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Effects of Red Light Intensity on Cultivation and Oxygen Production in Arthrospira platensis

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SENIOR THESIS APPROVAL

This Honors thesis entitled

“Effects of Red Light Intensity on

Cultivation & Oxygen Production in *Arthrospira platensis*”

written by

Abigail Roberts

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the requirements for completion of

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meets the criteria for acceptance

and has been approved by the undersigned readers.

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Date: 12/14/22

Abstract

Maintaining life sustaining resources during long-term space travel has encouraged scientists to turn their attention to the potential benefits of a cyanobacteria named *Arthrospira platensis*, commonly known as Spirulina algae. This experiment measures the oxygen production and cell population growth of two structurally different Spirulina cultures under two different levels of red light intensities, 8 μmol/m^2/s (high); 3 μmol/m^2/s (low). The cell population, oxygen produced, and oxygen produced per cell measurements were observed and recorded at three 24 hour intervals. It was found there was not a significant difference between high and low light intensities when considering the cell concentration data which indicated the red light intensities did not affect the cell concentration level. However, both the oxygen alone and oxygen per cell produced from the high and low light intensities were found to result in significant data differences. The mixed culture at 8 μmol/m^2/s produced the most oxygen (not factoring in O_2 per cell) on average for all three- 24 hour periods with the highest occurring at 52.3 cm^3 on day 2. The mixed cultures had a higher level of cell counts than the coiled cultures at the start of each experiment which ultimately skewed the results when looking at the O_2 cm^3 production alone. The coiled Spirulina cultures produced more O_2/cell at 8 μmol/m^2/s on average for all three- 24 hour time intervals with the highest occurring on day 1 at 4.92 x 10^{-4} O_2 cm^3/cell. A previous experiment using white light at 25 μmol/m^2/s produced 9.35 x 10^{-4} cm^3/cell. In contrast, the highest oxygen per cell production on average using red light was 8 μmol/m^2/s was 4.92 x 10^{-4} cm^3/cell. This indicated that, on average, white light was more efficient at producing oxygen. However, the 8 μmol/m^2/s red light intensity for coiled Spirulina produced a 24 hour period total of 9.28 x 10^{-3} cm^3/cell indicating that there is potential for red light to be just as efficient, if not more, than white light intensities.

Introduction

Arthrospira platensis has been a key superfood and medical aid since ancient times. As a photoautotroph, spirulina is able to recycle exhaled CO_2 and convert it to fresh O_2 through the process of photosynthesis and as a superfood can provide essential amino acids and vitamins (Mapstone et al. 2022). Additionally, its composition consists of seventy percent protein dry matter and includes a rich content of vitamin B complex, carotene, and ascorbic acid (Wolina et al. 2018). These nutritional benefits combined with its ability to absorb light and convert it to chemical energy provide ample reason why Spirulina continues to be studied as a space travel aid.

When considering the physiological challenges that space travel creates, astronauts can encounter a variety of health threats during missions. These physiological challenges include bone density depletion, muscular atrophy, loss of body mass, change in metabolic rate, cardiovascular degradation, impairment of immune function, and neurovestibular changes (Bernstein, 2021). Additionally, due to the increased exposure to radiation in space, the astronauts are at a greater risk of developing cancer and degenerative diseases including heart disease and cataracts (Mars, 2021). As more missions have been completed, researchers are more aware of the potential risks space travel can have on the body both during the mission and after returning to Earth. The key to combat these risks is finding proactive ways to prevent them from happening in the first place rather than having astronauts require treatment to recover upon return. This is ultimately where specific use of Spirulina comes into play. Since the space environment is known for making crops mutate, it is important to consider how microgravity, radiation, or volatiles in the spacecraft will affect the way Spirulina grows on a mission (Moore, 2020). Some of these mutations may include strange metabolites or a different nutritional

composition, however an important research study found that Spirulina is more resistant to radiation than other algae tested (Moore, 2020). This resistance in addition to its tendency to have consistent and predictable growth makes Spirulina a good candidate to act as a preventative measure to avoid the health hazards mentioned above.

Spirulina is specifically useful in two main ways. First, the nutritional value that it possesses is significant. One tablespoon of Spirulina includes approximately 20 calories, 4 grams of proteins, 1 gram of fat, 2 grams of carbohydrates, and 0 grams of sugar (Burgess, 2022). In fact, its protein levels are comparable to the protein received from eggs. A specific protein known as phycocyanin contributes to Spirulina's antioxidant properties. This is important in helping protect the body from cell damage and can also block tumor growth and kill cancer cells (Burgess, 2022). Another important antioxidant in Spirulina is beta carotene which is converted to Vitamin A to help protect eye health (Burgess, 2022). In addition to Vitamin A, Spirulina is also concentrated with zeaxanthin which is a pigment that helps reduce the risk of cataracts and age-related vision loss (Begum, 2022). Both beta carotene and zeaxanthin are especially important in space as Astronauts are more prone to cataracts and other permanent effects on the visual system. Spirulina also strengthens the immune system through its broad range of vitamins and minerals that help boost the production of white blood cells and antibodies to fight infections (Burgess, 2022). The second main way that Spirulina is useful is its photosynthetic nature. This means that it is able to photosynthetically convert N_2 and CO_2 while producing O_2 and organic compounds. Therefore, the metabolic waste in the cabin produced by the crew, like exhaled CO_2, can be recycled by Spirulina (Fais, 2022). It is Spirulina's ability to act as a reliable food source and repurpose metabolic waste as necessary oxygen that establishes the objective of this study.

Spirulina structure and genetic makeup plays a large role in why it possesses these benefits. It is a planktonic filamentous cyanobacterium that is composed of individual cells, and it grows in subtropical alkaline lakes. It can survive under a wide range of conditions, but it thrives at temperatures of approximately 35 ℃ and a pH of about 10 (Masojídek, 2008). The symbiotic blue-green algae is known for its multicellular cylindrical trichomes that consist of an open-left hand helix (Figure 1). These filaments undergo binary fission and will fragment into shorter segments which eventually develop into whole trichomes once completing the cycle. They have visible transverse cross-walls and lack cellulose cell walls. This allows Spirulina to be easily digestible (Karkos, 2010). The trichomes are typically 50-500 µm in length and 3-4 µm in width and are enveloped by a thin sheath. Spirulina will adapt to light intensity forming a straight rod structure or its characteristic spiral structure (Figure 2). This allows Spirulina to increase or decrease the concentration of light that it receives to perform photosynthesis and cell reproduction (Spirulina, 2021). These two structures are compared in the experiment by looking at how strictly coiled Spirulina or a mix of both coiled and straight differ in their oxygen and cultivation responses under different levels of light intensity.

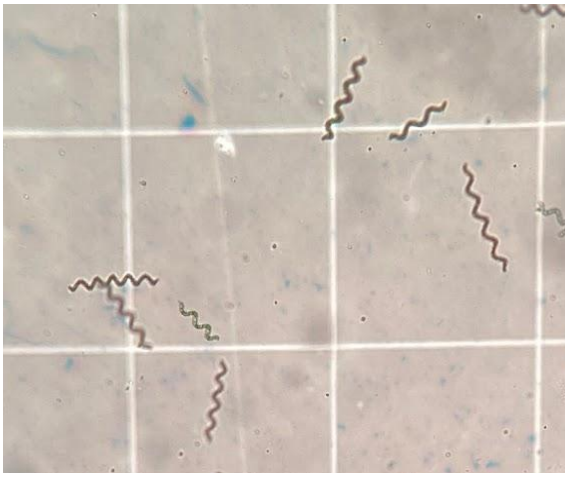

Figure 1. Microscopic image of sampled coiled (helical) shape of Arthrospira platensis. Sample taken from coiled only culture.

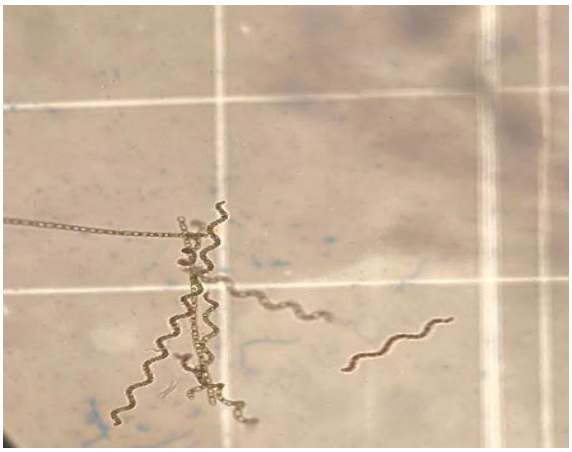

Figure 2. Microscopic image of straight structure (linear) shape of Arthrospira platensis. Sample taken from mixed culture.

To produce the chemical energy it needs to survive, cyanobacteria like Spirulina algae will perform photosynthesis. The starting reactants of this process include sunlight, carbon dioxide and water. During the reaction the light energy transports electrons from water in order to generate NADPH and ATP. These two molecules are then used to convert carbon dioxide into carbohydrates like glucose. It is these sugar molecules that are produced that contain the energy necessary for living things to survive. Photosynthesis also produces oxygen as a by-product. Cyanobacteria are very important organisms for the health and growth of many plants. For instance, cyanobacteria convert inert atmospheric nitrogen into an organic form like ammonia or nitrate which plants use for their growth. When the light intensity increases, photosynthesis will increase linearly with it. However, there is a point where the reaction becomes saturated, and a theoretical maximum will be reached.

In previous studies that looked at Spirulina's growth under specific light intensities it was determined that white light produced high oxygen production levels with increasing cell counts

over the 24, 48, and 72 hour periods. These results indicated that white light would be an effective energy source to grow the algae while on mission. However, the question remained if lower light intensities, like red light, would provide a similar or adequate amount of growth as its counterpart of white light. This is because the benefits of using red light rather than white would greatly impact the efficiency of space travel. Some of the benefits of using red light to grow the Spirulina include that it is more cost efficient and it consumes less energy. This is important when considering space travel because light sources that rely on high intensities of power ultimately use too many resources that are necessary and indispensable during space travel. If it were to be found that the Spirulina could be effectively grown at a similar, if not better, rate than the white light experiments then it could be assumed that red light may be a better resource for space travel.

As a result of Spirulina's numerous benefits, this study hopes to find the most efficient and effective light intensity for its growth so that space travel can potentially use it as a vital and effective resource on missions. Previous studies have determined that Spirulina grows well under white light and produces high O_2 concentrations. In an attempt to help reduce costs and potentially allow for operations to be completed at lower energy levels during missions, two different intensities of red light were used to grow the algae.

