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Manipulation of light wavelength at appropriate growth stage to enhance biomass productivity and fatty acid methyl ester yield using *Chlorella vulgaris*

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HIGHLIGHTS

- LEDs are suitable light source of microalgal cultivations.
- Blue light LED illumination led to significantly increased cell size.
- Red light LED illumination led to small-sized cell with active divisions.
- Innovative process with wavelength shift increased biomass and FAME.

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ABSTRACT

LEDs light offer several advantages over the conventional lamps, thereby being considered as the optimal light sources for microalgal cultivation. In this study, various light-emitting diodes (LEDs) especially red and blue color with different light wavelengths were employed to explore the effects of light source on phototrophic cultivation of *Chlorella vulgaris*. Blue light illumination led to significantly increased cell size, whereas red light resulted in small-sized cell with active divisions. Based on the discovery of the effect of light wavelengths on microalgal biology, we then applied appropriate wavelength at different growth stages; blue light was illuminated first and then shifted to red light. By doing so, biomass and lipid productivity of *C. vulgaris* could be significantly increased, compared to that in the control. These results will shed light on a novel approach using LED light for microalgal biotechnology.

1. Introduction

Due to the higher price of petroleum as well as environmental concerns regarding to the steep rise of carbon dioxide as a greenhouse gas, alternative bio-fuels have been received considerable attention worldwide (Hill et al., 2006). Among the various biomass sources, microalgae are considered to be one of the most promising feedstocks for biodiesel, due to their rapid growth and high lipid content. For these reasons, the development of biodiesel originated from microalgae has become a hot topic in recent years (Chisti, 2007).

Microalga could also offer several additional advantages not only limited to microalgal biodiesel but also include a wealthy of the other benefits. Firstly, microalgae could biologically capture and fix carbon dioxide during the process of photosynthesis. Secondly, an enormous variety of compounds with high values including antioxidants and potential medications could be also produced from microalgal biomass. Finally, microalgae could utilized as environmental agents, since microalga are capable of rapid uptake of nitrogen and phosphate in the wastewater. Therefore, microalgal biomass hold a great promise for future biotechnological applications and recently it has become reality with intensive effort and investment around the world.

In order to utilize microalgal biomass for above-mentioned purposes, it must be essential to obtain sufficient microalgal biomass. Therefore, microalgal cultivation is one of the most important processes for the use of microalgae to produce any substances of

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interest, particularly for biodiesel. Moreover, it has been well accepted that the cultivation process alone claims high production cost, restricting further industrialization or commercialization using microalgae. So far, most of research for the production of biodiesel using microalgal biomass was limited in the small scale. However, in order to achieve economic feasibility, efficient mass cultivation strategy with high lipid productivity remains a key challenge to microalgal biotechnology (Uduman et al., 2010).

As with photoautotrophic organisms, microalgae carry out the photosynthesis as a main metabolism for the acquisition of organic materials by using energy from light sources such as sunlight or artificial light. Therefore, multiple lines of evidences suggested that light is the most significant factor governing the entire process of mciroalgal cultivation. Based on different approaches to obtain light source, the strategy for microalgal cultivations could be broadly categorized into open-pond or photobioreactor. While open-pond system utilize free sunlight, microalgal cultivation in the closed photobioreactor with artificial light source have the indisputable advantages in that biomass productivity could be significantly increased, particularly for value added products, compared to those in open pond system. However, again, the supply of artificial light causes the most expensive cost to operate the photobioreactor. Moreover, light energy penetrated into microalgal suspensions was significantly decreased along with increase of light path-length at high microalgal cells concentrations, which acts as a bottleneck for microalgal cultivation. Therefore, it will be crucial to developing efficient process to maximize the utilization of light energy, thereby improving economic feasibility in microalgal cultivation process.

Recently, light-emitting diodes (LED) has been emerged as a replacement of traditional artificial light source. Compared to the conventional tubular fluorescence lamps, the recent developed LEDs light make significant advances in narrowing a specific wavelength with low power consumption. Additionally, LEDs are suitable light source of photobioreactor (PBR) for indoor mass cultivation due to their small chip size and long duration (Zhao et al., 2011). Due to these advantages, now, the applications of LEDs are actively extended to the field of microalgal cultivation (Wang et al., 2007). Because LEDs could provide a particular wavelength to illuminate microalgal culture, it will be pertinent to select LEDs for the purpose of adequate manipulation of microalgal cultivation. In accordance with this notion, a number of studies have addressed that there are optimum wavelengths for each of microalgal species, though contradictory results have been obtained about the influence of specific wavelength of LED on microalgal growth. Whereas a red light was the most effective for *Botryococcus braunii* (Baba et al., 2012), a blue light led to the best biomass productivity for *Nannochloropsis* sp. (Das et al., 2011). Similar contradictory results regarding specific wavelength for microalgal cultivation were obtained previously (Baba et al., 2012; Lee and Palsson, 1994). Hence, we still have limited reports regarding LED applications into the field of microalgal cultivations.

