The Slow Growth of *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a green unicellular algae that plays a significant role in the aquatic food chain and in particular, as a food source for many salmon species. *C. reinhardtii* is a unique organism that can grow through both photosynthesis as well as heterotrophic means by absorbing nutrients through their cell surface. Recent studies have used *C. reinhardtii* in fixing CO_2 due to their rapid photosynthetic capabilities. The focus of our study was to determine how different light wavelengths influence the growth patterns of *C. reinhardtii* for use in green technology. In particular, three light wavelengths were used to examine the growth rate of *C. reinhardtii*. The control used for this study was a white light treatment which was compared against the red and blue light treatments. Red light has a longer wavelength compared to blue light, whereas white light comprises all the wavelengths in the visible spectrum. This study was conducted over a 10 day growth period, where samples were collected every two to three days for a total of four sampling days. A hemocytometer was used to conduct the cell counts over the duration of this study and these values were used to determine the growth rate of *C. reinhardtii*. A one-way ANOVA test was used to analyze the overall growth rates of *C. reinhardtii* to determine if there were any significant differences in the growth rate between treatments. Growth rates for the white light, red light, and blue light treatment yielded averages of 0.0114 [cells/mL]/day, 0.1793 [cells/mL]/day, and 0.1047 [cells/mL]/day respectively. The statistical analysis yielded a p-value greater than 0.05, indicating that there was no difference found between the growth rates of each treatment. Despite our non-significant findings, further research in this field should be pursued for benefiting future green technology.

Introduction

Algae are responsible for fixing almost 60% of the earth's CO_2 through CO_2 concentrating mechanisms including biochemical and biophysical mechanisms which help transport CO_2 to maximize photosynthesis (Yang et al., 2021; Singh & Dhar, 2019; Giordano, Beardall & Raven, 2005). With the growing concern of climate change, recent studies in green technology have considered using algae to fix CO_2 through photosynthesis to reduce carbon

emissions (Paul et al., 2019). *Chlamydomonas reinhardtii*, a green unicellular algae, has been a model used to study photosynthesis for over 40 years, due to a combination of their similarity to vascular plants in photosynthetic function and their rapid growth rate (Hanikenne, 2003; Dent, Han & Niyogi, 2001; Rochaix, 2001). To further evaluate the efficacy of utilizing algae to reduce carbon emissions, it is important to understand how different light wavelengths might influence photosynthesis, and in turn, their growth rates. Ensuring sufficient biomass is crucial in considering the application for both green technology and the aquatic food chain, as *C. reinhardtii* contributes a significant role in supporting many fish, including Atlantic salmon as their primary food source (Norambuena et al., 2015).

Photosynthesis is not essential for *C. reinhardtii's* survival, since they are able to grow rapidly via photoautotrophic means in the light and slowly via heterotrophic means in the dark through acetate metabolization (Yang et al., 2021; Bell, 2012, Funes, Franzén, & Halphen, 2007). However, this biflagellate organism contains a single chloroplast that constitutes 40% of its cell volume which plays a role in *C. reinhardtii's* growth rate by influencing the timing of *C. reinhardtii's* cell division, thus regulating the rate of photosynthesis (Rochaix, 2001; Vítová et al., 2011). To best apply *C. reinhardtii* in future climate change solutions, increasing growth rate is necessary to maximize efficiency. This is seen from growth under high light intensity where *C. reinhardtii* was observed to grow at a much faster rate and with greater biomass production, when compared to growth under low light intensity (Bonente et al., 2012). Additionally, different wavelengths of light can also influence the rate of photosynthesis and growth rate. In efforts to maximize photosynthesis and *C. reinhardtii* growth rates to integrate algae into climate change solutions, the aim of this study is to determine how different light wavelengths influence the rate

of growth in *C. reinhardtii* cultures. In particular, we focus on the effects of red, blue, and white light on *C. reinhardtii's* growth rate.

H_O: *Chlamydomonas reinhardtii's* growth rate is not influenced by the different wavelengths of light (red, blue and white).

H_A: *Chlamydomonas reinhardtii's* growth rate is influenced by different wavelengths of light (red, blue and white).

