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Original article

The Influence of Light Reduction on the Growth of *Microcystis aeruginosa* and Variation of Environmental and Chemical Parameters in Large-scale Cultivation System

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Abstract Large-scale cultivation of *Microcystis aeruginosa* in different light conditions was conducted for verifying the cell growth in a greenhouse system. Environmental and chemical parameters of the large-scale culture medium were measured for analyzing the interaction between *M. aeruginosa* and its symbiotic bacteria. During cultivation, a difference in cell growth pattern was observed between control (natural light) and light-limited groups (reduction of blue, green, and blue/green light, respectively). Comparing the control group, the light reduced groups showed slow and delayed cell growth through the cultivation period. Also, there is differences in the consuming pattern of total nitrogen and total phosphorus which indicated that the possibility of interaction between *M. aeruginosa* and symbiotic bacteria.

Key words: light reduction, large-scale cultivation, *Microcystis aeruginosa*

INTRODUCTION

Harmful cyanobacterial blooming is a serious worldwide water pollution problem, especially in a freshwater environment, which is related to water management (Barros *et al.*, 2020), also human health (Codd *et al.*, 2020). Cyanobacterial blooming (blue-green algae) consists of surface forming genera (e.g *Anabaena*, *Aphanizomenon*, *Nodularia*, *Microcystis*), subsurface forming genera (e.g *Cylindrospermopsis*, *Oscillatoria*). *M. aeruginosa* is a representative cyanobacterium that can produce harmful cyanotoxin such as microcystin, anatoxin, and saxitoxin in freshwater circumstances (Metcalf *et al.*, 2020). The growth of *M. aeruginosa* in a natural environment is generally regulated by light intensity, pH, temperature, phosphorus, nitrogen, and other nutrients (Hozumi *et al.*, 2020; Nagao *et al.*, 2020). Although several specific conditions of temperature and chemical factors have been optimized in laboratory-scale experiments for the effective growth of *M. aeruginosa*, still unsolved questions for determining the optimal growth conditions are remained for several decades.

Natural sunlight including the ultraviolet light, infrared light, and visible light differs from laboratory artificial light which has been tested for the growth of microalgae in several studies. These studies reported that light intensity affects algal cell density, chemical components of cells, production of lipid contents and toxins (Tong *et al.*, 2011; Cheirsilp and Torpee, 2012; Wahidin *et al.*, 2013). Blue, red and white light can

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affect the growth, biomass, and lipid content of microalgae, and their influence efficiency can be differentiated depending on the kind of microalgae. The blue and green light is capable of increasing the total biomass and lipid contents of *Chlorella vulgaris*, *Nannochloropsis* sp., *Phaeodactylum* sp. and *Dunaliella* sp. (Jung *et al.*, 2019; Metsoviti *et al.*, 2020; Wei *et al.*, 2020). However, in the case of cyanobacteria, blue LED down-regulates the photosynthesis of *Synechocystis* sp. (Scott *et al.*, 2020). Energy absorption by photosynthesis organisms depends on their constructive pigments. Photosynthesis reaction centers in chloroplast absorbed various visible light, especially natural light of 600～700 wavelength (yellow to red color) is preferred for the efficiency of photosynthesis (Kommareddy and Anderson, 2003). The wavelength of 750 nm and above has an energy content difficult to mediate chemical changes, therefore radiant energy absorbed in this range only appears as thermal effects. Conversely, radiation of 380 nm and below brings only ionizing effects. Photosynthetic pigments; chlorophyll, carotenoid, phycobilins, and phycocyanin, have specific light absorption spectra that originated their constructive chemical compounds for photosynthesis (Kommareddy and Anderson, 2003). It is can say that constructive chemical compounds of photosynthesis organisms decide the preferred wavelength for the more efficient light absorbent. Most microalgae have both chlorophyll and carotenoid absorbing each suitable light wavelength for their photosynthesis and chlorophyll to carotenoid ratio influenced under abiotic (eg. temperature, light, pH and salinity, etc.) and biotic (eg. pathogen contamination and competition with other microorganisms) stress (Carvalho *et al.*, 2011). Considering with above, it is expected that the growth pattern of *M. aeruginosa* will be shown distinguishably by a different wavelength such as blue or green of natural visible light.