Furthermore, although some researchers attempted to utilize LEDs for microalgal growth, there has been no research to investigate and consider biological responses of microalgal cells upon the specific wavelength of LED illuminations, including the cellular physiology or gene expressions. To date, very few studies have only focused on the installment of simple LED illuminations for microalgal growth without knowing detailed cellular mechanisms upon specific LED wavelengths. The knowledge on these will become of great interest to the microalgal bioengineers and could be directly applied into microalgal biotechnology, such as mass cultivation in the photobioreactors.

Therefore, in this study, we examined the effect of specific wavelength of LEDs on microalgal biology including growth, cell physiology, fatty acid biosynthesis, and gene expressions using *Chlorella vulgaris*. Indeed, it turned out that red and blue wavelength has a specific influence on *C. vulgaris* biology. Considering the effect of LED wavelengths in particular, we then tried to manipulate red or blue wavelength illumination at appropriate stage of microalgal cell growth. These results demonstrated that adequate manipulations of LED wavelengths during the microalgal cell growth could lead to significant increase in biomass and lipid productivity. Here, we report a novel discovery of specific influence of LED wavelengths on microalgal biology and propose the putative process based on LED wavelength shift for the enhanced production of biomass as well as lipid using *C. vulgaris*.

2. Methods

2.1. Strain and culture condition

C. vulgaris was obtained from the UTEX culture collection. Inoculums were regularly prepared using modified JM medium (Thompson et al., 1988) in 500 mL Erlenmeyer flasks at 25 ± 0.5 °C under cool-white fluorescent lights (approximately 50 μmol m^{-2} s^{-1}). For the all experiments, cells were cultivated in 1000 mL Erlenmeyer flasks containing 600 mL JM medium. The initial cell density was adjusted to be 10^6 cells mL^{-1} and temperature was maintained at 26 ± 0.5 °C. Continuous illumination was supplied at an average light intensity of 100 μmol m^{-2} s^{-1} under either monochromatic blue (λ_{max} = 430–465 nm) or red (630–665 nm) light. To ensure sufficient aeration, cultures were bubbled with sterilized-filtered air at a flow rate of 100 mL min^{-1}.

2.2. Analysis

The UV/vis spectrophotometer (T60 U, korea) was used for measuring both optical density and the level of ROS staining.

To analyze chlorophylls, cells were centrifuged, washed twice with de-ionized (DI) water, and the pellet was dried for 24 h. Then, equal amount of dried cells was re-suspended in 100% MeOH at 50 °C for 50 min. After centrifugation at 12,000*g* for 5 min, supernatant was collected and absorbance was measured at 650 and 665 nm, respectively. The content of chlorophyll a, chlorophyll b, and the total chlorophyll content (mg/L) was calculated using the following equations:

$$\text{Chlorophyll a} = 16.5 \times A665 - 8.3 \times A650$$

$$\text{Chlorophyll b} = 33.8 \times A650 - 12.5 \times A665$$

$$\text{Chlorophyll}(a+b) = 25.5 \times A650 + 4.0 \times A665$$

2.3. Growth measurements

Algal growth was measured with optical density at 660 nm with a UV/vis spectrophotometer (T60 U, korea). The number of cells was determined by direct counting with a haemocytometer (Hausser Scientific, Horsham, PA) under an OPTINITY microscope (KB-500, korea). The dry cell weight was measured by filtering the algal suspension through a pre-dried and pre-weighed, 0.45 μm cellulose nitrate membrane filter (Whatman, USA) and drying in an oven at 80 °C for 24 h. The specific growth rate (μ) was calculated based on the following equation.

$$\mu = (lnX_1 - lnX_0/t_1 - t_0)$$

where X_1 and X_0 were the biomass concentration (g L^{-1}) at time t_1 and t_0, respectively.