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Effect of different irradiance levels on the growth of the cyanobacterium *Lyngbya majuscula*

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Abstract

Lyngbya majuscula is a prolific producer of secondary metabolites that are used in the pharmaceutical industry. As a photosynthetic organism, the effect of irradiance on its cultivation was studied to maximize algal growth for mass production. The cyanobacterium was subjected to different irradiances of 20, 45, 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ to determine its specific growth rate (SGR) in each treatment. Results showed the highest SGR under 20 and 45 µmol photons $m^{-2}s^{-1}$ during the exponential phase, showing a significant difference ($p < 0.05$) among all other treatments. Minimal growth rates were obtained under 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ and results showed that the SGRs under these treatments have no significant difference ($p > 0.05$) with the negative control; however, color changes were observed at these irradiances. These showed that *L. majuscula* prefers lower irradiances to maximize its growth, while higher irradiances are unideal.

Introduction. - *Lyngbya majuscula* is a filamentous marine alga that is a prolific producer of 196 novel and diverse secondary metabolites, whose genus is responsible for over 40% of all marine cyanobacterial secondary metabolites (1,2,3). These metabolites are carotenoids, proteins, and vitamins which are beneficial in the pharmaceutical industry and food technology for their antioxidative and antimicrobial properties [4].

Several factors can be modified to optimize the growth and secondary metabolite production of cyanobacteria. For instance, Burja et al. [2] investigated the effect of culture vessel configuration, growth conditions, and media composition and determined that culture conditions have the greatest effect on secondary metabolite production. One important culture condition [3,5,6] that can be modified is light availability. Studies [7,8] reported that algal growth is better under continuous lighting since growth is directly proportional to the length of the light exposure.

Although most studies investigate the relationship of light received by the algae to its growth, little research has been done regarding the optimization of the yield under continuous light exposure. There are claims that *L. majuscula* would yield highest growth under various light–dark cycles and among different levels of irradiation from ranges of 20 µmol photons $m^{-2}s^{-1}$ to 120 µmol photons $m^{-2}s^{-1}$ [3,9,10].

Previous research would suggest that the optimum growth of the algae *L. majuscula,* would be sustained under higher light intensities within the ranges of 180 µmol photons $m^{-2}s^{-1}$ to 320 µmol photons $m^{-2}s^{-1}$. This is due to the property of *L. majuscula* being a surface cyanobacterium; hence, it readily produces more carotenoids as a mechanism against photoinhibition [9]. However, Loogman [10] also observed general cyanobacterial death at 320 µmol photons $m^{-2}s^{-1}$. Low light irradiances have also been claimed as the optimum irradiance for other *Lyngbya* species such as *Lyngbya kuetzingii* and *Lyngbya stagnina,* where the irradiance values found were 20 µmol photons $m^{-2}s^{-1}$ and 45 µmol photons $m^{-2}s^{-1}$, respectively [11,12].

Realizing the need to optimize the growth of the algae for future use in the pharmaceutical industry, *L. majuscula* was chosen to be subjected to stress or bioprocess intensification through light irradiance. Mass cultivation of the algae was targeted because most drug candidates do not reach the pharmaceutical market due to the low availability or small yield of bioactive compounds [13].

This study aimed to determine at which irradiance between 20, 45, 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ the algal growth of *Lyngbya majuscula* is at a maximum. Specifically, it aimed to:

(i) assess changes in the color, pH, and temperature of *L. majuscula* during each treatment;

(ii) analyze the trend of algal growth in terms of dry weight and specific growth rate (SGR); and

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(iii) determine the highest specific growth rate by comparing the SGR values during each treatment using Planned Comparisons after One-Way Analysis of Variance (ANOVA)

Methods. - The methods were divided into 5 steps: (1) preparation of the alga, (2) culture of the alga in different irradiance levels, (3) determination of dry weight using gravimetry, (4) computation of specific growth rate, and (5) analysis of data.

Sample Collection. *L. majuscula* was purchased from the Southeast Asian Fisheries Development Center/Aquaculture Department (SEAFDEC/AQD) in Tigbauan, Iloilo. Samples were washed to remove residues and were subjected to a scale-up of 2 L after purification based on the standard protocol from the Handbook of Phycological methods [14].

