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The Effects of Light Intensity and Cell Structure on the Cultivation of Arthrospira platensis

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The Effects of Light Intensity and Cell Structure on the Cultivation of *Arthrospira platensis*

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"The Effects of Light Intensity and Cell Structure on the Cultivation of *Arthrospira platensis*"

written by

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and submitted in partial fulfillment of
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

ABSTRACT

As scientists explore further into space, more cost-effective resources are needed for long-term space travel. An interesting solution is *Arthrospira platensis*, a filamentous cyanobacteria high in proteins and nutrients, and known for its helical structure. In unfavorable conditions, coiled spirulina cells become straight. Spirulina converts carbon dioxide gas into pure oxygen and the different cell structures stimulate different responses in oxygen production and cultivation. In these experiments, 2.3 L containers of pure coiled spirulina and mixed (coiled and straight) spirulina were placed into 3 incubators with different light intensities: 51µmol/m^2/s, 25µmol/m^2/s, 12µmol/m^2/s. Each experiment length was 72 hours and the spirulina cultures' cell concentration, and oxygen production was measured at the 24, 48, and 72 hour mark. During observations, the coiled spirulina expressed a continuous cell concentration growth, whereas the mixed spirulina steadily declined over the 72 hours. Results of this experiment also suggest that coiled spirulina produces more oxygen at an intensity of 25µmol/m^2/s and can do so at a lower concentration while growing at a consistent rate. The mixed cultures appear to multiply faster in a lower light intensity, but the oxygen production remains low.

INTRODUCTION

Imagine you are an astronaut in space. Inky blackness stretches out for miles in front of your eyes, broken up only by the peppering of stars, asteroids, and distant galaxies. Only there is one galaxy growing nearer, closer. The details and colors more vivid than anyone has ever seen. This is the farthest man has been. Your awe is interrupted by alarms blaring around you. Warnings of dangerously low oxygen supplies flash on the main controls. There is nothing you can do. You continue to gaze out at the galaxy, still growing ever closer, as you breathe the little amount of oxygen left on your spaceship. If only you had more resources, better resources to support your journey all the way to this unknown galaxy. If only you had taken *Arthrospira platensis* with you…

This scenario is extremely dramatic, but *Arthrospira platensis*, better known as Spirulina, could save an astronaut's life in space. It might even provide the opportunity for man to travel farther than we have ever gone before. Spirulina is known as a superfood. Not only that, but when given the proper nutrients and resources, spirulina can produce sufficient amounts of oxygen. Spirulina sounds like an astronaut's best friend in space.

Why? Space travel is evolving. Technology is able to go farther than before and deeper into space. Missions are becoming more long-term proving it necessary to find a nutrient-rich option that is cost-efficient and multifunctional. Scientists have been turning to algae as a resource that can offer multiple different benefits for space travel (Menezes 2015). Spirulina is a natural algae high in nutrients and protein (Ciferri 1983). It is a cyanobacteria or "blue-green algae" utilized as a dietary supplement which can be used by astronauts in long-term space flight or missions (Menezes 2015). The interest in the algae is a result of its using less resources than livestock but still producing a reasonable amount of protein. It is a more cost-efficient option compared to livestock. Both of these things are important to consider in space travel, as space and resources are limited.

Spirulina algae can adapt to take a straight or coiled structure. Spirulina's natural, coiled structure is shown below in Figure 1. Its unnatural straight structure is shown in Figure 2, which also shows the coiled cells side by side for comparison. Difference in conditions and environmental stressors can cause coiled spirulina to become straight (Ciferri 1983). Usually, unfavorable conditions cause this structural change (Wang 2005). Coiled spirulina is slightly preferred over straight structured spirulina because the coiled has a slightly higher protein concentration than the straight spirulina (Noor 2008). Previous research also found that the growth yield and protein content of linear spirulina strands were much lower than that of their coiled counterpart (Wang 2005). Straight spirulina were found with a slightly higher fat content than the coiled spirulina.

Figure 1. Microscopic Image of Characteristic Helical Shape of Arthrospira platensis. Sample taken from the Coiled original culture.

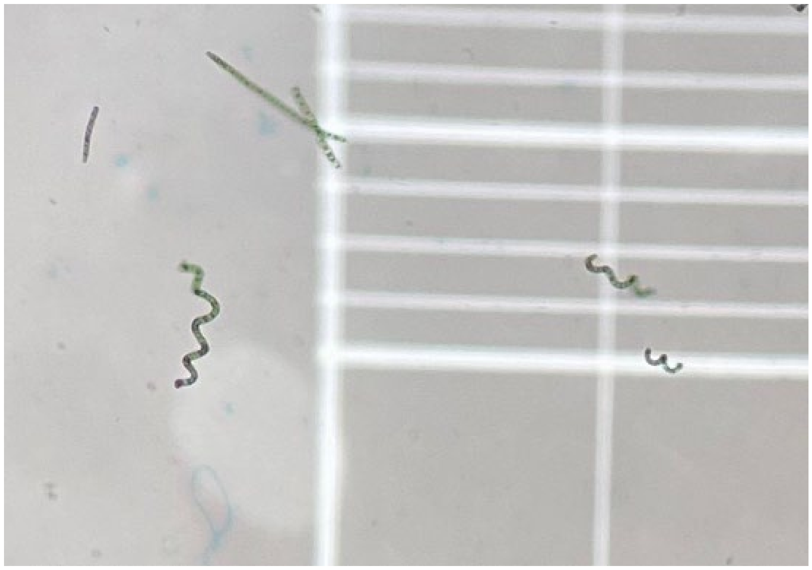

Figure 2. Microscopic Image of Spontaneous Stress-Response Conformation Change of Arthrospira platensis from Helical Structure to Linear Structure.

Another benefit of using spirulina is that it produces oxygen and removes carbon dioxide from its surrounding environment. Previous research has been done over the effect of reducing carbon dioxide availability on spirulina biomass production. The research has focused more on the amount of biomass produced rather than oxygen production from the spirulina itself. Some studies have shown that higher light intensities enable spirulina to have higher oxygen production rates (Qiang 1998). Other research on the effects of different light wavelengths on the growth of other species of algae have shown the algae's preference and better growth rate under blue light (Loong Teo 2014). There is almost no research available on the differences between coiled and straight spirulina and whether the different structured algae produce different amounts of oxygen. If there is a difference in the oxygen production rate and this rate is able to be amplified, spirulina would be an optimal choice as a long-term resource in space. Spirulina's ability to produce pure oxygen while continuing to need very limited resources could be a significant breakthrough in research for long-term space travel.

This research tested the difference in oxygen production based on the difference in the structure of the algae, as well as testing different light intensities with both structure types to determine a preferrable environment by the algae.

MATERIALS

Materials for these experiments used six oxygen-monitoring containers. Four holes were drilled into each of the clear 2.3 L containers and two collection tubes, one clear plastic straw, and a tube with a cap were glued onto their respective holes. The collection tubes, a 50 mL and a 15 mL, measured the oxygen produced by the algae. The plastic straw allowed the algae to push media out of the top of the straw as pressure within the container built up due to the produced oxygen.

Figure 3. Spirulina Container Example During Experiment.

The experiment was created with two differently structured spirulina cultures. The Mixed original culture was made from coiled and straight spirulina at a roughly 50/50 ratio. The Coiled original culture consisted purely of coiled spirulina. The original and experimental cultures were sustained using the Zarrouk nutrient media (Appendix A).

The experiments used two incubation chambers with different light intensities: 25µmol/m^2/s and 12µmol/m^2/s. The control variable was considered to be the coiled and mixed experimental cultures placed under a high light intensity of 51µmol/m^2/s. For this research, three repeated experiments were conducted.

METHODS

The original spirulina cultures were maintained at a pH of 10 at 30°C and given 50 mL of nutrient media daily. The six oxygen-monitoring containers were filled completely with spirulina media. Three were filled with Coiled media and the other three were filled with Mixed media. One of each culture type was placed in the three different light intensities: 51µmol/m^2/s, 25µmol/m^2/s, 12µmol/m^2/s. These light intensities are also referred to as high, medium, and low, respectively. The light was constant except for the 51µmol/m^2/s intensity cultures which had 12 hours of light and 12 hours of dark in 6-hour increments. This routine was modeled after the light schedule of the original cultures which also experience 12 hours of light and 12 hours of dark in 6-hour increments.

