Different Light Wavelengths and Oxygen Production in *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a green alga widely distributed in soil and freshwater systems that serves as a source of food and oxygen for salmon species. Our study examined the effect of different light wavelengths on *C. reinhardtii* oxygen production from photosynthetic activity. These different light wavelengths consisted of white light (control), red light, blue light, and green light that were shone on six vials of *C. reinhardtii* culture. It was predicted that white light would yield the highest concentration of oxygen while red light would produce the lowest concentration. These six vials were subjected to similar luminance and temperature conditions which were measured for oxygen concentration with a dissolved-oxygen probe connected to a TI-84 calculator. A one-way ANOVA test comparing oxygen production between different light treatment groups revealed there was a significant difference between the white light treatment group and the red, blue and green treatment groups. The test also revealed there was a significant difference between red and blue, red and green, and blue and green light oxygen production based on Tukey's multiple comparison test. The highest oxygen production was measured from the green light treatment group, while the lowest oxygen production was found in the red light treatment group.

Introduction

Chlamydomonas reinhardtii is a unicellular green algae that plays an integral role in the ecosystem. It is one of the most common autotrophs in freshwater systems around the world, providing oxygen to a plethora of organisms (Merchant et al. 2007). Furthermore, it was discovered that it is a main source of food for salmon, as ingesting small amounts of algae results in positive effects on fish growth performance (Norambuena et al., 2015). This is essential because salmon conservation is one of the main focuses for improvement in freshwater ecosystems. Salmon are considered a keystone species as it provides food security for wildlife and humans, especially for Indigenous communities (Nesbitt & Moore, 2016). Moreover, salmon spawning influences the composition, structure and functioning of freshwater systems during migration (Walsh et al., 2020). As oxygen concentration is a significant factor determining where

salmon spawn (Fellman et al., 2018), it is imperative to assess how oxygen production is affected in freshwater autotrophs, such as *C. reinhardtii*.

C. reinhardtii is a photoautotroph, which means that it undergoes photosynthesis to generate energy and produce oxygen. Photosynthesis is a process in which organisms like *C. reinhardtii* absorb light from a source and convert it into electrochemical energy that non-autotrophic organisms can use (Minagawa & Tokutsu, 2015). As a result of photosynthesis, oxygen is also released into the atmosphere that can be used for cellular respiration. As oxygen production by *C. reinhardtii* is dependent on light, it is productive to observe whether altering this variable would produce changes in oxygen production. It was found that the proteins required for photosynthesis in *C. reinhardtii* are activated depending on different wavelengths of light (de Mooij et al., 2016). This suggests that in order to produce more oxygen, we should look into which wavelength of light photosynthesis would be most efficient in *C. reinhardtii.*

This research was conducted to assess whether different wavelengths of light would result in different levels of oxygen production by *C. reinhardtii.* Our hypothesis was created based on results from previous studies. We hypothesized that white light would produce the highest oxygen concentration as it contains all of the wavelengths. According to a previous study, this variety in wavelengths would activate all of the proteins required for efficient photosynthesis in *C. reinhardtii* (de Mooij et al., 2016). Next highest yield of oxygen would be green light, as under strong white light it was found to penetrate deeper into photoautotrophic organisms than red or blue light, resulting in a more effective photosynthesis (Terashima et al., 2009). Finally, red light would produce the least amount of oxygen, as previous studies discovered that *C. reinhardtii* is more sensitive to blue light than red light (Oldenhof et al., 2006).

Methods

Our data was gathered by measuring oxygen concentrations produced by the organism *Chlamydomonas reinhardtii*. This culture was prepared by Mindy Chow, a laboratory technician at the University of British Columbia, to optimal conditions according to the "Chlamydomonas Maintenance" document. To create the master stock of *C. reinhardtii* as depicted in **Figure 1**, 200 mL of excess media was added to 1000 mL of culture creating a 5:1 dilution. This dilution was made to ensure that all of the 30 vials used in this experiment will be completely filled with the master stock, allowing each vial to have a relatively uniform concentration of *C. reinhardtii* culture. This master stock was used in all treatments.