Materials & Methods

Control Culture

The original Spirulina cultures were maintained in alkaline conditions with a pH of 10 at 30 °C and given 50 mL of Zarrouk's (Appendix A) nutrient media daily (Figure 4). The mixed culture consisted of a 50/50 ratio of coiled and straight Spirulina and was grown in a fish tank

labeled with a green marker. The coiled culture consisted of only coiled Spirulina and was grown in a fish tank with yellow marker. Two plexiglass covers were used to create a barrier for the algae so that unwanted contaminants were kept out. Each tank had an air pump at the bottom to keep the Spirulina unsettled and to help mix the culture to make sure the algae received optimal light. The two cultures were kept under a constant light intensity of 24 µmol/m^2/s for twenty-four hours a day. A litmus pH indicator was used to determine the pH of each culture and sodium bicarbonate powder was added as needed to maintain the optimum pH of 10 (Figure 3). Deionized water was added to a fill line when the cultures reached a thick green color and a point of density that prevented light penetration at the bottom of the tank. Cell counts of each culture were taken every other day using the Sigma-Aldrich hemocytometer. The cell count determined the culture's density and overall health. The amount of nutrient media added to each culture was dependent on the culture's growth. If cultures were high in cell count, 50 mL of nutrients were added every other day. If culture growth was lacking, 100 mL of nutrients were added daily until growth was consistent again. Once both cultures reached high and approximately equal cell concentrations, experiments were able to be conducted.

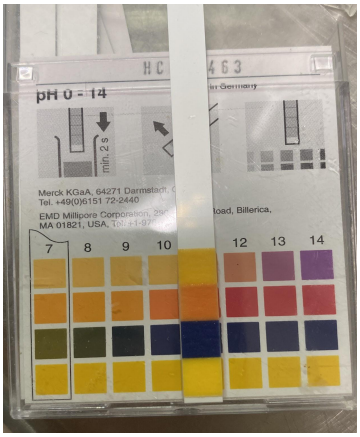

Figure 3. Litmus pH test of Spirulina culture. Test shows that the culture is at the desired pH of 10.

Figure 4. Original Spirulina cultures grown in alkaline conditions with pH of 10 at 30 °C. Mixed culture pictured on the left and coiled only culture pictured on the right.

Light Experiments

The four oxygen-monitoring containers were built using 2.3L plastic containers, two collection tubes, one clear hollow tube, and a wide hollow tube with a screw on cap (Figure 5). The 50 mL and 15 mL collections tubes were glued onto the container so that the algae could fill both tubes completely and had small openings at the top that could be easily closed using clay. The clear hollow tube allowed for algae culture to be displaced within the tube when the pressure within the container increased due to oxygen production. At the start of the experiment, the four oxygen-monitoring containers were filled completely with Spirulina culture (two filled with coiled culture and two filled with mixed culture) and placed in two incubator with two different light intensities: high light intensity (8 µmol/m^2/s) and low light intensity (3 µmol/m^2/s). Both light intensities were kept constant for the entire experiment and the incubators had covers to prevent other light from entering. A stir bar was used to agitate the Spirulina during the experiment and each container had one. The containers were sealed through hot glue and

constructed in a way that allowed easy access to culture cell samples, measurement of oxygen production, and nutrient insertion.

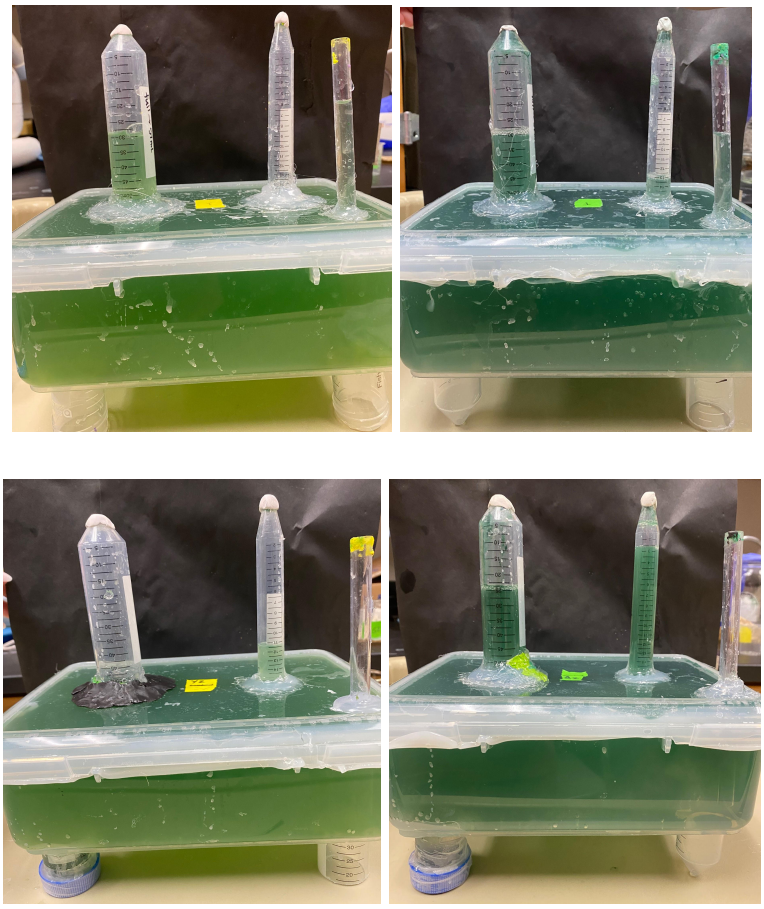

Figure 5. Example of oxygen production after 24 hours of light incubation. From top left to bottom right: coiled only culture at low light, mixed culture at low light, coiled only culture at high light, mixed culture at high light.

Two incubation chambers were used to grow the Spirulina cultures under the high light intensity, 8 µmol/m^2/s, and low light intensity, 3 µmol/m^2/s (Figure 6). Oxygen production was measured every 24 hours by measuring the amount of O_2 gas production in the 50 mL and 15 mL

tubes. After O_2 was recorded the containers were squeezed to release the O_2 and refill the two tubes with algae so that oxygen production could be recorded at the next 24 hour period without the O_2 from the previous 24 hours interfering. Culture cell counts were taken before starting the experiment and then after every 24 hour period by using a micropipette to pull samples from the containers and a hemocytometer to count the cells. Cell counts were replicated for each container three times from each sample and then averaged to determine a final cell count for each container. A micropipette was also used to administer 3 mL of Zarrouk's media to each container after each 24 hour period (Appendix A). Any leaks on the containers were noted during each experiment. After the 72 hour period, the experiment was complete and the containers were cleaned and repaired to prepare for the next experiment.

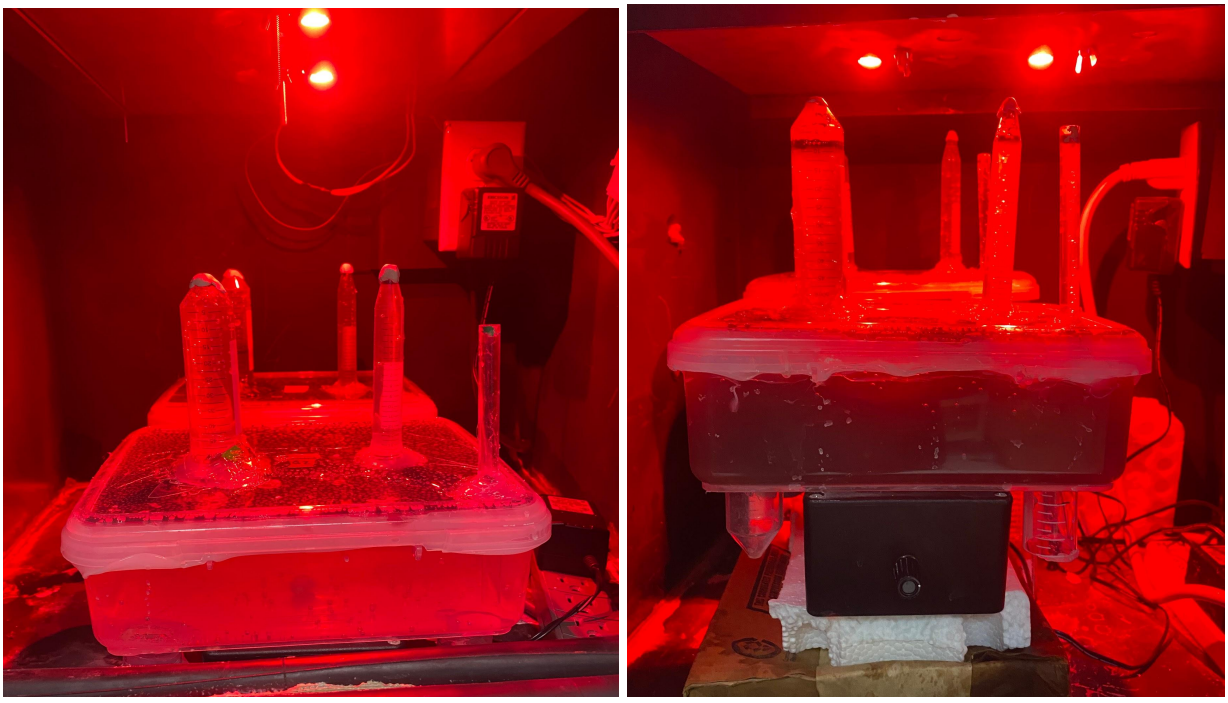

Figure 6. Experiment Container Under Light Intensity of 3 µmol/m^2/s (left) and 8 µmol/m^2/s (right).

Statistical Tests

Statistical tests were performed to see if different light intensities affect the cell concentration and oxygen production in Spirulina. The null hypothesis assumed there is no significant difference between the tested groups. To determine if the null hypothesis could be accepted or rejected, statistical tests were performed in R Studio (R Core Team, 2022). The data used for the statistical test were the recorded results over each 24 hour period of the cell concentration, oxygen production, and oxygen production per cell (Appendix B). Tests were completed to see if the assumptions of One-Way Analysis of Variance test (ANOVA) were met by using the Shapiro-Wilk Normality test and the Bartlett Test of Homogeneity of Variances Test. If the p-value was less than 0.05 for these two tests then the assumptions of ANOVA could not be met and there was a statistically significant difference within the data. A box plot was created to show a visual representation of the equal variance among the groups of the distribution of data and points outside of the box range indicated extreme values within the data set. A Q-Q plot with a regression line was created to show a visual representation of the distribution of data and any extreme values outside of the regression line indicated a potential lack of normality in the data. If the data was not normally distributed, a nonparametric Kruskal-Wallis Test was then run to determine if there was a statistically significant difference in the data. If the p-value was less than 0.05 for the Kruskal-Wallis test, then a post-hoc Kruskal-Wallis MC test using the pgirmess package was run to find which groups showed a statistical difference. For the results that met the assumptions of ANOVA and had a p-value greater than 0.05, a post-hoc Tukey Multiple Comparisons of Means 95% Family-wise Confidence Level test was run to show how large an observed difference must be for the multiple comparisons to be determined as significant.

Results

Cell Concentrations			
Test	Results		
One-Way ANOVA	F Value: 1.827	N/A	P-Value: 0.183
Shapiro-Wilk Normality Test	W: 1.5572	N/A	P-Value: 0.2121
Bartlett Test of Homogeneity of Variances Test	K Squared: 7.2291	Df: 3	P-Value: 0.06494
Tukey Multiple Comparisons of Means	Difference: -20420.83	N/A	P-Value: 0.247586

Table 1. Statistical Tests for the cell concentrations depending on the high and low light (8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$) intensities.

The assumptions of the One-Way ANOVA were met for the high and low light intensity cell concentrations. The results of the Shapiro-Wilk Normality Test had a p-value of 0.2121 and the Bartlett Test of Homogeneity of Variances Test had a p-value of 0.06494. Since the p-values were greater than 0.05 for both of these tests, a One-Way ANOVA was run and gave a p-value of 0.183. This p-value indicated that the null hypothesis could not be rejected and that there was not a statistically significant difference in the data for the cell concentrations when comparing the high and low light intensities. It can be assumed that any observed difference was potentially a result of sampling or experimental error.