Previous studies on using *C. reinhardtii* as a renewable biofuel source observed the most significant accumulation in triacylglycerols under red light, followed by green light (Goncalves et al., 2016; Gaytán-Luna et al., 2016). These studies further align with the discovery of red-orange wavelengths increasing *C. reinhardtii's* cell division and decreasing cell size, while blue wavelengths showed decreasing cell division and increasing cell size (Oldenhof, Zachleder, & Van den Ende, 2006). Lastly, when the two wavelengths were mixed, *C. reinhardtii* observed faster growth rates when the blue wavelengths were reduced (Li et al., 2021). Based on these previous studies, our purpose is to determine whether *C. reinhardtii* would experience faster growth rates when exposed to red wavelengths as opposed to blue and white wavelengths.

Methods

Culture Tube Preparation (Day 0)

An initial count of the *C. reinhardtii* stock was performed using a hemocytometer in order to determine the initial concentration. The *C. reinhardtii* culture was diluted with an autotrophic *C. reinhardtii* medium in a 250 mL flask so that 1.0×10^5 cells/mL were present. All surfaces were cleaned with ethanol prior to transferring the stock to 15 mL tubes. A total of three different light treatments were used: red (R), blue (B) and white light (C), which acted as the

control. Three replicates for each treatment (red, blue, and white light) were prepared in 15 mL tubes, which were wrapped with either red acetate film, blue acetate film, or cheese cloth to obtain a desired light intensity of approximately 16 flux. The tops and bottoms of the tube were left unwrapped as each end was covered with an opaque covering which would prevent light penetration from occurring. Once all the test tubes were prepared, 10 mL of the diluted *C. reinhardtii* solution were transferred to each test tube. An open flame was used throughout the process of transferring the stock to maintain sterile conditions.

Incubation

The culture tubes containing 10 mL of the Chlamydomonas diluted culture were put in the incubator where light intensity was set to 100 μmol $m^{-2} s^{-1}$. The tubes remained in the incubator at 25°C over a 10 day period.

Fixation and Sampling Day (Day 1/2/3/4)

Sampling was conducted every second or third day over 10 days for a total of 4 sampling days. For each sampling day, three samples from each culture tube were taken for a total of 27 sampling tubes. Each tube was labeled with their respective treatment wavelength (R/B/C), replicate number (ex: IIa where II represents which replicate, and a represents the sample) and the sampling day (1/2/3/4). To fix the cells, 10 μL of potassium iodide was added into each of the 27 sampling tubes (3 samples from each treatment). From each tube, 100 μL of *C. reinhardtii* was pipetted and transferred into the 27 sampling tubes. Once all the samples were taken, they were stored in the fridge at approximately 4°C to prevent bacterial growth. The remaining culture tubes were placed back in the incubator to allow for continuing growth. These steps were repeated for day one, two, three, and four.

Calculating Growth Rate and Statistical Analysis

Cell count was performed using a hemocytometer after the four sampling days using 10 μL of the resuspended cell mixtures. The goal of each cell count was to count approximately 100 cells from each sample within a respective chamber in the hemocytometer. After the cells were counted, cell concentrations of each replicate were calculated. Growth rate was determined using the concentration of *C. reinhardtii* from each day and a one-way ANOVA test was used to analyze the overall growth rates of *C.reinhardtii* to determine if there were any significant differences in the growth rate between treatments.

Results

Over a period of 10 growth days, the average concentration of four sampling days were taken for each treatment group. The averages of each treatment group were taken and plotted on their own individual growth plots to compare the deviations of each replicate. Slopes for each replicate in the control, red wavelength, and blue wavelength group were taken and the averages for each group were taken in order to conduct a one-way ANOVA test.

