND ABSORPTION

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Abstract

This paper discusses the results of improved optical probe that works according to optical fluorescence and absorption phenomena for measuring the phytoplankton suspension concentrations. Measurements are made on the *Scenedesmus* sp. culture. The laboratory test has shown that range of concentration from 10^4 up to around 1 x 10^6 cells/mL; fluorescence intensity at $\lambda = 685$ nm; and logarithmic of transmission intensity at $\lambda = 405$ nm are proportional to the cell concentration linear with proportional constant $\gamma = 4 \times 10^{-5}$ and $\beta = -2 \times 10^{-7}$ mL/cell respectively.

Abstrak

Penyempurnaan Probe Optik untuk Mengukur Konsentrasi Suspensi Phytoplankton dengan Memanfaatkan Fluoresensi dan Absorpsi Cahaya. Pada makalah ini dilaporkan hasil rancang bangun penyempurnaan probe optik untuk mengukur konsentrasi suspensi phytoplankton. Dari hasil pengujian skala laboratorium dengan menggunakan kultur *Scenedesmus* sp. ditunjukkan bahwa untuk rentang konsentrasi dari 10^4 hingga di sekitar 1 x 10^6 sel/mL intensitas fluoresensi pada $\lambda = 685$ nm memiliki hubungan yang linier terhadap konsentrasi sel dengan konstanta proporsianl $\gamma = 4 \times 10^{-5}$ mL/sel. Sementara itu untuk rentang konsentrasi yang sama, logaritma intensitas pada $\lambda = 405$ nm berbanding lurus dengan konsentrasi sel dengan konstatnta proporsional $\beta = -2 \times 10^{-7}$ mL/sel.

Keywords: optical probe, optical fluorescence, optical absorption, phytoplankton concentration

1. Introduction

In order to obtain optimal results in fishery, various aspects need to be considered. Other than the exact physical and chemical parameter in water, food concentration dissolved in water also needs to be optimal. In fishery, natural food (phytoplankton) plays an important role. Phytoplankton is microscopic plant that drifts in water [1]. Besides functioning as fish food, this microscopic plant also protects fish from direct sunlight and produces oxygen [2]. Therefore, it is important to continuously monitor its concentration.

Several types of device configuration to measure the concentration of phytoplankton suspension have been developed [3-7]. Some of them utilize the phenomenon of optical absorption and fluorescence, while others take advantage of holography method involving sophisticated and expensive equipment. However, in Indonesia, most practitioners in fisheries and phytoplankton cultivation still measure phytoplankton concentrations manually, by relying on the ability of the eyes to observe the color and turbidity of water. These measurements are not exact since the results are strongly influenced by light intensity, the sharpness of the observer's eyes and other dissolved particles. In the culture process, phytoplankton concentration measurements are carried out by taking phytoplankton suspension samples and counted using a hemacytometer and hand counters with a microscope. Motivated by this situation, we decided to design a device to measure the phytoplankton suspension concentration based on the optical properties of phytoplankton. In this regard, several efforts have been made [8-10] by developing two laboratory-scale optical sensor configurations which utilized a combination of absorption and scattering phenomena using a laser diode 690 nm, as well as absorption, scattering and fluorescence, using a light source 690 nm laser diode and halogen lamp. Using monoculture *Chlorella* sp. as objects, the measured intensities were

shown to have a consistent linear relationship with *Chlorella* sp culture suspension. Continuing these studies, in order to simplify the design, a portable optical sensor design, which utilized the phenomenon of absorption by using a single light source, laser diode 690 nm has been developed. Using *Scenedesmus s*p. dissolved in medium bold basal (MBB) and natural water [11-12], the experiment showed that the logarithm of the measured normalized transmission intensities also has consistent linear relationship with the cell concentration. Next, for the application in fresh water, an optical probe using two light emitting diodes, 635 and 690 nm, and four detectors, has also been developed based on absorption characteristic of phytoplankton intensity [13]. This probe can detect the cell concentration from about 5×10^4 up to at around 1×10^6 cells/mL.

Meanwhile, the optical characteristics test showed that *Scenedesmus* sp. has fluorescence intensity at around 685 nm when it is excited by laser diode 405 nm. This characteristic is specific, different to other materials commonly found in aquaculture [14]. Previous studies also demonstrated that plankton has a specific optical absorbance spectrum in the wavelength range from 300 up to 800 nm with dominant peaks at around 400, 635 and 685 nm. The magnitudes of this intensity depend on cell concentration [8]. The most dominant peak is mainly caused by the content of *Chlorophyl* a [15]. Phytoplankton are mixed together with other microorganisms and detritus drifting in water. This condition affects the light absorption measurement. Thus, it needs to be considered.

In this paper, we report the improvement of previous optical sensor design [13] by developing new optical probes design to simplify and enhance the detection sensitivity. Different from previous probe, the optical probe works based on fluorescence and absorption properties of phytoplankton. The probe is designed in a more compact size, and it is easier to be operated.

