The effect of increasing light intensity on oxygen production in *Chlamydomonas reinhardtii*

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Abstract

The single-celled green alga, *Chlamydomonas reinhardtii*, is a commonly used model organism, especially in studies involving photosynthesis. Our study focuses on the effects of varying light intensities on oxygen production by *C. reinhardtii.* There were four replicates of wild-type *C. reinhardtii* per light intensity treatment (480, 1000, 3160, and 5150 lux). Each one was exposed to its treatment for 1.5 hours and oxygen concentrations were measured before and after each treatment. Overall, we observed an increasing rate of oxygen production with increasing light intensity. A one-way ANOVA test resulted in a *p*-value of 0.0248. It is likely the significant differences arise between the 480 lux and 3160 lux treatment and between the 480 lux and 5150 lux treatment since the 95% confidence intervals of the means for oxygen production at these light intensities do not overlap. Increasing light causes an increase in the photosynthetic rate most likely due to light's stimulatory effect on Photosystem II of *C. reinhardtii*.

Introduction

Chlamydomonas reinhardtii is a model organism used in multiple studies around the world, particularly in those involving photosynthesis. *C. reinhardtii* is structurally and behaviourally related to vascular plants, but has a much simpler cellular structure, which makes it easier to study their photosynthetic processes (Dent, Han and Niyogi 2001). Each cell includes a large chloroplast, pyrenoid and an eyespot, composed of photoreceptors that perceive light (Levine and Ebersold 1960). This single-celled green alga is found all over the world in oceans, freshwater and soil (Levine and Ebersold 1960). It possesses two flagella that enable the organism to move directionally (Levine and Ebersold 1960).

Photosynthesis is the process by which photoautotrophs, like *C. reinhardtii,* capture light to acquire energy. Scientists have found that alterations in the process can lead to the production of different by-products. An example that is currently a prevailing focus of study is the production of H_2 by *C. reinhardtii.* In an anaerobic environment with the absence of sulfur

nutrients, *C. reinhardtii* changes its morphology to photosynthetically increase its production of molecular hydrogen, a popular element of biofuel (Tamburic *et al.* 2012). Therefore, the rate and conditions of photosynthesis of this organism is relevant for current and future studies in, for instance, biofuel production.

During the light-dependent reactions of photosynthesis, electrons from water are transferred to oxygen to form O_2 (Figure 1). Therefore, O_2 is the final electron acceptor as well as a major product of photosynthesis (Ignjatovic 1968). Consequently, oxygen release is often measured to calculate the rate and magnitude of photosynthesis.

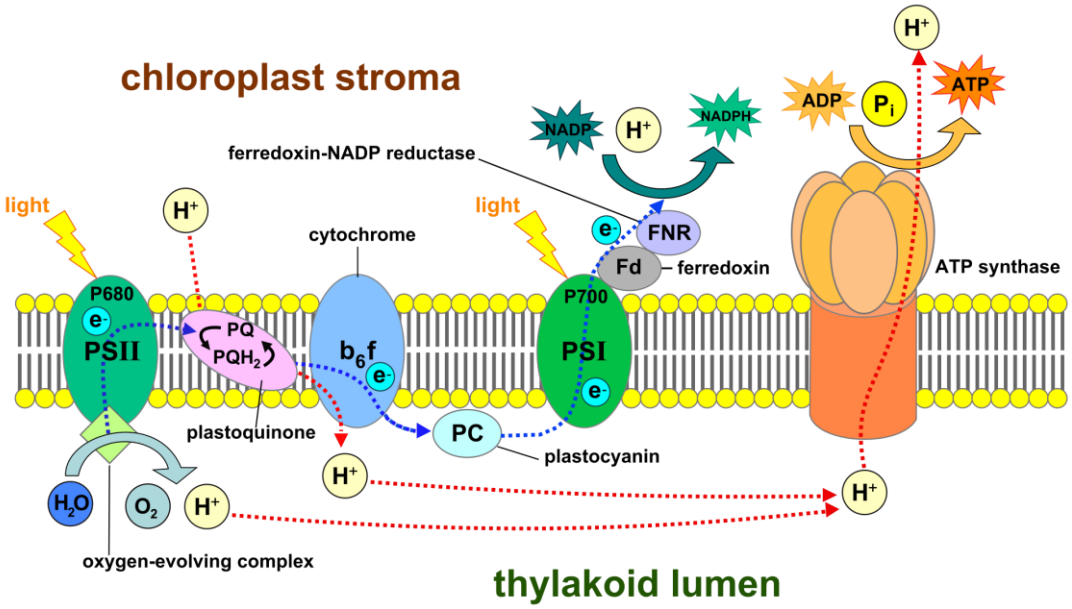

Figure 1. The electron transport chain of the light dependent reaction. Light, as shown by the lightning symbol, initiates this process. As shown near the left of the lumen, the electron is transferred from H_2O to O_2. (Wikipedia)