Interactions between cyanobacteria and its symbiotic bacteria are related to their metabolic systems. Through N_2 fixations and organic compound decomposition, bacteria can supply inorganic C, N, P, and other nutrients to cyanobacteria (Ramanan *et al.*, 2016). Also, cyanobacteria contribute as a source of organic compounds such as proteins and large molecular weight carbohydrates as well as molecular oxygen for bacterial metabolites. However, specific details of the interaction at molecular levels are unclear phenomena although previous studies showed the symbiotic interactions between bacteria and algae (Thompson *et al.*, 2013; Cooper *et al.*, 2015; Perera *et al.*, 2019). In this study, we investigated the effect of limited natural light (by blue, green, and blue/green light reduction) on cell growth of *M. aeruginosa* in large-scale cultivation as well as the changes of environmental and chemical parameters.

MATERIALS AND METHODS

1. Seed cultivation

Seed culture of *M. aeruginosa* sp. FBC-A141 was obtained from Nakddongang National Institute of Biological Resource (NNIBR, Korea) used in this study. The culture was maintained using BG-11 medium under laboratory conditions at 20ºC under 50 μmol m^{-2} s^{-1} light intensity using fluorescent light and 14/10 light-dark cycle.

2. Large-scale cultivation of *M. aeruginosa* sp. in filmed acryl tank in a greenhouse

M. aeruginosa was cultivated under 50 cm (diameter circle shape) × 1500 cm tall uncolored acryl tank in an indoor greenhouse (Fig. 1). The initial seed culture of *M. aeruginosa* was grown in a 2-L mini-column until cell density was reached to 2.0×10^7 cells mL^{-1}, and then transferred into the four different filmed tanks containing 100-L of 1/2 BG-11 medium. Using the blue, green, and mixed (blue with green) films, the acryl tank covered tightly and measured natural light intensities inside of each of the tanks 3 times for static analysis. Representative days for one sunny and one cloudy day were selected separately for natural light measurement during the cultivation period (Table 1). The algal culture was agitated with a paddle wheel system during day sampling to prevent settling and an air burble maker was set to enhance the supplement of CO_2. Microscopic analysis was carried out daily to check the purity of cell culture. From the 28th of July 2020 (set ‘0’ day), 3 times in a week, environmental parameters were analyzed and recorded for 16 days until 12th August 2020.

3. Flow cytometric analysis for live-cell density

Culture medium obtained from four testing tanks, was analyzed using Guava® easyCyte™ flow cytometer (Luminex Corporation). 200 μL of culture medium was stained using Fluorescein diacetate (FDA, Sigma-Aldrich, final con. 2 μM) for 10 min in dark. A Guava® easyCyte™ flow cytometer was used with an excitation light of the blue (488-nm) laser and the gain controls set to 1.30 (forward scatter), 1.00 (side scat-

Fig. 1. Large-scale cultivation system in this study (Control, blue, green and blue/green groups in order, from the left).

Table 1. Measured light intensity of control and light reduces groups during the cultivation periods. (unit: μmol m^{-2} s^{-1})

	Day 0∼14	Day 15
Control group	33.65 (7.2), 40.82 (10.9), 16.51 (3.9)*	64.78 (1.6), 100.92 (24.8), 23.15 (4.1)
Blue group	5.7 (1.5), 5.57 (1.9), 2.36 (0.4)	22.2 (2.9), 18.22 (2.7), 3.99 (1.0)
Green group	3.53 (1.0), 7.69 (2.0), 2.09 (0.3)	13.72 (4.5), 16.4 (1.1), 5.7 (0.3)
Blue/green group	1.2 (0.3), 1.66 (0.1), 0.66 (0.2)	1.56 (0.3), 3.16 (0.6), 0.48 (0.1)

*Measured at 9 am, 12 pm, 5 pm (Mean ± SD, n = 3).

ter), 1.54 (green fluorescence), 1.61 (yellow fluorescence), and 1.30 (red-B fluorescence). Samples of 200 μL were analyzed in 96-well flat-bottom plates (Corning Life Sciences) with automatic mixing of each well for 5 sec at high speed before sampling. For analyzing the state of cells, a cluster of *M. aeruginosa* was selected on plotting coordination (forward scatter and side scatter) concerned cyanobacterial cell size (Fig. 3(a)). Indeed, concerning the emission of chloroplast (red range), a cluster of *M. aeruginosa* was selected again in forward scatter and red fluorescence coordination (Fig. 3(b)). Selected cyanobacteria clusters are counted concerning the fluorescent intensity on plotting coordination (green fluorescent and forward scatter) and live-cell numbers are calculated automatically (Fig. 3(c)). During every sampling, flow cytometric analysis for *M. aeruginosa* cells was demonstrated with all samples and replicated at least 3 times (n = 3) for static analysis.

