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Enhancement of biomass, lipids, and polyunsaturated fatty acid (PUFA) production in *Nannochloropsis oceanica* with a combination of single wavelength light emitting diodes (LEDs) and low temperature in a three-phase culture system

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ABSTRACT

A three-phase culture system combining blue (465 nm) light-emitting diode (LED) wavelength as the first phase, green (550 nm) as the second phase, and temperature stress as the third phase was applied to a *Nannochloropsis oceanica* culture in 14-L photobioreactors. Microalgal growth promotion parameters were optimized in the first phase, followed by green LED stress for lipid production in the second phase. Maximum biomass and lipid production values of 0.75 gdcw L^{-1} and 57.6% (w/w) were obtained at an aeration rate of 0.50 vvm, with a light intensity of 250 μmol m^{-2} s^{-1} and 24:0 h light/dark cycle. Culture temperatures of 15, 10 and 5 °C were applied in the third phase, where temperature stress induced the production of monounsaturated and polyunsaturated fatty acid synthesis in *N. oceanica*. The production of α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid increased by 52% (w/w), 96% (w/w), and 77% (w/w), respectively, at 5 °C in the third phase.

1. Introduction

During the past century, polyunsaturated fatty acids (PUFAs) have become well known as nutrients beneficial to human health (Chew et al., 2017). The ω-3 PUFAs, α-linolenic acid (ALA, $C_{18}H_{30}O_2$), eicosapentaenoic acid (EPA, $C_{20}H_{30}O_2$), and docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) have been reported to contribute to heart disease prevention, brain development, and eye health, and to protect against age-related diseases (Martins et al., 2013; Wen and Chen, 2003; Rajaram, 2014). The traditional source of ω-3 PUFAs is fish. However, the supply of ω-3 PUFAs produced by fish and the current global fish harvest are uncertain and potentially insufficient to supply ω-3 PUFAs for the global population (Ryckebosch et al., 2012). Marine microalgae, which are ω-3 PUFA primary producers, are therefore under consideration as an alternative source of ω-3 PUFAs. *Nannochloropsis* species are among the most promising marine microalgae in both fuel and food supplement production due to their high photosynthetic efficiency and lipid productivity (Ma et al., 2016). Although several studies have shown that *Nannochloropsis* species produce high amounts of cell biomass and lipids, the majority have been laboratory-based, with a maximum working volume of 1 L (Ma et al., 2014; Ra et al., 2016).

Photobioreactors (PBRs) use highly energy efficient light-emitting diodes (LEDs) with low energy consumption to overcome economic barriers to microalgal culture studies. However, it is uncertain whether the standard LED chip can supply sufficient light intensity to penetrate cultures with depths exceeding 20 mm (Taisir et al., 2016). To solve this light distribution problem, internal illumination sources have been applied. However, light quality measures including wavelength, light intensity, and photoperiod remain critical parameters in microalgae cultivation.

The specific light wavelengths generated by LEDs at proper light intensity and photoperiod have been shown to enhance both biomass production and lipid accumulation in various microalgae. Ra et al. (2016) reported that *Nannochloropsis* species produced a maximum cell biomass under a blue LED wavelength rather than under other single LED wavelengths, and that increased light intensity led to increase in cell biomass production. However, an excessive light intensity 150 μmol m^{-2} s^{-1} caused a reduction in *Nannochloropsis* species cell

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biomass. Similar to light intensity, Wahidin et al. (2013) demonstrated that an increased light period during the photoperiod light/dark cycle always led to an increase in microalgal cell biomass production; however, excessively long light periods induced photoinhibition. Also, there is a study mentioning that the high light intensity such as 220 μmol m^{-2} s^{-1} and continuous light period showed the positive effect in enhancement of microalgal cell biomass production (Merseck et al., 2005). Therefore, a suitable light intensity and photoperiod are required for culture light condition optimization.

Another critical parameter is aeration rate, which can improve biomass production in microalgae culture. CO_2 is the main carbon source of microalgae and is captured via aeration during culture mixing (Mirón et al., 2000).

Microalgae accumulate high lipid content under stress conditions (Minhas et al., 2016). Among environmental stress factors, the use of green LED wavelengths is a convenient method that has proven effective in driving lipid accumulation in *N. oceanica* (Ra et al., 2016). However, the effects of green wavelength stress have never been optimized or demonstrated at the PBR scale. Besides green wavelength stress, culture temperature stress also produces lipids with a high PUFA content. High cell biomass is usually obtained by increasing culture temperature (González-Fernández et al., 2012). In contrast, low culture temperature tends to produce lipids with high PUFA content. However, there have been few studies reporting the effects of low temperature on PUFA accumulation in cell bodies (Minhas et al., 2016).

In this study, *N. oceanica* was cultured in 14-L PBRs with a three-phase culture system. A correlation analysis of growth promotion factors including the aeration rate, light intensity, and photoperiod (light and dark cycle) of a blue LED wavelength (465 nm) and cell biomass production was performed for the first phase. The effects of the light intensity and photoperiod of green LED wavelength (550 nm) on lipid accumulation were explored in the second phase. Finally, the influence of different culture temperatures on ω-3 fatty acid accumulation was evaluated in the third phase.