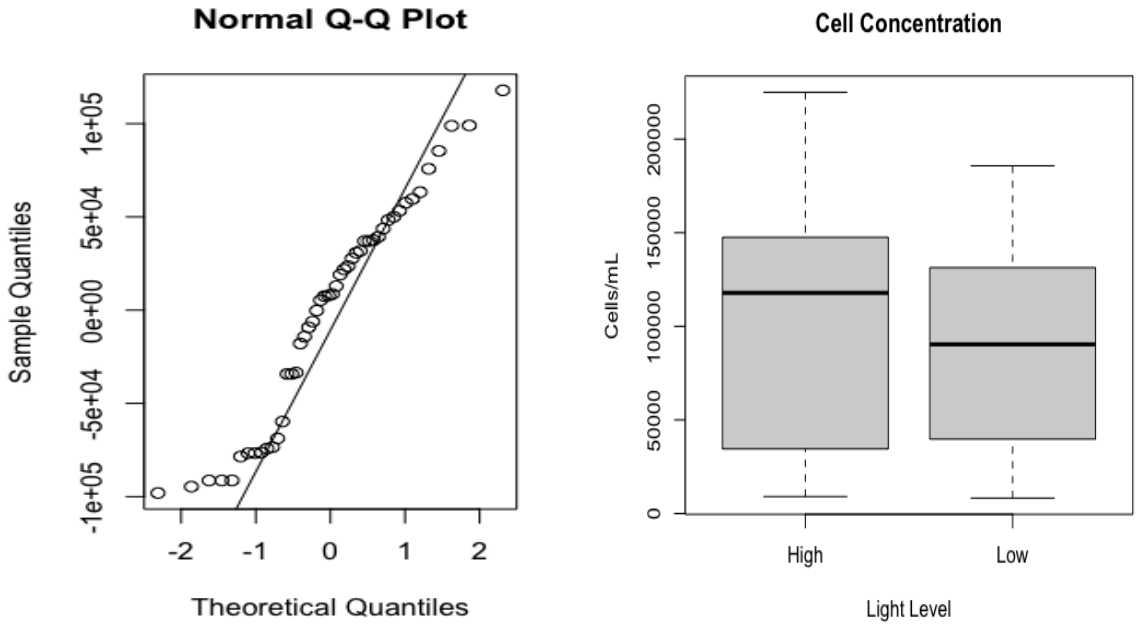

Figure 7. Left: Normal Q-Q plot of cell concentrations (cell/mL) by light intensities. Right: Box plot of cell concentrations (cell/mL) depending on the high and low light intensities (8 µmol/m^2/s and 3 µmol/m^2/s).

The Q-Q plot expressed that the cell concentrations for high and low light intensities were normally distributed because the points followed the regression line and there is no skew of the data present (Figure 7). The box plots display the variance among the high and low light intensity groups for the cell concentrations (Figure 7). Both of these plots demonstrate further that the cell concentration data was not statistically significant to claim that there were any differences between the high and low light intensities.

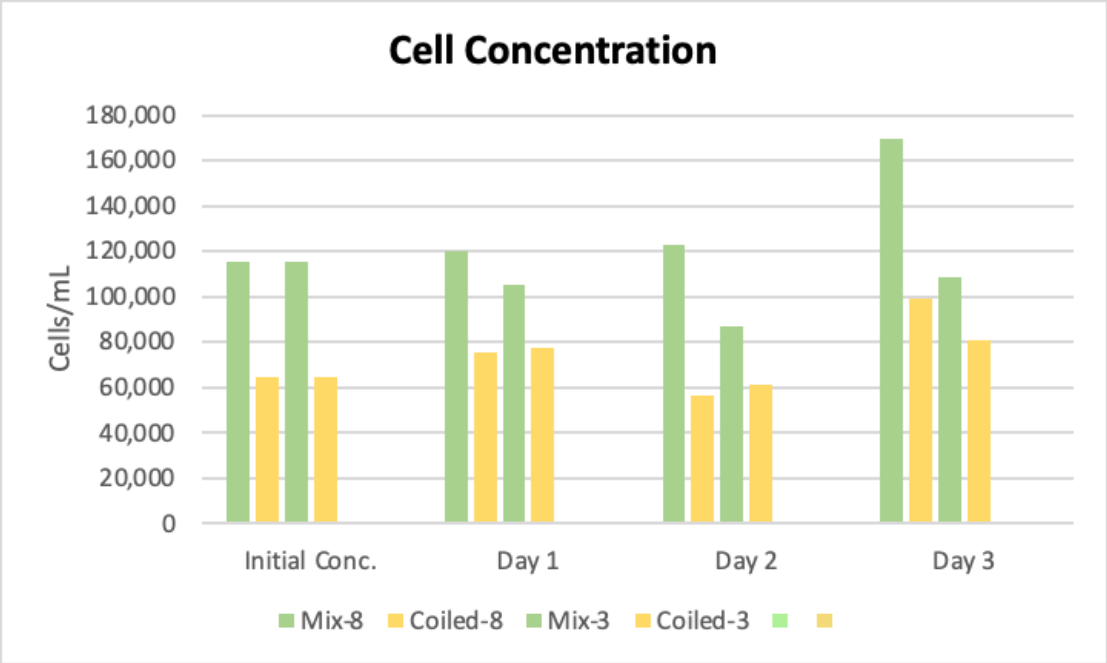

Figure 8. This bar graph shows the cell/mL concentrations for the high and low light intensities (8 µmol/m^2/s and 3 µmol/m^2/s) for both the mixed and coiled cultures over the 24 hour periods (Day 1, Day 2, and Day 3).

After the first 24 hours of incubation, all of the cultures saw an increase in cell count except for the mixed culture in the low light intensity (Figure 8). For day 2, the second 24 hour period the cell concentrations decreased except for the mixed culture at high light intensity. After the third 24 hour period, day 3, the cell concentrations increased for all cultures. The greatest amount of growth in cell population was found after the third 24 hour period on day 3 for the coiled culture at high light intensity. It should be noted that the initial cell counts on average for the mixed and coiled cultures were significantly different. The mixed culture started at a much higher concentration than the coiled cultures and the deficit should be considered when comparing data. Although differences in cell concentrations were recorded, the statistical

analysis indicated that there were not any significant differences among the groups that were valid (Table 1).

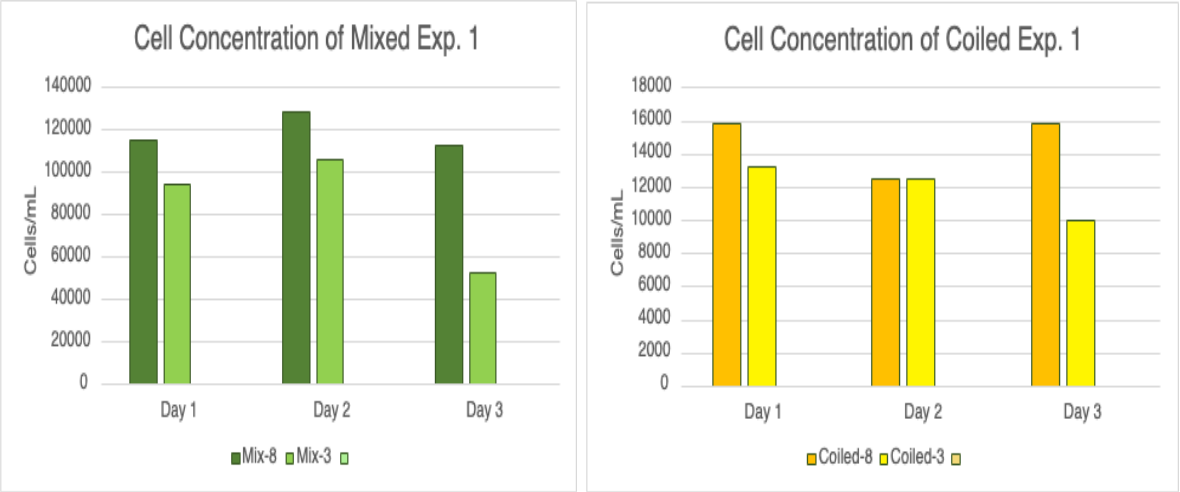

Figure 9. Experiment 1 cell concentrations for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

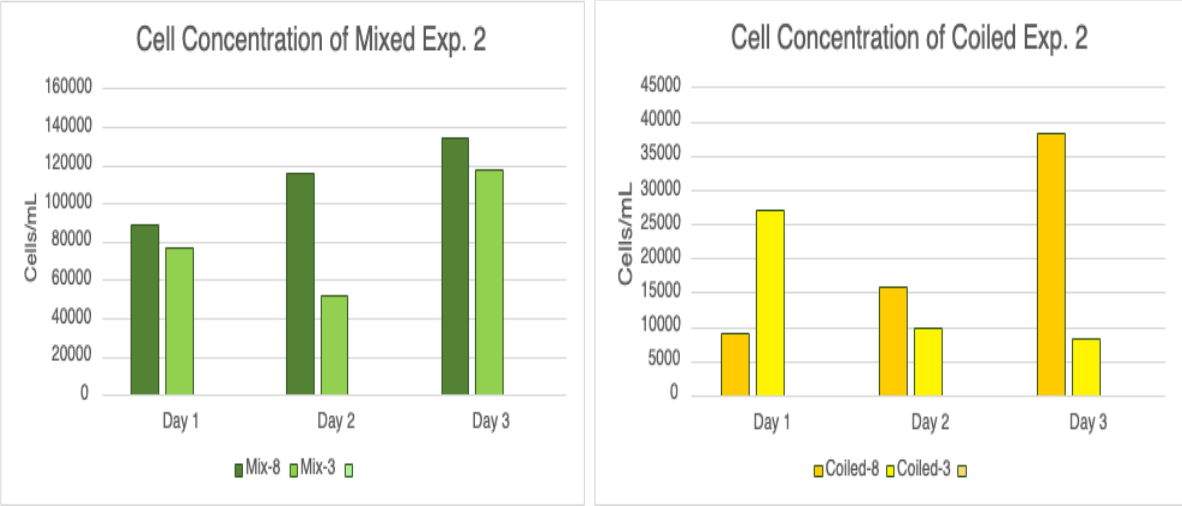

Figure 10. Experiment 2 cell concentrations for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