Figure 4. Experiment Containers Under Light Intensity of 12μmol/m²/s

Figure 5. Experiment Containers Under Light Intensity of 25μmol/m²/s

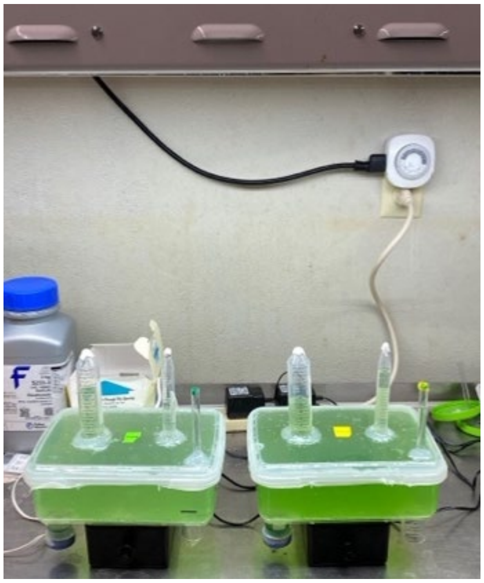

Figure 6. Experiment Containers Under Light Intensity of 51μmol/m2/s

The smaller experimental cultures were given 3mL of nutrient media daily during the length of the experiments. At the 24-, 48-, & 72-hour marks, the cultures were taken out of the incubators, a media sample was taken, and oxygen measurements recorded. The cell concentration (cells/mL) was measured from the samples using a microscope and the Sigma-Aldrich hemocytometer. After the 72-hour mark when observations were recorded, the containers were cleaned and ready for the next experiment. It was noted that leaks in the container did occur throughout the experiments but did not interfere with observational data. If leaks occurred, they were repaired after the container was cleaned and before the next experiment.

RESULTS

After the data from all three experiments was collected, the overall experimental averages were calculated from the data sets in Appendix B. These have been translated into the graphs below for easier representation.

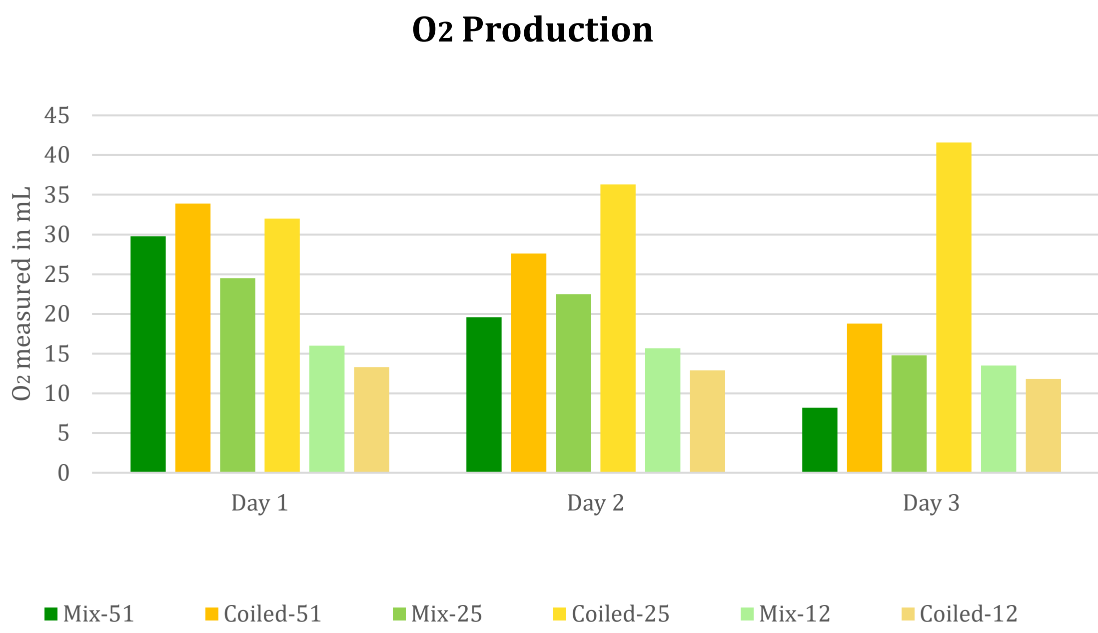

Figure 7. Average Oxygen Production of the Spirulina Experiments.

The graph above is the average oxygen production of both mixed and coiled experimental cultures in high, medium, and low light intensities. The calculated averages demonstrates that coiled spirulina produced more oxygen overall. The most oxygen was produced by the coiled spirulina in the 25µmol/m^2/s on day 3 (Coiled-25).

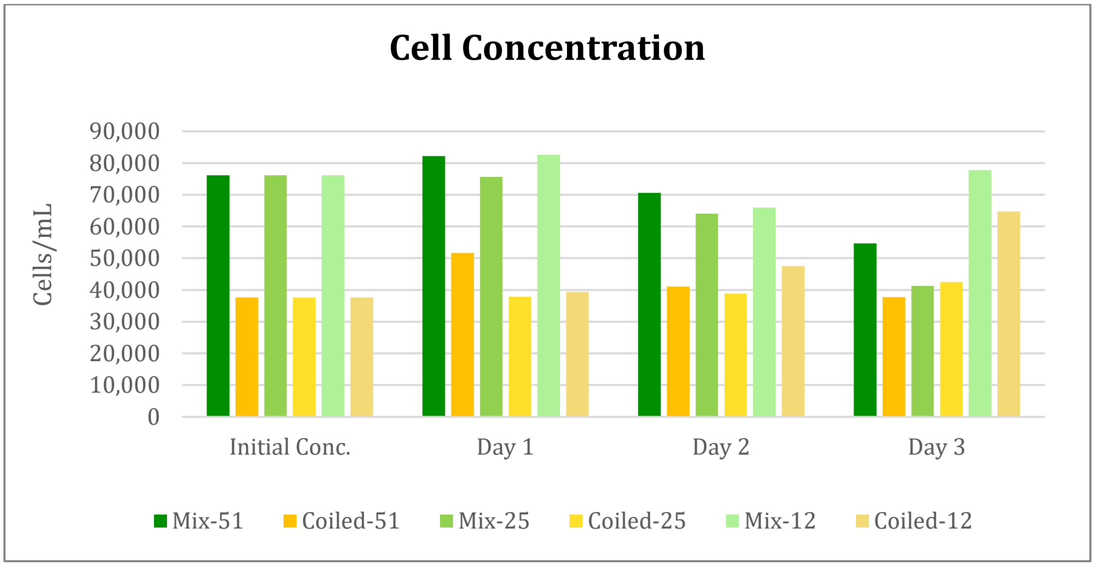

Figure 8. Average Cell Concentration of the Spirulina Experiments.

The graph above shows the average cell concentration per mL of both mixed and coiled experimental cultures in high, medium, and low light intensities. These averages illustrate that the mixed spirulina cultures had higher cell concentrations, but the initial concentration started much higher. The coiled spirulina cultures showed more consistent growth in their cell concentration whereas the mixed cultures showed decline in cell concentration over the three-day period.