4. Measuring environmental parameter

Environmental parameters in the experimental system were measured during the cultivation periods. Temperature, dissolved oxygen (%), conductivity, pH, and turbidity, and salinity were recorded (see Fig. 2) at all sampling days using ProDSS Multiparameter Water Quality Meter (YSI Inc.).

5. Chemical composition analysis

For verifying the utilization of nitrogen and phosphate by microorganisms, each 2-L of culture medium which filtered with a membrane filter (pore size 3.0 μm) was used chemical composition analysis for total nitrogen (TN), total phosphorus (TP), BOD, and COD (Fig. 2). These measurements were performed by WONIL CHEMICAL & ENVIRONMENT CO., LTD (Korea).

RESULTS AND DISCUSSION

1. Environmental and chemical parameters

Environmental and chemical parameters such as temperature, pH, DO, BOD, COD, TN, and TP were changed during

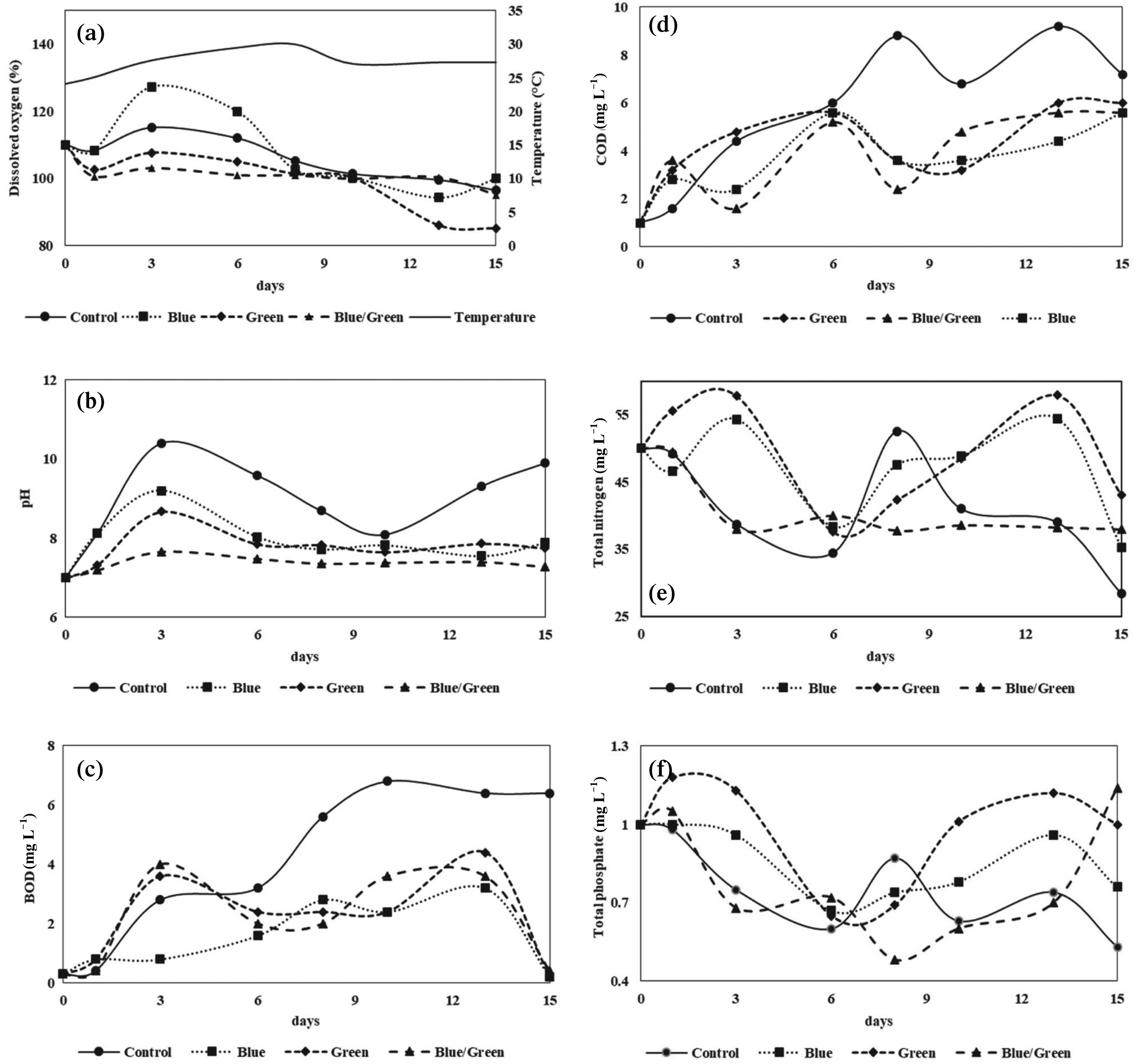

Fig. 2. Measured environmental and chemical parameters during the cultivation periods. (a) Temperature and DO, (b) pH, (c) BOD, (d) COD, (e) TN and (f) TP (not supported Mean ± SD).

the large-scale cultivation for *M. aeruginosa* with (control group) and without natural light reduction (blue group, green group, and blue/green group) (Fig. 2). The day-time temperature of cell culture increased from 24ºC to 30ºC during the six-teen cultivation days. Since for burble maker, DO value was not significantly changed during the cultivation days through the four groups.