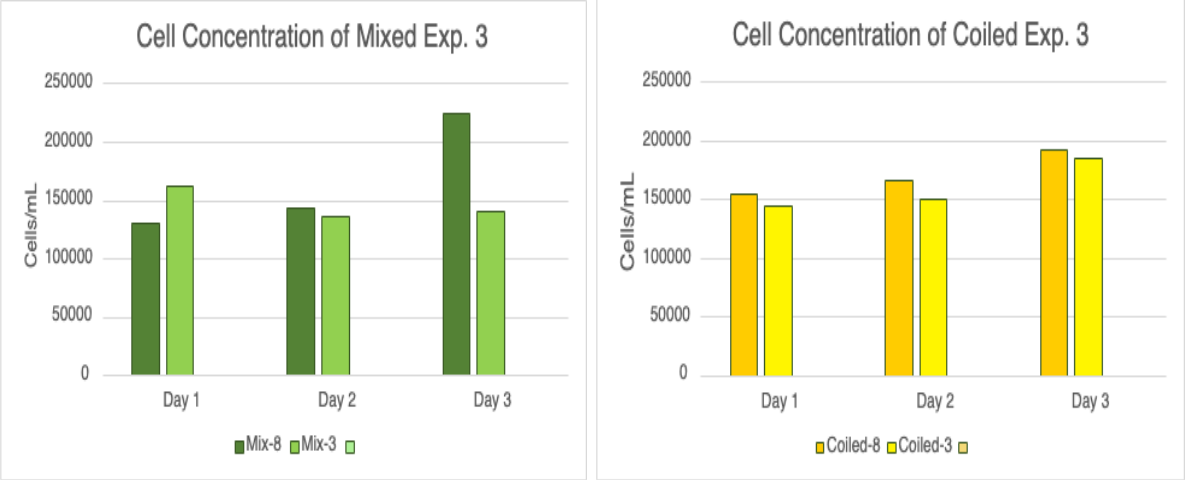

Figure 11. Experiment 3 cell concentrations for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

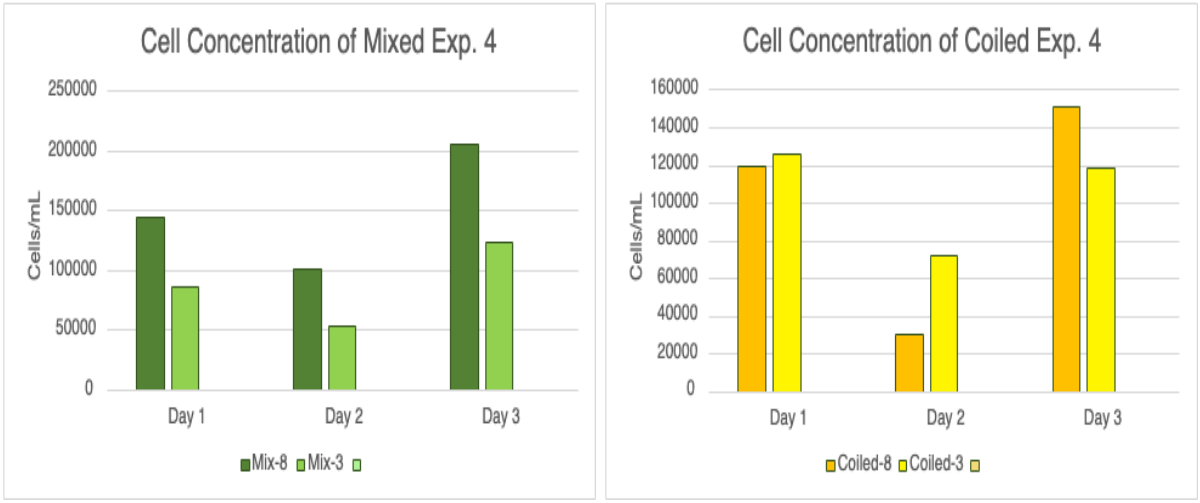

Figure 12. Experiment 4 cell concentrations for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

The highest total cell concentration was found to be 225,000 cell/mL during Experiment 3 for the mixed culture on day 3 at 8 µmol/m^2/s (Figure 11). The greatest increase in cell count between 24 hour periods was 120,075 for Experiment 4's day 3 coiled culture at 8 µmol/m^2/s

(Appendix C). Based on the statistical test results, the data for cell concentrations of all four experiments did not have a significant difference between the high and low light intensities. Cell concentration values also varied depending between experiments because the original culture's cell concentration did not remain constant and the health of culture varied at different points throughout experimentation. The coiled culture also started at a lower concentration than the mixed culture in each experiment.

Oxygen Production			
Test	Results		
Shapiro-Wilk Normality Test	W: 0.85427	N/A	P-Value: 2.828×10^{-5}
Bartlett Test of Homogeneity of Variances Test	K Squared: 15.938	Df: 3	P-Value: 0.001168
Kruskal-Wallis Rank Sum Test	Chi-Squared 15.841	Df: 1	P-Value: 6.888×10^{-5}
Kruskal-Wallis MC	High-Low Significant Difference: TRUE		

Table 2. Statistical Tests for the oxygen production (cm^3) depending on the high and low light intensities (8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$).

The assumptions of the One-Way ANOVA were not met for the high and low light intensity oxygen production because results of the Shapiro-Wilk Normality Test had a p-value of $0.2.828 \times 10^{-5}$ and the Bartlett Test of Homogeneity of Variances Test had a p-value of 0.001168 (Table 2). Since the p-values were less than 0.05 for both of these tests, a One-Way ANOVA could not be conducted and a nonparametric Kruskal-Wallis Rank Sum Test was conducted instead to give a p-value of 6.888×10^{-5}. This p-value indicated that the null hypothesis could be

rejected and that there was a statistically significant difference in the data for the oxygen production when comparing the high and low light intensities. A Kruskal-Wallis MC test was run to determine that the high and low groups were significantly different in the oxygen production.

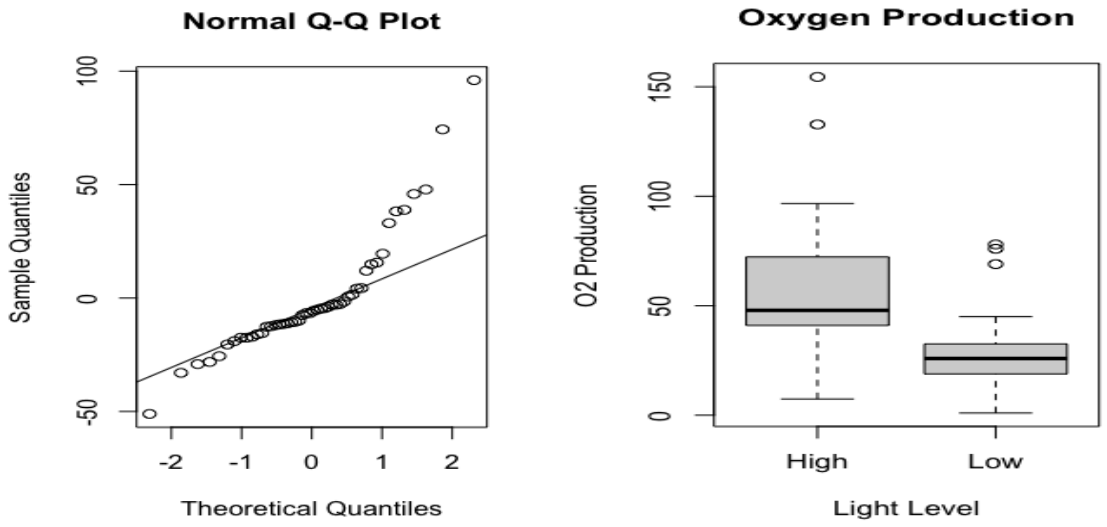

Figure 13. Left: Normal Q-Q plot of oxygen production (cm^3) by light intensities. Right: Box plot of oxygen production (cm^3) depending on the high and low light intensities (8 µmol/m^2/s and 3 µmol/m^2/s).

The Q-Q plot shows that the data for oxygen production is not normally distributed because there are points lying outside of the regression line. This indicates that the data is skewed (Figure 13). The box plot shows that there is significant variance among the high and low light intensity groups for the oxygen production (Figure 13). There are also points lying outside of the box range indicating extreme values in the data. Both of these plots demonstrate statistically significant results of the Shapiro-Wilk Normality test and Bartlett Test of Homogeneity. The oxygen production (cm^3) data was statistically significant and there were valid differences between the high and low light intensities.

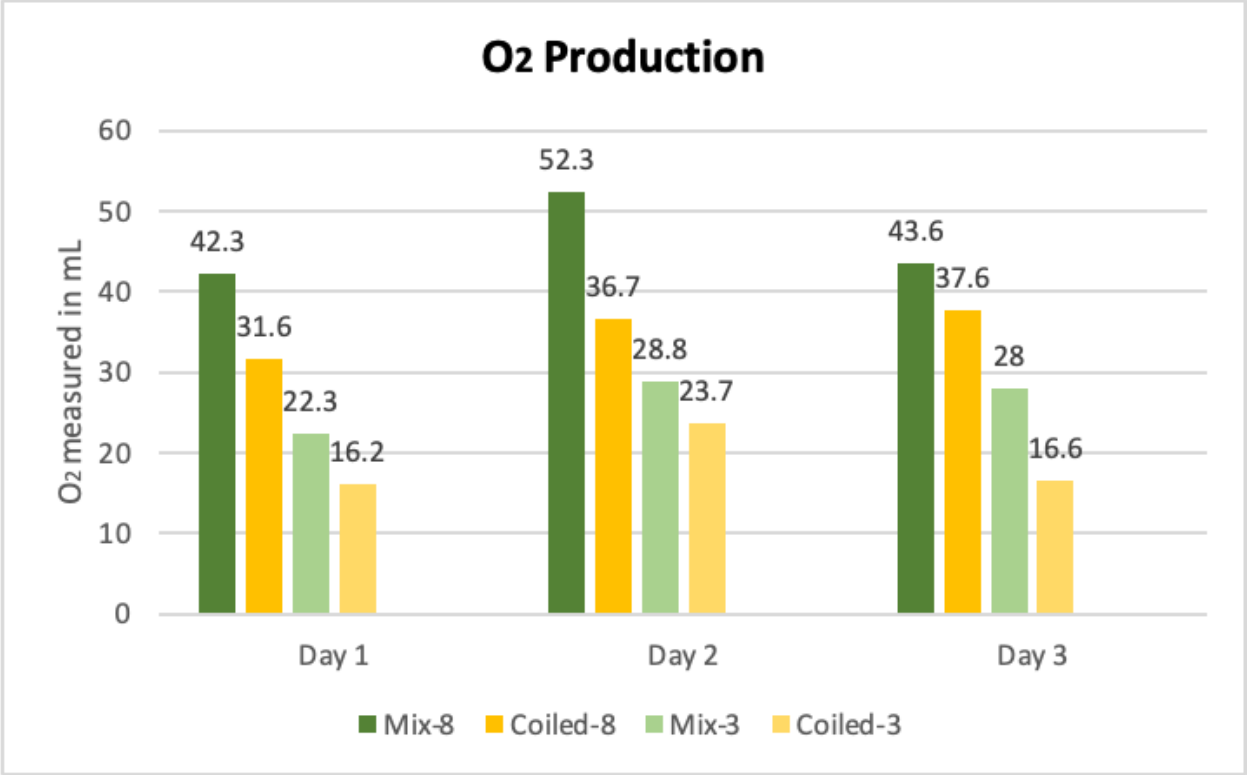

Figure 14. This bar graph shows the oxygen production (cm^3) for the high and low light intensities (8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$) for both the mixed and coiled cultures for each 24 hour period (Day 1, Day 2, and Day 3).