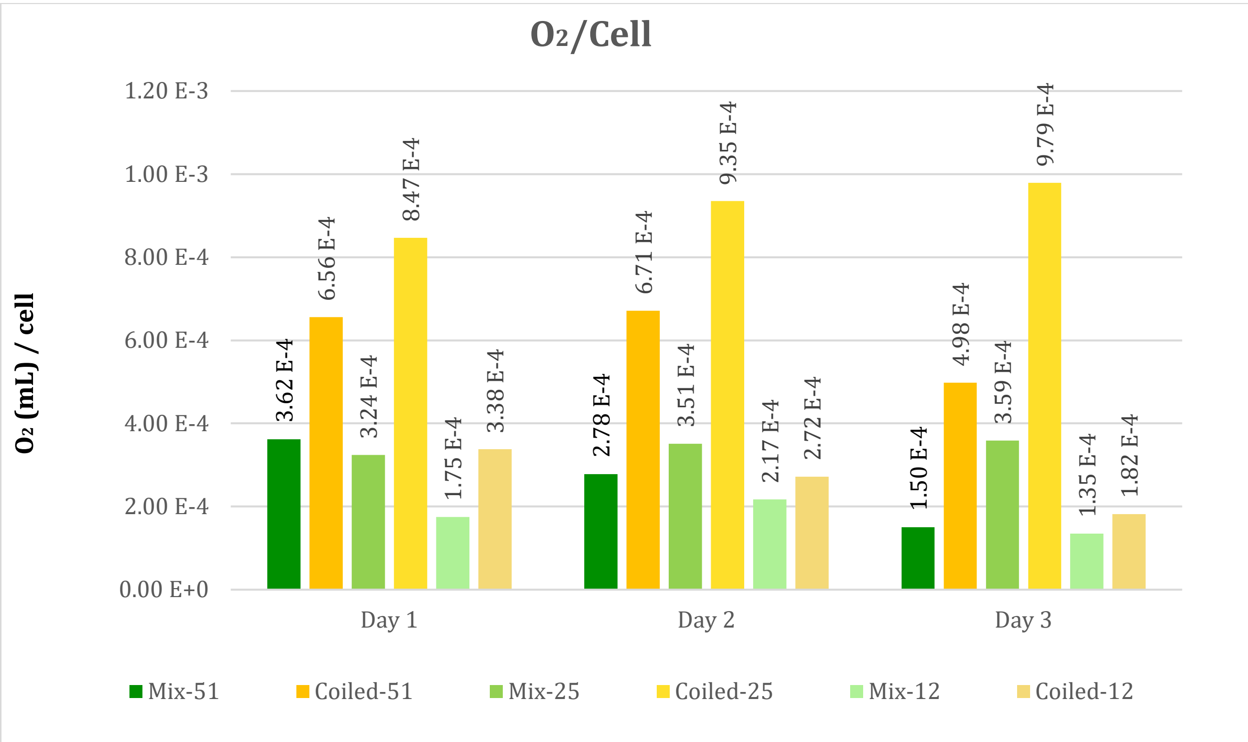

Figure 9. Average Amount of Oxygen Produced Per Cell of the Spirulina Experiments.

This graph represents the average oxygen produced per cell of both mixed and coiled experimental cultures in high, medium, and low light intensities. This data was calculated by dividing the oxygen produced (in mL) by the cell concentration. The full data set is shown in Appendix C. The data indicates that coiled spirulina produces more oxygen than the mixed culture containing coiled and straight structured spirulina.

After collecting all three components of data from each of the three experiments conducted, a series of statistical tests were run to determine the normality, validity, and significance of the findings of the research. To test for assumptions, normality needed to be tested first. This was done by running Q-Q plots with a Q-Q line, along with a formal test using Shapiro Wilk. The next component tested was equal variance. Bartlett's test was run for this purpose and then checked utilizing a visual box plot. The first dataset tested was the cell concentration values from the three experiments.

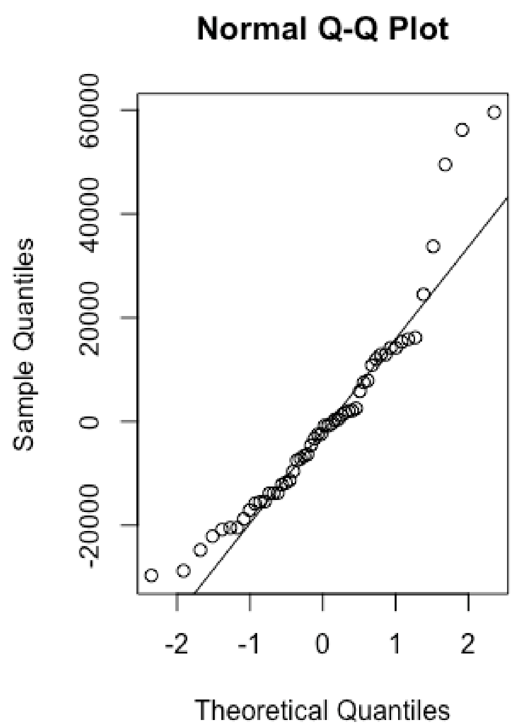

Figure 10. Normal Q-Q Plot for Cell Concentration vs. Light Intensities.

The Q-Q Plot above illustrates some normality within the data. However, it does appear to be skewed on both sides. This can be seen as the data points, which are the dots plotted in the figure, stray from the regression line. The next test performed was the Shapiro-Wilk Normality

Test. This test yielded a p-value of 0.000553, which is far below the value of 0.05, meaning this test also shows abnormality in the dataset (Table 1).

A boxplot of the data demonstrates that the interquartile ranges of the cell concentrations at each light intensity level are different, and that there are distinct outliers present in the results (Figure 7). This leads to further evaluation Bartlett test, which results in a p-value of 0.0006612 (Table 1). This p-value is also below 0.05, meaning that variance within the cell concentration results is not equal. These combined test values do not work with ANOVA assumptions, so the nonparametric Kruskal-Wallis Rank Sum Test was performed next. This test gives a p-value of 0.5361, which is above 0.05 (Table 1). With this test, it can be concluded that the differences between the cell concentration medians from the different light intensities were not statistically significant.

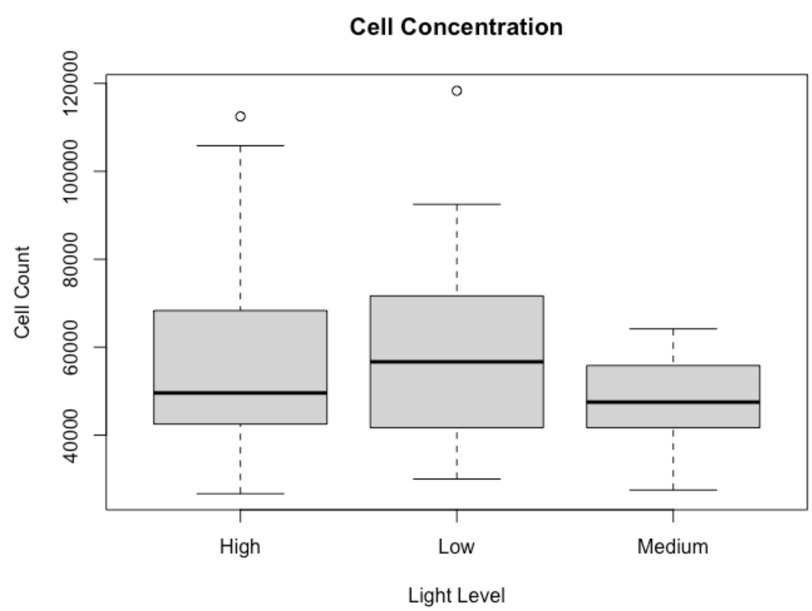

Figure 11. Box Plot of Cell Concentration Dataset.

Test Type	P-Value
Shapiro-Wilk Normality Test	0.000553
Bartlett Test of Homogeneity of Variance Test	0.0006612
Kruskal-Wallis Rank Sum Test	0.5361

Table 1. P-Values from Statistical Tests on Cell Concentration Data Based on Differing Levels of Light Intensity.

The oxygen production data set was also tested for normality using a Q-Q Plot with a Q-Q line (Figure 8). The plot below indicates that the data is slightly left skewed, but otherwise normal. The Shapiro-Wilk test resulted in a p-value of 0.5082, meaning that the values are normal (Table 2).

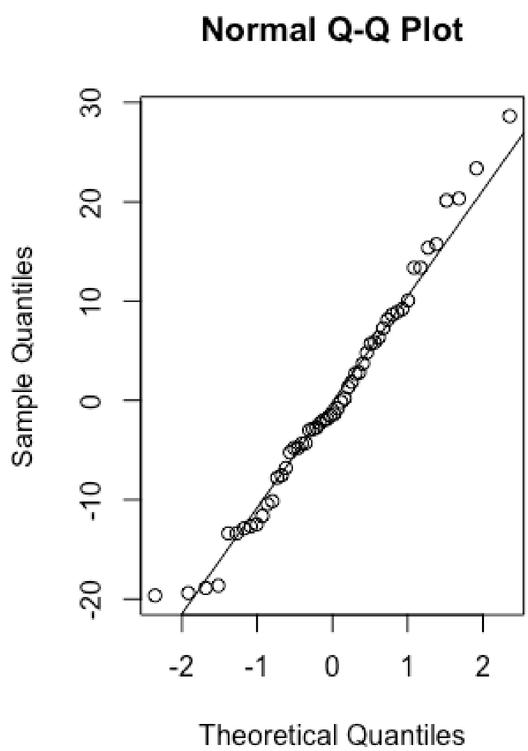

Figure 12. Normal Q-Q Plot for Oxygen Production vs. Light Intensities

Test Type	P-Value
Shapiro-Wilk Normality Test	0.5082
Bartlett Test of Homogeneity of Variance Test	0.02012
Kruskal-Wallis Rank Sum Test	0.003353
Kruskal-Wallis Multiple Comparison Test	Low-Medium Significant Difference: TRUE

Table 2. P-Values from Statistical Tests on Oxygen Production Data Based on Differing Levels of Light Intensity.

