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Effects of Light Intensity on the Oxygen Production of Arthrosprira Platensis

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SENIOR THESIS APPROVAL

This Honors thesis entitled

"Effects of Light Intensity on the Oxygen Production of *Arthrosprira Platensis*"

written by

Savannah Edwards

and submitted in partial fulfillment of
the requirements for completion of
the Carl Goodson Honors Program
meets the criteria for acceptance
and has been approved by the undersigned readers.

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Date: 4/27/20

Abstract

Space travel is challenging due to the depletion of resources as missions become lengthier. The cyanobacteria, *Arthrosprira platensis*, commonly known as Spirulina, has the potential to resolve this problem. In order to determine the effects of light intensity and nutrient availability on oxygen production and cell population growth, experiments were run on Spirulina at different light intensities and different nutrient levels. Spirulina cultures were grown in white light boxes with different light conditions (10 μmol m^{-2} s^{-1}, 18 μmol m^{-2} s^{-1}, and a light-dark cycle with 18 hours of 18 μmol m^{-2} s^{-1} per 24 hour period). Oxygen production and cell population measurements were recorded every 24 hours for 72 hours. Statistical tests were run to determine if any differences were significant. High intensity light significantly produced 62.6429 cm^3 more oxygen than low intensity light. There was no significant difference in oxygen production between high intensity light and the light-dark cycle nor was there a significant difference between cell population increase in any of the light intensities. There was no significant difference in the oxygen production or the cell population growth between high and low nutrient groups. There was also no significant statistical interaction between the nutrient levels and light intensity. Results indicate that Spirulina produced more oxygen in 18 μmol m^{-2} s^{-1} light. This indicates that Spirulina could not only be used as a food source, due to its high protein and vitamin content, but also as a way to consume carbon dioxide and produce oxygen due in space due to its photosynthetic nature.

Introduction

Arthrospira plantesis is a multicellular cyanobacteria commonly known as Spirulina. Spirulina has a history of being used as a source of protein, minerals, and vitamins in various parts of the world. It contains between 60 and 70 percent protein by dry weight. It is an excellent source of β-Carotene, vitamin B12, tocopherols, and iron. Spirulina lacks cellulose walls, which makes it easily digestible by humans. (Karkos et al. 2008).

Spirulina is a photosynthetic cyanobacteria that thrives in waters characterized by high levels of bicarbonate and therefore a high pH. Spirulina can survive under a wide range of conditions, with optimum growth conditions being 35-37 °C and a pH of about 10. The fact that Spirulina thrives at high temperatures and an alkaline pH allows for the maintenance of monoalgal cultures and the prevention of contaminants (Belay et al. 2010).

Spirulina exists as an arrangement of multicellular, helical trichomes that are solitary and move freely in solution. The trichomes are arranged in an open left-hand helix. The helix diameter of the trichomes vary from about 30 to 70 µm while the helix pitch varies from 12 to 72 µm. Spirulina *plantesis* usually exists as short trichomes with 5 to 7 coils (Tomaselli et al. 1997). Environmental factors, such as temperature and the chemical composition of the water they grow in, affect the helical shape. The trichomes may reversibly transition from their characteristic helical shape to a spiral shape or irreversibly to a straight rod. The observed spiral shape is due to variations in geometry even observed in monospecific populations. Conversion of trichomes to a straight rod is common, and it has been suggested to occur due to a mutation. Spirulina cells divide via binary fission. The trichome fragments into shorter segments. These shorter segments

enlarge and mature and then enter a cycle to develop into whole trichomes (Tomaselli et al. 1997).

Photosynthesis in cyanobacteria converts light energy into the chemical energy that it needs to survive. Light energy is used to transport electrons from water to generate ATP and NADPH which are used to convert CO_2 to carbohydrates. The evolution of oxygen occurs as a byproduct of this process. In cells grown in low intensity light, oxygen evolution coupled with carbon dioxide fixation is lower than in high light grown cells (Mohhanty et al. 1997). Growth of Spirulina platensis became saturated at a light intensity of 150–200 µmol $m^{-2}s^{-1}$. In the dark, oxygen evolution and by extension, carbon fixation will be negative due to respiration. As light intensity increases, the rate of photosynthesis increases linearly. There comes a point when photosynthesis reaches a maximum and becomes saturated (Vonshak et al. 1997).