The pH value increased after 2 days through the cultivation days in all groups (Fig. 2(b). CO_2 can present in liquid culture as chemical formations of CO_2, carboxyl acid, and methanoate anion. Cyanobacteria consumed CO_2 for the dark reaction of photosynthesis generally leads to the alkalizing of the culture medium (Axelsson, 1988). During the cultivation periods, alkalizing of the the pH value represent the cell growth of cyanobacteria is under the exponential phase. Compared with the other groups, pH value of the control group was getting higher dramatically in 3 cultivation days, and it can indicate that the growth rate and volume of the control group were

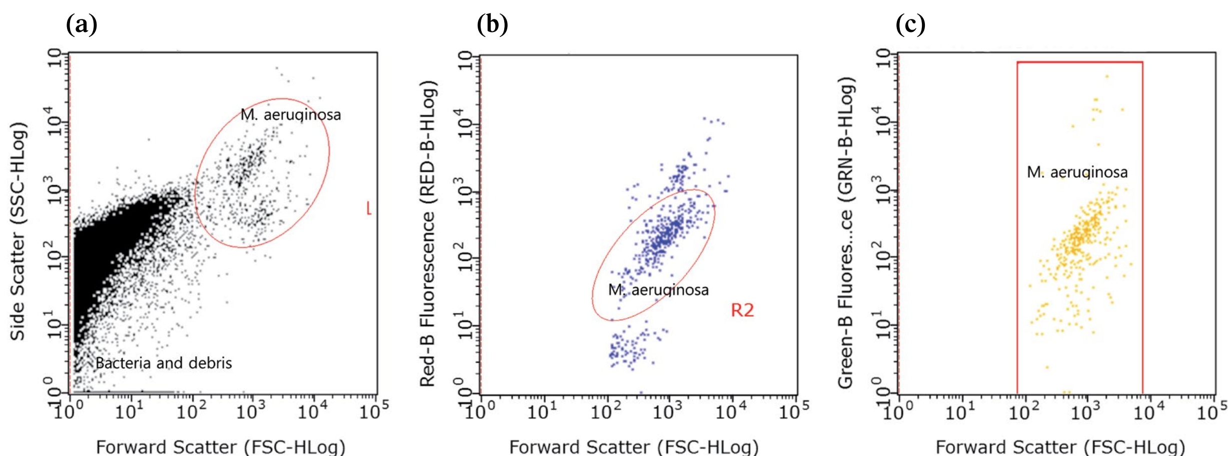

Fig. 3. Quantification for *M. aeruginosa* using Guava flow cytometry (X and Y axis are arbitrary units). (a) Forward scatter and side scatter, (b) Forward scatter and Red-B fluorescence, (c) Forward scatter and Green fluorescence.

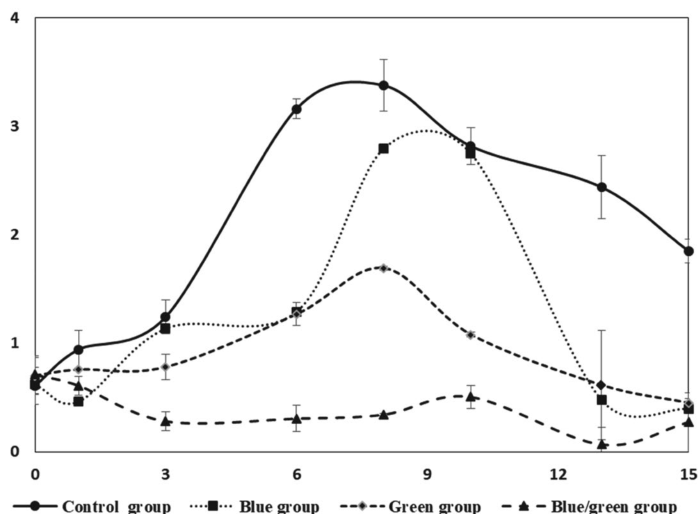

Fig. 4. Cell growth of *M. aeruginosa* in large scale cultivation for control and light reduced groups. Live cell density measured using Guava software 1.1. All sample tested three-times for statist analysis (Mean ± SD).

