The Inhibition Efficiency of Blue Light And Light Intensity On The Growth Rate of *Microcystis Aeruginosa*

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Abstract

Lake eutrophication is associated with the occurrence of cyanobacterial blooms which have a negative effect on other organisms. Several studies demonstrated that blue LED irradiation inhibits the growth rate of cyanobacteria *Microcystis aeruginosa,* while the efficiency varies from study to study. In this paper, the focus was on the effects of light intensity on the growth of *M. aeruginosa* because the light intensity used in the precious studies varied from 12 to 45 μmol photons m^{-2} s^{-1}. Growth experiment of *M. aeruginosa* was conducted with 32 μmol photons m^{-2} s^{-1} blue light and fluorescent light, and the results were compared with the findings of previous reports. Furthermore, co-culture of *M. aeruginosa* and diatom *Nitzschia palea* was also prepared. The growth rate of *M. aeruginosa* was 0.33 day^{-1} and 0.11 day^{-1} under fluorescent light and blue light, respectively. The blue light dropped the growth rate by 67%. Compared with previous studies, the inhibition efficiency seemed to be the best at 20 μmol photons m^{-2} s^{-1}. The growth rate of *N. palea* was 0.62 day^{-1} and 0.36 day^{-1} under fluorescent light and blue light, respectively. Since the efficiency of *N. palea* by blue light (42%) was smaller than that of *M. aeruginosa,* blue light is considered to be a countermeasure to cyanobacterial blooms.

Introduction

Lake eutrophication is one of the most serious environmental problems around the world (Bhagowati & Ahamad 2019) and is associated with the occurrence of water blooms (O'neil et al. 2012). The increase of atmospheric CO_2 gas concentration promotes the growth of cyanobacteria *Microcystis aeruginosa* (Ma et al. 2019). Consequently, the occurrence of water blooms will increase around the world due to global warming.

Water blooms have a negative impact on various organisms. For instance, fish are killed because of oxygen depletion caused by cyanobacterial respiration during night time and the clogging of their gills (Jewel et al. 2003). Other examples include the inhibition of photosynthesis of aquatic plants due to shading (Casanova et al. 1999) and loss of algal biodiversity owing to the dominance of *Microcystis* (Magrann et al. 2012). Furthermore, they can produce odorous substances such as 2-Methylisoborneol (MIB) and geosmin, and harmful compounds known as microcystin and anatoxin (Izaguirre et al. 1982; Watanabe et al. 1988; Edwards et al. 1992).

The growth of cyanobacteria is related to environmental conditions such as pH, temperature and nutrients. Moreover, the interspecific interactions are key to whether cyanobacteria become the predominant species in aquatic ecosystems. For instance, *M. aeruginosa* suppressed the growth of green algae *Scenedesmus obliquus* at pH 7–9, whereas the growth of *M. aeruginosa* declined in co-culture with *S. obliquus* at pH ≦ 6 compared with a monoculture (Yang et al. 2018). Zhang et al. (2013) reported that green algae *Quadrigula chodatii* has a strong inhibitory effect on the growth of *M. aeruginosa* above 25°C. Amano and Machida (2013) reported that the co-culture with the three species at PO_4-P = 0.5 mgL^{-1} led to the predominance of *M. aeruginosa*, while *Scenedesmus quadricauda* completely dominated at PO_4-P = 0.1 mgL^{-1}. Benthic diatom *Nitzschia palea* had better growth performance than *M. aeruginosa* in high nitrogen concentration medium, while *N. palea* did not to exert inhibition effects in lake water which had less nutrients than the growth medium (Watanabe et al. 2019). Thus, algal species competing with *M. aeruginosa* do not always inhibit the

growth of *M. aeruginosa*. In order for green algae and diatom to exert inhibition effects on the growth of *M. aeruginosa*, it is effective to artificially provide a good environment, in which they have a competitive advantage over *M. aeruginosa*.

