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THE LONG-TERM EFFECT OF LIGHT INTENSITIES IN DIFFERENT TEMPERATURES CAN PLAY A CRUCIAL ROLE IN SUPPRESSING *MICROCYSTIES AERUGINOSA*

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Abstract. *Microcystis aeruginosa* toxicity is an increasing problem for human and animal health. Therefore, this study is designed to evaluate the long-term effect of light intensities in different temperatures to eradicate *M. aeruginosa*. *M. aeruginosa* was acclimated at 30°C and 20°C in two incubators for 24 days. After 24 days of acclimatization, the first samples were collected (known as zero days), and the second samples were collected seven days later for the five light intensities, 10, 30, 50, 200, and 600 µmol m^{-2} s^{-1}. The samples were collected to analyze optical density (OD_{730}), H_2O_2 concentration, protein content, chlorophyll-a (Chl-a) content, and the antioxidative enzyme activities of catalase (CAT) at 30°C and 20°C. To homogenize *M. aeruginosa* cells, five to eight 3mm beads (BMS Inc., Japan) were used with the appropriate buffer and vigorously shaken. The zero days collection sample is identical because preliminary light intensities were changed during the collection time in each temperature. In the seven-day exposure compared to zero-day, H_2O_2 concentration and protein content showed a parallel variational increasing trend until 200 µmol$m^{-2}s^{-1}$ of light intensity, then decreasing afterward at each temperature and were higher at 30°C than 20°C. Chl-a drastically decreased from 50 µmol m^{-2} s^{-1} to 200 µmol m^{-2} s^{-1} and continued thereafter in the seven days of treatment. Higher H_2O_2 concentration decreases Chl-a content from zero days to seven days in both temperatures. CAT activity is proportionate to H_2O_2 and H_2O_2/protein content in both temperatures in various light intensities. Photoinhibition occurs at 200 µmol m^{-2} s^{-1} after seven days of exposure at 30°C and 20°C as algal biomass (H_2O_2/protein) decreases. Algal biomass is higher at 30°C than 20°C indicating 30°C is the more favorable temperature for them to grow. Therefore, H_2O_2 can be used as a possible biomarker. These results indicate that high light intensity (~ 200 µmol m^{-2} s^{-1}), becomes oxidative stresses in both temperatures that can reduce algal biomass. These findings can be used as a nonchemical approach to eliminating *M. aeruginosa*.
Keywords: *Microcystis aeruginosa*, Hydrogen peroxide, temperatures, protein content, light intensities

1 INTRODUCTION

Many countries experience the bloom of cyanobacterium *Microcystis aeruginosa* in eutrophic lakes and reservoirs, including Japan (Dokulil and Teubner 2000; Ma and Wang 2021; Ohtsuka 2000; Ozawa et al. 2003). *M. aeruginosa* has been reported as toxic to mammals (Carmichael 1981; Gorham 1964a; Schwimmer and Schwimmer 1968). Some of the strains of *M. aeruginosa* produce hepatotoxins called microcystins (Otsuka et al., 1999 and 2000; Rantala et al., 2004; Tillett et al., 2001; Yoshida et al., 2008), which cause hepatocellular carcinoma (MacKintosh et al., 1990; Yoshizawa et al 1990). Various factors, such as temperature and light, affect the distribution and abundance of cyanobacteria (Cao et al. 2006, 2008; Joehnk et al. 2008; Asaeda et al. 2022a).

Compared to other algae species, *M. aeruginosa* can proliferate at high temperatures, making cyanobacteria prevalent in summer blooms (Drake et al., 2010). The preferable growth temperature for *Microcystis spp* is from 24°C to 34°C (Ganf, 1974). It is suggested that changing water temperature can effectively control cyanobacterial blooms (Chu et al. 2007). However, there has been little documentation of the species-specific effect of temperature change on their growth.

Cyanobacteria are directly dependent upon light. Stress may be caused by moderate light changes (Welkie et al. 2019). Cyanobacteria rely almost exclusively on light for energy, so responding to various light intensities is essential (Asaeda et al., 2022a). A lot can be learned about photosynthesis and biotechnological applications of cyanobacteria based on how they respond to various light intensities (Flombaum 2013; Oliver 2016; Leblanc Renaud et al. 2011). The light intensity changes within a day also affect the habitat preference of cyanobacteria (Saha et al. 2016). Through an elaborate electron transport pathway, cyanobacteria carry out photosynthesis and respiration with the help of solar energy (Lea-Smith et al., 2006). Excess solar energy produces reactive oxygen species (ROS), including superoxide radicals (Latifi et al., 2009; Raja et al., 2017), and damages cellular components, such as the D1 protein. This protein is responsible for recovering the damaged photosynthesis apparatus (Gill and Tuteja 2010; Nishiyama, Y., Murata, 2014; Weerakoon et al., 2018). Antioxidant activities detoxify superoxide radicals into the water through superoxide dismutase (Asada et al., 1999; Ma and Gao 2010; Rastogi et al., 2010; Sharma et al., 2012), and photoinhibition occurs. Thus, excessive solar radiation inhibits the proliferation of cyanobacteria. In the ecology of cyanobacteria in water, even though solar radiation intensity is highest at the surface, photoinhibition plays an important role in reducing cyanobacterial biomass (Harrisn and Smith 2013; Salonen et al., 1999).