The bar graph demonstrates the differences in oxygen production for the mixed and coiled cultures at the high and low light intensities for each 24 hour period. The mixed culture at 8 $\mu mol/m^2/s$ produced the most oxygen on average for all three days. The 8 $\mu mol/m^2/s$ light intensity produced more oxygen on average than the 3 $\mu mol/m^2/s$ for the mixed and coiled cultures for each 24 hour period. The most oxygen produced on average was 52.3 cm^3 on day 2 for the mixed culture at 8 $\mu mol/m^2/s$. Since this is not considering oxygen produced per cell, the mixed culture produced more oxygen on average at each 24 hour period measurement (Figure 14).

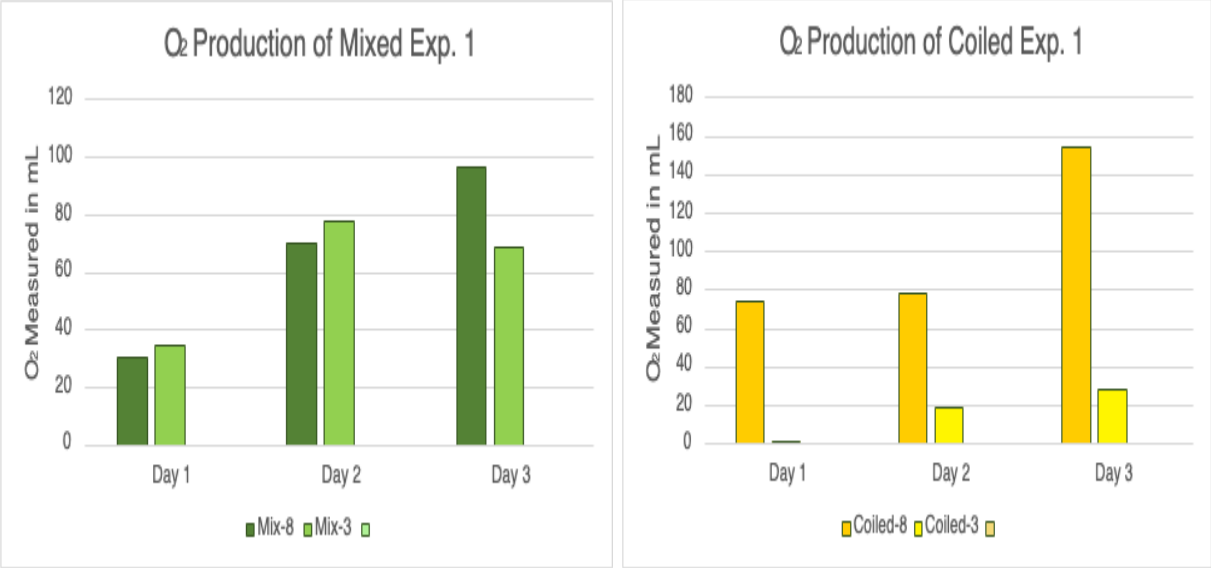

Figure 15. Experiment 1 oxygen production (cm^3) for the mixed and coiled cultures at the 8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$ for three 24 hour periods (Day 1, Day 2, Day 3).

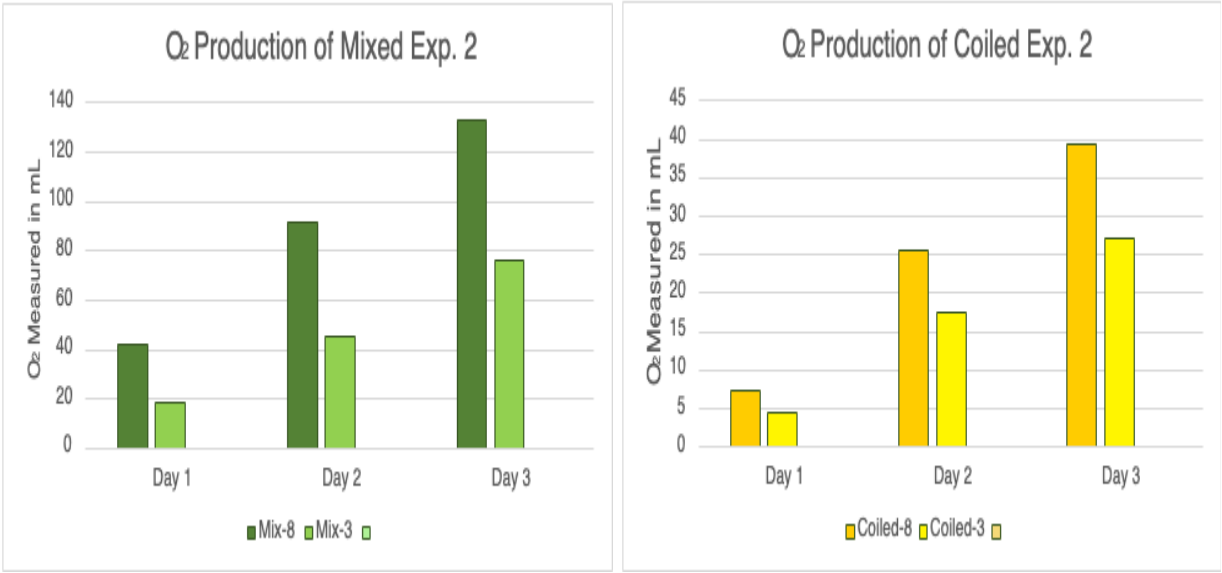

Figure 16. Experiment 2 oxygen production (cm^3) for the mixed and coiled cultures at the 8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$ for three 24 hour periods (Day 1, Day 2, Day 3).

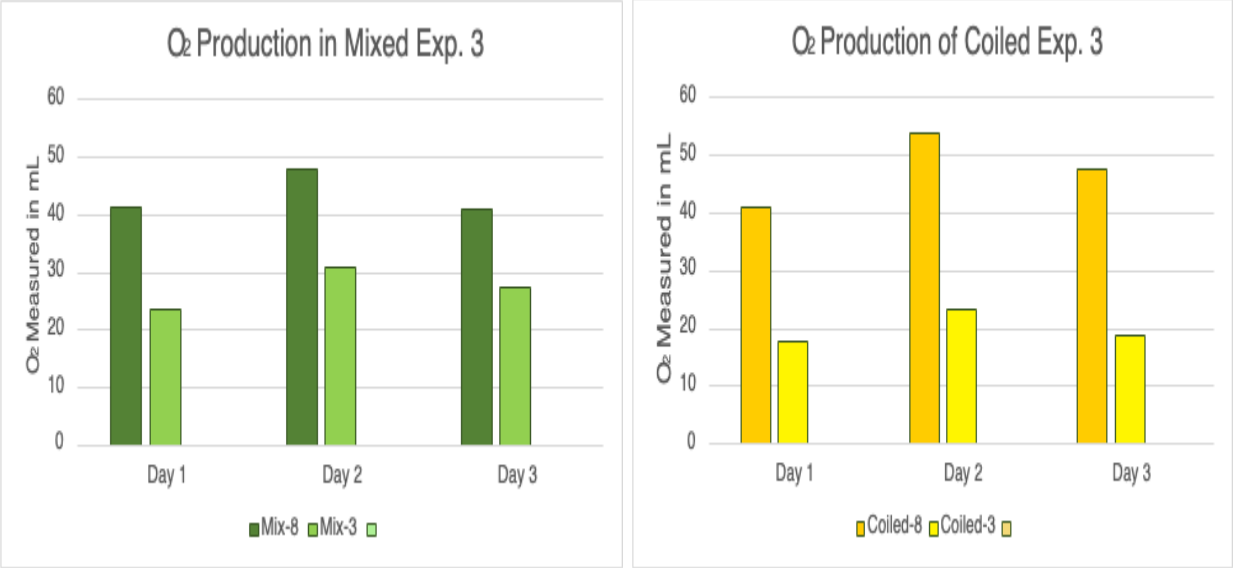

Figure 17. Experiment 3 oxygen production (cm^3) for the mixed and coiled cultures at the 8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$ for three 24 hour periods (Day 1, Day 2, Day 3).

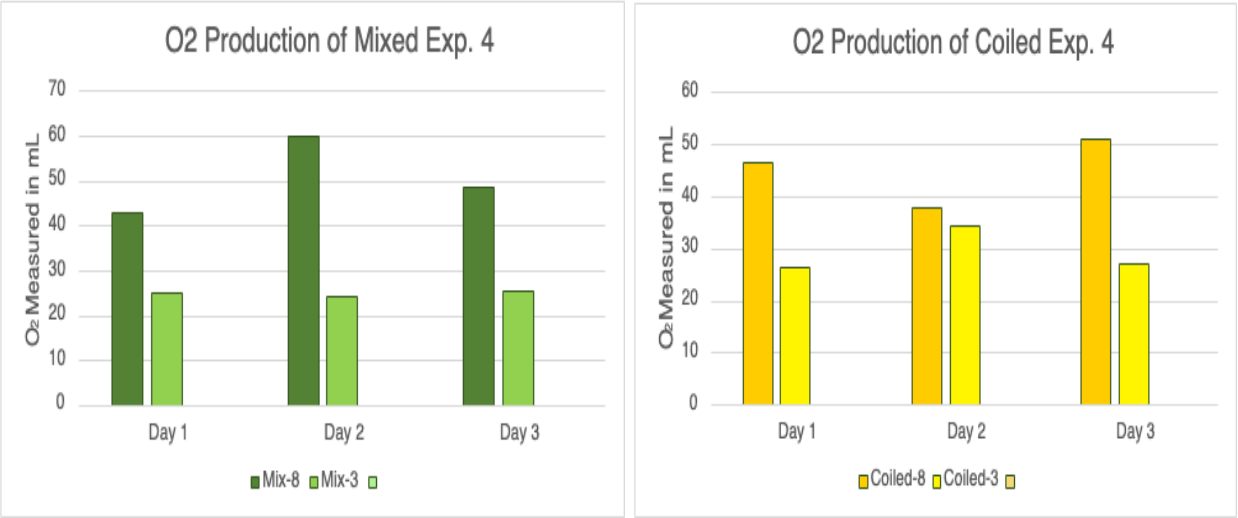

Figure 18. Experiment 4 oxygen production (cm^3) for the mixed and coiled cultures at the 8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$ for three 24 hour periods (Day 1, Day 2, Day 3).

The oxygen production was found to have a statistically significant difference between light intensities. In each of the graphs, except experiment 1 mixed, the 8 $\mu mol/m^2/s$ produced

more O_2 than the 3 µmol/m^2/s light intensity (Figure 15). The most oxygen was produced during Experiment 1 by the coiled culture under 8 µmol/m^2/s at 154.5 cm^3 (Appendix B).

Oxygen Production Per Cell			
Test	Results		
Shapiro-Wilk Normality Test	W: 0.59242	N/A	P-Value: 2.57×10^{-10}
Bartlett Test of Homogeneity of Variances Test	K-Squared 65.422	Df: 3	P-Value: 4.074×10^{-14}
Kruskal-Wallis Rank Sum Test	Chi-Squared 4.0833	Df: 1	P-Value 0.04331
Kruskal-Wallis MC	High-Low Significant Difference: TRUE		

Table 3. Statistical tests for the oxygen production per cell (cm^3/cell) depending on the high and low light intensities.