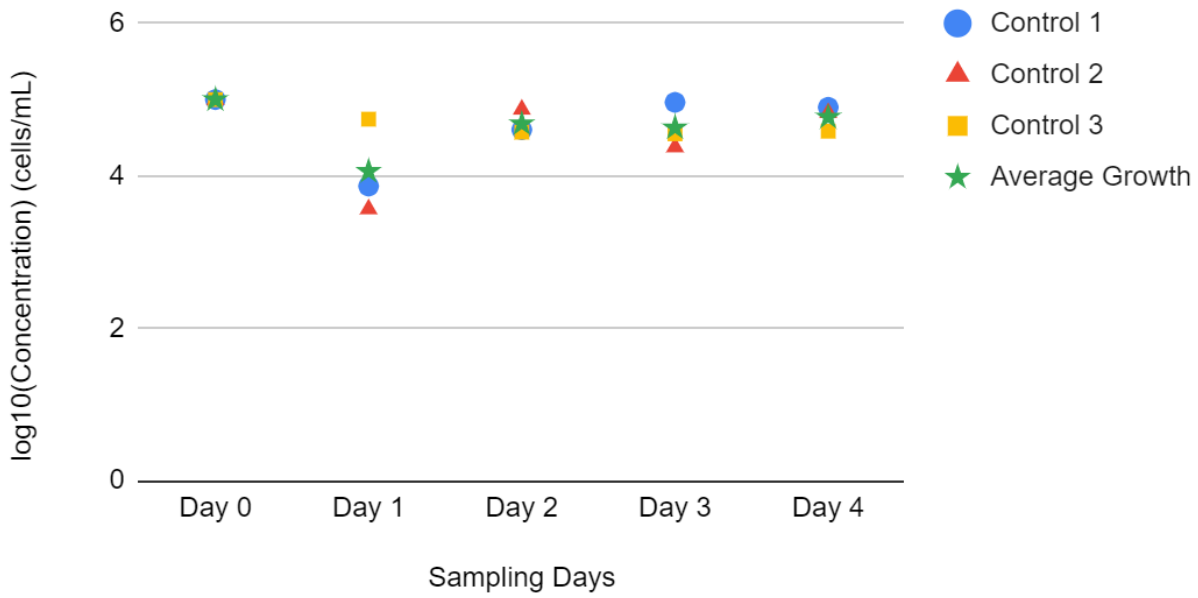

Figure 1. Concentrations for *C. reinhardtii* under natural light growing conditions. The concentrations (cells/mL) on the y axis have been converted to logarithm values to generate a straighter line. A total of three replicates (n = 3) were conducted under these conditions. The blue dots indicate replicate one, the red triangles indicate replicate two and the yellow squares indicate replicate three. The green star represents the average growth of each treatment.

From the growth plot of the control group, minimal growth can be observed based on the relatively straight line (figure 1). On day one, an observed dip can be seen compared to day zero for all replicates. After day one, we began to see growth on days two to four based on the higher concentrations. Despite this, day four yielded a lower final concentration compared to the initial concentration on day zero.