Our research aimed to measure the effects of different light intensities on the rate of photosynthesis by *Chlamydomonas reinhardtii*, measured by the release of oxygen. We predicted that increasing light intensity would increase the release of oxygen. Generally photosynthesis in photoautotrophs increases as light intensity increases until saturation is reached and beyond that

photosynthesis decreases due to photoinhibition (Ryther 1956). Photoinhibition is the process by which excess light damages and hinders the activity of photosystem II (PS II), thereby decreasing the efficiency of photosynthesis. However, *C. reinhardtii* is capable of protecting itself against photoinhibition since it is able to alter the activity of its PSII to minimize damage by excess light. As a result, it can increase the range of light intensities at which it can photosynthesize effectively (Schuster, Timberg and Ohad 1988). For this reason, we expect that photoinhibition will not be a prominent factor in our research, considering our choice of light intensities, with the maximum being 5150 lux (Sorokin 1958). Our alternate hypothesis was that increasing light intensity has an effect on the O_2 production of *C. reinhardtii*. Our null hypothesis was that increasing light intensity has no effect on O_2 production by *C. reinhardtii*.

Methods

Treatment Set-Up

We set up our experiment by preparing four treatments of varying light intensities. Each light intensity, except for the 480 lux, was set up with a lamp at a height appropriate for the level of light intensity desired. We also had four water baths and thermometers prepared for each treatment to allow us to monitor and control the temperature throughout our experiment. Ice was kept nearby in case the lamps caused our water baths to warm. We were able to achieve a room light intensity of 480 lux, and a light intensity of 1000, 3160, and 5150 lux with the lamps adjusted at different heights as shown in Figure 2.

Figure 2. Left: One of the treatment set-ups at 5000 lux with replicate and control pairs placed in the water baths. Right: An image of the folded mesh cloth between the lamp and the water bath needed to obtain 1000 lux.

Replicate and Control Set-Up

For our replicates and controls, we obtained 700 mL of stock cell culture of wild-type *Chlamydomonas reinhardtii* and 600 mL of standard medium. We then measured the concentration of *C. reinhardtii* using a haemocytometer, a clicker, and an Axio microscope, obtaining an average of 263 cells/mL. A DinoXcope was also used to capture images seen from the microscope (Figure 3). After obtaining our counts, we prepared our replicates and controls for each of the four treatments. We filled 16 27-mL vials with *C. reinhardtii* in their medium and filled another sixteen vials with our stock medium that would serve as our controls. There were four replicates and four controls per light intensity treatment. We measured and recorded the initial oxygen concentration of each replicate and control with an oxygen meter. After each measurement, we re-filled each vial either with *C. reinhardtii* or standard media due to the

spillage that occurred when taking the measurements and to minimize the air bubbles that could have resulted. The replicates and controls were then placed in pairs into the water baths for 1.5 hours. Each pair was placed in their respective light intensity treatment approximately 3-4 minutes after the other and each treatment was staggered by approximately 15 minutes to allow enough time to obtain final oxygen concentrations at the end of each treatment.

Figure 3. Image showing fixed *Chlamydomonas reinhardtii* cells from our original stock solution on the haemocytometer at 100X total magnification.

Final Data Collection

At the end of the 1.5 hours for each replicate and control pair, we measured the final oxygen concentration with an oxygen meter. Then, we extracted 100 μL from each replicate and placed this into a microcentrifuge tube, and fixed it with 10 μL of Iodine Potassium Iodide (IKI). Each sample was mixed by micropipetting the solution. After collecting the samples, we removed 10 μL and transferred it onto a haemocytometer to make the final cell counts.

Data Analysis

The data were analyzed using Microsoft Excel software. First, the oxygen produced for each replicate was calculated by subtracting the initial oxygen concentration from the final

oxygen concentration. This was done for the controls as well. To obtain a final oxygen production value, the difference in control oxygen concentration was subtracted from the corresponding replicate final oxygen concentration value in order to correct for any oxygen produced or lost during the measuring process. The final oxygen concentration for each replicate was then divided by the cell count calculated for that replicate in order to obtain the per cell oxygen production. The means were then calculated for the four values as well as the standard deviations and confidence intervals. The aforementioned procedure was done for each light intensity treatment resulting in four mean oxygen production values. The four means were then analyzed using a one-way ANOVA test.