The eradication or prevention of cyanobacteria growth has been the subject of numerous studies worldwide (Paerl et al. 2011; Rodríguez-Molares et al. 2014; Rajasekhar et al. 2012). The use of chemical methods to eliminate cyanobacteria is discouraged due to their secondary harm to ecosystems (Grandgirard et al. 2002; Lake et al. 2003; Jančula et al. 2011). Consequently, non-chemical approaches can be used, but they require more understanding of the relationship between cyanobacteria and the environment, their interaction with other species, and their responses to environmental stresses (Asaeda et al. 2022a).

Living organisms and their ecosystems play a crucial role in preventing or repairing the damage caused by climate change. Stress triggers the production of endogenous reactive oxygen species (ROS) (Caverzan et al., 2012; Sharma et al., 2012). The overproduction of ROS can be controlled by antioxidant activities. When the antioxidants' capacity to scavenge ROS is exceeded by oxidative stress, ROS imbalance will occur (Dumont et al., 2019; Ugya et al., 2020). Several different environmental stressors, such as various light intensities or temperatures (Leblanc Renaud et al. 2011; Wu et al. 2008), generate H_2O_2, superoxide radical ($O_2^{\cdot-}$), as well as the hydroxyl radical ($OH^{\cdot}-$) (Sharma et al. 2012; Asada et al. 2006; Satterfield et al. 1955; Zhou et al. 2006). Most of the ROS will be converted to H_2O_2. Some of them will be extremely dangerous, although antioxidant activities have also increased to reduce the risk of damage to the cyanobacteria cells (Asaeda et al. 2018, 2019, 2020, 2022a; Barnuevo and Asaeda, 2018; Rahman et al. 2023a, 2023b).

While light intensity and temperature have been studied independently by numerous researchers (Wu et al., 2008; Leblanc Renaud et al. 2011; Utkilen et al. 1992; Asaeda et al. 2020, 2021), little information is available about their combined effects on cyanobacterial growth. Therefore, the main objective of this study is to investigate the long-term combined effect of temperatures and different light intensities for cyanobacterial bloom treatment as a non-chemical approach.

2 MATERIALS AND METHOD

2.1 *M. aeruginosa* cell culture

M. aeruginosa (NIES-111) is a bloom-forming blue-green algae that usually form colonies obtained from the National Institute for Environmental Studies (NIES), Japan. The Autoclaved 100% BG 11 (Rippka, et al. 1979) medium was used for the *M. aeruginosa* culture throughout the experiment. The strain was cultured at 30°C (± 0.3°C) with white fluorescent light, containing a flux of 10–20 µmol m^{-2} s^{-1} in a 12 h:12 h light: dark cycle inside an incubator (Cool Incubator KMH-259 from AS ONE Corporation). Each culture was manually shaken twice a day during the light phase in the incubation period. *M. aeruginosa* was subcultured (Abeynayaka et al. 2017, 2018) until a sufficient amount was achieved.

2.2 Experimental procedure

A sufficient amount of *M. aeruginosa* was transferred into two incubators at 30°C (± 0.3°C) and 20°C (± 0.3°C) at 10-20 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for 24 days to acclimatize. To examine the long-term effect of light exposure in *M. aeruginosa* at 30°C and 20°C, five different light intensities- namely, 10, 30, 50, 200, 100 and 600 µmol m^{-2} s^{-1}, were introduced by white fluorescent light (Toshiba, Japan). An automatic timer (REVEX PT7, Saitama, Japan) was set to maintain 12 hours of light and 12 hours of darkness. The light intensities were measured using a quantum sensor (Apogee, MQ-200, United States) and adjusted uniformly in the media. Cyanobacteria culture flasks were gently shaken three times daily to ensure homogeneous exposure to the light environment. Samples were taken twice for analysis. The first samples were taken at 12:00 on the 24 days of acclimatization, considered zero days. Following the first sampling, a second sampling was conducted at 12:00 with a 7-day interval. Collected samples were allocated in 1.5 mL tubes, taking 1 mL of *M. aeruginosa* cells, and centrifuged at 10,000 rotations per minute (rpm) for 10 minutes at 4°C. The supernatant was removed and kept at -80°C for bioassays described below. *M. aeruginosa* cell wall cannot break down by using the buffer (phosphate buffer (pH7; 0.1M), NaOH solution for measuring H_2O_2 and protein respectively). Therefore, five to eight 3mm beads (BMS Inc., Japan) were added with buffer and vigorously shaken. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was used as an extract for further analysis. Each culture group was replicated three times to conduct the experiment.

2.3 Measurement of total soluble protein concentration

Minor modifications were made to (Abeynayaka et al., 2017)'s method to estimate total soluble protein concentrations. Cyanobacterial cells were unfrozen, and distilled water was used to wash the pellet once. The extraction procedure was performed using 1mL of 0.5 M NaOH solution using beads. The extraction was centrifuged at 4°C for 15 minutes at 10,000 rpm to pick up the supernatant. A Coomassie Bradford protein assay was used to measure protein content. After incubation at room temperature (25 ± 2°C) for 10 min, the absorbance was recorded at 595 nm using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan. Protein levels were determined by a series of known albumin concentrations.