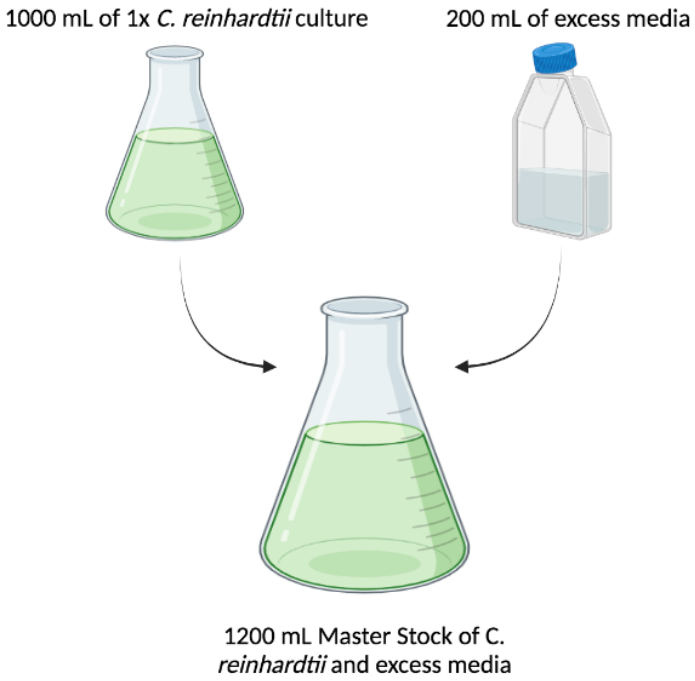

Figure 1. Creation of the *C. reinhardtii* Master Stock. This diagram depicts the amount of *C. reinhardtii* culture and excess media required to create the 5:1 dilution of the master stock. The final 1200 mL volume of master stock was sufficient in filling up all the treatment vials used in the experiment.

The initial O_2 concentration was then measured using samples without any treatment. This was conducted by pouring the master stock from the flask into six small vials that were immediately sealed to prevent fluctuations in O_2 concentration. Next, each vial was opened and the initial O_2 concentration was individually measured using a dissolved-oxygen probe connected to a TI-84 graphing calculator. These test vials were exclusively used to obtain the initial O_2 concentration. They were discarded after measurement since the produced O_2 has already been released into the atmosphere.

Treatment Set-up:

Four treatments of water baths were created and placed under white lamps as the light source. As seen in **Figure 2**, only three out of the four treatments were placed under a lamp as the regular light intensity of the room was measured to be 600 lux. This was used for the white light treatment, which was the control group. The light intensity of the other three lamps were then measured with the Lux mobile app to maintain a light intensity of 600 lux across all treatments, which was adjusted by moving the lamp closer to the water baths. To obtain a constant temperature across all four water baths, an Anova sous vide was connected to each bath to maintain the temperature to 25.5°C (**Figure 2**). If the temperature fluctuated, it was easily controlled by adjusting the settings on the sous vide to slightly heat up the baths to the same temperature. To prepare the light treatments, red, blue and green acetates were placed under three of the lamps to produce three treatment water baths. For the control group, clear acetate was used to keep the environment similar to the other treatments. This experimental set-up is demonstrated in **Figure 2**.