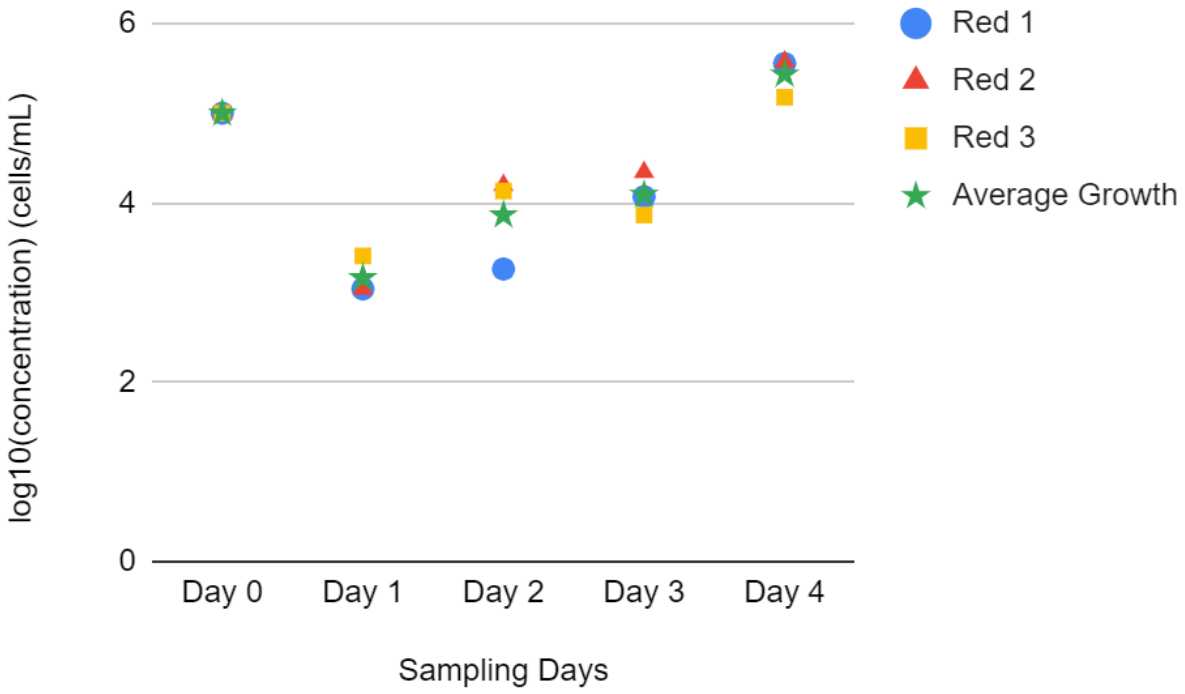

Figure 2. Concentrations for *C. reinhardtii* under red wavelength growing conditions. The concentrations (cells/mL) on the y axis have been converted to logarithm values to generate a straighter line. A total of three replicates (n = 3) were conducted under these conditions. The blue dots indicate replicate one, the red triangles indicate replicate two and the yellow squares indicate replicate three. The green star represents the average growth of each treatment.

Under red wavelength conditions, a decrease in concentration can be seen from day zero to day one, similar to the control (figure 2). After day one, gradual growth for all replicates can be observed based on the increasing slope. On day four, the average of all the final concentrations for the red wavelength treatment is greater than day zero's.

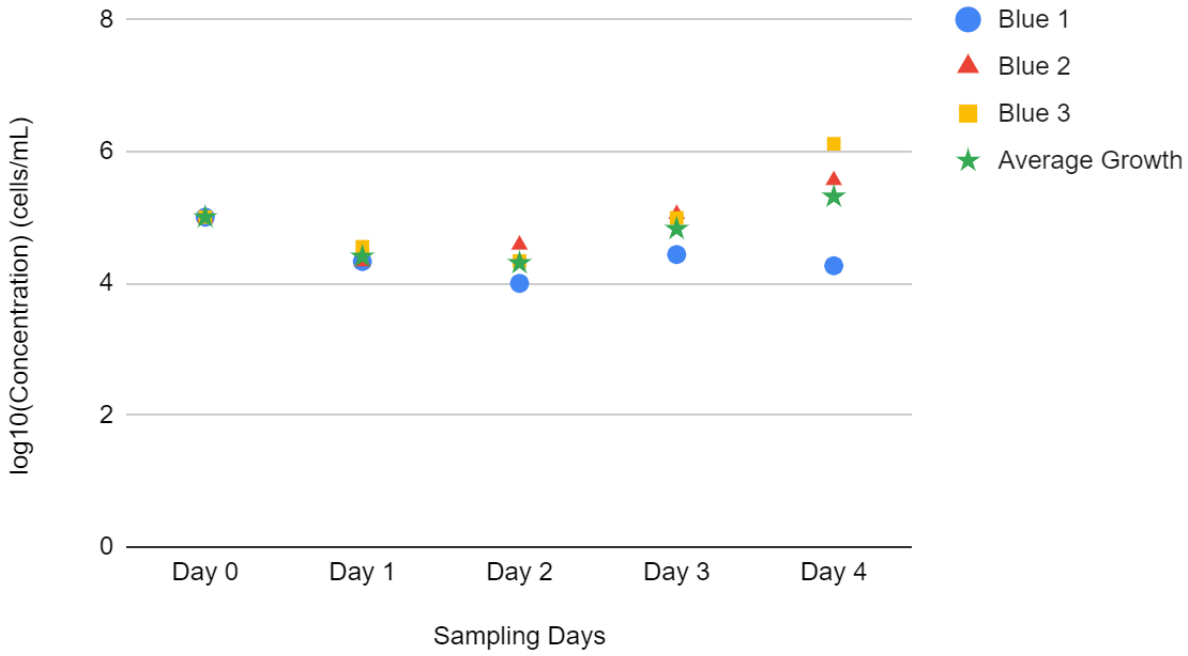

Figure 3. Concentrations for *C. reinhardtii* under blue wavelength growing conditions. The concentrations (cells/mL) on the y axis have been converted to logarithm values to generate a straighter line. A total of three replicates (n = 3) were conducted under these conditions. The blue dots indicate replicate one, the red triangles indicate replicate two and the yellow squares indicate replicate three. The green star represents the average growth of each treatment.