2. Materials and methods

2.1. Algal strain and culture medium

N. oceanica was obtained from the Korea Marine Microalgae Culture Center (KMMCC, Busan, Korea), pre-cultivated for 12 days in sterilized seawater, and then enriched with modified f/2 medium containing 75 mg $NaNO_3$, 5 mg $NaH_2PO_4{\cdot}H_2O$, 4.36 mg Na_2EDTA, 3.15 mg $FeCl_3{\cdot}6H_2O$, 0.02 mg $MnCl_2{\cdot}4H_2O$, 0.02 mg $ZnSO_4{\cdot}7H_2O$, 0.01 mg $CoCl_2{\cdot}6H_2O$, 0.01 mg $CuSO_4{\cdot}5H_2O$, 0.006 mg $Na_2MoO_4{\cdot}2H_2O$, 30 mg Na_2SiO_3, 0.2 mg thiamine-HCl, 0.01 mg vitamin B12, and 0.1 mg biotin per liter (Guillard and Ryther, 1962). The medium was contained in 2-L flasks. The experiment was carried out in 14-L PBRs at a working volume of 10 L and agitation speed of 100 rpm. *N. oceanica* was inoculated at a final cell concentration of 1×10^5 cells mL^{-1}. The culture process was divided into three phases to enhance biomass production in the first phase, lipid accumulation in the second phase and PUFA synthesis in the third phase.

2.2. Photobioreactor, illumination system and cooling system

Each PBR was made of Pyrex glass with an internal diameter of 200 mm and height of 450 mm (FMT ST series; Fermentec Co. Ltd., Chungcheongbuk, South Korea) (Fig. 1). Filtered air was supplied through a ring sparger at the bottom of the tank and the aeration rate was controlled by a rotameter. The agitation system consisted of 2-disk turbine impellers and a foam breaker. To enhance the mixing ability, three baffles were set at the bottom of the PBR. Temperature sensors were set in the PBR for temperature monitoring, and LED panels (28.5 cm × 38.6 cm × 4.4 cm), each consisting of 20 LED diodes spaced at 1-cm vertical and horizontal intervals (Luxpia Co. Ltd., Suwon, Korea), were installed outside of the PBR for external illumination. Three LED sticks contained within glass tubes were set inside the PBR for internal illumination. A blue LED wavelength (465 nm) was used in the first phase and green (550 nm) in the second and third phases. Light intensity was measured in the middle of the PBR by a light sensor (HD2102.2; Delta Ohm S.R.L., Padova, Italy).

Cooling system is composed of 40 L styrofoam box, silicone tube and peristaltic pump (MasterFlex Microprocessor 7524-10; Cole-Parmer, Illinois, United states). Cold water was used as a coolant for making 15 and 10 °C culture temperature conditions using dry ice in water. Ten% (w/w) NaCl solution was used for the maintenance of 5 °C culture temperature condition. Coolant contained in styrofoam box was supplied through the bioreactor by peristaltic pump. Temperature could be adjusted by adjusting of coolant flow rate.

2.3. Experimental design

The three-phase culture system was used to enhance cell biomass production, lipid accumulation and PUFA synthesis in *N. oceanica*. In the first phase, important growth promotion parameters including the aeration rate, light intensity and photoperiod of blue LED wavelength were examined. Aeration rates were varied (0.25, 0.50, and 0.75 vessel volume per minute, vvm) to determine the optimal aeration rate for cell biomass production. *N. oceanica* was then culture under the optimal aeration rate under blue LED light (465 nm) at varying light intensity (100, 150, 200, 250, 400, and 550 μmol m^{-2} s^{-1}) to determine the optimum light intensity for *N. oceanica* cell biomass production. The effect of photoperiod on *N. oceanica* growth was then explored at varying light/dark cycles (12:12, 18:6, and 24:0 h). Once the optimal culture conditions for cell biomass production had been determined, blue LED light was replaced by green LED light (550 nm) in the second phase. The optimal light intensity and photoperiod for green LED light to enhance lipid accumulation in *N. oceanica* were then determined, at the same treatment levels used for blue LED light, over a period of 3 days. Finally, the culture temperature was varied at the same treatment levels to examine the effect of low temperature on PUFA synthesis in *N. oceanica*. Samples for the determination of microalgae growth and total lipid content were collected at the same time everyday and all experiments were performed in triplicate.

2.4. Determination of microalgal growth

Cell biomass production (dry cell weight, dcw) was determined by an ultraviolet–visible spectrophotometer (Ultrospec 6300 Pro; Biochrom Ltd., Cambridge, UK) at an optical density of 680 nm (OD_{680}). The correlation equation ($R^2 = 0.98$) for *N. oceanica* dcw at OD680 was as follows:

$$\text{Dry cell weight of } N.\ oceanica\ (\text{g dcw L}^{-1}) = 0.24(OD_{680}) \quad (1)$$

2.5. Total lipid determination

A centrifuge (Supra 22 k; Hanil Scientific Inc., Gimpo, Korea) was used for *N. oceanica* cell collection at 9946 × g for 10 min; the collected cells were then washed twice with distilled water. *N. oceanica* cells were dried in a freeze dryer (SFDSM-24L; SamWon Industry, Seoul, Korea); 5 mL distilled water was added to 10 mg dried cell biomass, and the mixture was sonicated for 10 min using a sonicator (100 W, 20 kHz, 550 Sonic Dismembrator; Fisher Scientific Inc., Pittsburgh, PA, USA). Total lipid content was determined using methanol and chloroform following a modified solvent-based method (Bligh and Dyer, 1959), as follows:

$$\text{Lipid content}(\%\ \text{of dcw}) = \frac{(W_2 - W_1) \times 100}{\text{DCW}} \quad (2)$$

where lipid content is the cellular lipid content of the microalga (% of DCW), W_1 (g) is the weight of the empty 20-mL glass tube, W_2 (g) is the weight of the lipid-extracting 20-mL glass tube, and DCW (g) is the