The assumptions of ANOVA were not met for the high and low light intensity oxygen production per cell. The results of the Shapiro-Wilk Normality Test had a p-value of 2.57×10^{-10} and the Bartlett Test of Homogeneity of Variances Test had a p-value of 4.074×10^{-14} (Table 3). Like the tests for oxygen production, the p-values were less than 0.05 for both of these tests and a One-Way ANOVA could not be run. A nonparametric Kruskal-Wallis Rank Sum Test was run instead to give a p-value of 0.04331. This p-value indicated that the null hypothesis could be rejected and that there was a statistically significant difference in the data for the oxygen production per cell when comparing the high and low light intensities. A Kruskal-Wallis MC test was run to determine that the high and low groups were significantly different in the oxygen production per cell.

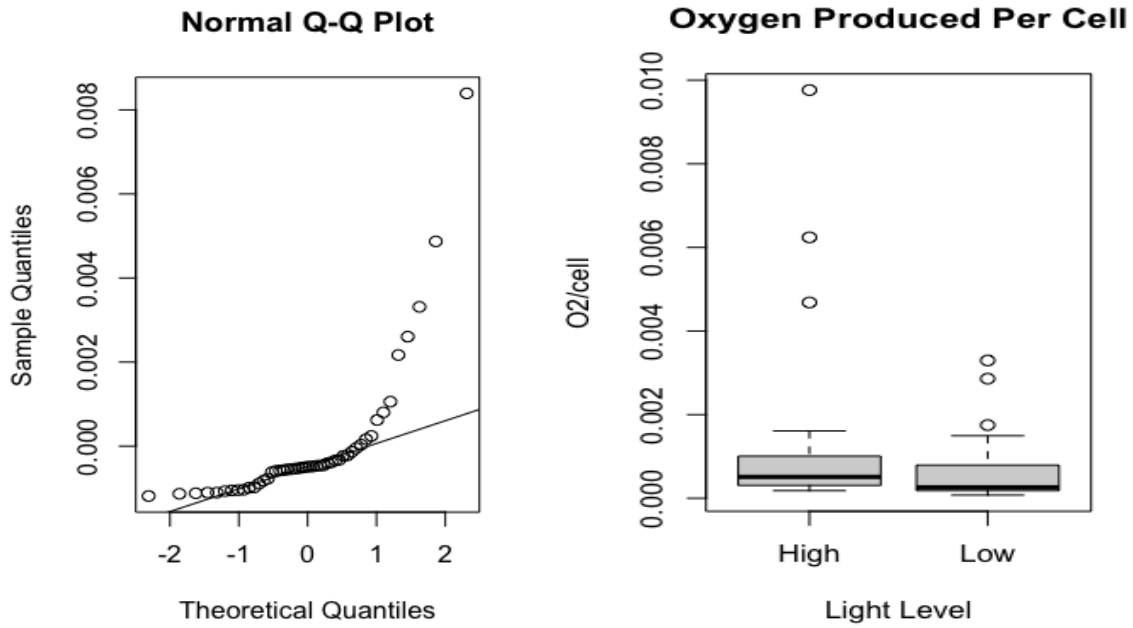

Figure 19. Left: Normal Q-Q plot of oxygen production per cell (cm^3/cell) by light intensities. Right: Box plot of oxygen production per cell (cm^3/cell) depending on the high and low light intensities (8 µmol/m^2/s and 3 µmol/m^2/s).

The Q-Q plot shows that the data for oxygen production per cell is not normally distributed and is skewed because there are points lying outside of the regression line at extreme values (Figure 19). The box plot shows that there is significant variance among the high and low light intensity groups for the oxygen per cell production (Figure 19). Both of these plots demonstrate statistically significant results of the Shapiro-Wilk Normality test and Bartlett Test of Homogeneity. The oxygen production per cell data was statistically significant and there were valid differences between the high and low light intensities.

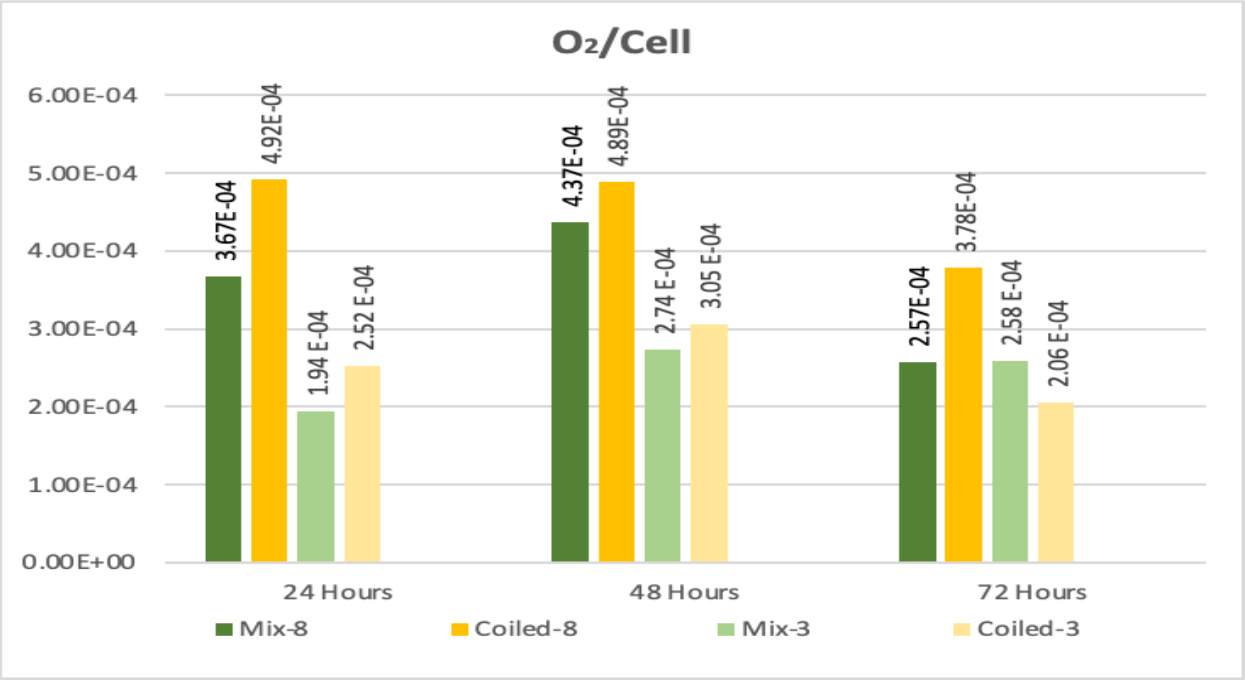

Figure 20. This bar graph shows the oxygen production per cell (cm^3/mL)for the high and low light intensities (8 µmol/m^2/s and 3 µmol/m^2/s) for both the mixed and coiled cultures for each 24 hour period (Day 1, Day 2, and Day 3).

As the statistical tests determined, the data for oxygen produced per cell was significantly different for the high and low light intensities (Table 3). The 8 µmol/m^2/s produced more oxygen per cell on average than the 3 µmol/m^2/s for both the mixed and coiled cultures at each 24 hour period except for day 3 for the mixed culture. When considering the amount of oxygen produced per cell for each culture it was determined that the coiled cultures produced more O_2 cm^3 per cell on average for both the high and low light intensities for all three 24 hour periods. This indicated that the coiled Spirulina produced more oxygen overall. The most oxygen on average was produced by the high light intensity coiled Spirulina on day 1 at 4.92 x 10^{-4} O_2 cm^3/cell.

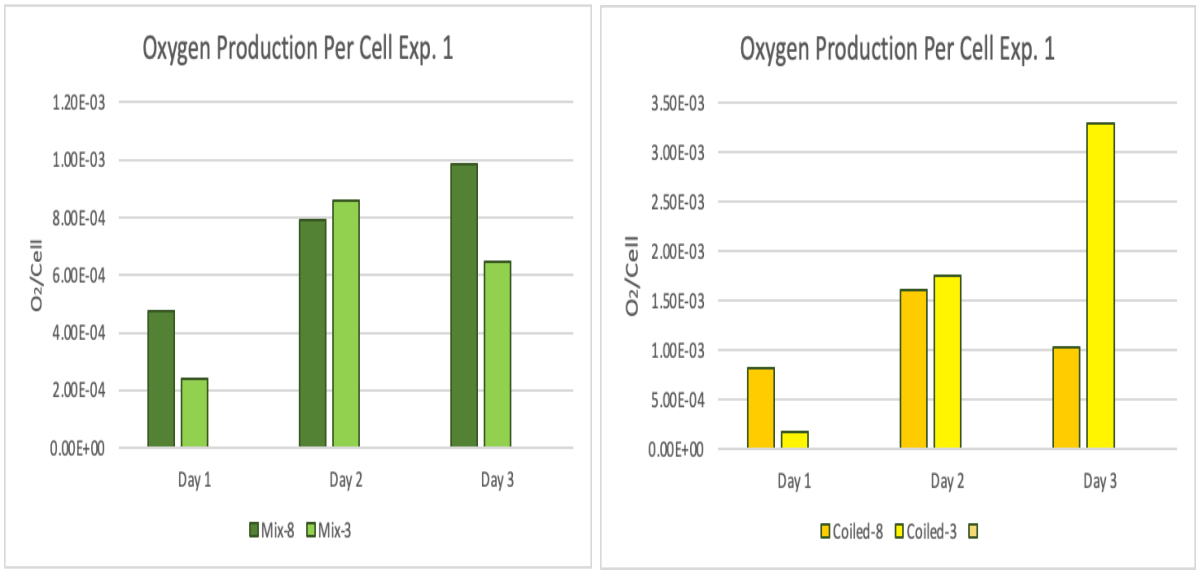

Figure 21. Experiment 1 oxygen production (cm^3/cell) for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

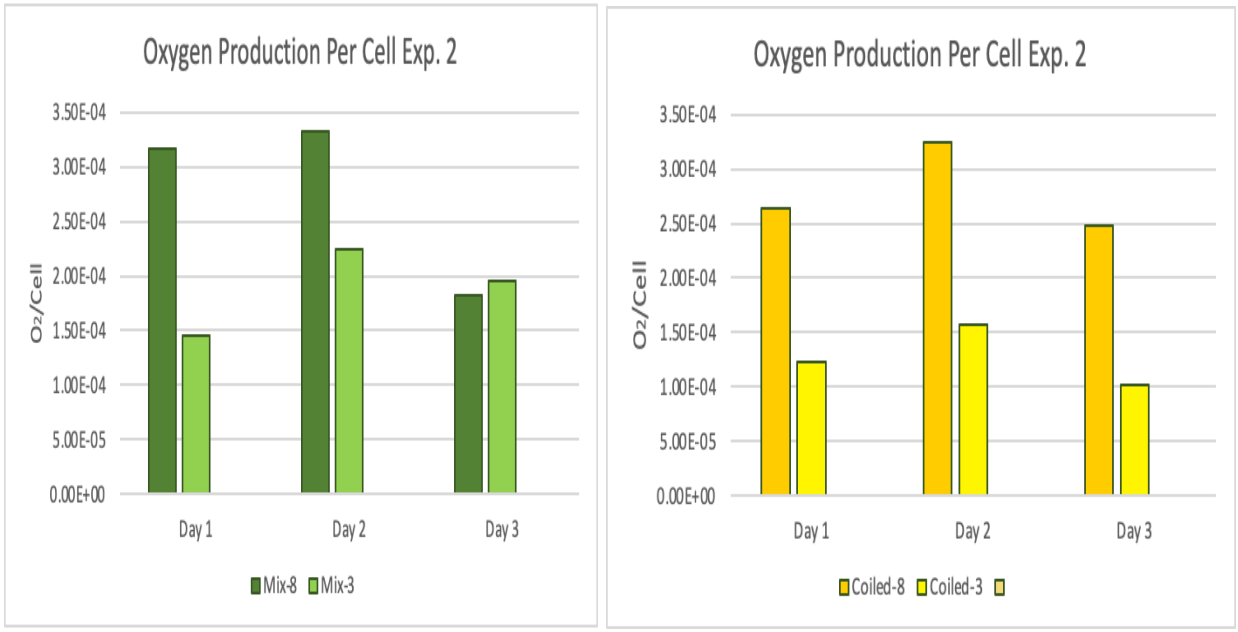

Figure 22. Experiment 2 oxygen production (cm^3/cell) for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