The oxygen production data represented in the boxplot below exemplifies interquartile ranges that are similar for both high and medium light intensities (Figure 9). However, the low light intensity, which, as mentioned previously, is 12μmol/m^2/s, shows a much different range than the others. The Bartlett test was performed next, which produced a p-value of 0.02012 (Table 2). This value is lower than 0.05, signifying that the variance of the data is not equivalent.

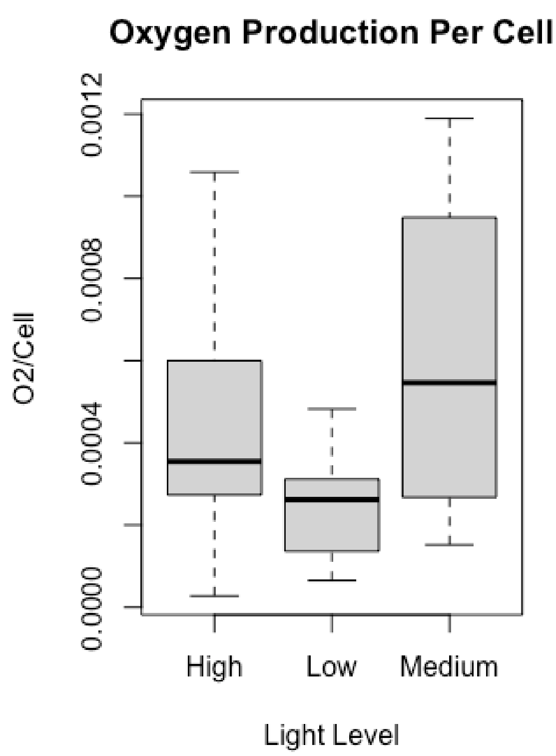

Figure 13. Box Plot of Oxygen Production Dataset.

Again, these values do not work with ANOVA assumptions, the nonparametric Kruskal-Wallis Rank Sum Test is used and emits a p-value of 0.003353, rejecting any notions that assume the population medians are equal (Table 2). Due to this rejection, a post-hoc test was run next. A Kruskal-Wallis Multiple Comparison Test revealed significant differences in the oxygen production of the experiment cultures under the low light intensity and the medium light intensity (Table 2).

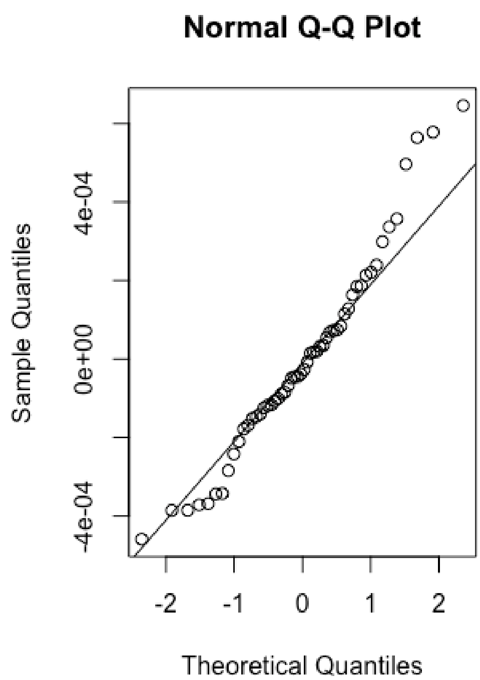

Figure 14. Normal Q-Q Plot for Oxygen Produced Per Cell vs. Light Intensities

The data set for the oxygen produced per cell was also tested for normality. The Q-Q Plot with a Q-Q line pictured above displays a small skew on both the right and left sides, but for the most part, the data appears to be normal (Figure 14).

Test Type	P-Value
Shapiro-Wilk Normality Test	0.1138
Bartlett Test of Homogeneity of Variance Test	0.0001098
Kruskal-Wallis Rank Sum Test	0.001566
Kruskal-Wallis Multiple Comparison Test	Low-Medium Significant Difference: TRUE

Table 3. P-Values from Statistical Tests on Oxygen Produced Per Cell Data Based on Differing Levels of Light Intensity.

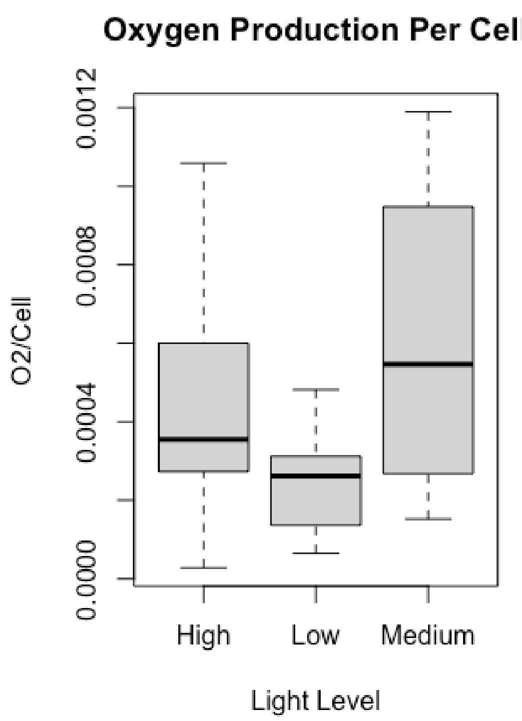

Figure 15. Box Plot of Oxygen Produced Per Cell Dataset.

By utilizing the Shapiro-Wilk Normality Test, the data produces a p-value of 0.1138, which is above 0.05, so the values appear to be normal (Table 3). Next, the data set was configured into a boxplot, where the figure demonstrates that the data's interquartile ranges are all unique (Figure 15). Using the Bartlett Test resulted in a p-value of 0.0001098 (Table 3). This value is much smaller than 0.05, which conveys there is high variance in the dataset for oxygen produced per cell within the different light intensities. The nonparametric Kruskal-Wallis Rank Sum Test is appropriate to use based on the values that these tests produced. This test also gives a p-value smaller than 0.05, which is 0.001566 (Table 3). This indicates that the population medians are not equal among the data set. This required a post-hoc test to be run on the data. A Kruskal-Wallis Multiple Comparison Test revealed significant differences in the oxygen produced per cell of the spirulina cultures under the low light intensity compared to spirulina cultures under the medium light intensity (Table 3).

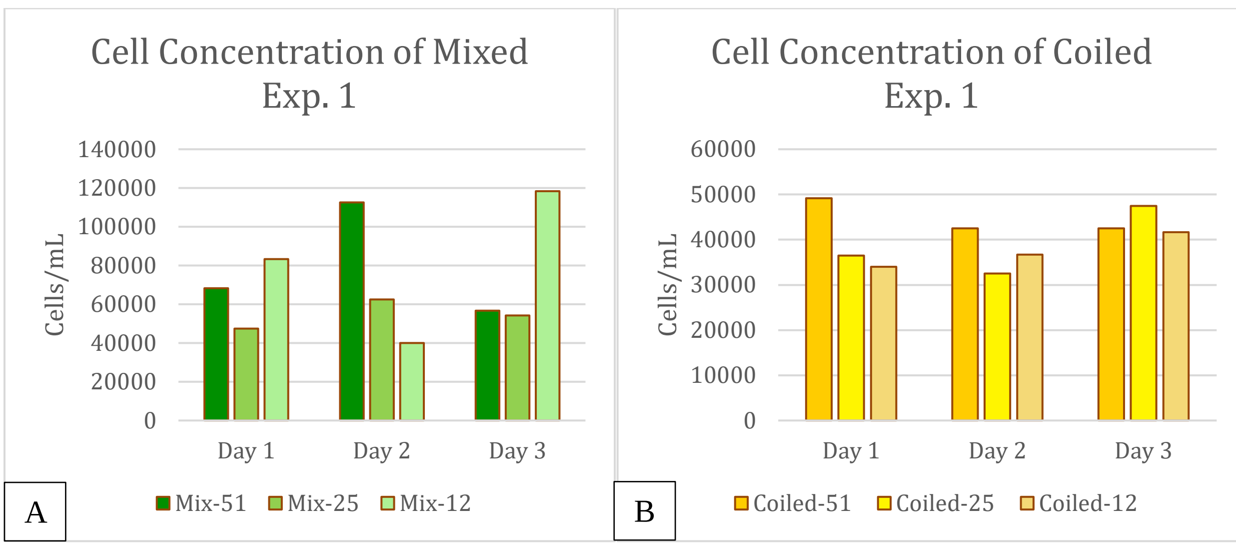

Figure 16. Experiment 1 Cell Concentration Results for (a) Mixed and (b) Coiled Cultures.