2. Methods

If light passes through a medium consisting of particles, the light intensity is attenuated due to the absorption and/or scattering process. Therefore, the attenuated light can be used to measure density and concentration of particles contained in the medium [16-17]. The formulation is known as the Beer Lambert Law:

$$I_0 = I_i \exp[-NA\alpha(\lambda)x] \quad (1)$$

with I_0= light intensity of light after penetrating medium as far as x, I_i = intensity of incident light, α(λ) = absorptivity coefficient depending only on the particle, A = diameter of the beam, and N = number of particles.

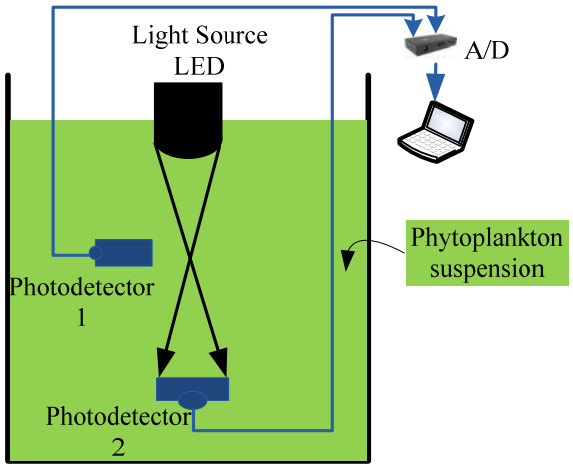

Figure 1. Experimental Set Up

Figure 1 shows the experimental set up to characterize the optical probe. Different from previous studies, the device uses a single light source and two detectors. The body is made of 2 mm black acrylic with 10 cm height, 3.5 cm wide and 2.5 cm thick. The phytoplankton suspension was placed in the black acrylic container to minimize reflections from the wall behind the container with dimensions of length, width and height, 11, 5 and 8 cm respectively. Furthermore, the optical probe was dipped into the container. As a light source, the LED 405 nm, 35 mW (HL6738MG) was chosen, while pin diodes (FDS 100) were used as detectors, respectively to detect the transmission and fluorescence intensity. Wavelength of the light source was selected based on the location of the dominant peaks in the optical absorbance characteristics of *Scenedesmus* sp. There were two detectors used: one detector was mounted in the direction perpendicular to the beam of LED light source to detect the fluorescence intensity of phytoplankton, while the other one used to measure the intensity of transmission was mounted in the direction to axis of LED light source. Fluorescence Intensity detector was coated with a red filter (Roscolux # 325) to eliminate the influence of light from the LED, while the other detector was coated with a blue violet filter (Roscolux # 368) in order to avoid the fluorescence light. The measurement of both detectors was carried out simultaneously using A/D converter PMD-1208LS, and afterwards it was processed by the computers.

In this experiment, *Scenedesmus* sp. (Subang culture suspensions isolates) was used. This plankton had been grown in Medium Bold Bassal (MBB) with 1260-3600 lux light intensity and 14-hour daily cycle of light and 10 hours of darkness. The culture room was maintained at a temperature range of 21-25 °C and air humidity 75-86%**.** The microscope observation showed that *Scenedesmus* sp. have ovalshape with a size from 8 up to 10 µm. To observe the relationship between the

intensity measured with the concentration of cells, samples with a concentration range from 10^4 up to at around 1×10^6 cells/mL were used. This range of concentrations represents the conditions commonly found in aquaculture and phytoplankton cultivation. All experiments were conducted in the Opto-Electrotechnique and Laser Applications Laboratory, Electrical Engineering Department, Faculty of Engineering, University of Indonesia.

Although there had been considerable scattering in the measuring container, the light transmission formulation was approached by Beer Lambert equation**.** By applying equation (1) for the transmitted light on the configuration of the optical probe, the following was obtained:

$$I_{Trans} = I_i \, T_l T_g^2 T_f \exp(-\alpha_{ap} x) \quad (2)$$

T_i = transmission factor of the focusing lens; T_g transmission factor of the detector glass protector; T_f coating filter transmission factor of the detector; α_{ap} = attenuation coefficient due to phytoplankton suspension (water and phytoplankton); and I_i = intensity of incident light. For constant value of I_i, T_l, T_g, T_f, α_a (attenuation coefficient due to water), and x (in this sensor was 3 cm), the equation can be rewritten into:

$$\ln I_{Trans} = - \beta \, C_p \quad (3)$$

It shows that the logarithm of the transmission intensity depended only on the plankton concentration C_p, with a proportional constant β.

For the materials with fluorescence properties, most of the absorbed light was reemitted by P_f. This emitted power was directly proportional to the energy extracted from light, and from [16-17]:

$$I_f = \varphi_f(\lambda) I_i \left[1 - e^{-NA\alpha(\lambda)x}\right] \quad (4)$$

I_f = power emitted by the phytoplankton; φ_f (λ) = constant ratio between the number of quanta emitted to the number of quanta absorbed; and I_i = incident intensity. For constant value of α_a and x and by applying Mac Laurin series on equation (4):

$$I_f = I_i \, T_g^2 \, T_f \, \varphi_f(\lambda) \, \beta C_p \quad (5)$$

Because of I_i, $\varphi_f(\lambda)$ and β were constant, as well as glass transmission factor and coating filter, and the equation can be rewritten as:

$$I_f = \gamma \, C_p \quad (6)$$

γ indicating the proportionality factor and C_p is the concentrations of plankton.