higher than other groups.

The increase of BOD and COD values of control group in 8 cultivation day was two-timed higher than other groups, it is possibly caused by inorganic and organic compounds produced by the bacterial and algal cellular metabolism (Kshirsagar, 2013). Consuming rate of total nitrogen and total phosphorus by the bacteria showed higher in control and blue/green groups than blue and green groups. Although the variation of the pH value was not observed in the blue/green group, the consumption rate of the TN and TP was higher in the control group. It indicated that the utilization of TN and TP by the bacteria (none cyanobacteria) present in the blue/green group and it may cause by low cell concentration of the *M. aeruginosa*. The value of TN and TP in the control group decreased in 2 to 5 days once, then recovered above TN and TP value of 0 day and decreasing again. Otherwise blue and green groups, the value of TN and TP increased first and decreased again occurring two-times. However, the blue/green group keeps decreasing through the cultivation periods, except TP value in 15 cultivation day. These results implied that natural light reduction can induce different growth rates of *M. aeruginosa* and consuming patterns in chemical compounds.

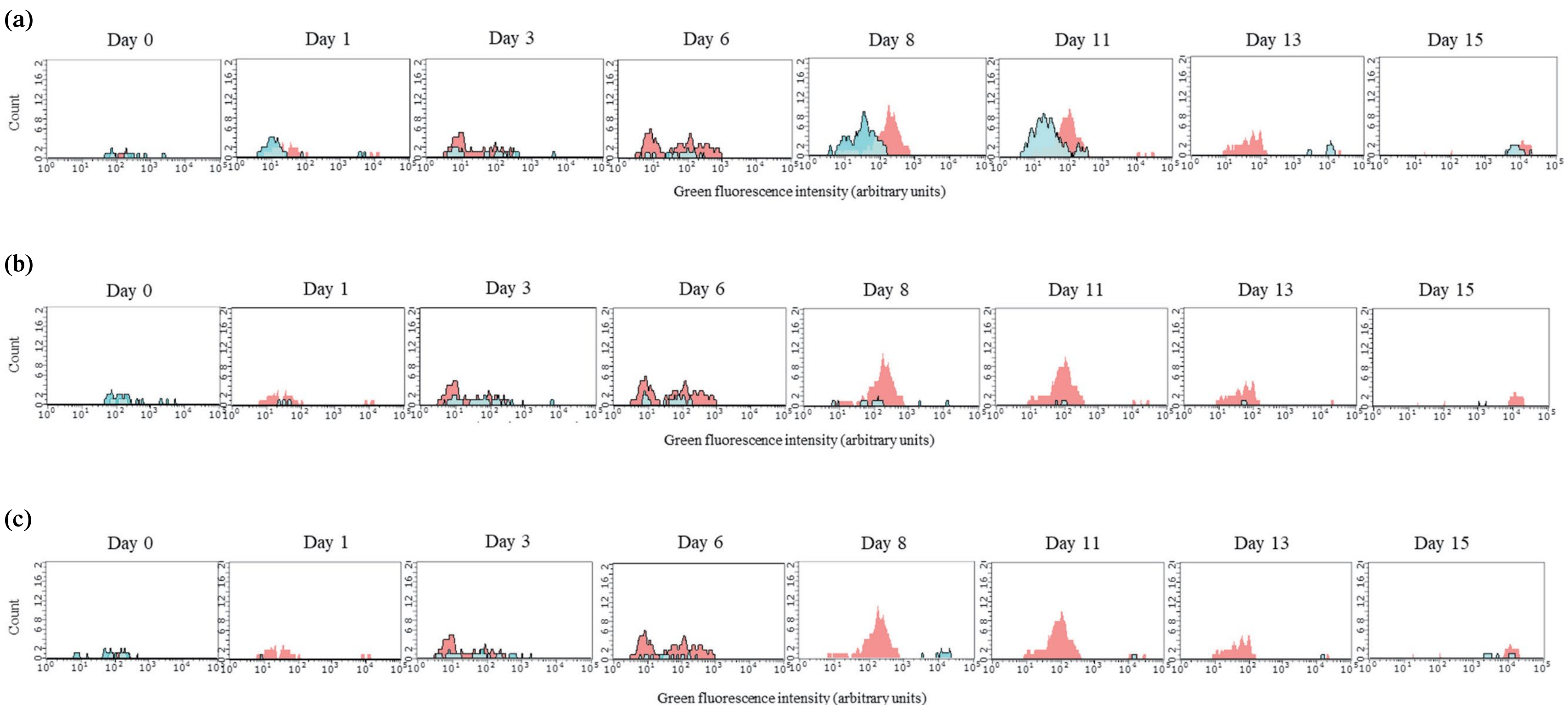

Fig. 5. Schematic diagram of growth tendency of *M. aeruginosa* during the large scale cultivation. X-axis representative intensity of green fluorescence using Guava flow cytometry. The total particle numbers are counted and showed in Y-axis during the six-teen cultivation periods. (a) Control group and blue group, (b) Control group and green group, (c) Control group and blue/green group.

2. Live cell analysis by flow cytometry

The Guava flow cytometer is a powerful statistic method with high precision and fast, automated processing for analyzing the cyanobacteria cells. Several studies implied that Guava flow cytometry is a useful method for analyzing the status of microalgae cells (Debenest *et al.*, 2010; Krediet, 2015). This method detects and counts cyanobacterial particles passing through a microcapillary tube based on their fluorescence and light scattering (www.millipore.com/easycyte). Also, this method can detect multiple ranges of light wavelength together and is capable of detecting dead and live cyanobacterial cells given their intrinsic chlorophyll fluorescence. Indeed, analyses of both size detecting and fluorescence intensity of stained cyanobacteria revealed a cluster of particles with high green fluorescence and a defined light-scattering values.