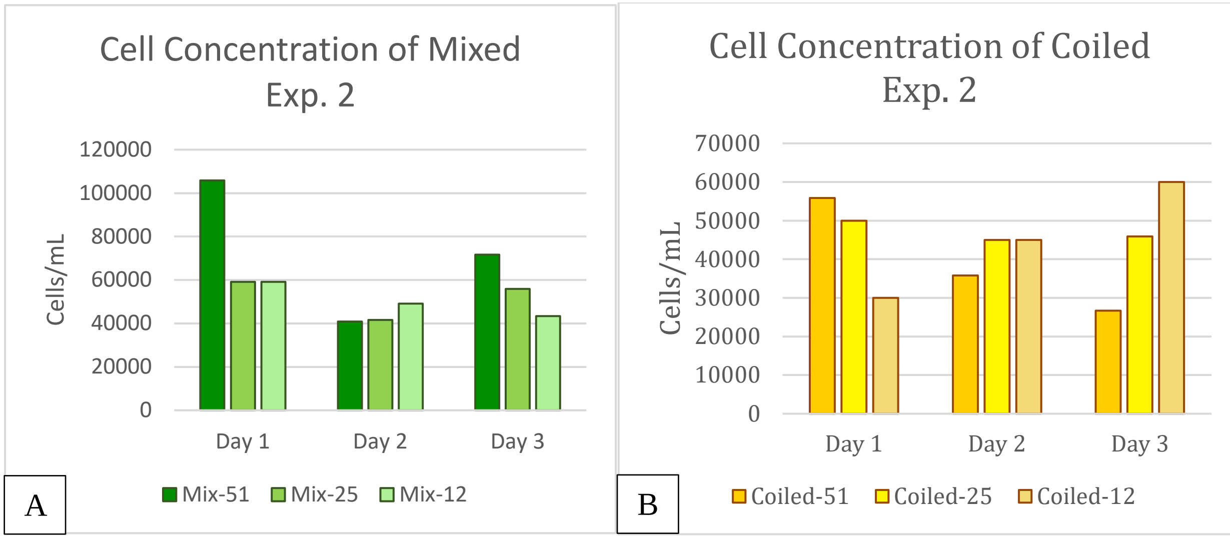

Figure 17. Experiment 2 Cell Concentration Results for (a) Mixed and (b) Coiled Cultures.

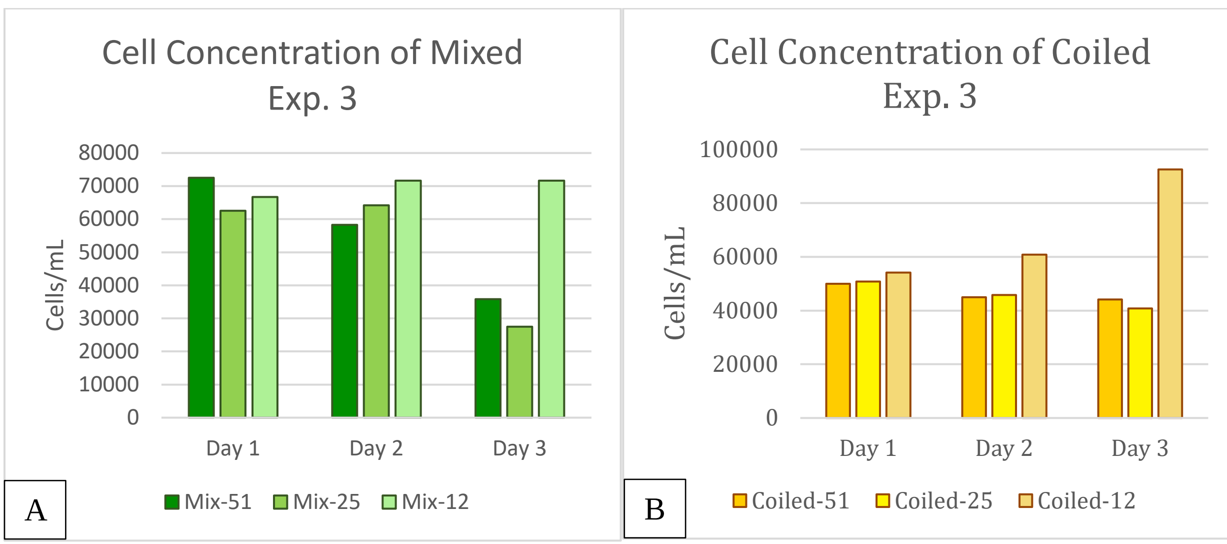

Figure 18. Experiment 3 Cell Concentration Results for (a) Mixed and (b) Coiled Cultures.

The graphs above display the cell concentration data from the three individual experiments themselves (Figure 16, 17, & 18). More fluctuation was seen in the concentrations of the mixed cultures than in the pure coiled cultures. It was also noted that the mixed cultures began with a higher cell concentration than the coiled cultures due to concentration differences in the original stock cultures.

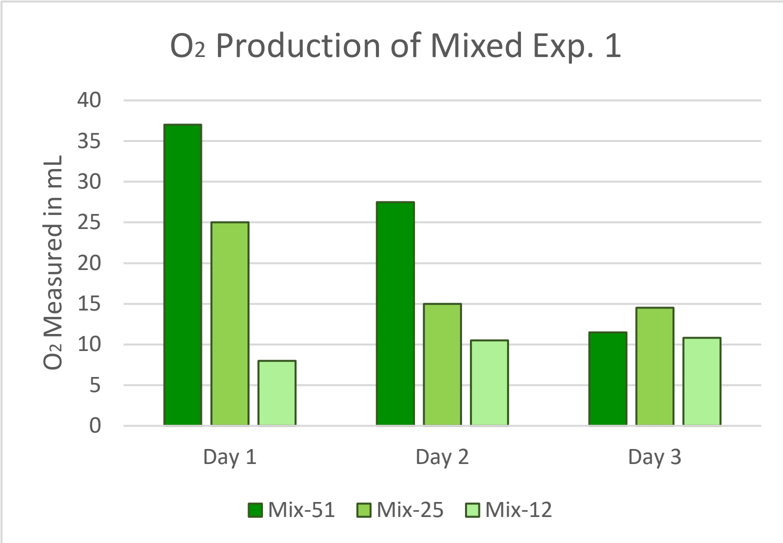

Figure 19. Experiment 1 Oxygen Production Results for Mixed Cultures.

These next graphs present the oxygen production data of all three experiments, and the oxygen produced was measured in milliliters (Figures 19, 20, 21, 22, 23, & 24). By looking at the oxygen production data from both mixed and coiled experimental cultures side by side, the difference in the oxygen production of the two structure types becomes clear. The coiled spirulina produced more oxygen than its mixed counterpart in each experiment (Figures 20, 22, & 24).

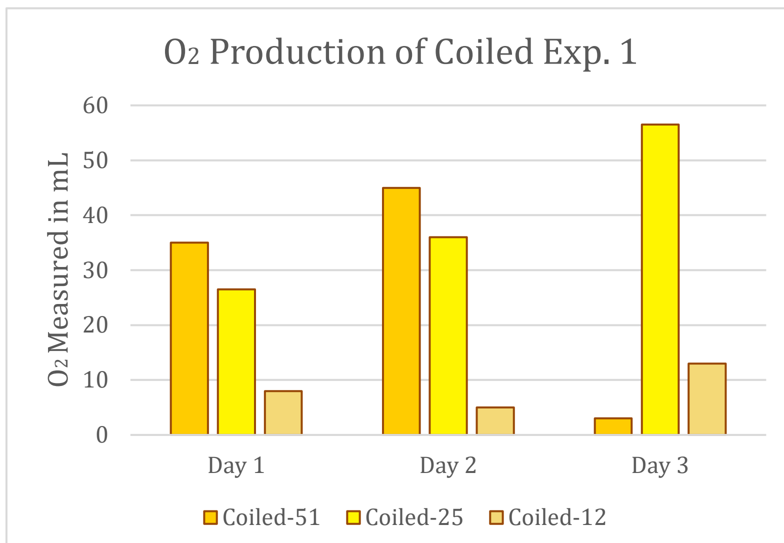

Figure 20. Experiment 1 Oxygen Production Results for Coiled Cultures.