Culture Set-up. Twenty-four (24) containers were each filled with 5 L algal cultures composed of 500 mL alga mixed with 4500 mL Ozonated Seawater-Conwy solution [15]. Five (5) lightboxes (92 cm x 31 cm x 66.5 cm) were made to contain four replicate containers each. The lightboxes had different irradiance levels: 20, 45, 110, 180, and 320 µmol photons $m^{-2}s^{-1}$, which were achieved using LED lamps (1 lamp = 2000 lumens). For the negative control, four replicate containers were not exposed to light.

The setup was irradiated for 24h throughout the 8-day culture. Irradiance was measured using a photometer (resolution: 0.1 µmol photons $m^{-2}s^{-1}$ in the range 0 - 1999 µmol photons $m^{-2}s^{-1}$), and one-point continuous aeration, pre-filtered (5 µm), was supplied by centralized pumps in the laboratory. The temperature and pH of the alga were recorded everyday using a temp/pH meter (resolution: 0.01 pH at range: 0.00 to 14.00 pH and 0.1°C at range: 0.0 to 100.0°C). The color of the alga in each treatment was also monitored everyday.

Algal Dry Weight. The biomass of *L majuscula* was measured in terms of dry weight using the Gravimetric method. The standard protocol set by the American Public Health Association (APHA) was followed [16]. One hundred (100) mL of the sample from each treatment replicate was vacuum filtered in pre-tared GF/F glass microfiber filters (0.7 µm pore size). Twenty (20) mL ammonium formate (Sigma Aldrich, 1M concentration with purity >99.0%) was then added to remove salt residues from the sample. The filter papers with alga residue were then oven-dried (Precision Scientific) at 60 °C for 2 hours, desiccated for 30 minutes, and then weighed using an analytical balance. This process was repeated until the net weight of the alga was obtained, with a difference of ± 0.0002 when weighed. Dry weight was calculated using the formula:

$$Dry\ Weight\ = \frac{W_1 - W_2}{mL},$$

Equation 1. Formula for Dry Weight

where W_1 = weight of the filter paper with the dried residue of the sample in mg, W_2 = tare weight of the filter paper in mg, and mL = volume of the sample.

Specific Growth Rate. Specific growth rate was calculated after obtaining the dry weight of the alga to determine the rate of algal biomass increase per day. It was computed using the formula [17]:

$$Specific\ Growth\ Rate\ (SGR) = \frac{ln\ (W_f) - ln(W_i)}{t}\ x\ 100,$$

Equation 2. Formula for Specific Growth Rate

where W_f = final weight in mg, W_i =initial weight in mg, and t = days of culture.

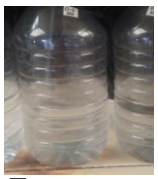
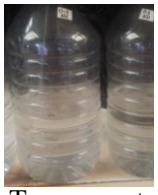
Data Analysis. Qualitative data were in the form of pictures of the treatment replicates each day. Quantitative data were in the form of algal dry weight (mg/mL), specific growth rate (d^{-1}), temperature (°C), and pH, where the mean and standard deviation in each treatment were computed. One-Way ANOVA with Planned Comparisons at 95% Confidence Interval was used to analyze the significant difference between samples. Tests of normality and homogeneity were also conducted to determine the conditions for the conduct of Planned Comparisons as post-hoc analysis.

Results and Discussion. - *Lyngbya majuscula* is a marine cyanobacterium that forms dense mats near the surface of the water, and its growth is dependent on numerous factors, with one of these being light availability [3,5,6]. The growth of the cyanobacterium in 8 days of culture was observed in different irradiances of 20, 45, 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ using LED lamps.

The exponential phase of the alga was first identified before being subjected to the different irradiances. This phase was chosen among all other phases of the cyanobacterial growth due to the rapid and frequent cell division that contributed to the maximum growth rate of the alga. In addition, the higher growth rate or biomass production rate will result in faster production of metabolites which are usually produced in the late exponential phase [18,19]. The alga showed the highest growth during the 4th day of culture, showing that the exponential phase can be observed from Day 0 to Day 4. After the 4th day, disintegration of the alga was observed, indicating that it has entered the stationary phase, leading to the death phase. A similar study on the culture of *L. majuscula* conducted by Mandal et al. [20] also showed the decay of the alga after its exponential phase of 3-4 days.

Morphological Color Changes. Changes in the color of the alga were observed in the different treatments, which indicate the presence of photosynthetic pigments that contribute to the color of the alga such as chlorophyll *a*, phycobilin, and carotenoids (Table 1). At low irradiances, the alga produced a dark-red violet color, possibly due to high concentrations of the photosynthetic pigment, phycoerythrin. In contrast, at high irradiances, the alga exhibited a dull yellowish-brown color, possibly due to the decrease in the phycobilin accessory pigment and chlorophyll *a* levels, and an increase in the carotenoid production [21].