A spacecraft is an artificial environment in which food, oxygen, and clean water must be provided and human waste, such as carbon dioxide, must be removed. Space travel is challenging due to the depletion of resources as missions become lengthier. Spirulina has the potential to resolve this problem. Spirulina is an excellent source of protein, vitamins, and minerals. It is also a photosynthetic organism, so it fixes carbon dioxide and releases oxygen. Spirulina could not only be used as a food source due to its high protein and vitamin content but as a way to consume carbon dioxide and produce oxygen due to its photosynthetic nature. The objective of my study was to determine the effects of white light intensity and nutrient availability on the oxygen production and cell population growth of Spirulina and optimize the growth conditions of Spirulina in anticipation of its use as a renewable resource for space travel.

Materials and Methods

Starting a Culture

Spirulina cultures were maintained in alkaline conditions with a pH of 10 at 28 °C with 60 mL of Zarrouk's (Appendix B) media given daily. Approximately two gallons of deionized water were added to a glass fish tank. Two heating elements were added to the water to heat the culture to 28 °C and maintain that temperature. An air pump was installed to agitate the Spirulina, as it tends to sink to the bottom of the tank and needs to be distributed throughout to receive an optimal amount of light. White lights were set up above the culture tank. The lights were set so that the culture received a light intensity of 18 μmol m^{-2} s^{-1} for 18 hours in a 24-hour period and 6 hours of complete darkness. Spirulina starter culture, obtained from Suncoast Marine Aquaculture, was added to the tank, along with necessary nutrients (Appendix B). Throughout experiments the ideal pH of 10 was maintained using sodium bicarbonate powder. The tanks were covered with plexiglass to keep contaminants out of the culture. For this two-gallon starter culture, 30 mL of Zarrouk's media was given daily, along with sodium bicarbonate if indicated by pH tests. To promote culture growth, the amount of water in the culture was doubled when the Spirulina became dense enough that light did not appear to penetrate to the bottom of the tank and the culture was a dark green color. Sixty milliliters of nutrient media was added daily after doubling the culture water. To monitor the progress of culture growth and to monitor the integrity of the culture, a cell count was taken every few days using a Sigma-Aldrich hemocytometer. Based on conclusions of previous experiments performed in Dr. Taylor's lab, the

ideal concentration of Spirulina cells is 3.0 x 10^4 cells/mL. Once the culture was dense enough to reasonably achieve this concentration experiments were started.

Light and Nutrient Experiments

Three liters of Spirulina in solution, from the previously mentioned culture, were placed into three-liter containers. A stir bar was placed in the solution to agitate the Spirulina throughout the experiment. The airtight container lids were modified to consist of a 50 mL conical tube open to the inside of the container and a small diameter tube open to the inside of the container and to the atmosphere (Figure 1). The rest of the volume of the container and tubes was filled up with deionized water and any air bubbles were eliminated. Putty was placed on the 50-ml conical tube to prevent the escape of gas. The small diameter tube was left open to the atmosphere (Figure 1) to allow any water, displaced by oxygen production, to escape the container. The cultures were placed in light boxes, and the light boxes were sealed off to eliminate any outside light from getting in. Every 24 hours, the cultures were given an amount of nutrient media appropriate for the experiment that is outlined below.

