The Impact of Varied Wavelengths of Light on the Growth Rate of *Chlamydomonas reinhardtii*

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Abstract

Light is integral for the growth of photosynthetic organisms. Specifically, photosynthesis occurs under a range of light wavelengths. *Chlamydomonas reinhardtii* is a microscopic algae which may act as a model organism for photosynthetic processes. *C. reinhardtii* was grown under blue, red, and white light to observe under which it grew most efficiently. We hypothesized that *C. reinhardtii* would grow best under white light, as white light contains all wavelengths of light under which photosynthesis occurs. Contrary to our predictions, *C. reinhardtii* grew best in red light. Performing the one-way ANOVA test produced a p-value of 0.1777, indicating that at a significance level of $\alpha = 0.05$, the difference in growth rates between the groups were insignificant. This study has important environmental significance, as microalgae make up a large part of their respective ecosystems and contribute to many biological cycles.

Introduction

Light is one of the factors required for the growth of photosynthetic organisms. The range of light under which photosynthesis occurs includes that of visible light between 380 nm and 750 nm (Carvalho et al., 2010). Above this range, the light wavelengths do not carry sufficient energy for the photosynthetic process, and below, the wavelengths have the tendency to ionize (Carvalho et al., 2010). Previous studies have observed the mixture of specific light wavelengths on the growth of photosynthetic organisms such as algae (Li et al., 2021). Other studies have focused on growth of photosynthetic species in response to differing light intensities (Bonente et al., 2012).

Microalgae are often used to study the effects of abiotic factors on metabolic processes as they have short generation periods and respond quickly to treatment conditionals (Hu et al., 2014). Specifically, *Chlamydomonas reinhardtii* is greatly representative of other species in regards to photosynthetic processes, and many studies have been conducted on *C. reinhardtii* because of this feature (Beel et al., 2012). As such, *C. reinhardtii* serves as a model for study of photosynthesis (Merchant et al., 2007). *C. reinhardtii* has two flagella and an eyespot, both of which aid the microalgae in seeking out the most desirable light levels and other environmental conditions (Choudhary et al., 2019). Additionally, *C. reinhardtii* is integral to its respective ecosystem, serving important roles in primary production, and acts as a major food source to species such as salmon (Muñoz et al., 2020; Norambuena et al., 2015). Furthermore, both salmon and microalgae, such as *C. reinhardtii*, are involved in the cycling of nutrients within their environment (Muñoz et al., 2020). Due to these roles in the maintenance of the health of ecosystems, it is important to understand the optimal growth conditions of *C. reinhardtii* so as to monitor the status of these systems over time.

Our study examines the growth rate of *C. reinhardtii* under different wavelengths of light to determine the optimal light colour under which it has the fastest growth rate. Over a period of around two weeks, *C. reinhardtii* was grown in blue (~450 nm), red (~700 nm), and white (~380-750 nm) light (Carvalho et al., 2010). Because white light contains all the wavelengths of light and thus the entire range under which photosynthesis occurs, we predict that *C. reinhardtii* grows best in white light (David & Whitehead, 2018). Our null and alternative hypotheses are:

H_O: There is no difference in growth rate between light wavelength conditions

H_A: The cells treated with white light had a greater growth rate than other light conditions.

Methods

For the duration that *C. reinhardtii* was handled, a sterilized environment was maintained by all group members through the use of lab gloves and disinfectant. When glassware was handled, an ethanol flame was used to sterilize rims to prevent contamination of the samples and cultures.

An initial stock solution of *C. reinhardtii* was used, which had an unknown concentration. To determine this concentration, 100 µL of the initial *C. reinhardtii* solution and 10 µL of IKI fixative solution were placed and mixed in an Eppendorf tube. 10 µL of the initial fixative-cell mixture sample was drawn and dispensed onto a hemocytometer and examined with a compound microscope. Initial cell concentration was counted using the grid guide on the hemocytometer, which represents area. Three replicas of the initial solution were counted to obtain an average and reduce error. The counting process was conducted by multiple group members to further ensure accuracy. To calculate the concentration of the *C. reinhardtii* stock solution, Equation 1 was used:

Equation 1: Concentration (Cell density/mL) =

(# cells counted/ # number of squares)(dilution factor of square)*1.1*

where the “squares” refers to the grid guide found on the hemocytometer used in the counting process.