Irradiation of blue light is known to promote the growth inhibition effects on cyanobacteria such as *M. aeruginosa*. Wyman and Fay (1986) reported that the growth rate of seven strains of cyanobacteria depends on the wavelength of light. The growth rate of *M. aeruginosa* under blue light (0.072 day^{-1}) was lower than that under fluorescent light (0.135 day^{-1}), meaning that the growth rate decreased by about 47% by changing the irradiation light colour. Tan et al. (2020) reported that the growth rate of *M. aeruginosa* decreased by about 24% by changing the light colour from fluorescent light to blue light. Watanabe et al. (2019) reported that the growth rate under fluorescent light and blue light was 0.27 day^{-1} and 0.00 day^{-1}, respectively. Although these studies demonstrated the inhibition effects of blue light on the growth rate of cyanobacteria, the inhibition efficiency varied from study to study. It is important for the application of the blue light in field conditions to identify the factor which affected inhibition efficiency of blue light on cyanobacterial growth.

In this paper, we focused on the effects of light intensity on cyanobacterial growth because the light intensity used in the previous studies varied from 12 to 45 µmol photons m^{-2} s^{-1}. We conducted the growth experiment of *M. aeruginosa* with 32 µmol photons m^{-2} s^{-1} blue light and fluorescent light, and compared the results with the previous reports. Furthermore, in preparation for the co-culture with *M. aeruginosa*, *N. palea* was also cultivated.

Materials And Methods

Algae cultivation

M. aeruginosa NIES-102 was obtained from The National Institute for Environmental Studies (NIES), Ibaraki, Japan. *N. palea* was isolated from Fujinohira dam, Saga Prefecture, Japan in 2015 where water blooms were present. *N. palea* was identified through an electron microscopic observation. Both species were cultivated in a WC medium (Guillard & Lorenzen 1972) at pH 8.0 autoclaved at 121℃ for 30 min, which was also used in all experiments. The incubates were performed at 25℃, 32 µmol photons m^{-2} s^{-1}, light-dark cycle of 12:12, and shaken once daily.

The effects of blue light irradiation on the monoculture of M. aeruginosa and N. palea

M. aeruginosa and *N. palea* were inoculated individually in 150 mL medium in a 300-mL Erlenmeyer flask, with an initial cell density of 4,000 cells mL^{-1} for each species. Algae were irradiated with a 3W blue LED (KASHINOKI SOGYO Co. Ltd., Tokyo, Japan) and fluorescent light (as the control) at 32 µmol photons m^{-2} s^{-1}, which is was not specifically assessed in the previous studies (Wyman and Fay, 1986; Watanabe et al. 2019; Tan et al. 2020). The light -dark cycle was 12:12. This experiment was conducted in triplicate at 25℃ until the stationary growth phase.

Analysis

By vigorous manual shaking and pipetting, *N. palea* was separated from the substrate and collected using a sterilized glass pipette. *M. aeruginosa* was collected without vigorous shaking. Cell density was counted every 2 or 3 days by using Optical Plastic Plankton Counters (Matsunami Glass Industry, Osaka, Japan) and a microscope (Olympus, BH2-RFCA). Using the cell density during the exponential growth phases, the specific growth rate was obtained by using the Eq. (1), where μ represents specific growth rate (day^{-1}), C_1 and C_2 represent the cell density ($cells\ mL^{-1}$) of *M. aeruginosa* and *N. palea* at the culture time of t_1 and t_2 (day), respectively.

$$\mu = 1/(t_2-t_1)\ \ln C_2/C_1 \quad (1)$$

A Student's t-test was carried out to compare the differences in the specific growth rate and the maximum cell yields in the monoculture between fluorescent light and blue light irradiated at 32 μmol photons $m^{-2}\ s^{-1}$.