2.4 Chlorophyll-a concentration analysis

Using (ISO 10260:1992; Gregor and Mars$alek, 2003)'s method, Chl-a concentrations were measured in cyanobacteria samples. The pellet was washed with distilled water once after each cyanobacterial cell was unfrozen, then extracted in 1.5 mL of 90% ethanol. After vigorous shaking, the mixture was kept at room temperature (25± 2°C) overnight in darkness. Then, each sample was centrifuged at 12,000 rpm, and the supernatant was measured with a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan) at the absorption wavelengths of 665nm and 649nm. The Chl-a content was calculated by using the following equation:

Chl-a = $(13.95*A_{665} - 6.88*A_{649}) *1.5$

2.5 Identification of cell growth by measuring OD_{730}

OD_{730} measurements were performed on each flask in order to determine the cyanobacteria growth rate. With a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan), OD_{730} was measured at a wavelength of 730 nm using a previously proposed methodology (Axler et al., 1994; Taras 1971)

2.6 H_2O_2 concentration measurement assay

A modified version of xylenol orange (eFOX) method was used to measure the H_2O_2 content of *M. aeruginosa* following the methods of (Queval et al., 2008; Cheeseman 2006). The homogenized cells were centrifuged at 10,000 rpm for 10 minutes at 4°C to extract the cellular H_2O_2. The supernatant (100 µL) was added to 1 mL of the assay solution containing 0.250 mM ferrous ammonium sulphate, 0.1 mM sorbitol, 0.1 mM xylenol orange, 1% ethanol, and 25 mM H_2SO_4, which had been deoxygenated with gaseous nitrogen to prevent artifact production in hydrogen peroxide during the reaction. After 15 minutes of reaction, the absorbance at 560 nm was measured by spectrophotometry. H_2O_2 content was calculated by a standard curve using a series of diluted solutions of commercial, high-grade 9.8 M H_2O_2 (Dautania et al. 2014; Asaeda et al. 2022a).

2.7 CAT assay

CAT activity was measured using the method described by (Aebi, 1984). A total of 1 mL of unfrozen cell pellets were homogenized in 1 mL potassium phosphate buffer (0.05 M, pH 7.0) containing 0.1 mM EDTA. The supernatant was collected as an enzymatic extract after centrifugation (12,000 rpm at 4°C for 10 min). The CAT activity was measured by reacting 15 µL of 0.75M H_2O_2, 920 µL of potassium phosphate buffer, and 65 µL of extract supernatant. Optical absorption was measured at 240 nm using UV mini-1240. The measurements were recorded every 10 seconds for 3 minutes, and the CAT activity was computed using a 39.4 mM/cm extinction coefficient.

2.8 Statistical Analysis

A one-way analysis of variance (ANOVA), followed by the Tukey test, was performed to check the statistical significance of variations among the means of sample groups. Significant differences between experimental groups of *M. aeruginosa* were evaluated using an independent sample t-test, assuming equality of variance. Pearson's correlation analysis was used to assess correlations among parameters. Statistical analyses were performed using IBM SPSS Statistics for MacBook, Version 28.0 (IBM Corporation, USA).

3 RESULTS

Due to preliminary light intensities changing during collection, the zero days collection in each temperature did not show too much fluctuation. Figure 1 shows the differences in OD_{730} between 30°C and 20°C regarding different light intensities. OD_{730} values significantly increased from 0 to 7 days at 20°C ($p<0.001$). In the 7 days at 30°C, OD_{730} decreased with increasing PAR. In the meantime, OD_{730} increased till 30 PAR at 20°C and took nearly the same value thereafter. A positive correlation was found between OD_{730} and different light intensities in 7 days between 30°C and 20° ($r=0.417$). Protein concentration substantially increased from 0 days to 7 days at 20 °C ($p<0.001$), whereas at 30°C, it slightly increased until 200 µmol m^{-2} s^{-1}, declined with 600 µmol m^{-2} s^{-1} (Figure 2). With respect to light intensity, after 7 days of light exposure, the protein concentration increased until 30 µmol m^{-2} s^{-1}, then constantly decreased with increasing light intensities, similarly in 30 °C and 20°C experiments. After 7 days of treatment, H_2O_2 concentration and H_2O_2/protein or algal biomass significantly increased in both temperatures ($p<0.001$ for each temperature) (Figure 3 and Figure 4). At 7 days, H_2O_2 and H_2O_2/protein concentration increased to 200 PAR at each temperature, and then it decreased with higher PAR. A significant positive correlation was observed from 30°C to 20°C between PAR with H_2O_2 ($r=0.859$ $p<0.001$)and PAR with H_2O_2/protein ($r=0.914$ $p<0.001$). In both temperatures, Chl-a increased until 50 PAR and continued to decrease with higher PAR (Figure 5). The Chl-a concentration is shown as a function of H_2O_2 of *M. aeruginosa* under different light intensities in Figure 6 in both temperatures. With 20°C, Chl-a concentration increased with less than 0.5 mmol/L of H_2O_2. However, with a higher concentration of H_2O_2, it decreased. With 30°C, Chl-a concentration slightly increased until 1 mmol/l of H_2O_2, then decreased with a higher concentration of H_2O_2. CAT activity significantly increased from 0 days to 7 days at 30°C ($p<0.01$) and 20°C ($p<0.01$) to decrease oxidative stress. CAT activity increased to 200 µmol m^{-2} s^{-1} at each temperature. CAT activity and temperatures showed a positive correlation (30°C and 20°C, $r=0.415$).

