Empirical and Simulative Evaluations of White Fluorescence-type Light Emitting Diodes as Algal Growing Light Sources Based on the Photosynthetic Oxygen Evolution by Synechocystis spp. PCC6803

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MATERIALS AND METHODS

Photosynthetic bacteria

As a model photosynthetic organism, cyanobacteria would be the best organism since the photosynthetic apparatus in this organism is very similar to the one found in plants (Jarvis, 2004). Among cyanobacteria, Synechocystis sp. PCC6803 (Fig. 1B) was chosen since it is one of the most highly studied strains capable of growth under both autotrophic and heterotrophic conditions (Ohkawa et al., 2000). Cells of PCC6803 were pre-cultured in BG-11 medium (at 23 ± 1°C) under a continuous fluorescent natural-white light at ca. 3000 lx. Culture was renewed at 2-week intervals.

Prior to experiments, cells were harvested by centrifugation at 3000 rpm for 10 min, then re-suspended in fresh medium and the optical density (OD) of the cell suspension was monitored at 680 nm by a spectrophotometer UV-1800 (Shimadzu Co., Kyoto, Japan). OD was adjusted to be 1.0, at which cellular density can be prepared to be approximately at 10^6 cells/ml. The cell suspension was then kept in darkness for 30 min.

Irradiation by LEDs

Three monochromic LEDs emitting single bands of light peaking at 430 nm, 450 nm, and 660 nm; and five different WF-LEDs as the light sources for algal photosynthesis based on the evolution of O2 by Synechocystis sp. PCC6803.

Keywords: cyanobacteria, LEDs, light quality, photosynthesis

INTRODUCTION

Historically, it has been elucidated that differently colored lights affect the growth and development in plants even in the 19th century. Sachs (1874), described in his book (originally published in 1868 Germany), a series of experiments using mineral supplemented candle flames as the source of “colored” lights for plant study. From the historical point of view, one of events which can be expressed as a technological singularity in the lighting technologies is the development of light-emitting diodes (LEDs) (Kawano, 2013). More than one-fifth of US electricity is used to power artificial lighting, therefore, LEDs based on group III/nitride semiconductors are bringing about a revolution in energy-efficient lighting (Pimputkar et al., 2009).

In agriculture and algal culture, development of key technologies or protocols requires a variety of basic and interdisciplinary researches. For an instance, development of novel lighting procedure for enhanced plant production require knowledge and skills in the field of plant molecular biology and plant physiology, background of agricultural science, and access to variety of emerging technologies such as designing and handling of novel lighting devices equipped with high brightness LEDs (HB-LEDs) and programmed and integrated circuit (IC) chips. Our team is now achieving such interdisciplinary academia-industry collaboration. On the course of development of novel photosynthetic devices, we frequently faced the difficulty in evaluation of artificial lighting approaches for plant and algal growth.

In the present study, we proposed both empirical (experimental) and simulative evaluations of chlorophyll-targeting monochromic LEDs and white fluorescence-type LEDs (WF-LEDs) as the light sources for algal photosynthesis based on the evolution of O2 by Synechocystis sp. PCC6803.
tuses were provided by iTEST Co. (Osaka, Japan). Prototypic sample WF-LEDs were kindly provided by Stanley Electric Co., Ltd. (Tokyo, Japan). Spectroscopic irradiance measurements were performed by using a P200-2-UV-VIS optic fiber (200 μm diameter) and USB 4000 optic fiber spectrophotometer (Ocean Optics, Inc., FL, USA) with Spectra Suite spectrometer operating software. Light intensity was simply modulated by adjusting the distance between the light source and the oxygen electrode chamber.

Monitoring of dissolved O2 concentration in the cyanobacterium culture

For monitoring the evolution of O2 from the cyanobacterium culture, a Clerk-type oxygen electrode (Oxygraph Plus System, Hansatech Instruments Ltd., Norfolk, UK) was used with some modification of oxygen electrode chamber (Fig. 2A, C, D). The electrode chamber was covered with black cardboard with an opening (optical window; diameter, 10 mm). Instead of a plastic lid of the electrode chamber, mineral oil (0.1 ml) was layered above the cell suspension (1 ml), so that radiation from LEDs position above the oxygen electrode directly reaches the level of algal cell suspension through the optical window. For each measurement, except for dark control, the cell suspension was subjected to a dark cycle of 300 sec followed by a light period of 1800 sec.