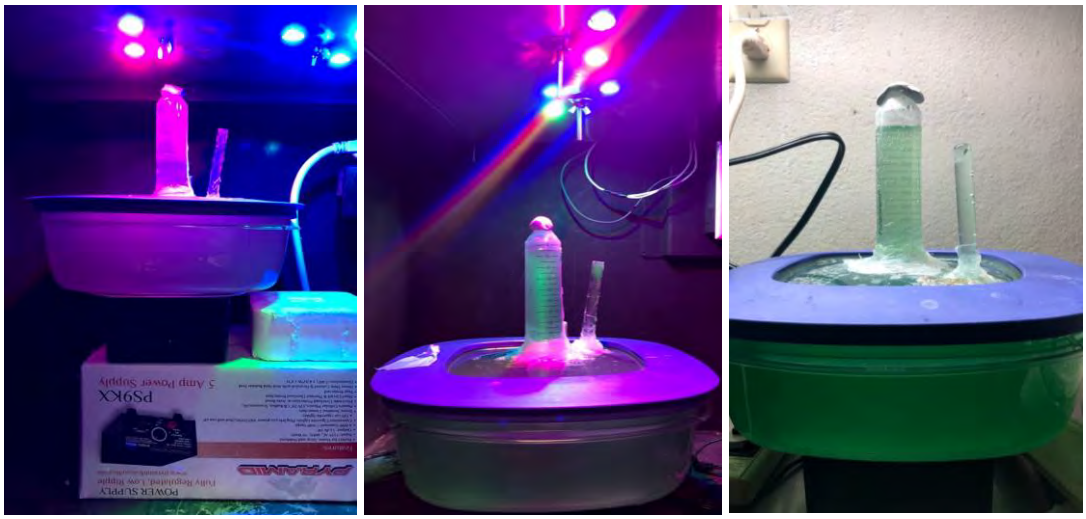

Figure 1 These photos show the setup of the Spirulina containing vessels during the high light intensity, low light intensity, and light-dark cycles (from left to right).

Three light conditions were tested; high light intensity (18 μmol m^{-2} s^{-1}), low light intensity (10 μmol m^{-2} s^{-1}), and a light-dark cycle with 18 hours of 18 μmol m^{-2} s^{-1} light and 6 hours of darkness in a 24-hour period. These light intensities are low when compared to something that is traditionally used to grow Spirulina. This is because light sources that produce such high light intensities use too many resources that are valuable and limited in space travel. Finding an ideal light intensity that does not use excess resources is essential to Spirulina's success as an oxygen source in space. Two different nutrient levels were tested; 2 mL of Zarrouk's media every 24 hours and 6 mL of Zarrouk's media every 24 hours. Oxygen production was measured every 24 hours by measuring the amount of gas in the 50 mL conical tube. A cell count was taken every 24 hours with a hemocytometer. Three replicates of each factor were run.

Statistical Tests

Statistical tests were run to determine how different light intensities and nutrient concentrations affect oxygen production and cell population increases in Spirulina. The total oxygen production and total cell population increase over 72 hours was recorded (Appendix A and Figure 2). All statistical tests were run in R (R Core Team 2018). The different nutrient levels were nested within each light intensity. Tests were run to determine if the assumptions of ANOVA were met. Shapiro-Wilk tests of each factor and Bartlett's test for homogeneity of variance were run. If these tests showed that the data fit the assumptions of an ANOVA, a two-way ANOVA was run to test if there was a difference in means between any of the treatment groups and if the independent variables interacted. If the results of the two-way ANOVA showed significant differences among groups, a Tukey's Honest Significant Test was run to determine which groups

differed among light levels. To provide a visualization of the data sets, a boxplot was generated in R, using the ggplot2 package (Wickham H 2016). An interaction plot was generated to visualize the interaction between the nutrient and intensity factors.