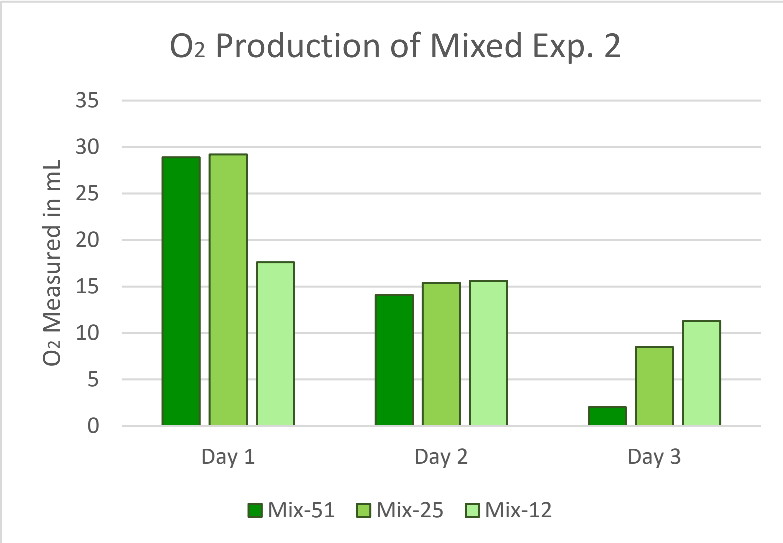

Figure 21. Experiment 2 Oxygen Production Results for Mixed Cultures.

Another highlight of this dataset is the differences in oxygen production of the cell cultures in the high, medium, and low light intensities. In Experiment 1, the mixed cultures exhibit the highest oxygen production in the experimental control equivalent, otherwise known as the high light intensity, which is an intensity of 51µmol/m^2/s (Figure 19). This trend continues for all three experiments. Although in experiment 2, the mixed cultures in the medium light intensity also produce an equivalent amount of oxygen (Figure 21).

When the data from each of the coiled cultures in the three different experiments is compared, the cell cultures under the medium light intensity of 25µmol/m^2/s produce the most oxygen out of all the experimental cultures, both mixed and coiled.

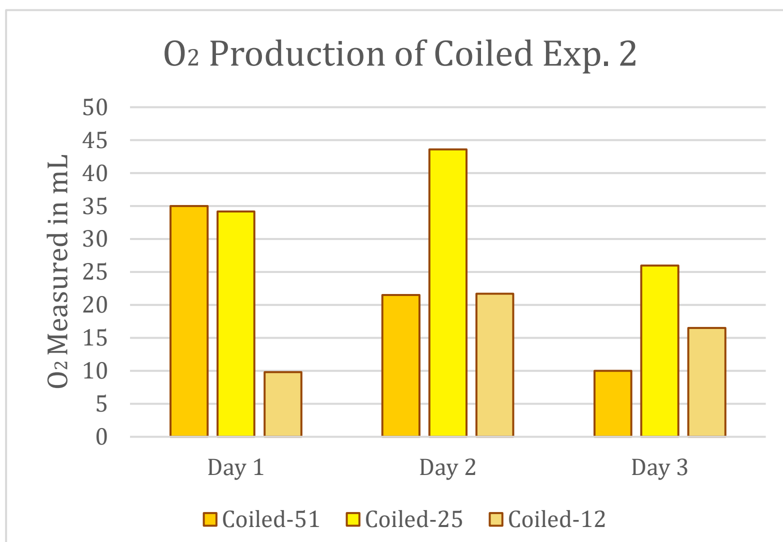

Figure 22. Experiment 2 Oxygen Production Results for Coiled Cultures.

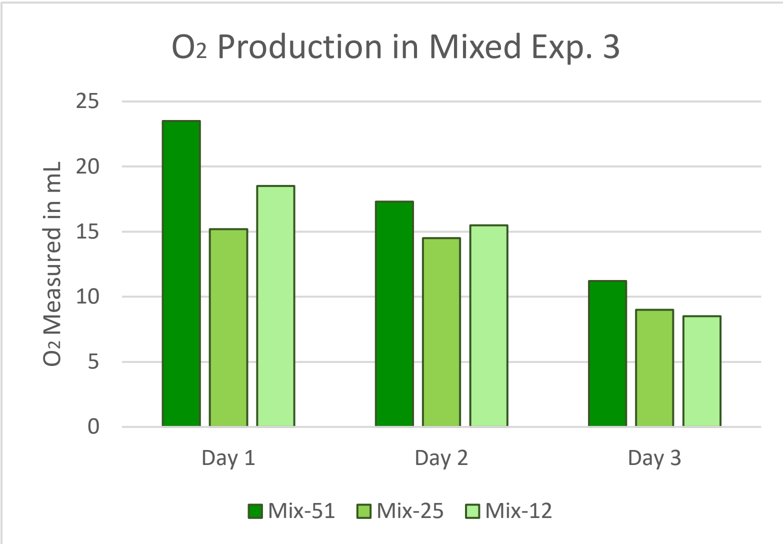

Figure 23. Experiment 3 Oxygen Production Results for Mixed Cultures.

With an amount of 56.5 mL on day three of the experiment, the coiled culture under medium light (Coiled-25) produces the highest amount of daily oxygen production of all collected experimental measurements (Figure 20, & Appendix B).

It is also noted that the cell cultures in the low light intensity of 12µmol/m^2/s produced the lowest oxygen measurements through the duration of the experiment.

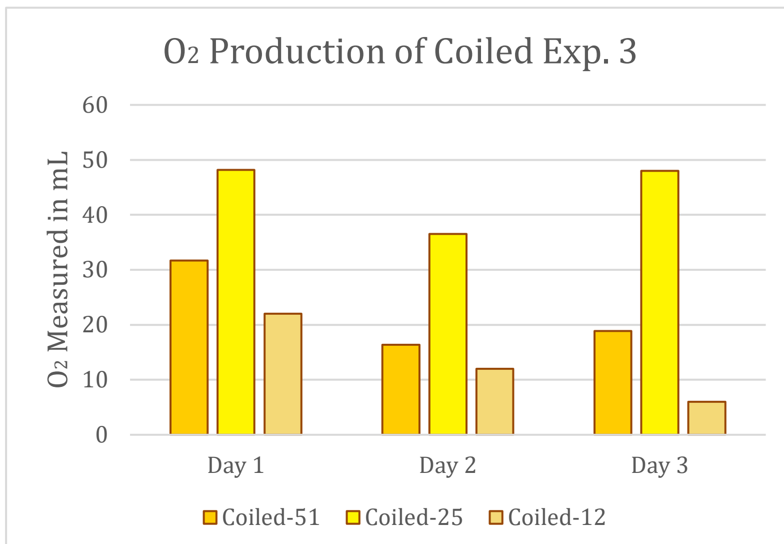

Figure 24. Experiment 3 Oxygen Production Results for Coiled Cultures.

Using the cell concentration data and amounts of oxygen produced data collected throughout the three experiments, the oxygen produced per cell was calculated for each of the data points. The results of this calculation allow the significant differences of each structure type to be seen more clearly. By observing the oxygen produced per cell graphs from experiment 1, the coiled cultures in every light intensity produced more oxygen per cell than the mixed cultures (Figures 25 & 26). This comparison of the oxygen produced per cell highlights the distingushing factor as cell structure.