Results

The effects of blue light irradiation on the monoculture of M. aeruginosa and N. palea

M. aeruginosa grew under fluorescent light until the end of the experiment and the growth rate was 0.33 day^{-1}, while they hardly grew under blue light. Exponential growth under blue light was observed up to day 8 (Fig. 1) and the growth rate was 0.11 day^{-1} which was significantly lower than that of fluorescent light (Student's t-test, $p < 0.01$). *N. palea* could grow under both light conditions. The specific growth rate for *N. palea* under fluorescent light (0.62 day^{-1}) was higher than that under blue light that was 0.36 day^{-1} (Student's t-test, $p < 0.01$). No significant difference was observed in the maximum cell yields of *N. palea* under fluorescent light and blue light (Student's t-test, $p > 0.05$).

Figure2 shows the growth rate obtained from each light intensity, including the previous studies, and Table1 shows each experimental condition. The growth rate for *M. aeruginosa* under fluorescent light showed an upward trend as the light intensity increased in the range of to 32 μmol photons $m^{-2}\ s^{-1}$ and the growth rate at 45 μmol photons $m^{-2}\ s^{-1}$ was about the same as at 32 μmol photons $m^{-2}\ s^{-1}$. Under blue light, the growth rate was 0.11 day^{-1} at 32 μmol photons $m^{-2}\ s^{-1}$. It was zero at 20 μmol photons $m^{-2}\ s^{-1}$ and was greater than zero at other light intensity. The growth rate for *N. palea* at 32 μmol photons $m^{-2}\ s^{-1}$ was higher than that at 20 μmol photons $m^{-2}\ s^{-1}$ under both light conditions.

Table 1 Culture conditionsof this study and the previous studies

Light intensity (μmol photons m^{-2} s^{-1})	Light-dark cycle	Temperature (°C)	Medium	Reference
12	16:08	20	BG-11 (diluted to 5 % by volume)	Wyman and Fay (1986)
20	24:0	25	WC	Watanabe et al. (2019)
32	12:12	25	WC	This study
45	12:12	25	BG-11	Tan et al. (2020)

Discussion

The growth curves of *M. aeruginosa* were significantly different under fluorescent and blue light. The specific growth rate for *M. aeruginosa* under fluorescent light (0.33 day^{-1}) was consistent with the one that Ohkubo et al. (1991) obtained from the same strain cultivated under similar conditions (0.32 day^{-1}). Compared with the results under the fluorescent light, the growth of *M. aeruginosa* was inhibited under blue light, and this result was in line with the previous studies (Wyman and Fay 1986; Watanabe et al. 2019; Tan et al. 2020). Luimstra et al. (2018) has reported that when phycobilisome-containing cyanobacteria *Synechocystis* sp. absorbed blue light and the efficiency of oxygenic photosynthesis and growth declined because of phycobilisomes. They can absorb light in the red range and work as accessory pigments to compensate for the deficiency of photons in photosystem Ⅱ under red LED. However, they cannot absorb light in the blue range, which created an imbalance in the electron transport energy between two photosystems under blue LED. Since *M. aeruginosa* contains phycobilisomes (Raps et al. 1985), it is considered that its growth was suppressed like *Synechocystis* sp.

The degree of the inhibition effects on the growth of *M. aeruginosa* by blue light differed among the studies. As shown in Table 1, the culture conditions were not consistent among each experiment. For instance, Wyman and Fay (1986) and Tan et al. (2020) used BG-11 medium at different concentration, suggesting available nutrient concentrations must have differed among the studies. It is known that the growth rate follows the Droop equation, which is a model that depends on the intracellular content, and the nutrient uptake rate is calculated by the Michaelis-Menten equation which depends on the nutrient concentration in the medium (Ducobu et al. 1998; Mikawa et al. 2016). Since the half-saturation constant for nutrient uptake is usually smaller than the concentration of the medium, it is considered that the growth rate does not differ depending on the type of the medium unless the nutrient is depleted. Li et al. (2014) demonstrated that there was no significant difference in the growth rate even when the medium with different nutrient concentration was used.