RESULTS AND DISCUSSION

Comparison of light emitting spectra

Cyanobacterium species principally utilizes chlorophyll a as the major antenna pigment (Rätsep et al., 2000) and therefore, light emitting spectra of LEDs employed here were measured and compared with the absorption spectrum of chlorophyll a (Fig. 1). By monitoring of chlorophyll a standard solution (10 μM), absorption maxima at
430 nm and 660 nm were observed (Fig. 1A). For evaluation of photosynthetic performance of LEDs, two types of LEDs namely, monochromatic LEDs and WF-LEDs were used. Light emitting spectra of a pair of chlorophyll a-targeting blue and red LEDs were shown to be emitting single band lights peaking at 430 nm and 660 nm, respectively (Fig. 1B).

In general, light emitting spectra of WF-LEDs differed in color temperature are consisted of two distinct peak of light emission, namely, the emission maxima at blue region (reflecting the excitation light from 450 nm LED chips) and green to red regions (reflecting the fluorescence).

Working hypothesis for performance of WF-LEDs
Among prototypic sample WF-LEDs examined, 2000 K WF-LED was most promising since 660 nm light component which is most active in chlorophyll a-driven photosynthesis was highest. In addition, evaluation of blue lights, one corresponding to Soret band of chlorophyll a and one corresponding to exciting light for WF-LEDs, is also within the scope of this study.

Light intensity-dependent increase in \( O_2 \) evolution
Photosynthetic \( O_2 \) evolution was performed using the experimental set-up shown in Fig. 2. All LED samples showed intensity-dependent increase in \( O_2 \) evolution as typical records of \( O_2 \) evolution (temporal profile) under irradiation of 6500 K WF-LED light at different intensity is compared in Fig. 2E.

Comparison of different LEDs
Photosynthetic performance by different LEDs were compared after determining the rate of \( O_2 \) evolution. As the effects of three single-colored monochromatic LEDs on photosynthetic \( O_2 \) evolution were compared (Fig. 3A), chlorophyll Q-band-targeting 660 nm light was show to be most active in induction of \( O_2 \) evolution by cyanobacterial cells. In contrast, impacts of chlorophyll B (Soret)-band-targeting 430 nm light showed low photosynthetic activity (ca. 1/5 of 660 nm light) which was as low as that of 450 nm light designed for excitation of fluorescent dye equipped on WF-LEDs (Fig. 3A). In Fig. 3 (A and B), broken lines and the symbol C indicate the level of compensation at which \( O_2 \) evolution and respiratory \( O_2 \) uptake are equal, thus apparently there is no change in \( O_2 \) level. At high intensities (>3 mW/cm²), level of \( O_2 \) evolution induced by two blue LEDs became slightly higher than the level of compensation.

By a series of prototypic WF-LEDs provided by Stanley Electric Co., Ltd. (differed in color temperature), only up to 4 mW/cm² were commonly manifested thus comparable, although four out of five WF-LEDs (2400, 4000, 5000 and 6500K) were able to produce much intensive light. Among five WF-LEDs examined (at 4 mW/cm²), 2000 K WF-LED was shown to be most active as photosynthesis-driving light source (Fig. 3B), as predicted by our preliminary working hypothesis. However, we have to admit that the photosynthesis-irradiance curves (PI-curves) for WF-LEDs were still incomplete largely due to the power limitation of the prototypic WF-LED samples available at the moment.

Simulation of light response curves (PI-curves)
For comparing the photosynthetic capacities of algae and green plants, a graphical representation of the empirically studied relationship between photosynthesis performance and light intensities (historically, solar irradiance) have been developed, which is now often referred to as light response curve or photosynthesis-irradiance (PI) curve. In 1976, Jasby and Platt have evaluated several candidate equations and concluded that an adaptation of well-known Michaelis-Menten equation \( V = \frac{V_{\text{max}} \cdot [S]}{K_s + [S]} \), previously proposed for enzyme kinetics, nicely reproduces the relationship between the light intensity and photosynthesis of marine algae (Platt and Jasby, 1976), and the Michaelis-Menten model now remains the standard for generation of PI-curves (Jasby and Platt, 1976; Marra et al., 1985).

The PI curve can be applied to both terrestrial and marine photosynthesis, but it is most preferably employed to explain the photosynthetic response to the changes in light intensity \( j \) in marine algae (Platt and Jasby, 1976). Aqueous photosynthetic organisms are important suppliers to the food web in the global hydrosphere and these organisms reportedly contribute up to 50% of total global carbon.
At lower light intensities, there is a roughly linear relationship between the velocity of photosynthesis ($P$) and a given light intensity ($j$) in most algae. However, at higher $j$, the algal cells are not capable of continuing evolution of $O_2$ at higher rate proportional to the increase in $j$. Thus, the incremental rate at which $O_2$-evoluting velocity increases gradually declines as $j$ approaches the maximal level that the algae can perceive without being damaged. Such PI curves must be mathematically reproduced using a hyperbolic model. As PI curve was originally invented as a derivation of the Michaelis-Menten kinetics, the model takes similar form as following:

$$P = P_{\text{max}} \cdot j / (K_j + j)$$

In this model: $P$ is the rate of photosynthetic $O_2$ evolution, $P_{\text{max}}$ is the maximal rate of photosynthetic $O_2$ evolution, $j$ is a given light intensity, and $K_j$ is a constant, which is the light intensity at which the photosynthetic $O_2$ evolution is half of $P_{\text{max}}$.