Results

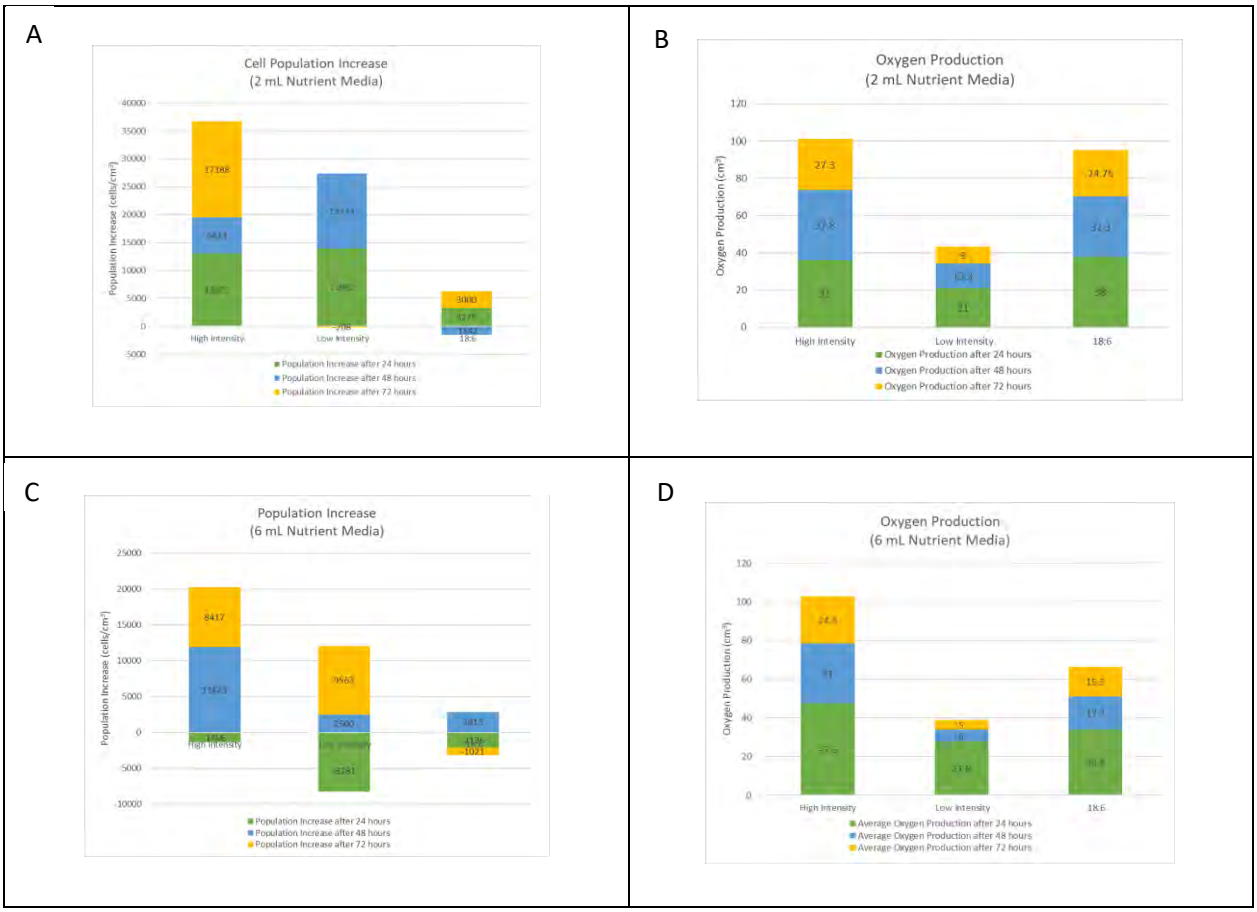

Figure 2 These graphs show the cell population increase and oxygen production over 72 hours in different light intensities and different nutrient availabilities. A) cell population increase with 2 mL nutrient media, B) oxygen production with 2 mL nutrient media, C) population increase with 6 mL nutrient media, and D) oxygen production with 6 mL nutrient media

Light and Nutrient Effects on Oxygen Production

The assumptions of ANOVA, normality and homogeneity of variance were met (Figure 3 and Table 1). Bartlett's test of homogeneity of variance showed that the variances within each

factor are equal (P=0.7868) within the nutrient factor and within the intensity factor (P=0.1473). The ANOVA indicated that within the intensity factor there was a significant difference (P=0.04090, F=3.986) in means between one or more pairs. There was not a significant difference (P=0.7410, F=0.113) in means between 2 mL nutrients and 6 mL nutrients nor was there a significant interaction (P=0.8898, F=0.118) between light intensity and nutrient availability (Figure 4). The Tukey posthoc test shows that the difference in means was between the high and low intensity light, with high intensity light producing 62.6429 cm^3 more oxygen than low intensity light (Table 2). There was no significant difference between high intensity and the light-dark cycle or between low intensity and the light-dark cycle.

Figure 3 Normal Q-Q plot of the residuals produced by the ANOVA of the oxygen data set. Data points fall along the Q-Q line and do not curve off at extreme values, indicating normality.

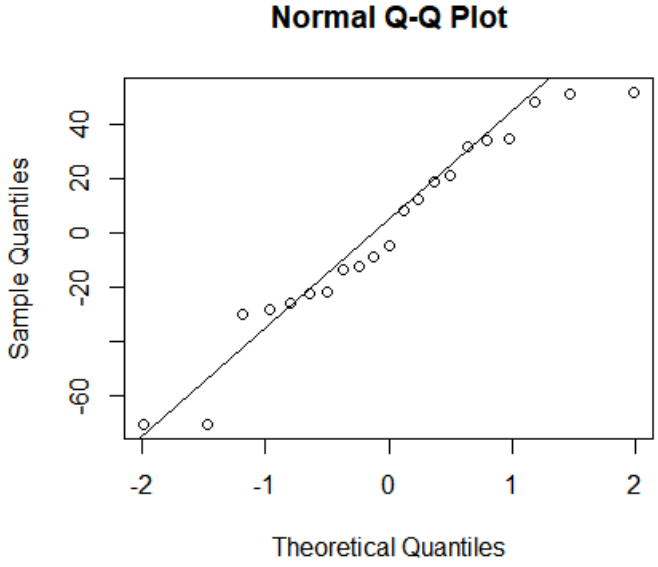

Table 1 Shapiro-Wilk normality test on all factors of oxygen data

Factor	W-value	p-value
2 mL Nutrients	0.9086	0.3026
6 mL Nutrients	0.92756	0.3062
High Intensity	0.85897	0.1482
Low Intensity	0.97332	0.9213
Light-dark Cycle	0.91395	0.4239