Temperature is also known to affect the growth of algae. It has been reported that the growth rate of *M. aeruginosa* increases with increasing temperature (You et al., 2018; Imai et al., 2009). Li et al. (2014)

demonstrated that the growth rate of *M. aeruginosa* was higher at 25 ℃ than that at 20 ℃. Therefore, the growth rate obtained by Wyman and Fay (1986) is expected to increase further when performed at 25 ℃ (as in this experiment). The light-dark cycle used in the culture is not the same. According to the experimental data by Zevenboom and Mur (1984), The growth rate of *M. aeruginosa* is about the same in the 12:12 and 24:0 light-dark cycles. Furthermore, the difference in growth rate due to different light-dark cycles is smaller under the light intensity which is insufficient to cause saturation with respect to the growth rate. As shown in Fig. 2, the blue light intensity such as 10, 20, and 32 μmol photons m^{-2} s^{-1} are not considered to have the intensity to cause saturation. If the relationship between the light-dark cycle and the growth rate is similar to that of fluorescent light, the difference in the growth rate under the blue light at different light-dark cycles is presumed to be small.

The growth rate may vary depending on the strain. Despite being cultivated under the same experimental conditions, which was in BG-11 at 25 ℃ under fluorescent light at 45 μmol photons m^{-2} s^{-1}, the growth rates of *M. aeruginosa* obtained from Li et al. (2014) and Tan et al. (2020) were different, indicating 0.60 day^{-1} and about 0.32 day^{-1}, respectively. On the other hand, the strain used in this study and Watanabe et al. (2019) were the same. The growth rate under blue light was zero at 20 μmol photons m^{-2} s^{-1} (Watanabe et al. 2019), while that at 32 μmol photons m^{-2} s^{-1} was 0.11 day^{-1}. Since the effect of the light-dark cycle can be assumed to be small, the increase in the growth rate of *M. aeruginosa* was due to the increase in the light intensity. The growth rate may vary depending on the strain, however, it is expected that the growth rate will increase as the blue light intensity increases. Overall, the observed difference in the growth rate of *M. aeruginosa* is determined by the light intensity. As shown in Fig. 2, it is considered that the growth rate tends to be at its minimum when the blue light intensity is around 20 μmol photons m^{-2} s^{-1}.

The specific growth rate for *N. palea* under fluorescent light (0.62 day^{-1}) was higher than that under blue light (0.36 day^{-1}). It was previously reported that the specific growth rate under blue light (0.23 day^{-1}) was higher than under fluorescent light, which was 0.21 day^{-1} (Watanabe et al. 2019). The difference of the specific growth rate under each light condition between the previous and current study is likely due to the difference in light intensity. Figure 2 indicates that the increase in the specific growth rate for *N. palea* in a light intensity was higher in fluorescent light than in blue light. The results showed that *N. palea* grew under blue light while *M. aeruginosa* did not, which was the same as the previous study for all conditions (Watanabe et al. 2019). Diatoms have a fucoxanthin which is a natural pigment (Wang et al. 2018). The adsorption of the fucoxanthins is optimal in the range of 480–560 nm, although there is some light absorption in the range of 420–470 nm, which represents the blue wavelength (Papagiannakis et. al. 2005). Similarly, Ohgai et al. (1992) reported diatom *Cocconeis* sp. grew drastically better in blue fluorescent light. Therefore, the growth of *N. palea* in blue light was promoted by fucoxanthin. However, the growth rate of *N. palea* under blue light at 32 μmol photons m^{-2} s^{-1} did not increase significantly. A fucoxanthin assists the growth of *N. palea* under blue light, but it may be slow to respond to increased light intensity. It should also be noted that no significant differences were detected in maximum cell yields between fluorescent and blue light conditions.