Results

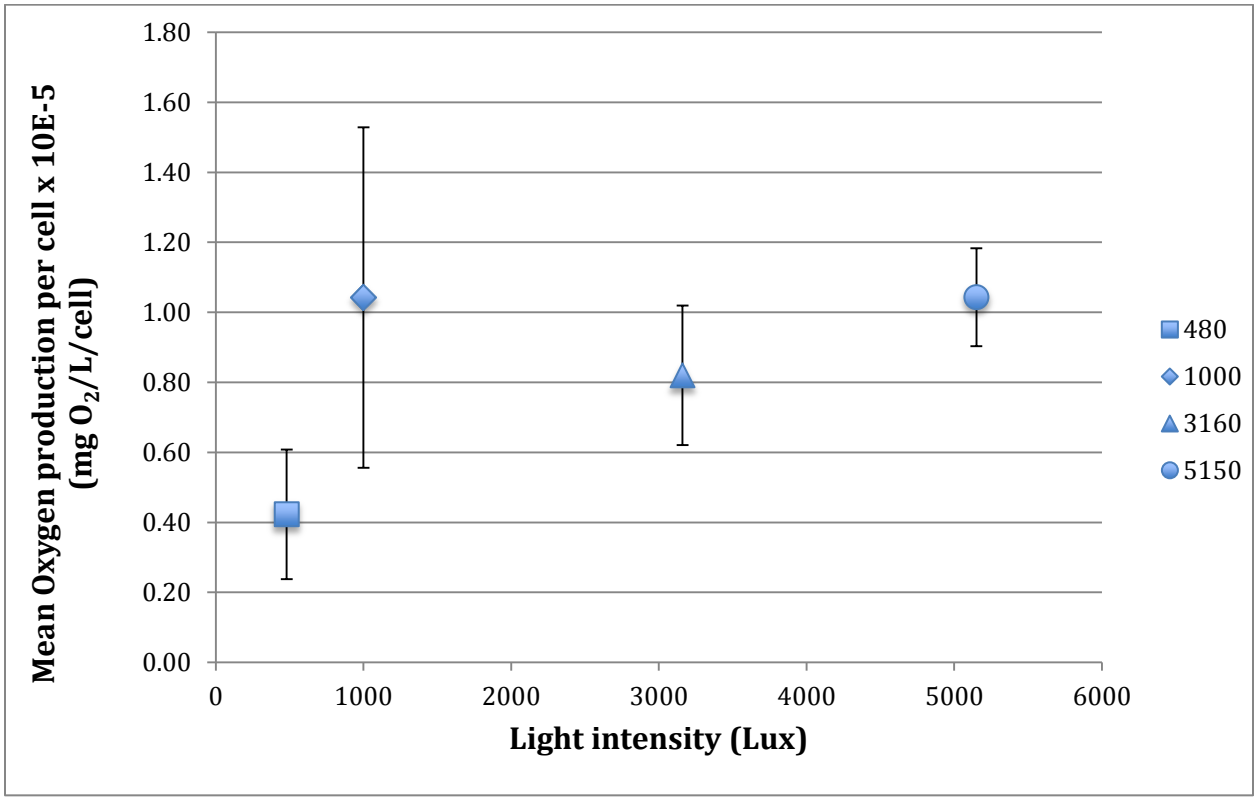

Figure 4. Effect of light intensity on the mean oxygen production per cell in *C. reinhardtii*. The points represent the mean oxygen production at different light intensities. Error bars represent the 95% confidence intervals of the means. p= 0.0248, (n=4).

Figure 4 shows there is an increase in mean oxygen production per cell from 480 lux to 5150 lux. There is a significant difference between the means of the four data points illustrated in Figure 4 as the *p*-value from the one-way ANOVA test is equal to 0.0248, which is less than 0.05. However, looking at specific means on the graph, there is likely a significant difference between the 480 lux treatment (4.231E-6 +/- 1.853E-6 mg/L/cell) and 3160 lux treatment (8.198E-6 +/- 1.994E-6 mg/L/cell) and between the 480 lux treatment (4.231E-6 +/- 1.853E-6 mg/L/cell) and the 5150 lux treatment (1.043E-5 +/- 1.400E-6 mg/L/cell) as their confidence intervals do not overlap. The largest difference between means is observed at the lowest light intensity treatment, 480 lux, and highest light intensity treatment, 5150 lux. Moreover, there is likely a lack of significant differences between the three highest light intensity treatments and the increase in oxygen production observed between these light intensities appears to be minimal (Figure 4).