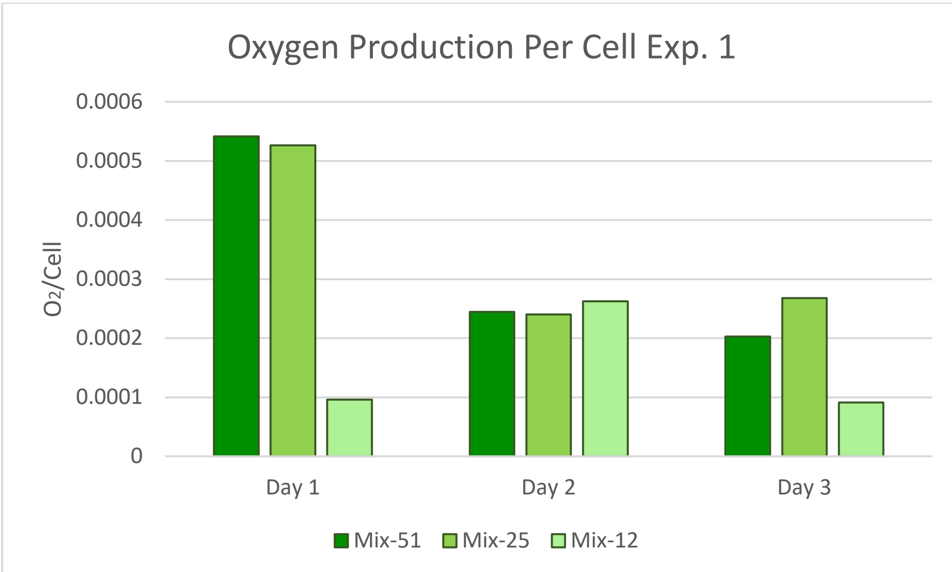

Figure 25. Calculated Oxygen Produced Per Cell of Mixed Cultures in Experiment 1.

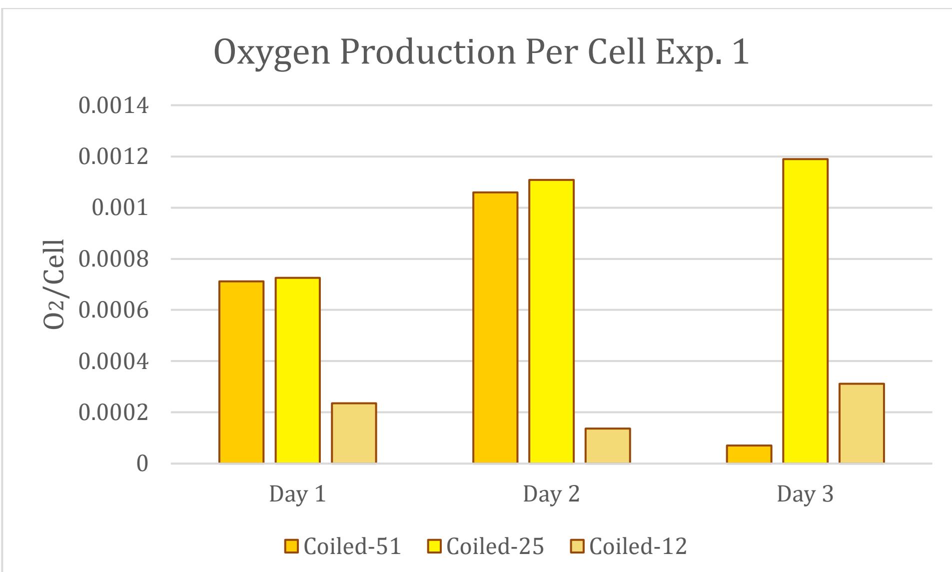

Figure 26. Calculated Oxygen Produced Per Cell of Coiled Cultures in Experiment 1.

As seen above, the coiled spirulina cultures produced sufficient amounts of oxygen at a much smaller cell concentration than the mixed spirulina cultures (Figures 25 & 26).

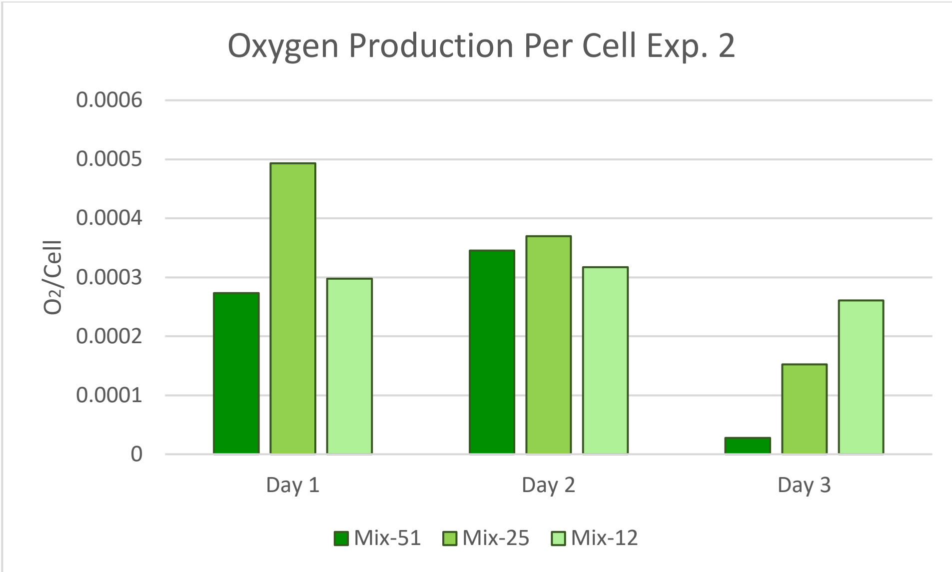

Figure 27. Calculated Oxygen Produced Per Cell of Mixed Cultures in Experiment 2.

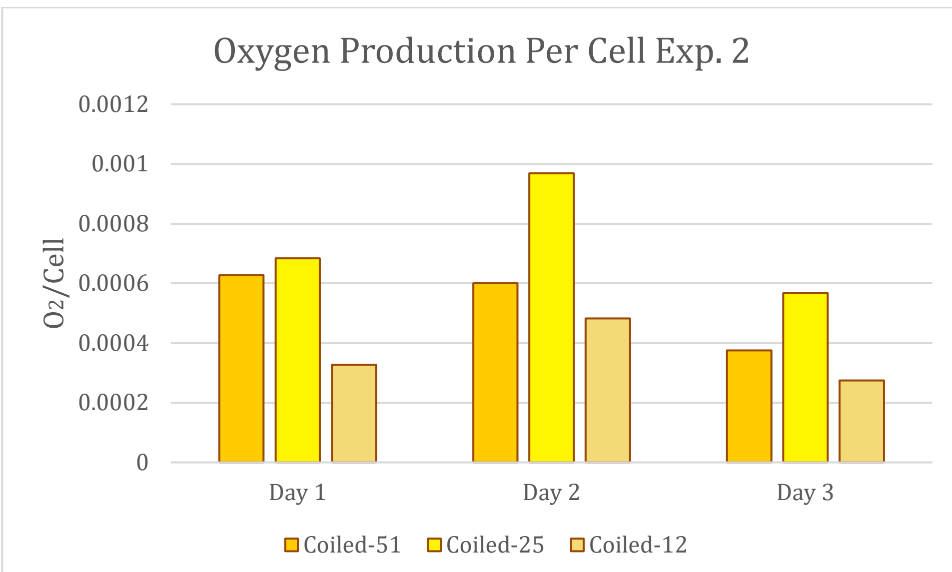

Figure 28. Calculated Oxygen Produced Per Cell of Coiled Cultures in Experiment 2.

In experiment 2, a small increase was seen in the amount of oxygen produced per cell from the mixed spirulina cultures (Figure 27). However, the coiled spirulina cultures still exhibit a larger and more substantial amount of oxygen produced per cell (Figure 28).

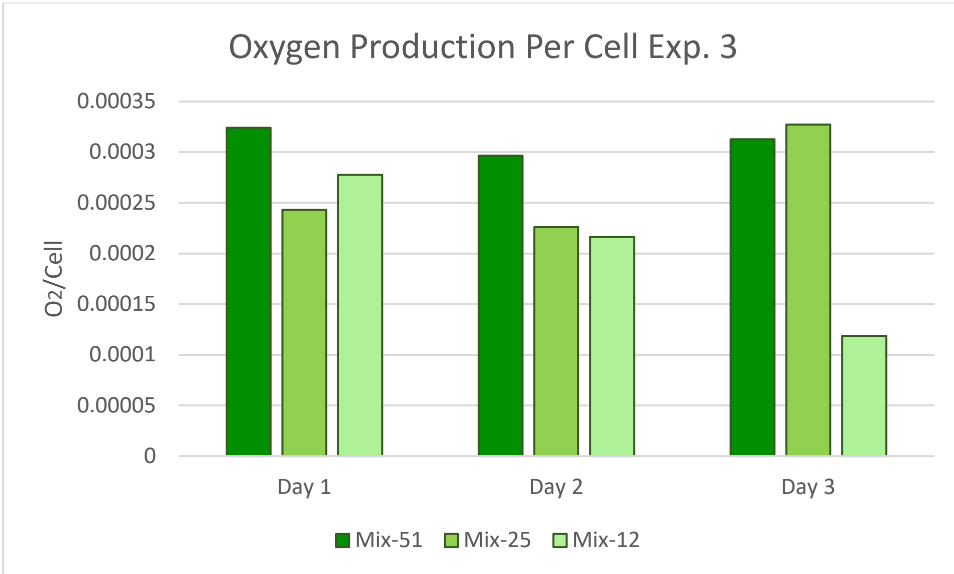

Figure 29. Calculated Oxygen Produced Per Cell of Mixed Cultures in Experiment 3.