In order to provide a good environment in which *N. palea* has a competitive advantage over *M. aeruginosa*, it is important to irradiate blue light at the intensity that can inhibit the growth rate of *M. aeruginosa* while promoting that of *N. palea*. The blue light intensity at 20 μmol photons m^{-2} s^{-1} is ideal as the growth rate of *M. aeruginosa* is zero while *N. palea* can still grow. When fluorescent light was replaced by blue light at 32 μmol photons m^{-2} s^{-1}, the growth rate of *M. aeruginosa* changed from 0.33 to 0.11 day^{-1}, meaning that the growth rate dropped by 67 % while that of *N. palea* dropped by only 42 %. Therefore, it is considered that blue light irradiation was useful in inhibiting the growth of *M. aeruginosa*, indicating that *M. aeruginosa* was in relatively disadvantageous conditions.

Conclusion

The inhibition efficiency of blue light on the growth rate of *M. aeruginosa* was related to the light intensity. The best light intensity was 20 μmol photons m^{-2} s^{-1}. It is considered that blue light irradiation could be a countermeasure to water blooms since the growth of *M. aeruginosa* was affected by the conditions.

Declarations

Ethics approval and consent to participate

Not applicable for us.

Consent for publication

Not applicable for us.

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

NM and IO prepared to do experiment such as making a medium, subculture of algae etc. PTN who had helped isolate *Nitzschia* proofread the English text. MF and TK were major contributor in writing the manuscript. AH and YI advised me on how to experiment. All authors read and approved the final manuscript.