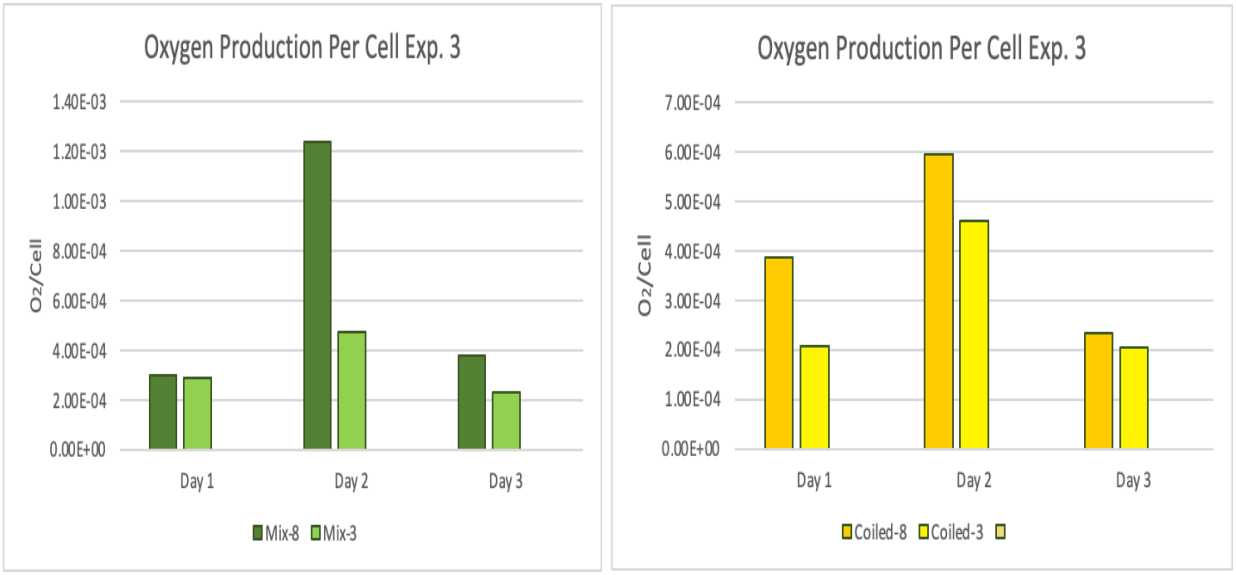

Figure 23. Experiment 3 oxygen production (cm^3/cell) for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

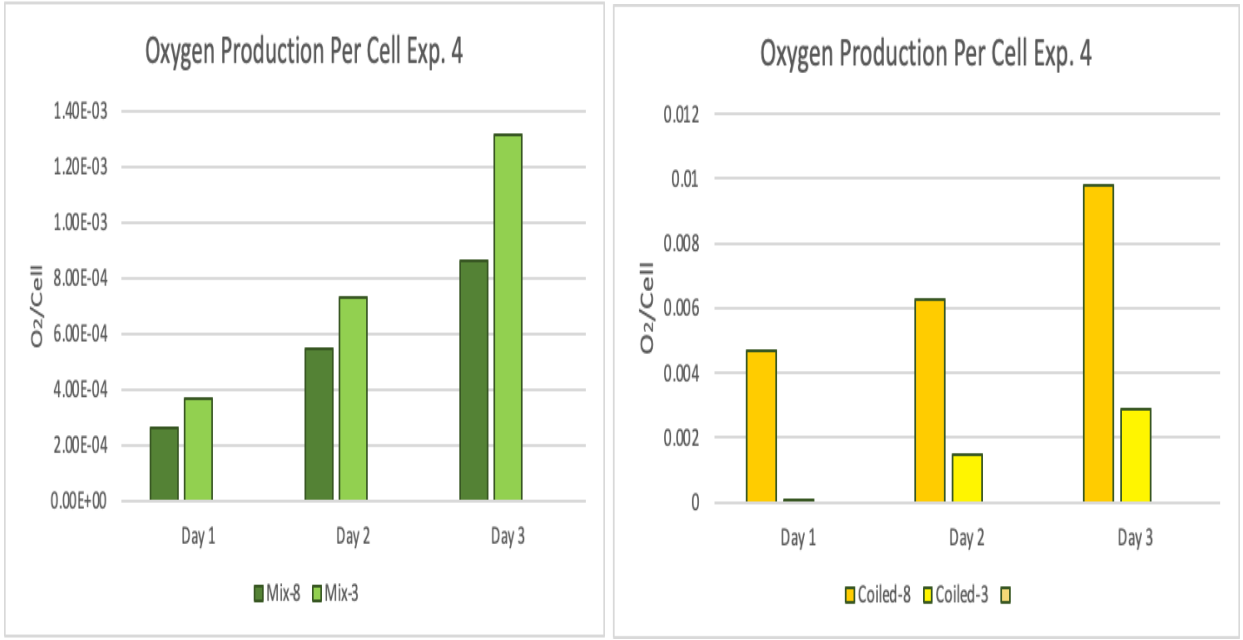

Figure 24. Experiment 4 oxygen production (cm^3/cell) for the mixed and coiled cultures at the 8 µmol/m^2/s and 3 µmol/m^2/s for three 24 hour periods (Day 1, Day 2, Day 3).

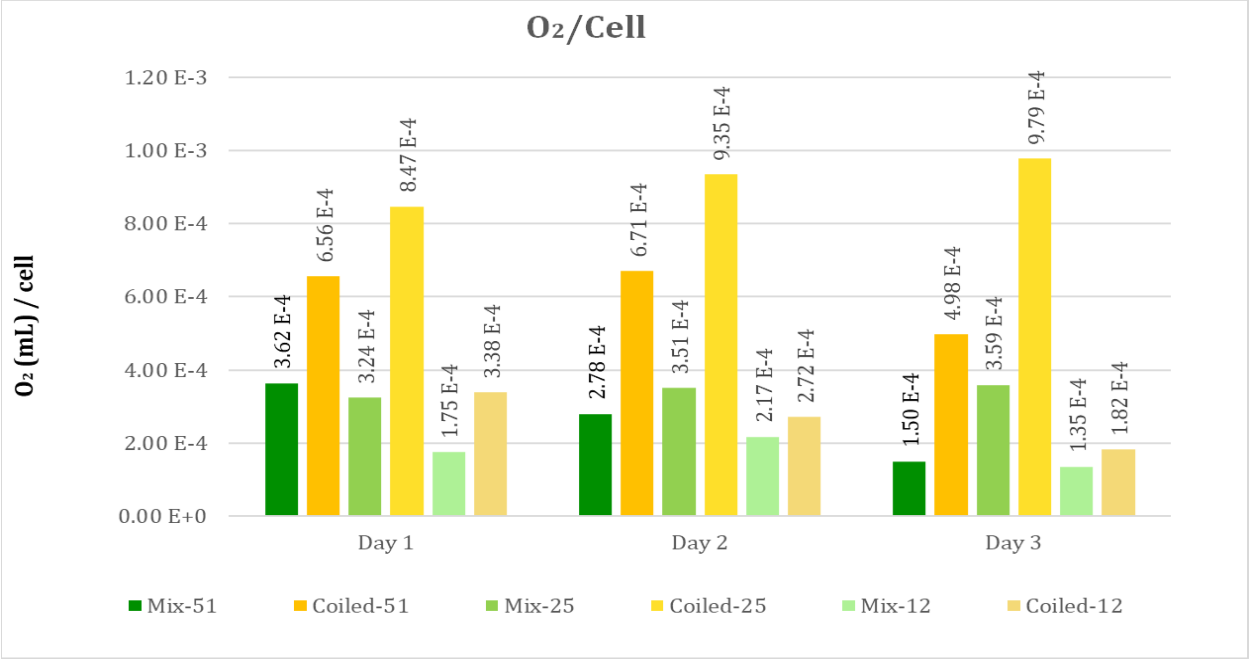

Figure 25. This graph shows the results of white light intensity results for oxygen production per cell (cm^3/cell) during a previous experiment. These results were used to compare the data of the red light intensities for oxygen production per cell.

When considering previous experiments with greater light intensities, 51 µmol/m^2/s, 25µmol/m^2/s, and 12 µmol/m^2/s, the high white light intensity of 25 µmol/m^2/s presented the highest oxygen produced per cell on average was 9.35 x 10^{-4} cm^3/cell by the coiled Spirulina at 25 µmol/m^2/s (Figure 25). While a coiled culture after 24 hours at 8 µmol/m^2/s produced on average 4.92 x 10^{-4} O_2 cm^3 per cell (Figure 20). Despite the 25 µmol/m^2/s white light intensity producing double the oxygen per cell on average, red light intensity at 8 µmol/m^2/s still produced a 24 hour period total of 9.28 x 10^{-3} in Experiment 4 which is greater than any total oxygen per production in the white light intensity experiments (Figure 24). The coiled Spirulina also consistently produced more oxygen when compared to mixed Spirulina after comparing results with other previous experiments using white light.

Discussion

The results of the statistical analysis for cell concentration for high and low light intensities show that there was not any significant difference between the two intensities. This could be due to the large difference in starting cell concentrations for the original mixed and coiled cultures. In future experiments a 50/50 ratio between the cultures for cell count could potentially result in a statistically significant difference in the cell concentrations for the cultures being tested. Accomplishing more even cell concentrations would also provide more accurate results for overall oxygen production. A significant difference may also have been detected had there been more statistical power and this should be considered in future experiments. If a potential correlation between cell growth rate and oxygen production of both of the Spirulina structure types were to be determined then it may also help find which Spirulina structure has a better oxygen production rate.

The statistical tests indicated that the high and low light intensities did have a significant difference in the oxygen production and oxygen production per cell. The box plot and Q-Q plot indicated that both did not have normally distributed data and that differences between light intensities were present. For oxygen production, the high light at 8 $\mu mol/m^2/s$ produced more oxygen on average than the 3 $\mu mol/m^2/s$. The mixed Spirulina at each 24 hour period produced more oxygen than the coiled Spirulina. The greatest average oxygen production was on day 2 at 52.3 cm^3 and the greatest total oxygen production was during Experiment 3 at 132.8 cm^3 on day 3. However, these results are skewed due to the initial concentrations of the mixed cultures being significantly greater than the coiled cultures. Further experiments with even initial concentrations would be ideal and would theoretically be consistent with the results of oxygen per cell

production. The oxygen per cell production was greatest at the high 8 μmol/m^2/s light intensity. By looking at the oxygen production per cell, the data can be seen with less skew.