Similar trends based on the red wavelength treatment can be observed in the blue wavelength treatment (figure 3). From the growth plot of each group, slight growth can be observed from the increasing log plots. In particular, the red wavelength (figure 2) and blue wavelength group (figure 3) seem to grow more compared to the control (figure 1) albeit minimally. Between the red and blue wavelength group, the average growth of the red wavelength group grows at a faster rate over the 10 day period. Based on the growth plots for each treatment, it can be seen that the final concentration for both the red wavelength and the blue wavelength treatment result in a higher concentration on day four, whereas the control treatment yielded a final count smaller than day zero. A feature from each plot shows that the day one count is lower than the day zero count.

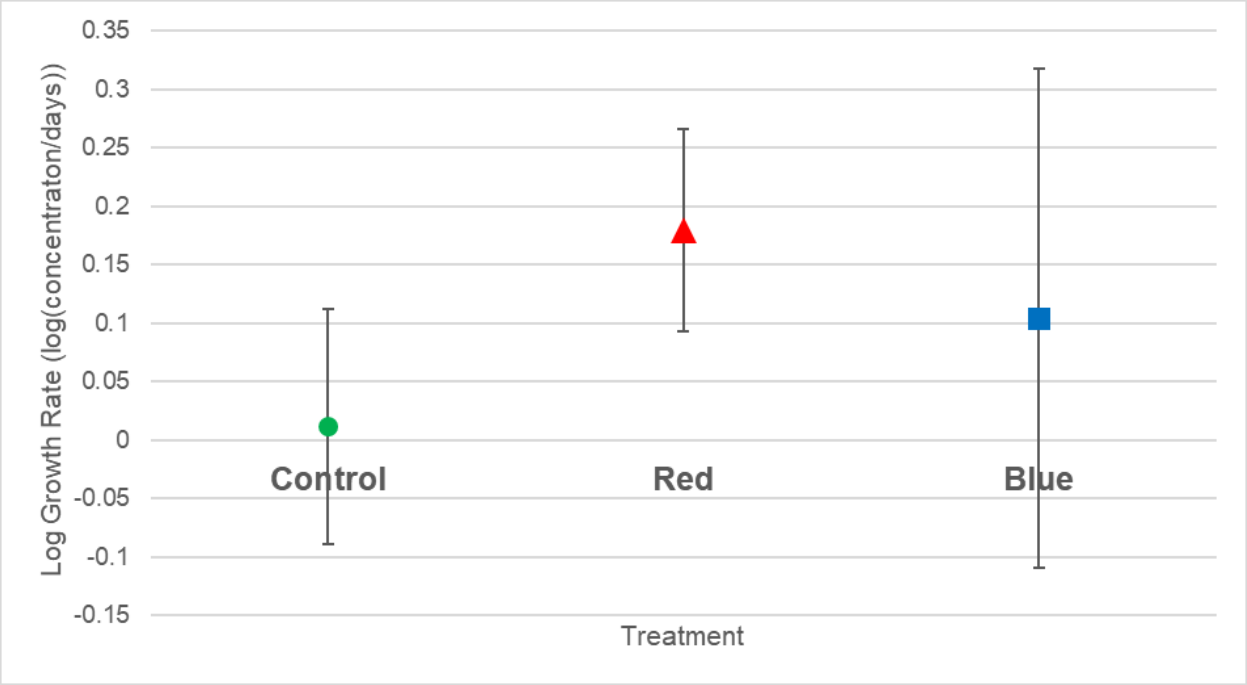

Figure 4. Dot plot of the mean growth rates for each treatment (n = 3, p >0.05). Each point on the dot plot shows the average growth rate from each treatment. The standard error bars represent the standard deviation of each treatment. There is no statistical difference between each group.