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Figures

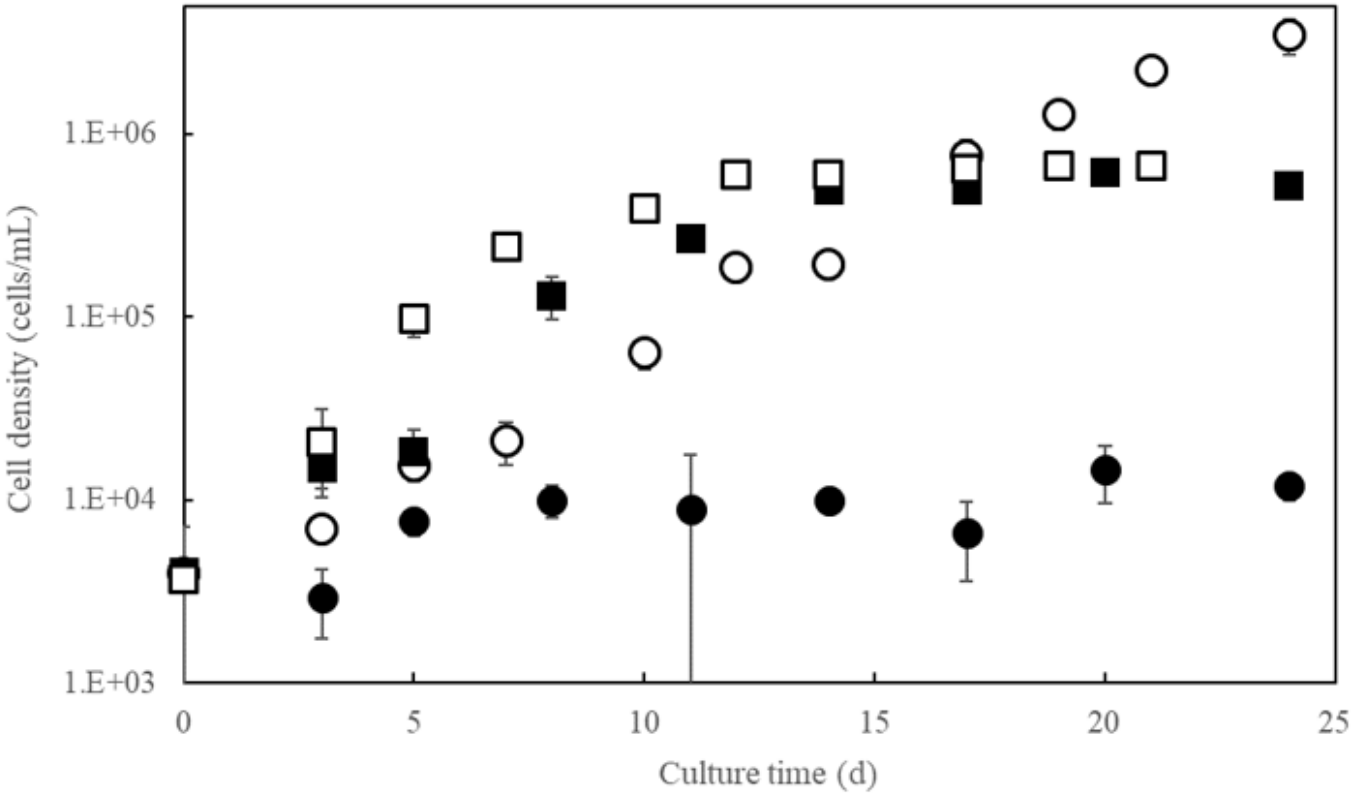

Figure 1

Change in cell density of M. aeruginosa and N. palea with time in monoculture under fluorescent light or blue light irradiation. Symbols: ● M. aeruginosa (fluorescent light), ● M. aeruginosa (blue light), □ N. palea (fluorescent light), ■ N. palea (blue light)

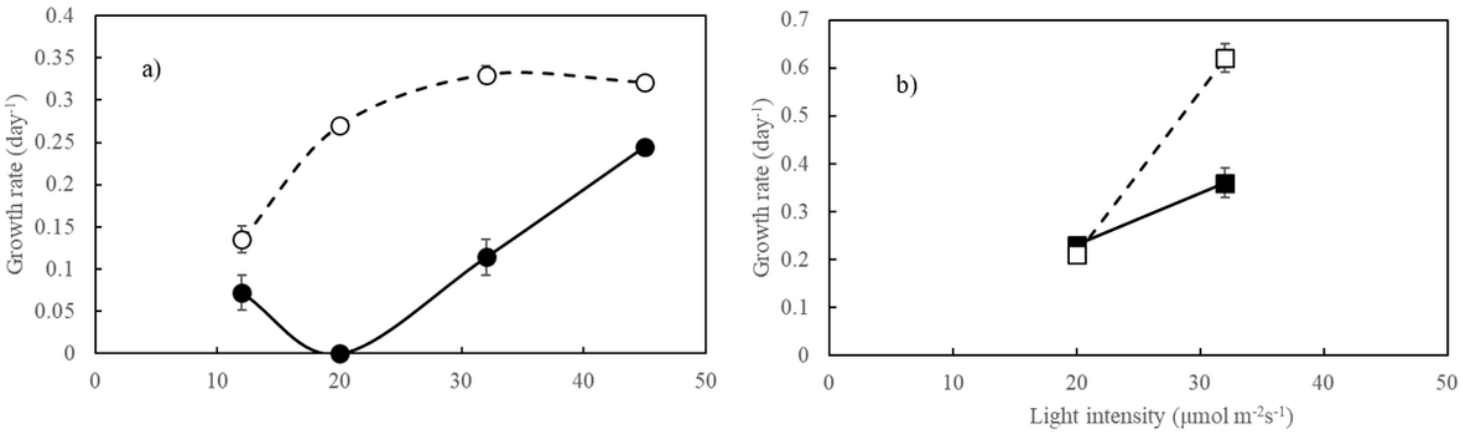

Figure 2

The growth rate for a) M. aeruginosa b) N. palea at each light intensity of fluorescent light and blue light. Symbols: ● M. aeruginosa (fluorescent light), ● M. aeruginosa (blue light), □ N. palea (fluorescent light), ■ N. palea (blue light). The growth rate at 12, 20, 45 µmol photons m-2 s-1 was obtained from Wyman and Fay (1986), Watanabe et al. (2019), Tan et al. (2020), respectively