Table 1. The color variation of the algae on day 0, 4, and 8 with color observations below the pictures.

Day	Irradiance (μmol photons $m^{-2}s^{-1}$)					
	0	20	45	110	180	320
0	 Transparent	 Transparent	 Transparent	 Transparent	 Transparent	 Transparent
4	 Transparent	 Dark-red	 Red	 Yellowish-brown	 Brown	 Light brown
8	 Transparent	 Dark-red	 Red	 Brown	 Brown	 Greenish-brown

The increase in the amount of carotenoids in the alga at high irradiances is necessary to protect cyanobacteria from photoinhibition. Carotenoids absorb excessive light energy that would damage the chlorophyll molecules. Excess absorption of irradiation of the cyanobacterium can lead to the formation of harmful reactive oxygen molecules through interaction with oxygen which would result in further damage of the photosystem II from photoinhibition [22]. These observations indicate that exposure to higher irradiances is closely linked to marked changes in the morphology of an alga.

A similar study by Mandal et al. [20] showed that *L. majuscula* under long exposure to high UV-B radiation formed yellowish sheaths. The alga under the negative control (0 μmol photons $m^{-2}s^{-1}$) experienced a color change from light red to transparent, showing no growth due to the inability of cyanobacterium to photosynthesize under the absence of light. These indicate that the alga exhibits color changes depending on the irradiance level.

Data Analysis. The dry weight and SGR of each of the treatments during the exponential phase were compared and analyzed. A significant difference existed among the groups at 95% confidence interval. After conducting Planned Comparisons, it was found that a significant difference exists between 20 μmol photons $m^{-2}s^{-1}$ and all other treatments, and 45 μmol photons $m^{-2}s^{-1}$ and all other treatments. However, no significant difference was found between the two treatments. Moreover, the treatments 110, 180, and 320 μmol photons $m^{-2}s^{-1}$ also showed no significant difference with each other and the negative control.

Table 2. The dry weight obtained at each irradiance level during the exponential phase.

Irradiance	Dry Weight (mg/mL)	
(μmol photons $m^{-2}s^{-1}$)	Day 0	Day 4
0	0.018 ± 0.005	0.024 ± 0.003[b]
20	0.018 ± 0.005	0.10 ± 0.04[a]
45	0.018 ± 0.005	0.08 ± 0.02[a]
110	0.018 ± 0.005	0.07 ± 0.04[b]
180	0.018 ± 0.005	0.04 ± 0.01[b]
320	0.018 ± 0.005	0.039 ± 0.003[b]

*where a and b indicate the groupings based on their significant differences.

Algal Dry Weight. Noticeable increase in average dry weight from Day 0 to Day 4 can be observed in each treatment as seen in Table 2. The highest biomass of *L. majuscula* during the exponential phase was observed under 20 μmol photons $m^{-2}s^{-1}$ among all treatments. The alga under 45 μmol photons $m^{-2}s^{-1}$ exhibited the second highest biomass. Previous studies have also identified both irradiance values as optimum algal growth values [6,22,23].

Specific Growth Rate. The alga under the treatment of 20 µmol photons $m^{-2}s^{-1}$ exhibited the highest SGR of 42.3 % d^{-1} during the exponential phase, while the alga under 45 µmol photons $m^{-2}s^{-1}$ exhibited the second highest SGR of 36.9% d^{-1} as shown in Figure 1. Minimal growth rate was also observed in treatments under 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ (Figure 1).

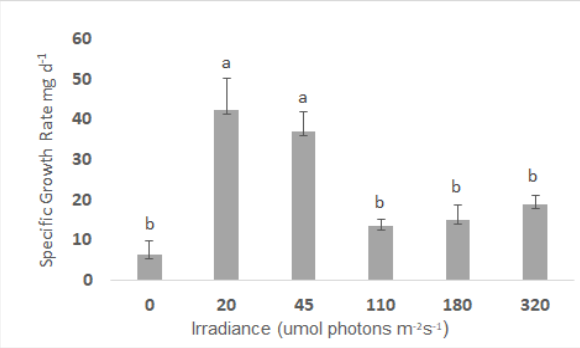


Figure 1. The Specific Growth Rate (SGR) of *L. majuscula* during the exponential phase (Day 0 to Day 4), where a and b indicate the groupings in which they are significantly different using Planned Comparisons after One-Way ANOVA ($p<0.05$).

pH and Temperature. Differences in irradiance caused small changes in the temperature and pH of each setup as shown in Figure 2.