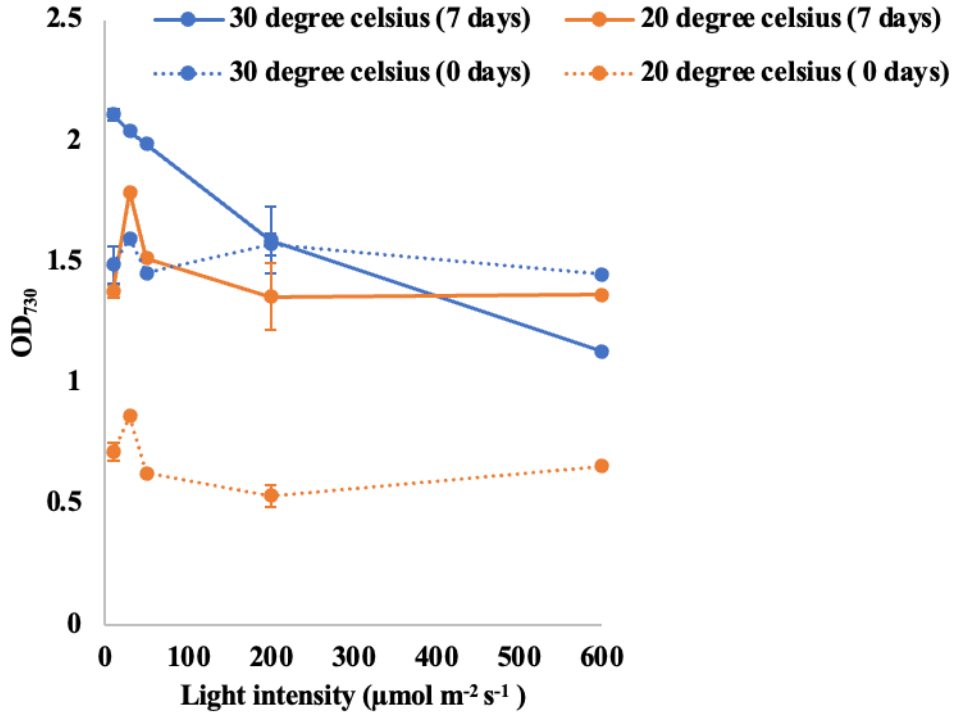

Figure 1. *M. aeruginosa* OD_{730} at 0 and 7 days at 30°C and 20°C. Solid lines indicate seven-day treatment, whereas dashed lines indicate 0 days. The error bars indicate standard deviations.

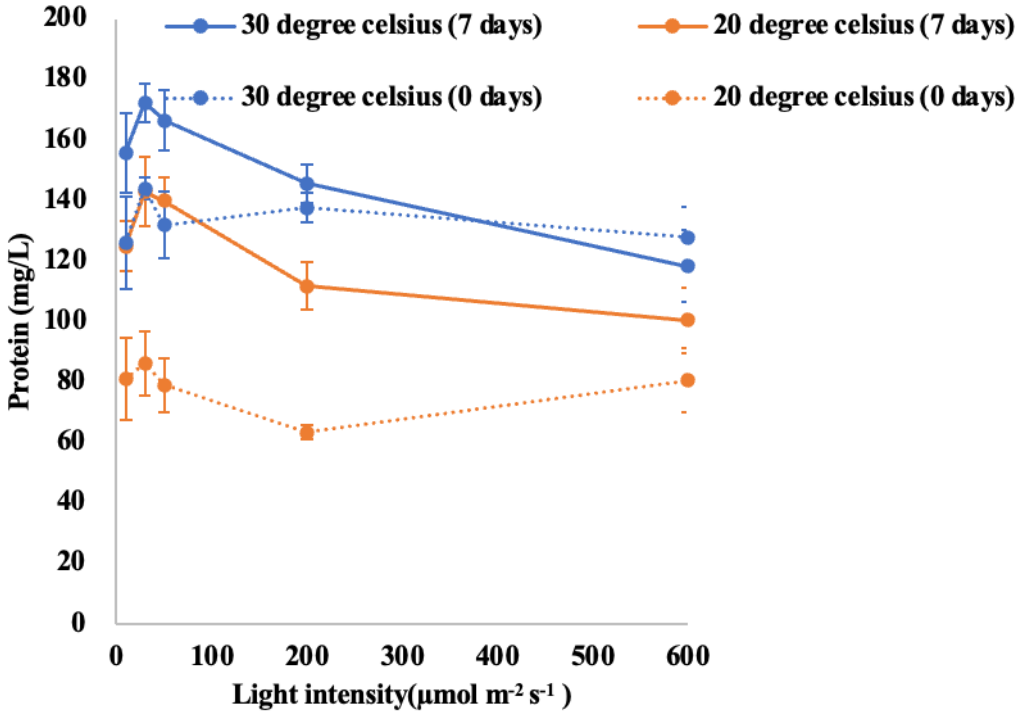

Figure 2. Changes in protein concentration concerning light intensity at 0 and 7 days of observing *M. aeruginosa*. Solid lines indicate seven-day treatment, whereas dashed lines indicate 0 days. The error bars indicate standard deviations.