Fig. 4 showed the fluctuation of live cell concentration of control and other groups. The maximum live cell concentration of the control group is twice higher than the green group, it may affected by a difference of 5 times higher light intensities through the day times (Table 1). However, the blue group which has light intensity eight-times lower than the control group, showed almost the same live-cell concentration compare with the control group on day 10. Control and green light reduced group reached in exponential phase in day 3, while the blue group reached in 5 cultivation day. Although there is not a significant difference in light intensity between blue and green groups, the starting day of the exponential phase was delayed 2 days in the blue group. This result suggested that different types of light can effects the growth rate of cyanobacteria even though their light intensities are similar.

Fig. 5 showed the variation of the cell status during the cultivation periods by comparing the control and other three groups (Red background: control group; blue background: other groups). The status of live cells showed a low level of green fluorescence until the start of the exponential day (day 6) through the four groups. While the control group showed strong fluorescence intensity of about 10^2, other groups showed weak fluorescence intensity. Even though the blue group reached a similar volume of the live cell compares with the control group, fluorescence intensity was 10^1 on day 8. Altogether, the control group showed intact and strong cell status than light reduced groups through the cultivation periods. Considering with chemical parameters and variation of cell growth of *M. aeruginosa*, it seems that most of TN and TP consumption in the blue/green group were facilitated by the cyanobacteria-associated bacterial community, not *M. aeruginosa*.

CONCLUSION

We investigated the effect on cell growth of *M. aeruginosa* in large-scale cultivation using natural light reduction with environmental, chemical parameters. Results showed that natural light reduction reduced the cell growth of *M. aeruginosa* and seem to there is differences in the cyanobacteria-associated bacterial community. However, the specific bacterial composition which has symbiotic relations with cyanobacteria and their functional module needs to investigate for further understating of cyanobacteria blooming in the ecosystem. Although the similar light intensity, blue and green groups showed different growth patterns that indicated that there is prefer light wavelength for the cell growth. Altogether, the light reduction can regulate the *M. aeruginosa* cell growth which capable of applied to the harmful cyanobacteria blooming. For further study, molecular genetic approaches for analyzing the related gene of cell growth and photosynthesis, and visualization of cell status during the different types of light irradiation.

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Author contribution statement Conceptulation: Chang Soo Lee and Eui-jin Kim Field survey: Taehui Yang, Ja-young Cho, Ha-jin Kang, Data analysis: Taehui Yang, Manuscript writing: Taehui Yang

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