A one-way ANOVA statistical analysis was conducted based on the average growth of each treatment and mean growth rates for each treatment were plotted with error bars representing the standard deviations (figure 4). Figure 4 shows that the red wavelength group grows at a faster rate compared to the other treatment groups. The average growth rate for the control, red wavelength, and blue wavelength treatment were 0.0114 (cells/mL)/day, 0.1793 (cells/mL)/day, and 0.1047 (cells/mL)/day, respectively. The one-way ANOVA test between each treatment group yielded a p-value of 0.4189 ($p > 0.05$). This reveals that there is no significant difference between each treatment group, so no further analysis was conducted.

Discussion

The goal of this study was to identify whether *Chlamydomonas reinhardtii*'s photosynthetic growth rate varies when exposed to different wavelengths. Our results showed that *C. reinhardtii* has no tendency to grow at a faster rate under red light, blue light or white light treatments. All our samples were cultured in separate tubes in a common incubator, and exposed to three different light treatments. We observed a slight growth in the samples exposed to red and blue wavelengths, and a very minimal growth in the control treatment group. Observationally, over the 10 days of growth, the *C. reinhardtii* samples exposed to red wavelengths appeared to have the highest growth rate.

A thorough statistical analysis of our results yielded no significant difference between the three treatment groups, implying that any observable changes in growth rates could be attributed to chance. Therefore, we fail to reject the null hypothesis of our study, since there is indeed no difference between treatment groups. This insignificance may be attributed to improper protocol in both dilution and counting methods in our study.

Our data showed that our initial solution (Day 0) had higher *C. reinhardtii* concentration than the first sampling day (Day 1). This can be attributed to improper counting of cells in our initial stock solution, or to an improper dilution calculation. Our initial concentration would not be 1.00×10^5 cells/ml as we had planned, but rather something lower. Unfortunately, we were unable to redetermine this initial concentration, since no samples were saved from the Day 0 solution to count again.

Furthermore, our data showed inconsistencies in counting within each treatment group. For instance, sample Blue 1 on Day 3 had a concentration of around 3.7×10^4 cells/ml, while sample Blue 3 on Day 3 had a concentration of 1.49×10^5 cells/ml. This difference of one order of

magnitude is immense, considering that both samples began from the same initial concentration and were exposed to the same blue light treatment. Inconsistencies of this kind can be attributed to the properties of our specimen. In fact, previous studies have established that unicellular organisms such as *C. reinhardtii* have cell aggregation inducing tendencies when exposed to changes in their environment (Schlesinger et al., 2012). Other errors that may have been introduced occurred during the counting of the samples, as this task was split amongst all members. Even though specific counting methods were established as a group, minor inconsistencies in counting technique could have led to inaccurate final counts. When diluting the stock solution to our desired initial concentration, the environment was drastically changed which may have stressed our specimen. When collecting samples of each treatment on each of the four collection days, a fixative was mixed with the sample to prevent further growth inside the counting tube. This additional change in environment may have also stressed *C. reinhardtii*, thereby inducing cell aggregation within the tubes. This phenomenon creates a few clumps of highly concentrated cells, and leaves most of the solution in the tube minimally concentrated. If three samples are taken from a tube with an aggregated cell culture, their counts may be inconsistent due to clumping. This can be prevented by resuspending each tube prior to each step in the dilution and counting protocol.

Finally, our data showed no differences in growth rate under different light wavelength treatments which fail to align with previous studies. Li et al. (2021) conducted a similar study where they found red-orange LED light exposure increased the growth rate of *C. reinhardtii*, in comparison to blue LED wavelength treatment. The errors noted above may have contributed to the differences between our results. In future studies, greater care should be considered upon transferring *C. reinhardtii* between stock solutions and sampling tubes to allow for samples and

replicates of equal concentration. Further work in this field with greater sensitivity to changes in concentration between sampling days, as well as analyzing growth rates under different light wavelengths would provide a more thorough study to this field.