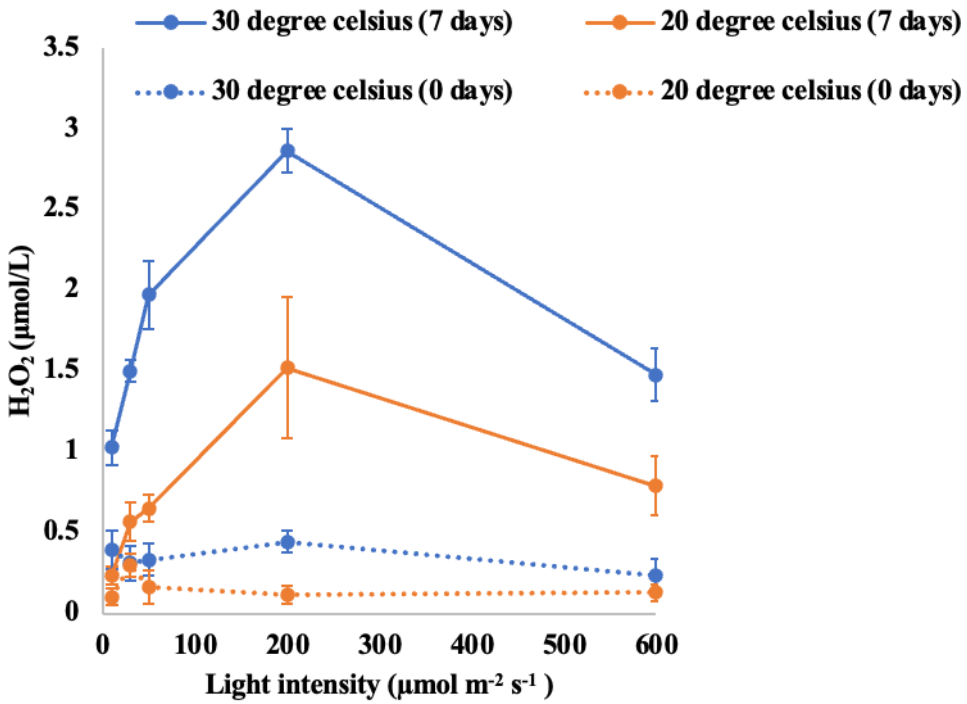

Figure 3. Changes in H_2O_2 concentration concerning light intensity in zero and seven days of observing *M. aeruginosa*. Solid lines indicate seven-day treatment, whereas dashed lines indicate 0 days. The error bars indicate standard deviations.

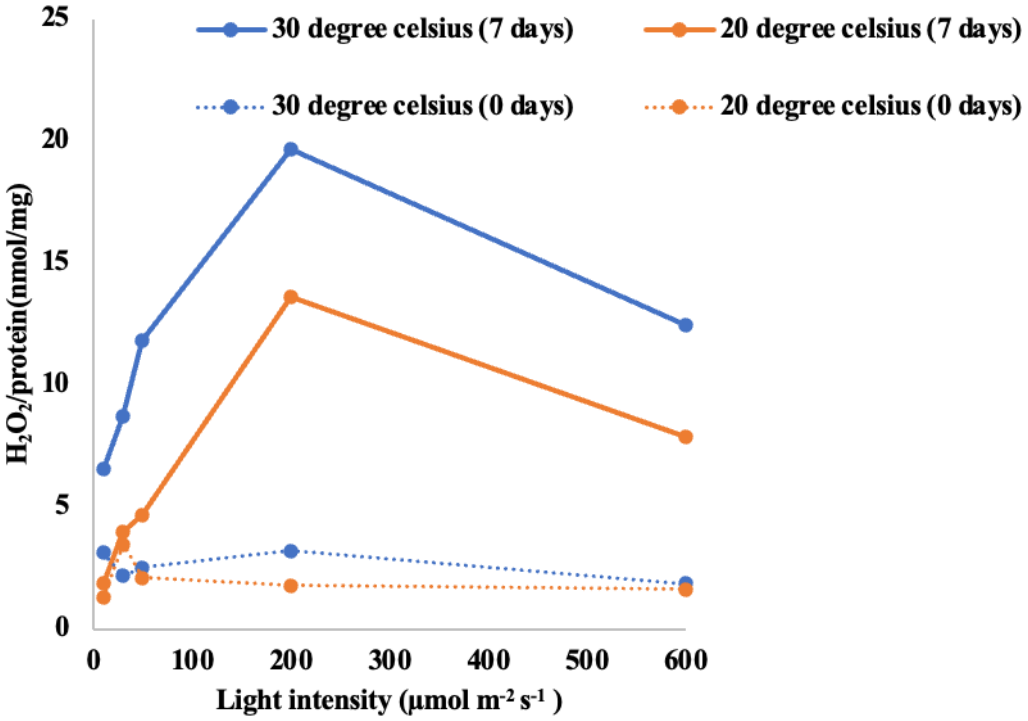

Figure 4. Changes in H_2O_2/protein concentration concerning light intensity at zero and seven days of observing *M. aeruginosa*. Solid lines indicate seven-day treatment, whereas dashed lines indicate 0 days. The error bars indicate standard deviations.