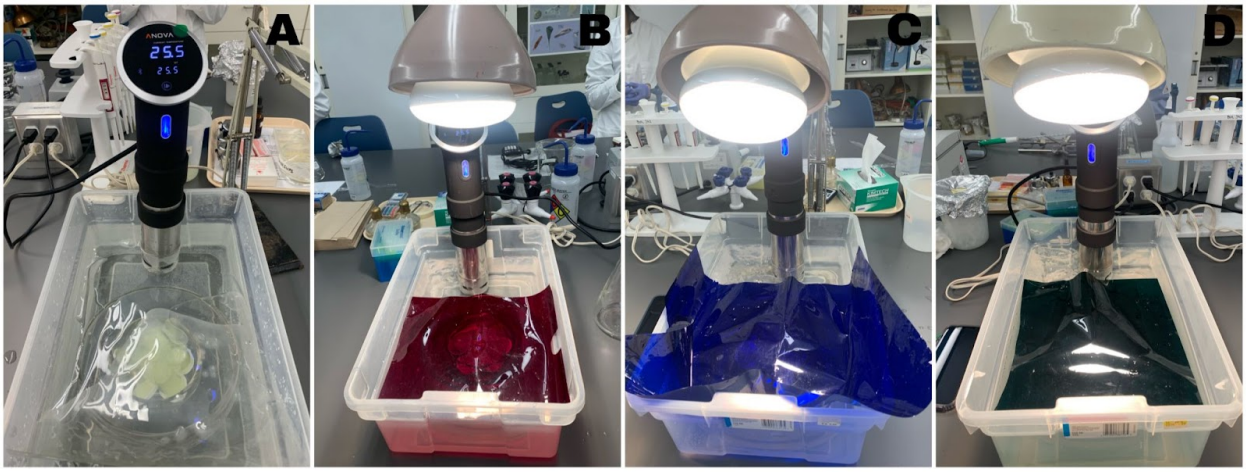

Figure 2. The Experimental Set-up of the Water Bath Treatments. Each bath was connected to an Anova sous vide to maintain a constant temperature. (A) The control group used ceiling light as the light source and had clear acetate. (B, C and D) Different coloured acetates were placed under white lamps with respect to each separate treatment.

After setting up the water baths, the remaining *C. reinhardtii* master stock was separated into 24 plastic vials, holding about 26 mL each. The vials were filled to maximum capacity and promptly sealed to prevent air bubbles and gas exchange with the atmosphere. Subsequently, in 5-minute intervals, six vials were placed in each of the four water baths to ensure O_2 concentration was obtained immediately after time elapsed. Each treatment was given 75 minutes to produce oxygen (**Figure 3**).

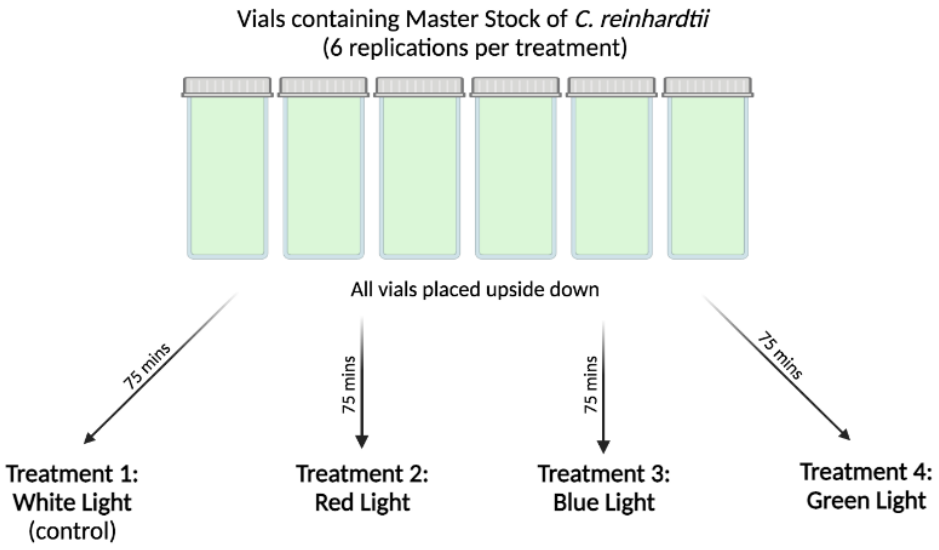

Figure 3. Experimental Setting for the Treatment and Control Groups. This diagram depicts the conditions of each treatment: white, red, blue, and green light. Each treatment consisted of 6 vials (n=6) and proceeded for 75 minutes. All vials were placed upside down to allow the light source to reach the *C. reinhardtii* cultures.

After the vials were taken out of the water baths in the order that they were placed, the O_2 concentrations were immediately recorded. The data obtained was then used for statistical analysis. The mean oxygen concentration was obtained for each treatment along with the standard deviations. This was demonstrated by using a box-plot to compare how different wavelengths of light affect oxygen production of *C. reinhardtii*. Next, we conducted a one-way Analysis of Variance (ANOVA) test to compare the means of four different treatments. The p-value obtained from this statistical test was used to determine if the difference between the groups were statistically significant. Lastly, a Tukey's multiple pairwise comparison test was used to discover which group results were significantly different.