3. Results and Discussion

Figure 2 shows the design of an optical probe. The test was conducted by dipping the optical probe into a container filled with phytoplankton suspension. The fluorescence and transmission intensities were measured simultaneously, and the results represented by the output voltage over concentration range from 0 (water without plankton) up to 1×10^6 cells/mL. For each concentration, the measurements were conducted three times, and then averaged. The results are shown in Figures 3 and 4. It can be seen that for the concentration range from 0 up to 1×10^4 cells/mL, the output of photo detector was relatively similar.

For higher concentrations, i.e. up to 1×10^6 cells the output of both detectors produces a consistent linear relationship with cell concentration. It shows that the relationship between the fluorescence intensity to cell concentration was linear with a proportionality constant of $\gamma = 4 \times 10^{-5}$ mL/cell. Therefore, it is in accordance with the theoretical formulation. On the contrary, the relationship between the logarithm of the transmission intensity to the concentration of phytoplankton cells decreased with an increase in the cell concentration with the proportionality constant $\beta = -2 \times 10^{-7}$ mL/cell. Thus, it is also in accordance with the theoretical formulation.

The experimental results show that the unknown concentration suspensions, both fluorescence and transmission intensity, had a linear relationship with

Figure 2. Optical Probe and Measurement Container

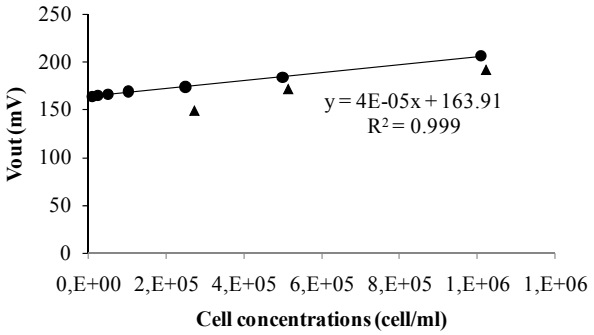

Figure 3. Fluorescence Intensities of *Scenedesmus* sp Culture Suspension, for the Concentration Range from 0 up to 10^6 cells/mL, (•) Experimental Results, (▲) Experimental Results with Unknown Cell Concentration

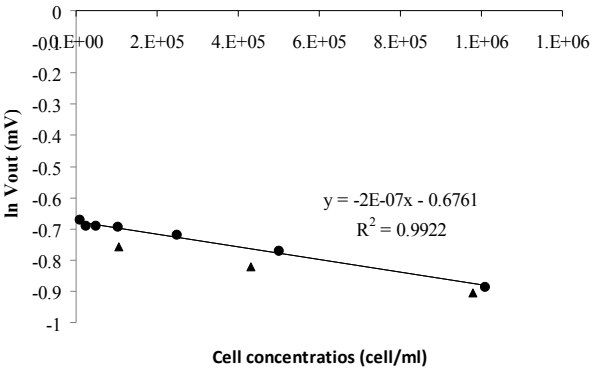

Figure 4. Transmission Intensities for the *Scenedesmus sp* Culture Concentrations, for the Concentration Range of 0 up to at Around 10^6 cells/mL, (•) Experimental Results, (▲) Experimental Results with Unknown Cell Concentration

the phytoplankton suspension concentration. This linear relationship was then used as the database. The next step was the testing of the optical probe using *Scenedesmus* sp suspension samples with unknown concentration. The procedure was similar to the filled in the measuring container, and then both of the detectors output voltages were measured simultaneously. These concentrations were also counted manually using a *hemacytometer* and hand counter with a microscope. The results are in accordance with the determination of the concentration, which is represented by the fluorescence and transmission detector output voltage with deviation at about 7% of reference value.

In the next research, the improved optical probe will be integrated to a signal processing circuits to construct a compact and low cost portable optical device. The device is designed to be able to display the phytoplankton concentration in cells/ml to ease the practitioners in the fisheries and phytoplankton cultivation.

4. Conclusions

The experiments using monoculture *Scenedesmus* sp. suspension show that the output voltage of improved optical probe, consisting 405 nm LED light source and two FDS 100 detectors, has consistent relationship with the cell concentration. Both fluorescence and transmission intensity have a linear relationship to the cell concentration. These results are in agreement with the theoretical formulation, with a proportional constants $\gamma = 4 \times 10^{-5}$ mL/cells and $\beta = -2 \times 10^{-7}$ mL/cell respectively.

It can also be concluded that the use of 405 nm LED and two pin diode FDS 100 detectors can enhance the sensitivity. The improved optical probe is able to measure the concentration phytoplankton from about 1×10^4 up to at around 10^6 cells/mL, and it is better than the previous probe. This range of concentrations is in agreement with concentration range commonly found in aquaculture and phytoplankton cultivation.

Acknowledgment

This research was supported by Directorate General of Higher Education Grants (Hibah Bersaing) under contract No. 2828/H2.R12/PPM.00 Penelitian/2011.

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