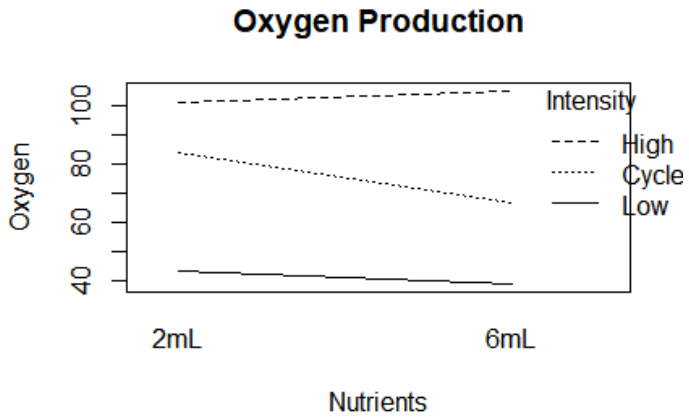

Figure 4 Interaction plot of oxygen data. The lines are parallel to each other, indicating that there is no interaction between nutrients and light intensity.

Table 2 Tukey HSD test of nutrient and intensity factors of oxygen data, High intensity light produced a mean of 62.6429 cm^3 more than low intensity light. *indicates a significant value

Factor	p-value	Difference
High-Cycle	0.40153	29.5000
Low-Cycle	0.32187	-33.1429
Low-High	0.03252*	-62.6429
6mL-2mL	0.74095	-45.1956

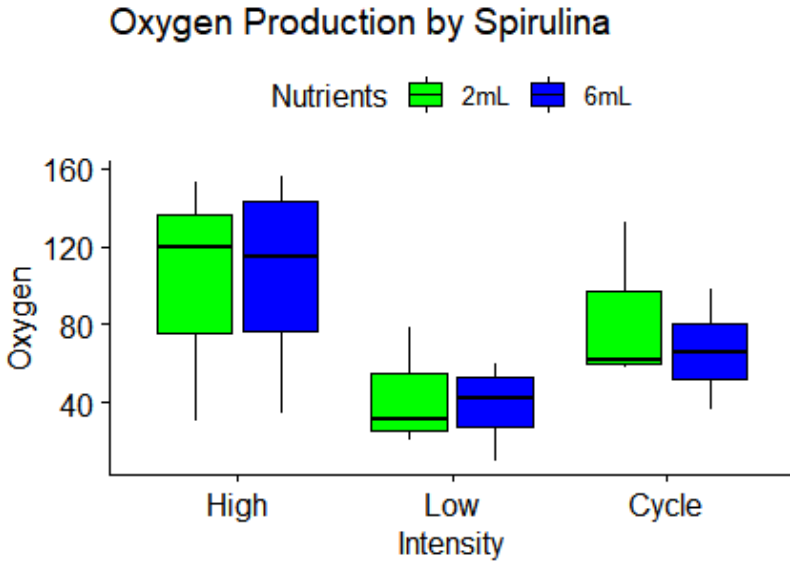

Figure 5 Boxplot of oxygen production data. Oxygen production is expressed in cm^3.

Light and Nutrient Effects on Cell Population

Using cell population size data, assumptions of ANOVA were met with square root transformed data. Shapiro-Wilk test of normality show that each factor is normal (Figure 6 and Table 3). Bartlett's test of homogeneity of variance showed that the variances withing each factor are