Results

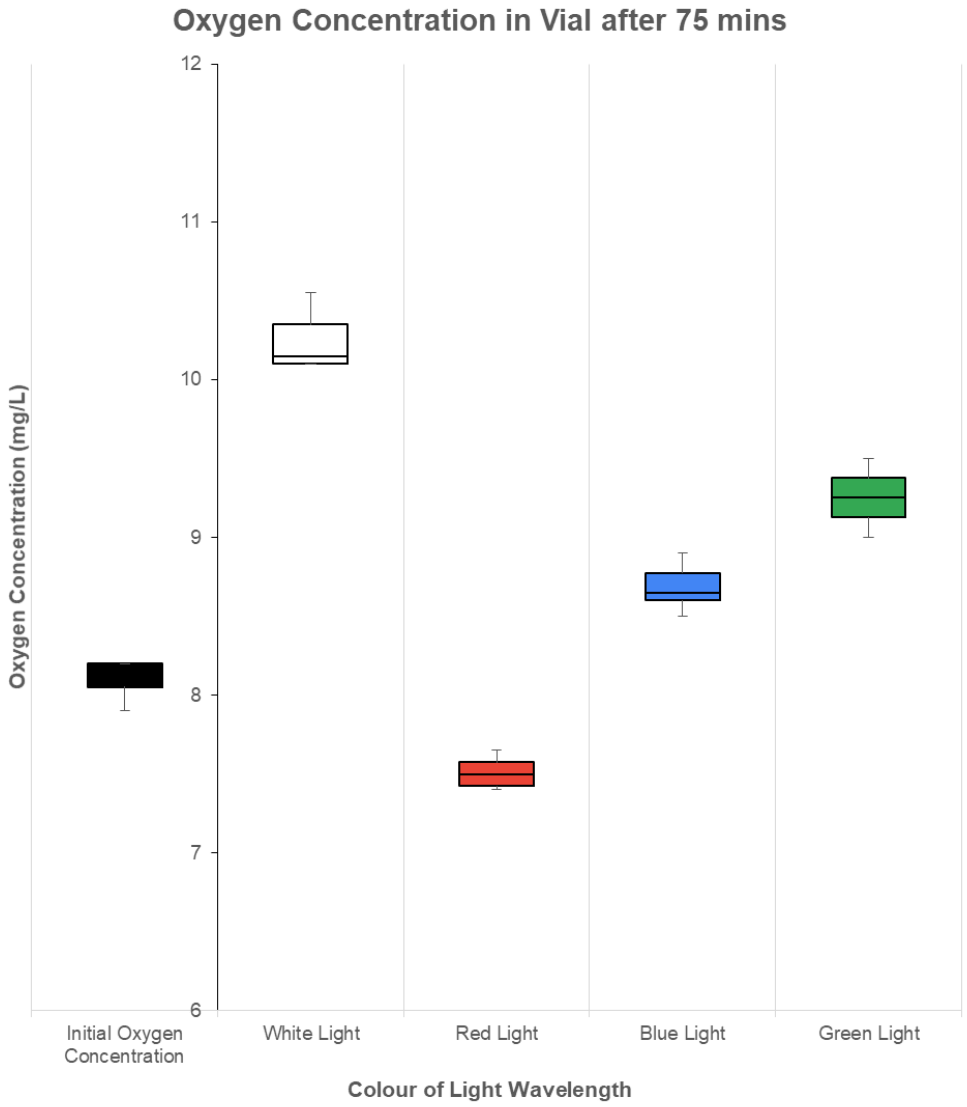

Figure 4. Boxplot displaying the effect of light wavelength on vial oxygen concentration produced by *C. reinhardtii* (n = 6). Rectangles represent the interquartile ranges within the dataset of each treatment. Upper and lower whiskers indicate the minimum and maximum points within each treatment dataset respectively. Black lines bisecting each rectangle represent the median of each treatment dataset. Initial oxygen concentration is shown here for comparison. A one-way ANOVA test results in a p-value < .00001, F-value = 144.362, and n = 4.