A volume of 200 mL diluted solution was desired for experimentation. The dilution volumes were calculated with the initial concentration of the solution containing *C. reinhardtii* using Equation 2:

Equation 2: C1V1=C2V2

Using our calculations, 1.881 mL of *C. reinhardtii* solution and 58.199 mL of medium were mixed for a final volume of 60 mL with a concentration of 1×10^5 cell/mL. Once the solution was diluted, it was divided into nine test tubes of 6 mL of solution each.

C. reinhardtii was grown under three different light conditions: white light as a control, red light, and blue light. Each treatment was replicated three times, for a total of nine test tubes (Fig. 1). The test tubes specific to each treatment were wrapped in a material so that the sample would only grow under a specific wavelength of light. The test tubes of the white light group were wrapped in cheesecloth, while the red and blue treatment groups were wrapped in translucent coloured plastics. To allow the samples to grow under similar light intensities, the samples were wrapped so as to permit only a range of around 10 - 20 lux through to the sample for growth.

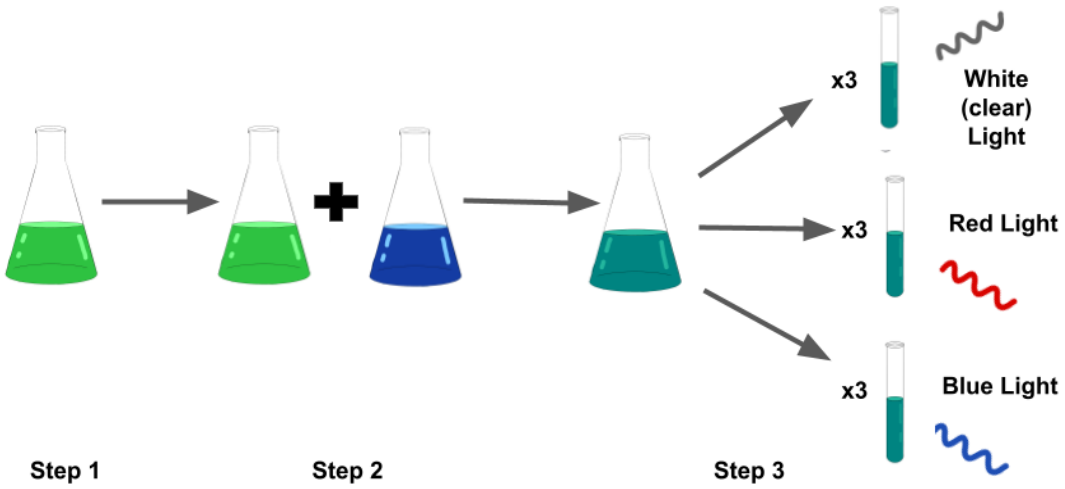

Figure 1: diagram describing dilution of *C. reinhardtii* stock solution into sample test tubes. Step 1: the concentration of the initial stock solution was determined. Step 2: 1.881 mL of the stock solution was mixed with 58.199 mL of growth medium to obtain 60 mL of 1 x 10^5 cell/mL solution. Step 3: the solution was distributed into nine test tubes of 6 mL and divided into white, red, and blue light treatment groups.

The samples were allowed to grow in an incubator at 25°C. The incubator was equipped with a light and the samples were placed in glass beakers and set an equal distance away from it. The fixed samples drawn for cell counting were stored in a refrigerator, and organized based on the day of sampling, colour, and replicate number.

Samples were drawn on days 3, 5, 8, 10, 12, 15, and 16 for a total of seven sampling days over a period of about two weeks. The same method was used as with the initial sample: 100 µL of sample solution was mixed with 10 µL of IKI fixative and stored in the refrigerator. At the end of the sampling period on day 16, the fixed samples were counted using the same methods as was used when determining the initial stock concentration of *C. reinhardtii*. Once the data was collected, growth rates of the samples were compared using statistical analysis through an ANOVA test.