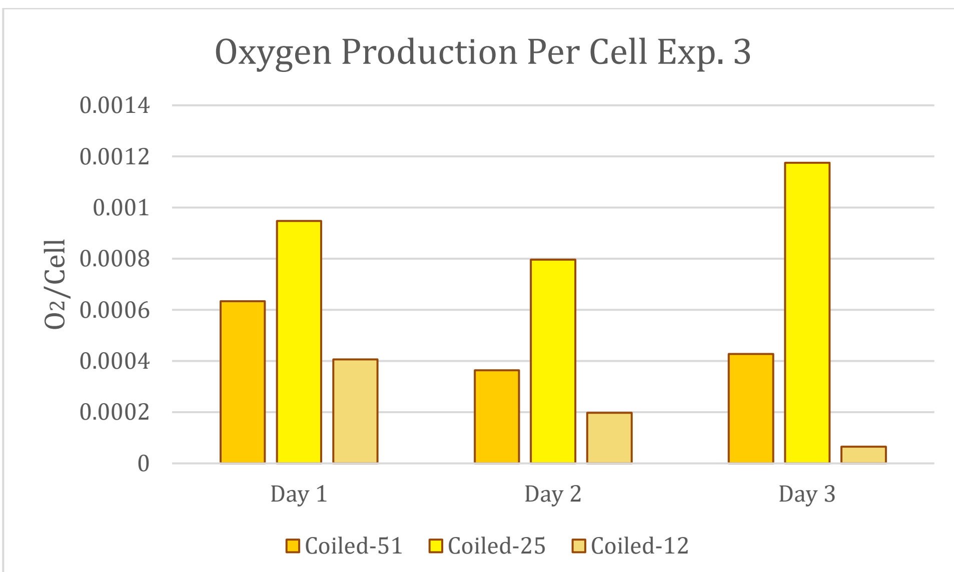

Figure 30. Calculated Oxygen Produced Per Cell of Coiled Cultures in Experiment 3.

In experiment 3, the same trend in data is observed (Figure 29 & 30). The coiled spirulina cultures show a much larger amount of oxygen produced per cell, and the mixed spirulina cultures show miniscule amounts of oxygen produced per cell. The amounts produced by the mixed cultures in experiment 3 are even smaller than the usual trend with no observations of any amounts above 3.5 x 10^{-4} (Figure 29 & Appendix C).

DISCUSSION

Based on these experiments, it seems as though the cellular structure of the algae impacts the amount of oxygen produced by the spirulina cells. The cause of this variance could be an indication that the helical spirulina take in more carbon dioxide and are able to photosynthesize more efficiently than its straight structured counterpart. The coiled spirulina produced the most oxygen overall with an amount of 56.5 mL in the 25μmol/m^2/s light intensity on day three of experiment 1, which was also 1.189 x10^{-3} mL of oxygen produced per cell (Figure 20, Appendix B, Figure 26, & Appendix C). The coiled spirulina expressed a continuous cell concentration growth, whereas the mixed spirulina steadily declined over the 72 hours. Straight spirulina are found to have lower protein content than coiled spirulina. The higher protein content in coiled spirulina allows the cells to absorb more energy.

For further research, the structurally different spirulina should be analyzed at a deeper level to understand why this oxygen production difference exists. Past protein analyses on coiled and straight spirulina reveal differences in their proteins. Two specific bands show up strongest in straight spirulina and two different protein bands show up strongest in the coiled spirulina (Wang 2005). The two structures express different levels of these four proteins, which could carry a link to the difference seen in oxygen production. In addition, former research pulled DNA from straight and coiled spirulina, which concluded that there are significant variations in their DNA (Wang 2005). The combination of all these distinctions could be the reason why the coiled spirulina cells produce more oxygen. In the future, further research on these specific proteins and their link to photosynthetic activity could reveal concrete proof of coiled spirulina maintaining higher oxygen production rates.

Another aspect to consider is the coiled and straight cells' responses to the analyzed variables of light intensity. The coiled spirulina is shown to thrive better at higher light intensities which is a further suggestion that photosynthesis occurs at a higher rate in coiled spirulina. The coiled cells are able to take in more energy from the light source possibly due to the structure itself. The spirulina cells are helical; therefore, the full intensity of the light does not hit the entire cell at the same time. There are parts of the structure that remain somewhat protected from the intensity, meaning that portions of the cell are allowed rest from the light. This could permit the cell to refocus energy intake to where the light is hitting and take in more nutrients and carbon dioxide in the places where the cell is somewhat sheltered from the light. This theory has not been proved, nor is there previous research on the idea.

CONCLUSIONS

The Coiled-25 culture, which was the coiled spirulina in the 25μmol/m^2/s light intensity, produced the most oxygen per cell. The highest daily amount of oxygen produced by the Coiled-25 culture was an amount of 56.5 mL on day three of the experiment. The Mixed-25 culture produced only an amount of 37mL of daily oxygen production, which was the highest amount produced from any of the mixed spirulina. The significance of these findings indicates that coiled spirulina appears to produce much more oxygen at a lower cell concentration than the mixed culture. The coiled spirulina also expressed a continuous cell concentration growth, whereas the mixed spirulina's cell concentrations steadily declined in each of the three-day experiments.

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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 vanadinate
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	Cell Concentration			O2 Production		
Exp. 1	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Mix-51	68333	112500	56667	37	27.5	11.5
Mix-25	47500	62500	54166	25	15	14.5
Mix-12	83250	40000	118333	8	10.5	10.8
Coiled-51	49166	42500	42500	35	45	3
Coiled-25	36500	32500	47500	26.5	36	56.5
Coiled-12	34000	36667	41667	8	5	13
Exp. 2	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Mix-51	105833	40833	71667	28.9	14.1	2
Mix-25	59166	41667	55833	29.2	15.4	8.5
Mix-12	59166	49166	43333	17.6	15.6	11.3
Coiled-51	55833	35833	26667	35	21.5	10
Coiled-25	50000	45000	45883	34.2	43.6	26
Coiled-12	30000	45000	60000	9.8	21.7	16.5
Exp. 3	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Mix-51	72500	58333	35833	23.5	17.3	11.2
Mix-25	62500	64166	27500	15.2	14.5	9
Mix-12	66667	71667	71667	18.5	15.5	8.5
Coiled-51	50000	45000	44166	31.7	16.4	18.9
Coiled-25	50833	45833	40833	48.2	36.5	48
Coiled-12	54166	60833	92500	22	12	6

Appendix C

Experiment	Oxygen Produced Per Cell		
Exp. 1	**Day 1**	**Day 2**	**Day 3**
Mix-51	0.00054147	0.00024444	0.00020294
Mix-25	0.00052632	0.00024	0.0002677
Mix-12	9.6096E-05	0.0002625	9.1268E-05
Coiled-51	0.00071187	0.00105882	7.0588E-05
Coiled-25	0.00072603	0.00110769	0.00118947
Coiled-12	0.00023529	0.00013636	0.000312
Exp. 2	**Day 1**	**Day 2**	**Day 3**
Mix-51	0.00027307	0.00034531	2.7907E-05
Mix-25	0.00049353	0.0003696	0.00015224
Mix-12	0.00029747	0.00031729	0.00026077
Coiled-51	0.00062687	0.00060001	0.000375
Coiled-25	0.000684	0.00096889	0.00056666
Coiled-12	0.00032667	0.00048222	0.000275
Exp. 3	**Day 1**	**Day 2**	**Day 3**
Mix-51	0.00032414	0.00029657	0.00031256
Mix-25	0.0002432	0.00022598	0.00032727
Mix-12	0.0002775	0.00021628	0.0001186
Coiled-51	0.000634	0.00036444	0.00042793
Coiled-25	0.0009482	0.00079637	0.00117552
Coiled-12	0.00040616	0.00019726	6.4865E-05