As the irradiance increased, the temperature also increased. The excess energy formed from the reaction during the absorption of chlorophyll *a* photons is turned into heat; thus, the higher the irradiance, the more heat is transferred. According to Ras et al. [24], increasing temperature, above optimal conditions, in the outdoor production of algae may result in the decrease in the growth of the alga.

As shown in Figure 2, the pH in each setup was ≈ 8 which indicated growth and efficient CO_2 retention of the algae. A pH level closer to 8 corresponds to normal CO_2 concentration in a saltwater environment [25].

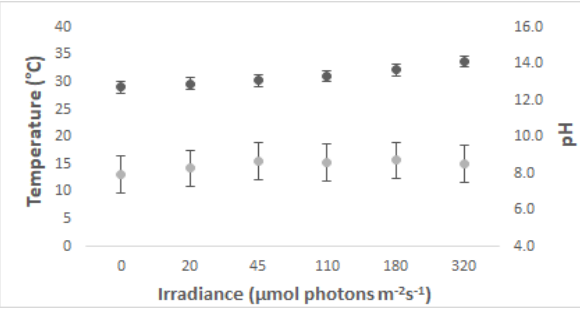


Figure 2. The average temperature and pH obtained in each treatment for 8 days of culture.

Effect of irradiance on growth. Individual dry weights and specific growth rates of the alga were significantly affected by the irradiance they were exposed to. For both 20 and 45 µmol photons $m^{-2}s^{-1}$, the alga exhibited higher dry weight and SGR values than the other treatments. This indicates that *L. majuscula* prefers low irradiances possibly because of the low maintenance rate of cyanobacteria - requiring only little energy to maintain their cell structure and function. Van Liere & Mur [9] compared the maintenance energy requirements in *Oscillatoria agardhii*, a cyanobacteria, with those of eukaryotes [22]. It was shown that maintenance requirements in cyanobacteria are much smaller than that of eukaryotes under limiting light. In addition, cyanobacteria at low irradiances have the capability to broaden the overall absorption band in order to balance the two antenna pigments responsible for their photosynthesis [6]. A study conducted by Yin et al. [26] on *Lyngbya wollei*, a close relative of *L. majuscula*, exhibited optimum growth at 22 µmol photons $m^{-2}s^{-1}$. Similarly, in a study conducted by Zhang et al. [12] on *Lyngbya kuetzingii,* the alga had its optimum growth under 20 µmol photons $m^{-2}s^{-1}$ while the study on *Lyngbya stagnina* by Jindal et al. [11] resulted in the highest exopolysaccharides and protein production under 45 µmol photons $m^{-2}s^{-1}$ in continuous light [11,12].

The minimal growth exhibited at irradiance levels of 110, 180, and 320 µmol photons $m^{-2}s^{-1}$ indicates that higher irradiances limit the growth of the alga. This is a minimizing response of the alga to the effects of photoinhibition caused by the excessive formation of Reactive Oxygen Species (ROS) [26]. Consequently, the results in the data analysis showed that the SGR values at high irradiances do not have a significant difference with each other and the negative control due to the cyanobacterium not being able to grow in the absence and at high light intensities.

Limitations. Due to time constraints, sampling was done only on days 0, 4, and 8; hence, daily growth rate of the alga was not assessed. No repetitions of the experiment were also made to further narrow down the irradiance values.

Conclusion. - *L. majuscula* exhibited maximum growth at 20 µmol photons $m^{-2}s^{-1}$ (29.7 °C, 8.28 pH) and 45 µmol photons $m^{-2}s^{-1}$ (30.25 °C, 8.67 pH). It can therefore be concluded that the species prefers low irradiance to maximize its growth. High irradiance, on the other hand, limits its growth.

Recommendations. - Information in this paper can be used as a basis for future studies in determining the irradiance that will result in the maximum growth of *L. majuscula*. The researchers recommend further studies between the irradiance levels of 20 µmol photons $m^{-2}s^{-1}$ and 45 µmol photons $m^{-2}s^{-1}$, with equal intervals, to exactly identify the most ideal irradiance level of maximum growth of the alga. Lastly, the researchers would also like to recommend daily sampling to determine the daily growth rate of the alga which is necessary to model a cyanobacterial growth curve.

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