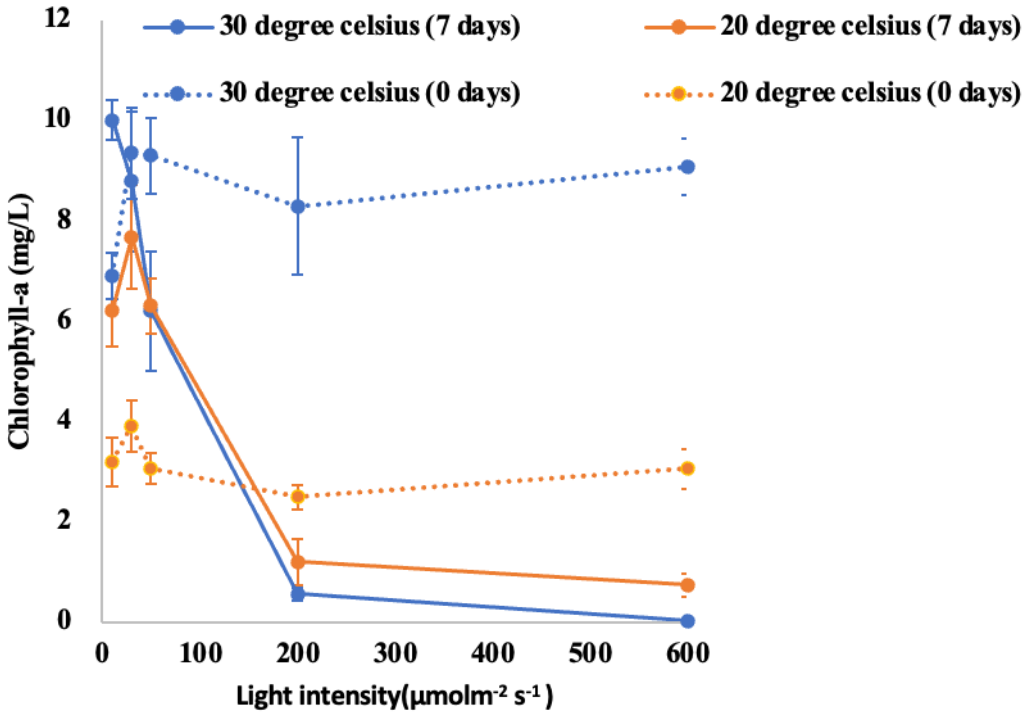

Figure 5. Changes in chlorophyll-a content concerning light intensity in zero and seven days of *M. aeruginosa* observation. Solid lines indicate seven-day treatment, whereas dashed lines indicate 0 days. The error bars indicate standard deviations.

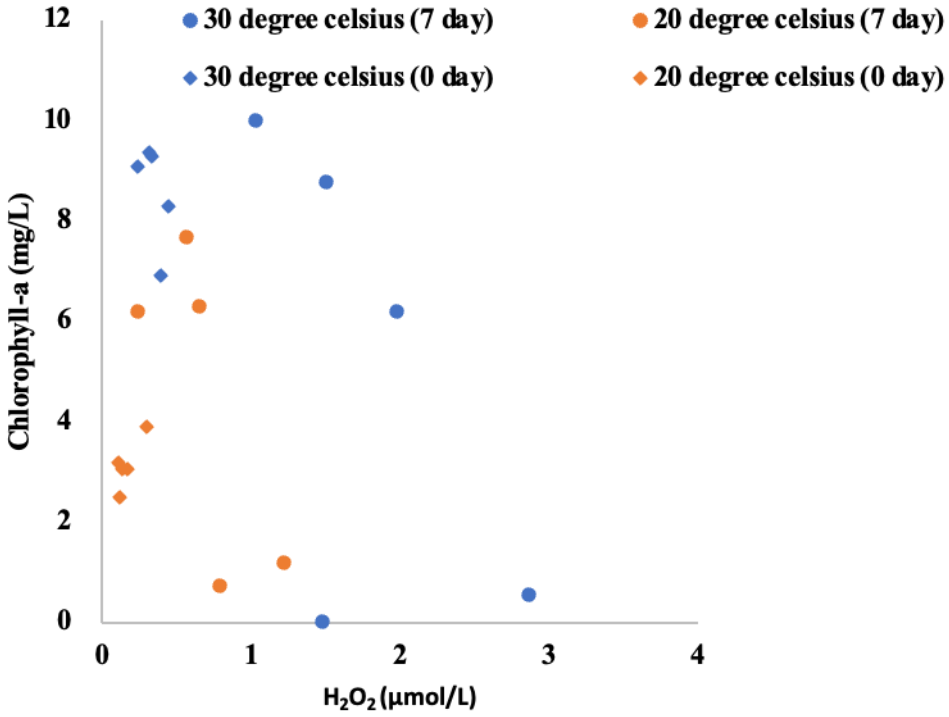

Figure 6. Changes in chlorophyll-a content concerning light intensity at zero and seven days of *M. aeruginosa* observation. Different symbols are used to separate 0 and 7 days of exposure. The error bars indicate standard deviations.