Results

The study was done in order to observe the growth trends of *C. reinhardtii* amongst the three treatment groups of differing wavelengths. The initial cell samples in the stock solution that were counted were 150, 148, and 136. This gave us an average of 145 cells in a 0.05 x 0.05 mm area on the hemocytometer. Using this average, we found that the initial concentration of stock solution was 3.19 x 10^6 cells/mL. The average concentration of *C.reinhardtii* cells in each treatment group was plotted on a growth curve as a function of time in days (Fig. 2).

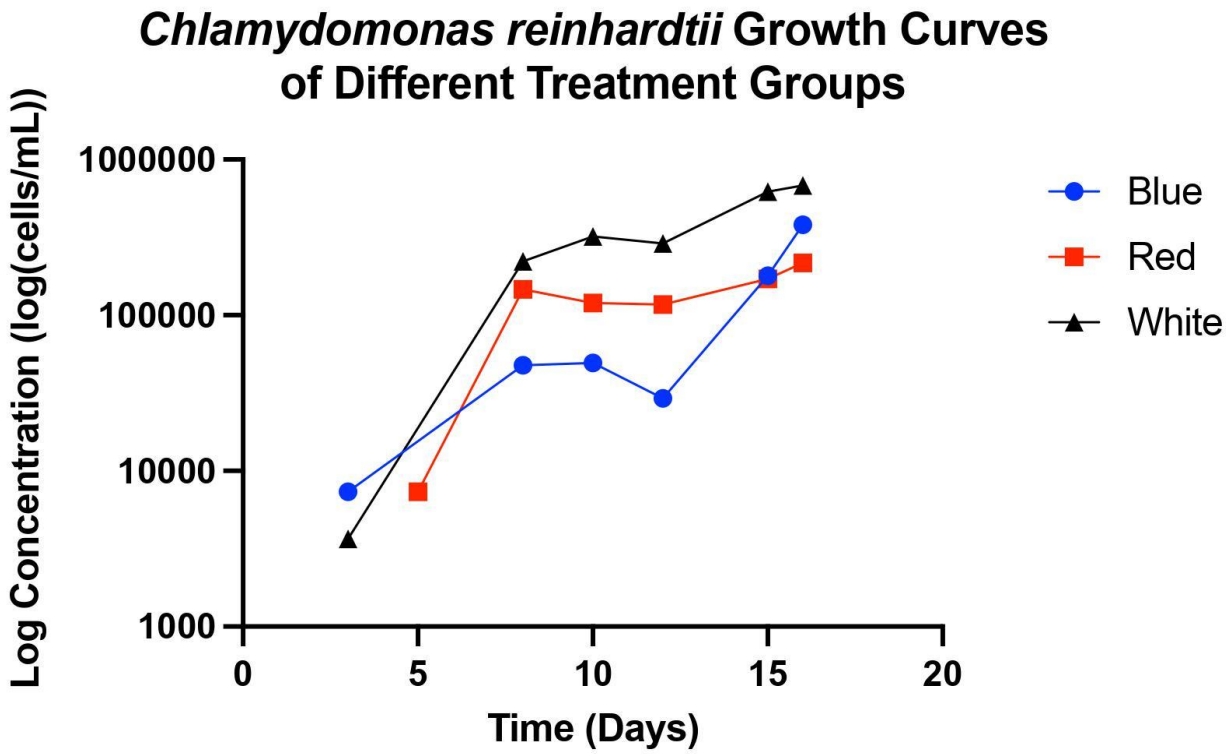

Figure 2: *C. reinhardtii* growth curves of the three treatment groups with n = 3 over a 16 day period. The y-axis corresponds to the cell concentration per milliliter. Each point is an average of the cell counts of all three replicates for each of the three treatment groups, conducted by various group members.