The coiled Spirulina produced more oxygen per cell for each 24 hour period. Although there is no definite reason behind coiled Spirulina's greater O_2 output there are some theories that suggest why. First, the coiled Spirulina assumes a helical conformation allowing it to distribute the amount of light it receives throughout its cell. In contrast, straight Spirulina is forced to receive the intensity of light at the same level at all times. This helical conformation could potentially allow for energy to be concentrated at certain points on the cell that absorbs more nutrients and CO_2. It has been found that the straight conformation is typically favored in artificial laboratory settings (Silli, 2012). This may be important for scientists to consider when trying to grow cultures for space travel since the coiled Spirulina is the desired conformation for maximum O_2 production. Further research as to why the straight conformation is formed by Spirulina could help cultivate more cultures with coiled only in the future. When considering space travel, the coiled structured Spirulina would be the more efficient culture to grow while in space travel.

After comparing results with previous experimentation, it was determined that with further research and experimentation red light intensity could compete with a white light intensity for best oxygen production output. For instance, although the higher light intensities, like 25 μmol/m^2/s, in previous experiments produced almost double the O_2/cell at 9.35 x 10^{-4} , the 8 μmol/m^2/s red light intensity still produced on average 4.92 x 10^{-4} which is considered a moderate amount. Additionally the 8 μmol/m^2/s still produced a greater total amount of oxygen per cell during Experiment 4 with a value of 9.28 x 10^{-3} . If explored further with multiple replications of the results, it could be determined that red light could aid as a cost-efficient

resource. These results are encouraging because they show that the Spirulina can grow and produce oxygen in light intensities that are lower than the traditional growth conditions. This could ultimately result in red light as a preferred source of light for space travel because of its maximization of energy at lower intensity. Statistical analysis on white light intensities versus red light intensities would be vital in determining a true significant difference within the data and should be used for future experiments.

The protein composition of Spirulina plays an important role in the nutritional value of the algae. Red light has been found to increase the production of an important nutritional protein within Spirulina called phycocyanin. With this information, it could be determined that red light increases phycocyanin production in Spirulina which would ultimately increase the nutritional value of the algae for astronauts while on mission. For instance, ingesting phycocyanin provides impressive antioxidant and anti-inflammatory properties to the body. A phycocyanin protein extraction and quantification procedure would allow for the protein production to be compared between both the coiled and straight Spirulina structures as well as red light intensity's effect on its production. Further experiments should consider including this as part of the study.

Conclusions

The results of this experiment suggest that Spirulina grown in red light intensities, 8 $\mu mol/m^2/s$ and 3 $\mu mol/m^2/s$ have the potential to produce oxygen at moderately sufficient amounts and potentially greater amounts than white light intensities. Space travel could ultimately benefit from the use of these lower intensities because the red light intensities like 8 $\mu mol/m^2/s$ use less valuable energy when compared to the high white light intensities like the 25 $\mu mol/m^2/s$. The coiled structure of Spirulina consistently produces more oxygen than coiled and

straight Spirulina combined (mixed). Although there is a lack of understanding as to why, further experimentation could provide more reasoning behind the coiled structure's photosynthetic benefits. A high red light intensity of 8 μmol/m^2/s produced more oxygen than the low 3 μmol/m^2/s and indicated that there is potential for equal or greater oxygen output than Spirulina grown at white light intensities like 25 μmol/m^2/s. However, further experiments with a larger number of replications could provide more evidence on oxygen production between these intensities.

References

Begum, J. (2022, September 21). *Spirulina: Dosage, eye health, Oral Health, and more*. Medical News Today. Retrieved November 23, 2022, from https://www.medicalnewstoday.com/articles/324027#eye-health

Bernstein, J. (2021, May 18). *Reaching for the stars*. We Are The New Farmers. Retrieved November 22, 2022, from https://www.new-farmers.com/blogs/news/nasa-Spirulina-space-superfood

Burgess, L. (2022, May 4). *Spirulina: Dosage, eye health, Oral Health, and more*. Medical News Today. Retrieved November 23, 2022, from https://www.medicalnewstoday.com/articles/324027#nutrition

Fais, G., et al. (2022, April 28). *Wide range applications of Spirulina: From earth to `space missions*. Marine drugs. Retrieved November 23, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9143897/

Karkos, P. D. (2010, October 19). *Spirulina in clinical practice: Evidence-based human applications.* Evidence-based complementary and alternative medicine : eCAM. Retrieved November 23, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3136577/

Mapstone, L. J., et. al (2022, March 19). Cyanobacteria and microalgae in supporting human habitation on Mars. Biotechnology Advances. Retrieved November 22, 2022, from https://www.sciencedirect.com/science/article/pii/s0734975022000428

Mars, K. (2021, February 3). *What happens to the human body in space?* NASA. Retrieved November 23, 2022, from https://www.nasa.gov/hrp/bodyinspace

Masojídek, J. (2008, August 6). *Mass cultivation of freshwater microalgae*. Encyclopedia of Ecology. Retrieved November 23, 2022, from https://www.sciencedirect.com/science/article/pii/B9780080454054008302

Moore, T. (2020, December 4). *Superfood goes to space*. News. Retrieved November 23, 2022, from https://blogs.ifas.ufl.edu/news/2020/12/04/superfood-goes-to-space/#:~:text=The%20Spirulina%20will%20grow%20in,samples%20for%20analysis%20on%20Earth.

Sang Vo, T. (2015, May 8). *Nutritional and pharmaceutical properties of Microalgal Spirulina*. Handbook of Marine Microalgae. Retrieved November 23, 2022, from https://www.sciencedirect.com/science/article/pii/B9780128007761000194

Spirulina, F. (2021, December 12). *Spirulina under the microscope - freshly Frozen Spirulina*. Fresh Australian Spirulina. Retrieved November 23, 2022, from https://www.freshSpirulina.com.au/spirulina/spirulina-under-the-microscope/

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Stal, L. (2015, December 23). *Nitrogen fixation in cyanobacteria - Stal - Wiley Online Library*. Wiley Online Library. Retrieved from https://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0021159.pub2

Sili, C. (2012). *Bashan Foundation*. Retrieved November 22, 2022, from http://www.bashanfoundation.org/contributions/Vonshak-A/vonshakarthorspira.pdf

Wollina, U., Voicu, C., Gianfaldoni, S., Lotti, T., França, K., & Tchernev, G. (2018, January 10). *Arthrospira platensis - potential in dermatology and beyond*. Open access Macedonian journal of medical sciences. Retrieved November 22, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5816296/

Appendix A

Zarrouk's Nutrient Media

Solution A
500 ml deionized water
1.43 g Boric acid
0.905 g Magnesium (II) chloride tetrahydride
0.110 g Zinc sulfate
0.040 g Copper Sulfate
0.050 g Molybdenum oxide
Solution B
500 mL deionized water
0.01145 g Ammonium vanadate
0.04800 g Chrome alum
0.02390 g Nickel sulfate
0.02200 g Cobalt nitrate
Growth Media
4 L deionized water
32 g Sodium bicarbonate
2.0 g Dipotassium hydrogen phosphate
10 g Sodium nitrate
4.0 g Potassium sulfate
0.8 g Magnesium sulfate
sulfate 0.16 g Calcium chloride
0.04 g Ferrous sulfide
0.32 g EDTA 4.0 mL
4.0 mL solution A
4.0 mL solution B

Appendix B

Experiment	Population (cells/mL)				Oxygen (cm^3)			Oxygen per cell (cm^3/cell)		
Experiment 1	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	115,000	115,000	129,000	112,500	30.3	70.5	96.7	4.76E-04	7.90E-04	9.85E-04
Coiled-8	25,000	15,825	12,500	15,825	74.1	78	154.5	8.15E-04	1.61E-03	1.03E-03
Mix-3	115,000	94,250	105,750	52,500	34.5	50.6	69	2.39E-04	8.57E-04	6.47E-04
Coiled-3	25,000	13,250	12,500	10,000	1	18.7	28.6	1.67E-04	1.75E-03	3.29E-03
Experiment 2	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	67,000	89,750	115,750	134,875	42.5	91.5	132.8	3.17E-04	3.33E-04	1.82E-04
Coiled-8	7,500	9,075	15,825	38,350	7.4	25.5	39.5	2.64E-04	3.24E-04	2.48E-04
Mix-3	67,000	77,500	52,500	117,500	18.5	45	76	1.45E-04	2.25E-04	1.96E-04
Coiled-3	7,500	27,000	10,000	8,250	4.5	17.5	27.2	1.23E-04	1.57E-04	1.02E-04
Experiment 3	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	152,500	130,750	144,250	225,000	41.4	48	41	2.98E-04	1.24E-03	3.81E-04
Coiled-8	211,750	155,500	166,750	192,500	41	54	47.8	3.88E-04	5.94E-04	2.34E-04
Mix-3	162,500	162,500	136,750	140,000	23.5	30.8	27.5	2.89E-04	4.73E-04	2.30E-4
Coiled-3	211,750	144,250	150,000	185,750	17.7	23.5	19	2.09E-04	4.60E-04	2.04E-04
Experiment 4	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	125,750	144,250	101,000	206,000	43	60	48.5	2.63E-04	5.47E-04	8.60E-04
Coiled-8	126,000	120,000	30,750	150,825	46.5	38	51	4.68E-03	6.24E-03	9.76E-03
Mix-3	125,750	86,500	53,250	124,250	25	24.5	25.5	3.66E-04	7.28E-04	1.31E-03
Coiled-3	126,000	126,000	72,500	118,500	26.3	34.3	27.2	7.55E-04	1.49E-03	2.86E-03

Appendix C

Experiment	Population (cells/mL)				Oxygen (cm^3)		
Experiment 1	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	115,000	0	14,000	-16,500	30.3	70.5	96.7
Coiled-8	25,000	-9,175	-3,325	3,325	74.1	78	154.5
Mix-3	115,000	-20,7500	56,500	-53,250	34.5	50.6	69
Coiled-3	25,000	-11750	-750	-2500	1	18.7	28.6
Experiment 2	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	67,000	22,750	26,000	19,125	42.5	91.5	132.8
Coiled-8	7,500	1,575	6,750	22,525	7.4	25.5	39.5
Mix-3	67,000	10,500	-25,000	65,000	18.5	45	76
Coiled-3	7,500	19,500	-17,000	-1,750	4.5	17.5	27.2
Experiment 3	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	152,500	-217,750	13,500	80,750	41.4	48	41
Coiled-8	211,750	-56,250	11,250	25,750	41	54	47.8
Mix-3	162,500	0	-25,750	3,250	23.5	30.8	27.5
Coiled-3	211,750	-67,500	5,750	35,750	17.7	23.5	19
Experiment 4	**Initial**	**24**	**48**	**72**	**24**	**48**	**72**
Mix-8	125,750	18,500	-43,250	105,000	43	60	48.5
Coiled-8	126,000	-6,000	-89,250	120,075	46.5	38	51
Mix-3	125,750	-39,250	-33,250	71,000	25	24.5	25.5
Coiled-3	126,000	0	53,500	46,000	26.3	34.3	27.2