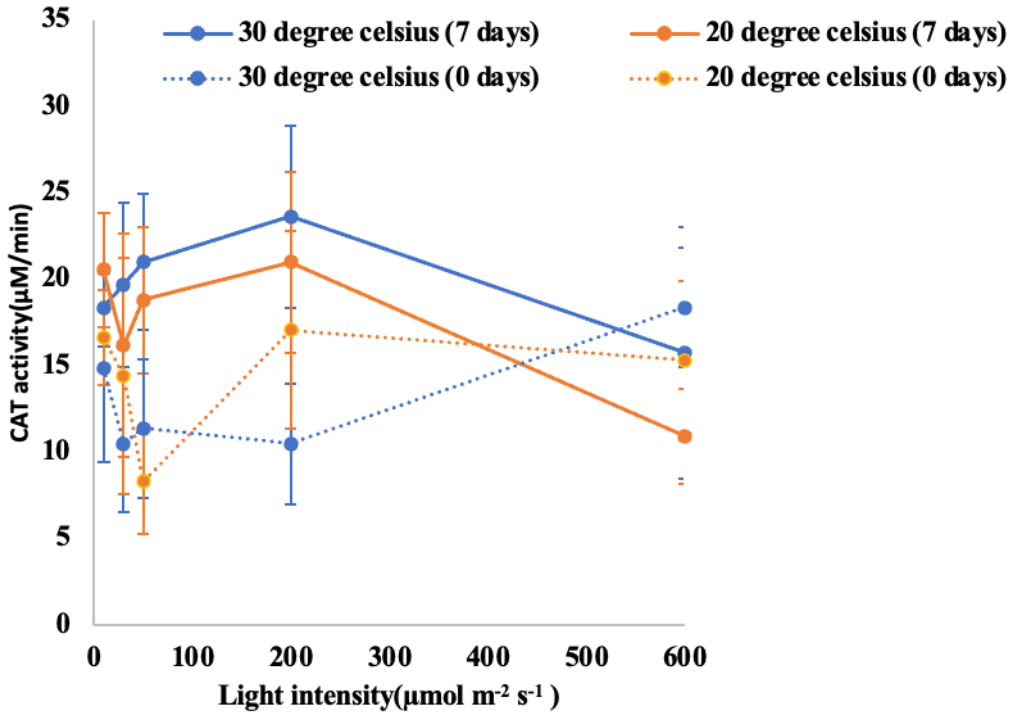

Figure 7. Changes in CAT activity concerning light intensity at zero and seven days of *M. aeruginosa* observation. Solid lines indicate seven-day treatment, whereas dashed lines indicate zero days. The error bars indicate standard deviations.

4 DISCUSSION

The accumulation of ROS is reported to increase in parallel with increased abiotic stress (Vesterkvist et al., 2012; Preece et al., 2017; Pham et al., 2018). In the present experiment, two abiotic stresses were applied, temperatures (30°C and 20°C) and low to high light intensities (10 to 600 µmol m^{-2} s^{-1}). Though H_2O_2 is produced under normal environmental conditions, its production is accelerated under high light intensity. In natural water, cyanobacteria are exposed to various abiotic stresses that enhance oxidative stress, producing H_2O_2, which may deteriorate cyanobacterial biomass by producing hydroxyl radicals. It is not necessarily cumulative that H_2O_2 is produced due to different abiotic stresses (Mittler et al., 2006; Saints et al., 2010). However, the H_2O_2 concentration was enhanced with increasing PAR intensity at a certain stage and decreasing at lower temperatures (Figure 2).

A significant positive correlation was found between OD_{730} and protein content after seven days of exposure at 30°C ($r=0.853$, $p<0.001$) and 20°C ($r=0.696$, $p<0.01$). This relationship indicates that the protein content is a proper biomass reference. It is reported that one-third to one-half of the cell biomass is composed of protein (López et al., 2010). Therefore, H_2O_2/protein is an appropriate indicator of H_2O_2 content in a cell.

4.1 Effect of light intensities and temperatures

M. aeruginosa exhibited a fluctuating response in different PAR. *M. aeruginosa* reached maximum H_2O_2 concentration at 200 µmol m^{-2} s^{-1} at both temperatures and decreased with higher light intensities. Both parameters' light intensities and temperatures were stressful mainly due to photosystem-produced ROS, and it will be converted to H_2O_2. The present results indicated that photoinhibition occurs even with 200 µmol m^{-2} s^{-1}; however, in the field observation, photoinhibition occurred when the light intensity exceeded 1000 µmol m^{-2} s^{-1} (Whitelam and cold 1983; Machová et al., 2008; Harel et al., 2004). Although surface colony receives high intensity of solar adiation, it declined relatively quickly inside water, particularly in the water rich in cyanobacterial biomass. Thus, the cyanobacteria colony does not receive high solar radiation directly. In addition, they avoid the highest solar radiation in a day, by migrating to deeper zones (Molle et al., 2005, Athukorala et al., 2010; Cañedo-Argüelles et al., 2013; McLellan et al., 2017), likely to avoid the high solar radiation and the oxidative stress before the recovery of homeostasis via the increasing antioxidant activities (Athukorala et al., 2010).

The growth of *M. aeruginosa* is significantly decreased by temperatures between 30°C and 20°C ($r=0.752$, $p<0.01$), similar to other studies (Chu et al. 2007; Imai et al. 2009; You et al. 2018). At the same

time, temperatures significantly increased both H_2O_2 and H_2O_2/protein concentration with each light intensity (30 to 20, $p<0.01$) until 200 PAR. In contrast, a positive correlation between CAT and H_2O_2 with temperatures (30°C and 20°C, $r=0.415$) was also found. These results indicate that *M.aeruginosa* maintains higher metabolism, whether it is regarding oxidative stress or antioxidant activities, with higher temperature, and it brings about a high growth rate with high temperature.

4.2 Effect of total protein content

The total protein content decreased with increasing light intensities, particularly after 200 PAR, and chlorophyll-a extremely decreased even after 30 µmol m^{-2} s^{-1} both at 30°C and 20°C. At the same time, H_2O_2/protein decreased from 200 PAR onwards (Figure 2). The reduction of chlorophyll- a concentration is due to the breakdown of photosynthesis components, such as photosystems I and II (Kura-Hotta et al., 1987), phycobilisomes (Raps et al., 1985; Walsh et al., 1997), and it decreases photosynthetic production. Phycobiliprotein synthesis is reduced in *M. aeruginosa* when light intensities exceed 200 mol m^{-2} s^{-1} to protect against the absorption of excess light energy and protein degradation by proteases. (De Oliveira et at., 2014; Pojidaeva et al., 2004; Rosales-Loaiza et al., 2008). This reveals that *M. aeruginosa* has a defense mechanism to prevent cell damage from light and temperatures. The negative trend of total proteins with increasing light may also have influenced microcystin content (Kaebernick et al., 2000).