Based on these growth curves a few observations can be made. The red treatment group does not have a point on day 3 due to the cell count of all three replicates being zero. This is the same reason that the white and blue treatment groups do not have a point on day 5. These results could be due to counting and sampling errors which are discussed later on. Although the white treatment had the least amount of cells on day 3, it ended up having the most on day 16. The growth rates of the red and white treatment groups from day 3 to day 8 are similar, whereas the blue treatment group seemed to have gone through exponential growth on days 12 to 15. On day 16, the cell concentration of all 3 treatment groups yield similar values.

The growth curve is useful in observation of the overall trends in the response of growth rate of *C. reinhardtii* to different light wavelengths over the course of the experiment. However, in order to determine if the differences amongst the three treatment groups were significant, an ANOVA test was performed on the log of the cell concentrations over the period of 16 days. A graph was produced in order to showcase the growth rate of each of the three treatment groups (Fig. 3). The data points highlight the mean and the error bars correspond to the respective standard deviations of each group. The graph showcases the similarities present in the growth rate, such as the means, which are shown to be within 0.0378 units of each other. The mean growth rate of the blue treatment group is 0.1051 (cells/mL)/day with a standard deviation of 0.0141 (cells/mL)/day, the mean growth rate of the red treatment group is 0.1290 (cells/mL)/day with a standard deviation of 0.0097(cells/mL)/day, and the mean growth rate of the white treatment group is 0.0912(cells/mL)/day with a standard deviation of 0.0334 (cells/mL)/day. The one-way ANOVA test between the three treatment groups reveals

an F-value of 2.336, and a p-value of 0.1777. Since the test revealed that at a significance level of α = 0.05, the differences in growth rates among the three different groups were insignificant, no further statistical tests were conducted.

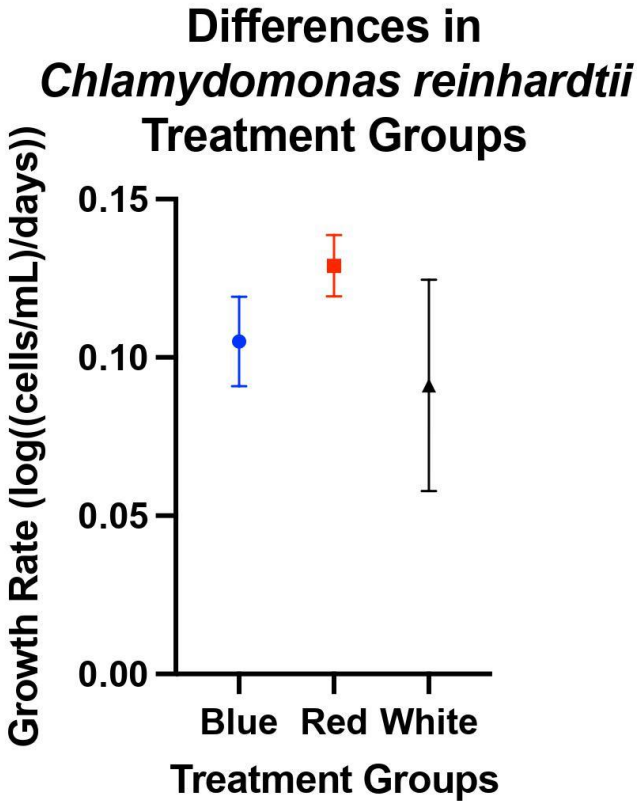

Figure 3: Differences in the log mean growth rate of *C. reinhardtii* between the three treatment groups. (n=3, F-value= 2.336, p-value=0.1777). The differences were not significantly different as alpha was set to 0.05.