As illustrated in Figure 4, the 95% confidence intervals for the mean oxygen production of the 480, 3160 and 5150 lux treatments are relatively similar compared with the 95% confidence interval of the 1000 lux treatment which is very large in comparison and has a value of +/- 4.82E-6 mg O_2/L/cell.

Qualitatively, we observed a dark green ring in the replicate vials when exposed to the different light intensities. *C. reinhardtii* moved to the top of the vials closest to where the light was hitting each vial; therefore, the intensity of the dark green ring increased over the time allotted for the experiment and throughout the four light intensity treatments.

Discussion

After analyzing our data using a one-way ANOVA test, we determined our *p*-value to be 0.0248. This *p*-value is less than 0.05; therefore, we can reject our null hypothesis and provide support for our alternate hypothesis, which states that light intensity has an effect on O_2 production in *C. reinhardtii*. We predicted that we would see an increase in O_2 production with an increase in light intensity since light is required to fuel the photosynthesis process. This seems to be the overall result since we observed an increase in O_2 production between our room light intensity with both the 3160 lux and 5150 lux light treatment. This is interpreted as an increase in the photosynthetic rate per cell.

A reasonable interpretation of our data is that the *C. reinhardtii* cells in the 3160 and 5150 lux treatments had higher photosynthetic rates than those in the 480 lux treatment. Therefore, our experiment shows an increase in the photosynthetic productivity of *C. reinhardtii* with higher light intensity. This agrees with previous research that reveals that *C. reinhardtii*'s photosynthetic rate increases with light intensity (Ryther 1956). Ryther (1956) explains how photosynthetic marine species react when exposed to light intensities within their natural habitat range. He was able to show that when exposed to higher light intensities up to 5000 lux, there is an increase in productivity amongst many of these marine algal species.

Moreover, light provides the energy required for photosystem II (PSII) to extract electrons from water (Melis 2000). PSII drives these electrons through the different components of the electron transport chain where they are transferred to oxygen, the final electron acceptor (Melis 1998). This process generates a proton gradient that is drawn on as an energy reservoir to create ATP (Hippler 1998). Although the mechanism has not been fully elucidated, it is generally accepted that the flow of protons from this reservoir enables ATP synthase to catalyze

the synthesis of ATP (Hippler 1998). Since light initiates this essential chain reaction, an increase in light intensity should also increase the photosynthetic rate of *C. reinhardtii* which is what we observed.

The effect of light on marine algal photosynthesis has been studied extensively and produces a photosynthesis-irradiance (PI) curve (Marra 1985). PI curves increase with light intensity before eventually plateauing at higher light intensities (Marra 1985). While our data seem to demonstrate the increasing portion of the PI curve, it does not appear to show a leveling off of *C. reinhardtii*'s photosynthetic rate.

The photosynthetic rate of *C. reinhardtii* will stop increasing when it reaches a point of light saturation at which time it will begin to plateau and eventually decrease due to photoinhibition (Ryther 1956). Performing a further study to observe *C. reinhardtii* under higher light intensities could determine at what point light inhibits the photosynthetic process. The future study could measure oxygen before and after the light treatment; however, the light intensities would range above 5000 lux since previous studies indicate that photosynthetic rate begins to plateau at higher intensities (Sorokin 1958).

Also, despite the fact that we obtained significant results, there are still some areas of uncertainty in this experiment. The main factor that we had to consider while doing our experiment was temperature because we did not want the *C. reinhardtii* to die due to heat exposure under the heat of the lamp. We did our best to solve this problem by keeping the cells in a water bath that was being constantly being monitored. We tried to maintain the water temperature at 22 °C by adding ice as needed however the temperature would fluctuate +/- 2 degrees occasionally. The second degree of uncertainty was fluctuating light intensity. The light intensity of each treatment was measured as we set-up our experiment and before the *C.*

reinhardtii vials went into the water bath; however, no measurements were taken in between because the light meter would have interfered with the lamp. Therefore, we are unsure if the light intensities of each treatment stayed constant throughout the experiment.

Lastly, the 95% confidence intervals for all treatments were very similar, except for the 1000 lux treatment, which had a lot more variation. Our experimental design for this treatment may have affected it differently than the others since, as seen in Figure 2; we needed to use a mesh cloth to obtain the 1000 lux treatment. This cloth may not have been uniform throughout; therefore, different vials may have received more light than others.

Conclusion

In conclusion, we were able to reject our null hypothesis and support our alternate hypothesis, which states that light intensity has an effect on O_2 production in *C. reinhardtii*. In our experiment, we used O_2 production as a measure of photosynthetic rate. Therefore, an increase in light intensity will increase the O_2 production, or the photosynthetic rate, of *Chlamydomonas reinhardtii*.

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