Conclusion

In conclusion, the statistical analysis calculated a p-value of 0.4189, indicating there was no significant difference between the red, blue, and control treatments on *Chlamydomonas reinhardtii*'s growth rate. Therefore, the results from this research do not align with results from previous studies. Although the growth rate in the red wavelength treatment appears to be slightly faster compared to the other treatments, the statistical analysis on the overall growth between treatments signifies that there is no strong indication of faster growth. These results are significant because it could indicate that not one specific wavelength will maximize photosynthesis in *C. reinhardtii*. In consideration to future applications of *Chlamydomonas reinhardtii* in green technology as a solution to climate change, further research can be conducted to determine the most efficient wavelengths to be incorporated in maximizing photosynthesis and growth rates. These results provide significant contributions to CO_2 fixation while maintaining the algae biomass necessary to support the aquatic food chain, including many salmon species.

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experiment, preparing our organism and equipment, and teaching us the fundamental techniques and skills necessary to conduct our research.

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Appendix

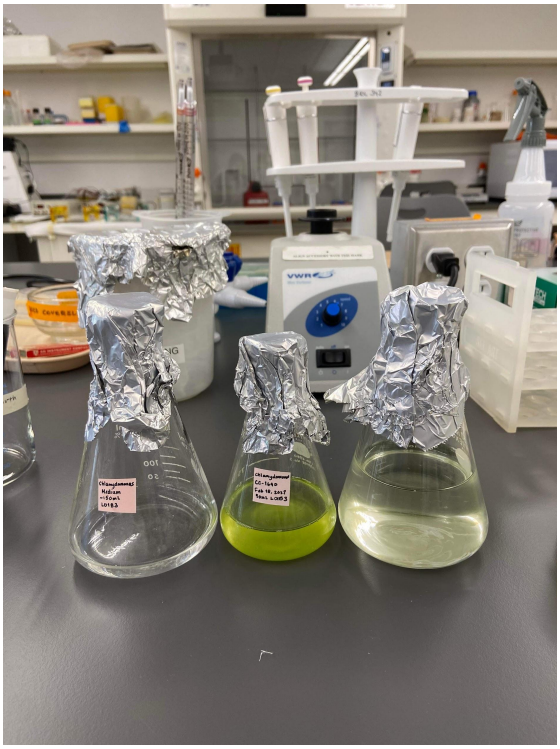

Figure 5. From left to right, the Chlamydomonas autotrophic growth medium, the Chlamydomonas stock solution and the diluted Chlamydomonas solution.

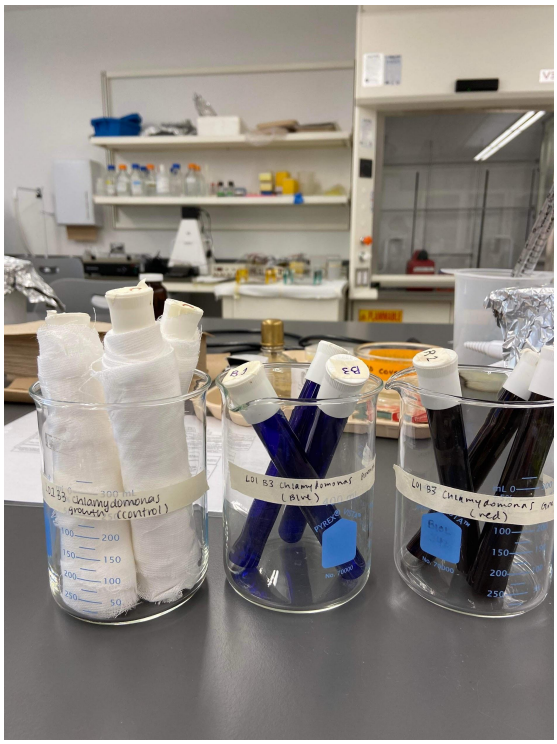

Figure 6. From left to right, the control treatment wrapped in cheesecloth, the blue wavelength treatment wrapped in blue acetate, and the red wavelength treatment wrapped in red acetate.