Discussion

Using a one-way ANOVA test between the means of the growth rates of the light treatments, we found a p-value of 0.1777. Our significance level was α = 0.05, thus, we fail to reject the null hypothesis since there is no significant difference in the mean

growth rate between the three treatment groups. The nature of the similar growth rate of the white light group, compared to the other treatment groups was inconsistent with our predictions. White light is composed of all light wavelengths within the visible light range (David & Whitehead, 2018). Photosynthesis is favoured within the entirety of the visible light range, thus, the white light treatment should have contributed to more availability of favourable light wavelengths for the growth of *C. reinhardtii* (Carvalho et al., 2010). The white light produced the lowest mean growth rate, followed by the blue treatment group and lastly the red treatment group which produced the greatest mean growth rate. This also goes against prior literature on *C.reinhardtii* as blue light and red light have been known to decrease photosynthetic efficiency and are suboptimal for cultivation (Mooij et al., 2016).

The experimentation process may have contributed to some sources of error, which could have impacted the results of the study. One such error could be a result of differences between the cell counting process of the researchers conducting the study, as instances including clustering of *C. reinhardtii* cells were not uncommon. As this method is subject to individual bias, it is possible that our results could have been influenced. Another error that occurred during the counting process was the inconsistent method of mixing the samples. This may have led to the clusters that formed that were previously discussed. Furthermore, as a group, no initial counts of the test tubes were taken on day 1 of the experiment. The initial concentration of the stock that was then diluted was recorded but not the initial count of each of the 9 test tubes. This could be a major reason why the results of our study did not correspond to prior literature which led

us to reject the null hypothesis, which was that the impact of light does not have a difference on the growth rate of *C. reinhardtii*.

There was some difficulty in counting the cells and after observing prior literature, this could be due to the placement of our cells in the incubator (Mooij et al., 2016). All test tubes were placed in the same spot, directly under the light source for the entirety of the 16-day study. One study notes that under bright light, microalgae absorb more light energy than can be converted into light energy (Mooij et al., 2016). This could be one of the reasons for the low cell counts recorded, as C. reinhardtii performs best under soft, low light conditions (Mooij et al., 2016). This would make sense as to why the white light had the greatest standard deviation amongst the treatment groups as well, as it is possible that more light was able to pass through the cheesecloth, despite efforts to limit this.

Alongside sources of error, there are some limitations within this study that could have impacted the results. The major limitation in interpreting our data is the nature of the sample size used. This experiment used a small sample size, which contributes negatively to the assumptions of the ANOVA test. This condition could have impacted the results of the ANOVA test, which could lead to false inferences between treatment groups. Another limitation to our study was the characteristics of the materials used to supply specific wavelengths of light to the treatment groups. Cheesecloth was used in the white light group to attempt to replicate the muted light intensity that was present in the coloured plastic sheets, however, there is a possibility that the light intensity was nevertheless different from the other treatment groups. Should this study be repeated, a larger sample size paired with one researcher counting the *C. reinhardtii* cells and a

consistent material for wavelength restriction should be used to obtain the most accurate results. A measurement for the respective lux in each test tube should also be noted so that the only variable present in the study is the specific wavelength of light to which the *C. reinhardtii* cells are exposed.

Conclusion

In this study we looked at the effects that different light wavelengths had on the growth rate of *Chlamydomonas reinhardtii.* Three treatment groups were chosen, which were white, blue and red light conditions, with three replicates each. Samples were taken between days 3 and 16 of the experiment, after which the cells were counted. An ANOVA test produced a p-value of 0.1777. At a significance level of $\alpha = 0.05$, we can conclude that there was no significant difference in the growth rates of the three treatment groups. This study failed to reject the null hypothesis that the difference of light wavelength has no effect on the growth rate of the microalgae *C. reinhardtii*. This was against our predictions that the white light treatment group would have the greatest growth rate. The white light treatment group had the lowest growth rate, while the red light treatment had the greatest growth rate. Further research should examine the growth rate of *C. reinhardtii* over a longer period of time with a greater sample size and recording placement of replicates in an incubator. With more accurate growth curves, better inferences on the growth rate of *C. reinhardtii* can be made between the different light treatment groups.

Acknowledgments

We recognize that we are on the ancestral, traditional, and unceded territory of the Musqueam people. We would like to thank Dr. Celeste Leander, Tessa Blanchard, Melanie Warren, Mindy Chow for their assistance and advice on this project. We would also like to thank the University of British Columbia for allowing us to conduct this research.

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