OD_{730} of *M. aeruginosa* in temperature also showed a fluctuation at 20°C; meanwhile, it started decreasing from 10 µmol m^{-2} s^{-1} to 600 µmol m^{-2} s^{-1} at 30°C in the seven days, respectively. In addition, it showed that *M. aeruginosa* was capable of surviving and proliferating continuously, despite its relatively weak growth rate and strict preference for 200 mol m-2 s-1, and experienced high stress under higher light intensities. However, the observed increases in protein content with increasing light stress suggest that there was a deviated stress response for *M. aeruginosa*. The increased protein content can be associated with the upregulation of stress-related protein, as the oxidative stress was enforced due to the elevated H_2O_2 content with increasing light intensity (Babele et al., 2019). However, further research focused on the upregulation of stress proteins is necessary to confirm this phenomenon.

4.3 Effect of antioxidant activity

By triggering the antioxidant defense system in cyanobacteria, the oxidative stress response mechanism protects them from adverse environmental conditions (Liu et al., 2017). Oxidative stress and antioxidative enzymes are disturbed by abiotic stress (De Silva and Asaeda, 2017; Rastogi et al., 2010). In the present study, high light intensities (200 to 600 µmol m^{-2} s^{-1}) led to a reduction in H_2O_2 concentration, and low light intensities (10 to 200 µmol m^{-2} s^{-1}) augmented it. *M. aeruginosa* showed increasing trends with CAT compared to zero to 7 days in each temperature. However, when testing the regression relationships between H_2O_2/protein and antioxidant enzymes, CAT activity was found to have a strong relationship at both temperatures. This confirms that CAT played a stronger role in antioxidant activity. The antioxidant balance also declined under higher stress. The CAT/protein activity with H_2O_2/protein concentration of *M. aeruginosa* under different light intensities is proportionate in both temperatures. CAT/protein values increased with the increment of H_2O_2/protein (Figure not shown).

4.4 Potentiality of using H_2O_2 as a bio-marker

Many studies have been conducted on the lethal concentration of H_2O_2 in cyanobacteria, mainly via incubation in laboratories, with the endorsement of different concentrations of H_2O_2 (Derakhshan et al., 2018; Shekoohiyan et al., 2013; Drábková et al., 2007a; Bouchard et al., 2011 Leunert et al., 2014; Foo et al., 2020). Laboratory incubations under different H_2O_2 concentrations provided the lethal H_2O_2 dosage for cyanobacteria in the past, which implies that cyanobacterial biomass is degraded with higher H_2O_2 concentrations. In the present study, because chlorophyll-a can be expressed as protein, we used protein content instead of chlorophyll-a content as an indicator of biomass (Marsac 1977).

H_2O_2 is generated in the photolysis process of dissolved organic carbon in water by UV (Cooper and Lean 1989; Scully et al., 1996). A water body's nutrient content increases its production rate of H_2O_2 due to its widespread distribution (Herrmann 1996). Thus, naturally generated H_2O_2 may be able to control cyanobacterial biomass in water and contribute to the decline of cyanobacteria. The protein content in water declined with an increasing H_2O_2/ protein concentration until 200 PAR (Figure 4). A higher protein level was not observed with higher H_2O_2 concentration levels in the present study (Figure 2). CAT activities are directly proportional to H_2O_2 and H_2O_2/ protein concentration with the increasing PAR at both temperatures and then decrease afterward. Therefore, the growth of cyanobacteria is suppressed by the generation of higher H_2O_2 levels.

4.5 Introducing an environmentally favourable approach to algal treatment

As a non-chemical method to control *M. aeruginosa*, high light exposure levels and temperature fluctuation is highly appreciated compared with chemical methods. However, the hypothesis of the method, such that low-light exposure suppresses the growth of cyanobacteria, is opposite to the present results. Supposedly, artificial water mixing in lakes and reservoirs (Visser et at., 2016) is not necessarily efficiently operated. In the case of introducing the system, *M. aeruginosa*'s both low- and high-light vulnerability can be carefully considered. The most efficient destratification process must be designed from light intensity and temperature distribution points beforehand (Asaeda and Imberger, 1993; Imteaz and Asaeda, 2000). In order to control *M. aeruginosa*, there is a possibility to introduce other types of practical methods, such as exposing deep water to high lights without raising the temperature. Methods should be developed to illuminate the water column beyond the tolerable light intensity level for *M. aeruginosa*.

5 CONCLUSION

M. aeruginosa was well-suited to illumination ranges of 30 to 50 $\mu molm^{-2}s^{-1}$. Beyond the optimal light intensities, the growth of *M. aeruginosa* was reduced, particularly after 200 PAR. Both temperatures significantly affect H_2O_2 concentration and protein concentration. H_2O_2/protein and CAT/protein also increased until 200 µmol m^{-2} s^{-1} and decreased to 600 µmol m^{-2} s^{-1} at seven days in both temperatures. *M. aeruginosa* demonstrated a greater tolerance for high light intensities. It could be possible to develop control mechanisms based on high-light intensities, which might lead to lower growth rates, or to improve the existing methods that rely on low-light intensities. This could help to control cyanobacteria in water bodies effectively.

6 DATA AVAILABILITY STATEMENT

The authors declare that the data will be available for everyone upon reasonable request.

7 COMPETING INTERESTS

The authors declare that there is no conflict of interest.

8 AUTHOR CONTRIBUTIONS

M.R. made experiments and chemical analyses and completed the first draft. T.A. contributed to the design of the experiment and the revision of MS. H.D.L.A. contributed to experiments along with M.R. K.F. extensively revised the manuscript.

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