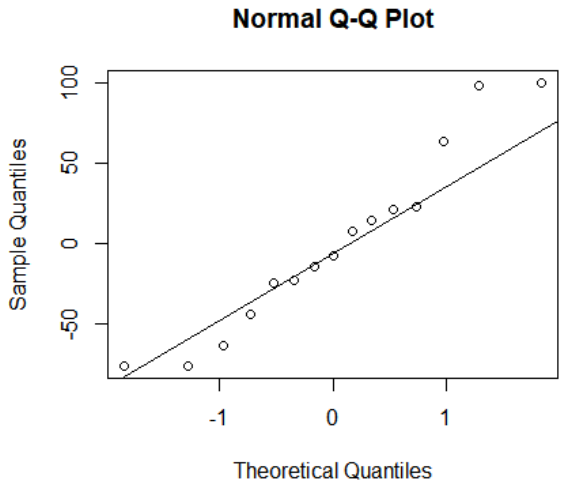

Figure 6 Q-Q plot of residuals of cell population data. Data points fall along the Q-Q line and do not curve off at extreme values.

equal within the nutrient factor (P=0.8146) and within the intensity factor (P=0.4838). The ANOVA indicated that there was no significant difference in means within the nutrients factor (P =0.301, F=1.203) or the intensity factor (P=0.221, F=1.794) nor was there any interaction between nutrients and light intensity (P=0.855, F=0.160, Figure 7).

Table 3 Shapiro-Wilk normality test on factors of square root transformed cell population data

Factor	W-value	p-value
2 mL Nutrients	0.85772	0.1139
6 mL Nutrients	0.86047	0.1528
High Intensity	0.92258	0.5241
Low Intensity	0.97267	0.8921
Light-dark Cycle	0.94028	0.656

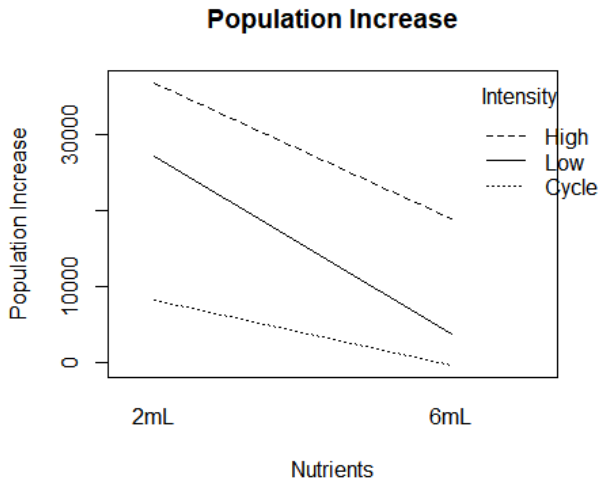

Figure 7 Interaction plot of cell population data. The lines are parallel to each other, indicating that there is no interaction between nutrients and light intensity.

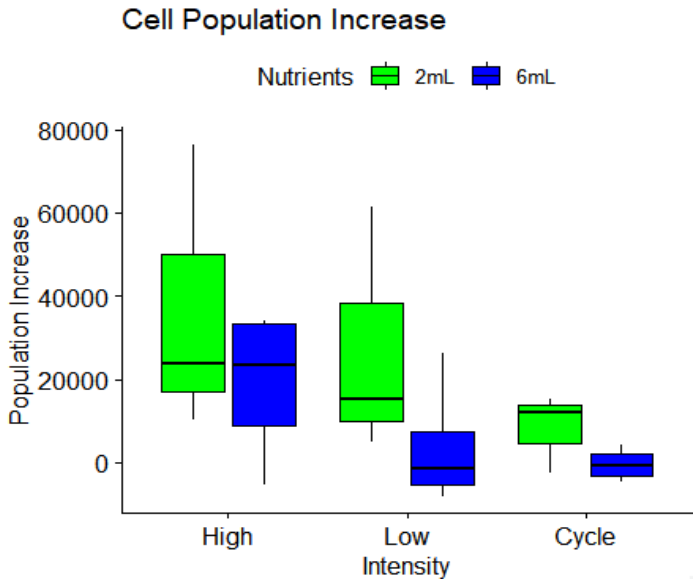

Figure 8 Boxplot of cell population data, population increase is expressed in cells/cm^3, Low intensity is 10 μmol m^{-2} s^{-1}, High intensity is 18 μmol m^{-2} s^{-1}, Cycle is 18 hours of 18 μmol m^{-2} s^{-1} light in each 24-hour period