The initial oxygen concentration of six vials were measured before four separate subsets of six vials were used in each 75 minute treatment, including the white light control (n = 6). The

mean initial oxygen concentration was measured to be 8.117 mg/L with a standard deviation of 0.133. After each treatment was completed, the largest mean oxygen concentration after 75 minutes was the white light control with a value of 10.3 mg/L and a standard deviation of 0.316 mg/L. The second highest mean oxygen concentration was produced under green light with a value of 9.267 mg/L and a standard deviation of 0.216 mg/L. The third highest mean oxygen concentration was produced under blue light with a value of 8.733 mg/L and a standard deviation of 0.250 mg/L. The fourth highest mean oxygen concentration was produced under red light with a value of 7.517 mg/L and a standard deviation of 0.117 mg/L. All treatments and the control yielded a higher mean oxygen concentration compared to the initial oxygen concentration except for the red treatment, which had reduced mean oxygen concentration after 75 minutes.

A one way ANOVA test between the mean oxygen concentration of the control and other three treatments with an alpha level of 0.05 from *Social Science Statistics* yields a p-value of < .00001 and an F-value of 144.362. This reveals a significant difference in mean oxygen concentration across all four treatment groups. A Tukey's multiple pairwise comparison test was used to determine which treatment groups were significantly different from each other. The test revealed the mean oxygen concentration between the control and all other treatment groups, red and blue, red and green, and blue and green treatment groups were significantly different from each other ($p < 0.05$).

Discussion

The one-way ANOVA test that was conducted yielded a p-value < .00001. Our p-value being less than 0.05 enables rejection of the null hypothesis of there being no significant difference in the mean oxygen concentration across the four treatments (white, red, blue, and green light). Results from a Tukey's multiple pairwise comparison test allowed us to determine

that the difference of mean oxygen concentration between the control group and each of the other treatment groups (red and blue, red and green, and blue and green treatment groups) were statistically significant.

Of the three colour treatments, the highest mean oxygen concentration was produced by white light, followed by green light, blue light, then red light. This result came out as we had expected in the initial stages of our experiment. We hypothesized that if the oxygen production in *Chlamydomonas reinhardtii* were to be affected by light wavelength, the highest oxygen production would be yielded by white light and the lowest oxygen production would be yielded by red and blue light. This prediction was based on prior literature that presented findings that colours having weaker absorption properties, such as green and yellow light, are linked with increased higher photosynthesis efficiency (de Mooij et al., 2016). Furthermore, another study discovered evidence that green light has the ability to penetrate further into the leaves than red or blue light does beneath strong white light; the absorption of green light by chloroplasts in turn, leads to an increase in photosynthesis (Terashimi et al., 2009). This is contrary to a correlation found between decreased photosynthesis efficiency and when blue and red light were the most strongly absorbed wavelengths in the photosynthesis of *Chlamydomonas reinhardtii* (de Mooij et al., 2016).

Two unexpected results were that the oxygen concentration of the cells exposed to red light is lower than the initial temperature, and the oxygen concentration of the cells exposed to green light is the second highest. A possible reason for the oxygen concentration being lower after exposure to red light is that the cells consume more oxygen than they are producing. The cultures exposed to red light produce less oxygen than the rest of the treatments, consuming more oxygen for cellular respiration. For the results of the cells exposed to green light, we

initially predicted that this would have the lowest oxygen concentration. This is because green light is usually reflected by autotrophic cells and gives the organisms a green colour. However, the green light treatment group had the second highest oxygen production, which is in tune with our revised hypothesis after reading an external source by Terashimi et al. (2009). Another limitation to our study could stem from the measurement for initial oxygen concentration. Only six vials were used for the initial oxygen concentration which were discarded after use. Therefore, there could have been differences between the initial oxygen concentration measured and the actual initial oxygen concentration of the vials exposed to the light treatments.

Conclusion

Chlamydomonas reinhardtii plays an essential role in aquatic ecosystems. The results show that each wavelength of light has a different impact on oxygen production by *Chlamydomonas reinhardtii.* Exposure to white light produces the highest water oxygen content, followed by green light, blue light and lastly, red light. Further studies of the factors that influence oxygen production and other *Chlamydomonas reinhardtii* behaviours allow for a better understanding of aquatic ecosystems, from the smallest organism upwards. This experiment allows us to understand the optimal light conditions for oxygen production in *C. reinhardtii* which is beneficial for the development of freshwater ecosystems and salmon conservation.

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