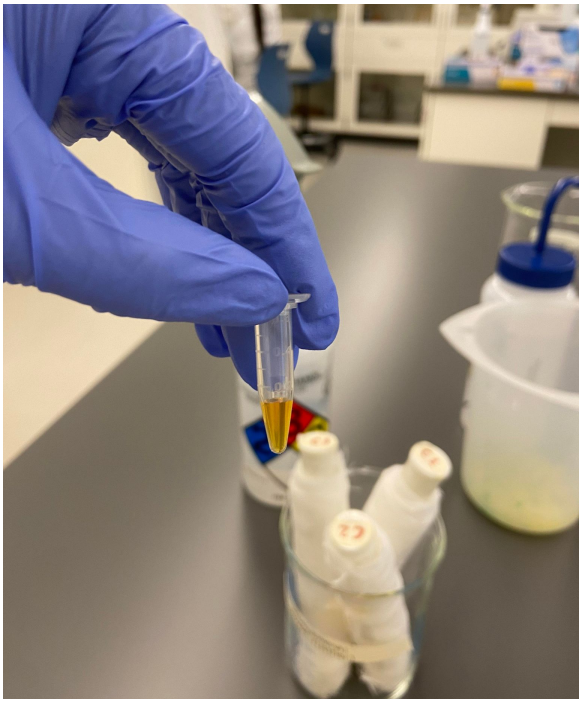

Figure 7. An example of a sampling tube containing 10 uL of potassium iodide fixative plus 100 uL of a *C. reinhardtii* sample.

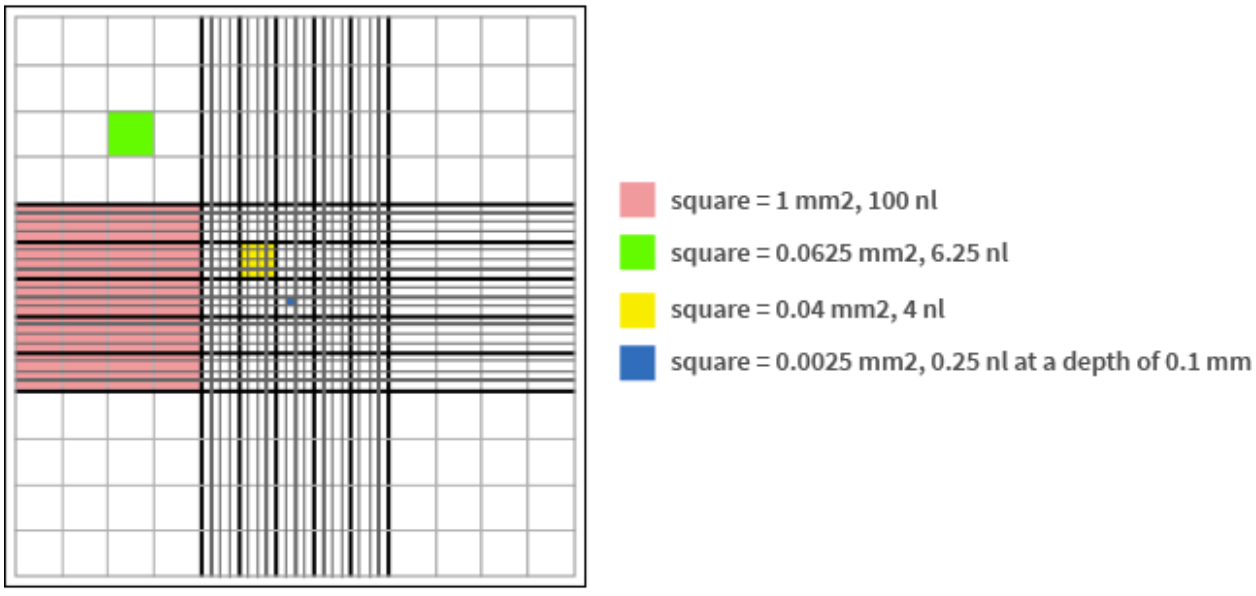

Figure 8. A schematic representation of a hemocytometer grid (Logos Biosystem, n.d.).

Example calculation to determine initial and final concentrations of the *Chlamydomonas reinhardtii* using a hemocytometer:

Concentration = $\frac{Average\ of\ cells\ counted}{\#\ of\ squares\ counted}$ $*$ $square\ size$ $*$ $dilution\ factor$

Concentration = 88.3 cells/1 red square * $1x10^4$ * 1.1 = $9.72x10^5$ cells/mL