Discussion

The results show that there is a significant difference in oxygen production between high and low intensity light, with high intensity light producing 62.6429 cm^3 more oxygen over the 72-hour period than low intensity light. This could be explained by the fact that in higher intensity light there is more light energy available for Spirulina to convert to the nutrients it needs and release oxygen as a byproduct. There was not a significant difference among oxygen produced by Spirulina in any of the other treatments. The lack of difference between the light-dark cycle and the high intensity light could be due to the fact that the light intensity of the light-dark cycle was the same as that in the high intensity, but the Spirulina was exposed to the light for fewer hours per day. There does appear to be differences here, but there was not enough statistical power to detect differences. The results suggest that high intensity is the best light intensity for stimulating oxygen production, but the light-dark cycle stimulates a moderate amount of oxygen production,

which may be beneficial because it uses less energy than the high intensity lights. This was a preliminary analysis, and further experiments with a larger number of replicates will provide a higher statistical power to detect differences. There was not a significant difference among treatments for cell population growth. In order to detect a significant difference, more statistical power may be needed. The results show that there is no significant difference in oxygen production between 2 mL of nutrients added daily and 6 mL of nutrients added daily. It was expected that the Spirulina would produce more oxygen in the higher concentration of nutrient media. This was expected because the nutrient media (Appendix B) contains a substantial amount of sodium bicarbonate, which Spirulina uses to fix as carbon dioxide. This process allows the cyanobacteria to undergo photosynthesis and produce oxygen as a biproduct. The nutrient media also has all the other chemicals needed for Spirulina growth and photosynthesis. It was expected that increasing the amount of necessary chemicals available to the cyanobacteria would increase the rate of photosynthesis and the rate of oxygen production. This was not the case. One reason for this could be that the 2 mL of nutrient media that acted as a control was the ideal amount of nutrient media. Any other media added could not be used by the cyanobacteria because of a yet to be determined threshold. To answer this question, more research is needed to find an ideal nutrient concentration and determine if there is a point in which there is an excess of chemicals that Spirulina cannot utilize for photosynthesis. The cell population increase was found to be the same for each nutrient concentration. This was not expected because Spirulina uses atmospheric carbon dioxide to grow and divide. This atmospheric carbon dioxide is provided in the nutrient media, so it was expected that providing the Spirulina with more carbon dioxide would result in a greater cell population increase. These results could be because the 2 mL of

nutrient media had the ideal amount of carbon dioxide, and Spirulina did not have the ability to utilize anymore, so the extra nutrient media was excess. The Spirulina could also be at too high of a concentration to grow effectively in the space and water it is given to grow in. More research is needed to test this. If larger containers are used with less Spirulina culture in them, adding more nutrients may allow the cyanobacteria to grow better.

Results of these experiments indicate that Spirulina grown in a higher light intensity, 18 μmol m^{-2} s^{-1}, produces significantly more oxygen than Spirulina at lower light intensities and Spirulina in the presence of a light-dark cycle. These are promising results and shows that Spirulina can still be grown and produce oxygen in a light intensity lower than in traditional growth conditions. This research also suggests that a light-dark cycle produces a moderate amount of oxygen, which has the potential to be beneficial to space travel because it uses less valuable energy than a constant high light intensity. Space travel relies on utilizing resources, including energy, in a way that maximizes efficiency. The ability of Spirulina to grow and prosper in a light intensity that does not use excess resources is vital to its success as a source of oxygen and food in space.

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Appendix A

Intensity	Population (cells/mL)	Nutrients (mL)
High	34000	6
High	33125	6
High	-5250	6
High	13667	6
Low	26250	6
Low	1250	6
Low	-4375	6
Low	-8000	6
Cycle	4375	6
Cycle	-2500	6
Cycle	-4625	6
Cycle	1375	6
High	76250	2
High	10300	2
High	23871	2
Low	61250	2
Low	5000	2
Low	15100	2
Cycle	-2500	2
Cycle	11,975	2
Cycle	15400	2

Intensity	Oxygen(cm^3)	Nutrients(mL)
High	139	6
High	91	6
High	34	6
Low	10	6
Low	51	6
Low	34	6
High	156	6
Low	60	6
Cycle	57	6
Cycle	98	6
Cycle	74	6
Cycle	36	6
High	30.5	2
High	120	2
High	153	2
Low	21	2
Low	31	2
Low	78	2
Cycle	62	2
Cycle	132	2
Cycle	58	2

Appendix B

Zarrouk's Nutrient Media

Solution A	500 mL deionized water
	1.43 g Boric acid
	0.905 g Magnesium (II) chloride tetrahydrate
	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
	0.16 g Calcium chloride
	0.04 g Ferrous sulfide
	0.32 g EDTA
	4.0 mL solution A
	4.0 mL solution B