In order to generate simulative PI curves based on limited number of empirical data (partly due to the lack of higher light intensities manifested by WF-LED samples), we decided to apply the Michaelis-Menten-inspired equation proposed by Platt and Jasby, namely, $P = P_{\text{max}} \cdot j / (K_j + j)$. For this purpose, $P_{\text{max}}$ values and $K_j$ values must be obtained through least-sized experiments.

In this study, double-reciprocal plots were employed for graphically determining the $P_{\text{max}}$ values and $K_j$ values for monochromic LED light (Fig. 4A) and WF-LED lights (Fig. 4B), by analogy to enzymatic analyses known as Lineweaver-Burk plot. The idea for applying Lineweaver-Burk plot for elucidation of the non-enzymatic biological phenomena in vivo was obtained from earlier works studying the interactions between natural auxin and synthetic (Kawano et al., 2003) or fungal auxins (Jambois et al., 2005) in living plant materials.

In fact, the equation of Platt and Jasby can be transformed as follows

$$P = (K_j + j) / P_{\text{max}}$$

$$P = K_j j / P_{\text{max}} + 1 / P_{\text{max}}$$

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**Fig. 4** Simulated photosynthesis-irradiance (PI) curves for $O_2$ evolution in cyanobacterium culture under different light qualities. By analogy to enzymatic analyses, double-reciprocal plots were employed for graphically determining the $P_{\text{max}}$ values and $K_j$ values for monochromic LED light (A) and white fluorescence type-LED lights (B). In order to generate simulative PI curves based on limited number of empirical data, the equation by Platt and Jasby, namely, $P = P_{\text{max}} \cdot j(K_j + j)$ was applied by using $P_{\text{max}}$ values and $K_j$ values obtained by double-reciprocal plots (C).

**Fig. 5** Observed and predicted photosynthetic $O_2$ evolution in cyanobacterium culture exposed to LED-based irradiance. (A) Comparison of photosynthetic $O_2$ evolution under irradiance at 4 mW/cm² performed by monochromic and WF-LEDs. Both experimentally measured and simulative values are presented. (B) Correlation between empirical and simulative evaluations for photosynthetic $O_2$ evolution under irradiance by monochromatic and WF-LEDs ($n=57$). Partial data presented in Fig. 3 (especially the data points with higher irradiance) were plotted against newly simulated values based on the equation: $P = P_{\text{max}} \cdot j / (K_j + j)$.
Assuming $P^{-1} = y$, $j^{-1} = x$

\[ y = K_j x \cdot P_{\text{max}} + 1 / P_{\text{max}} \]

Therefore, following conclusions can be withdrawn, $P_{\text{max}} = 1 / y$ (where $x = 0$) and $K_j = -1 / x$ (where $y = 0$).

These are what Lineweaver-Burk plot graphically tells us.

Eventually, simulative PI curves were generated based on $P_{\text{max}}$ values and $K_j$ values obtained by double-reciprocal plots (Fig. 4C). Then, observed and predicted photosynthetic O$_2$ evolution in cyanobacterium culture exposed to plots (Fig. 4C). Then, observed and predicted photosynthetic O$_2$ evolution in cyanobacterium culture exposed to natural irradiance were compared (Fig. 5A).

Experimental and simulative data for photosynthetic O$_2$ evolution performed under irradiance at 4 mW/cm clearly indicated that 660 nm monochromic LED was best acting as photosynthetic light sources. Among WF-LEDs, performance by 2000K WF-LED was highest, thus confirming the hypothesis.

Lastly, accuracy of simulative data was assessed by analyzing the correlation with empirical data (Fig. 5B). As high correlation coefficient ($r^2 = 0.9930$) was obtained through plotting of 57 data points, we can conclude that newly proposed simulative protocol for assessment of photosynthetic availability of artificial light sources with limited power, based on the equation: $P = P_{\text{max}} \cdot j / (K_j + j)$, is reliable.

These data further encourage us to test the availability of the simulative protocol in various photosynthetic organisms including agriculturally important crops and vegetables.

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