Low Light vs. High Light,
Photoautotrophic Growth vs. Mixotrophic Growth
Optimal yield for Chlamydomonas reinhardtii

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Dates of Experiment:
Photoautrophic: 7/27/15-7/31/15
Mixotrophic: 8/9/15-8/17/15

Introduction

In 2014 alone, the United States consumed 6.95 billion barrels of petroleum products. That is an average of 19.05 million barrels a day. Unfortunately, of this large number only 5% are biofuels[1]. Limited resources and other externalities of producing corn and soybean make it difficult to fully rely solely on plant-based biofuels. With population growth, less arable land, and limited freshwater resources, utilizing crop-based biofuels seem like an unfeasible long term resource. Microalgae is a viable alternative source of biofuel energy. They utilize less resources that humans depend religiously upon and is still carbon neutral. Chlamydomonas reinhardtii's extensively sequenced genome, and the vast amount of research already conducted on it make it a model organism for biofuel research. They can even grow in mixotrophic, photoautotrophic and heterotrophic conditions. Thus, Chlamydomonas' light and carbons factors need to be established to depict a clearer method in optimizing lipid and biomass production.

Purpose

The objective is to determine a method to maximize lipid and biomass yield in order to enhance microalgae biofuel production. To determine the best method of growth, the growing conditions of the microalgae were manipulated, providing them with either high or low light and mixotrophic or photoautotrophic growth. To determine the biomass population, growth curves were measured. In order to identify the lipid yield, Nile Red was measured under a fluorescence microscope. To determine its photosynthetic efficiency and the healthiness of the cells, PAM was also measured. Other tests were conducted to detect the state of the microalgae.

Methods and Set-Up

Throughout the experiment microalgal growth and sampling occurred in four separate photobioreactors that were specifically constructed for this experiment. Each sample was kept in a photobioreactor with a starting volume of 1.3 L. These reactors were placed in racks to stabilize them. They were also placed inside a tank filled with DI water. There were two biological replicates each for high and low light.

With photoautotrophic growth, Chlamy was grown in MASM (Modified Artificial Seawater Media). With mixotrophic growth Chlamy was grown in TAP (Tris Acetate Phosphate) media. During mixotrophic growth, the media contained organic carbon through the use of acetate. Lipid accumulation was not induced during both experiments.

[1] "Frequently Asked Questions," *U.S. Department of Energy*, 1 Aug 2015, <http://www.eia.gov/tools/faqs/faq.cfm?id=33&t=6>.

Types of Light Used for C. reinhardtii Growth

Table 1. The table below describes which sample contained the manipulated condition of high or low light throughout both experiments.

Sample	Light Measurement
Sample 1	Low Light
Sample 2	Low Light
Sample 3	High Light
Sample 4	High Light

Table 2. The table below depicts the daily conditions of the photo bioreactors and the time of sampling during photoautotrophic and mixotrophic growth.

Factors	Photoautotrophic Growth				
Date	7/27/15	7/28/15	7/29/15	7/30/15	7/31/15
Time Taken	9:00 AM	9:00 AM	9:30 AM	9:00 AM	1:00 PM
Temperature (C)	23	23	23	23	23
Relative Humidity (%)	100	100	100	100	100
CO_2	5000 ppm	5000 ppm	5000 ppm	5000 ppm	5000 ppm
Flow Rate of Humidicers (ml/min)	300	300	300	300	300

Factors	Mixotrophic Growth						
Date	8/9/15	8/10/15	8/11/15	8/12/15	8/13/15	8/14/15	8/17/15
Time Taken	Inoculation	9:45 AM	9:00 AM	9:00 AM	9:00 AM	8:45 AM	1:00 PM
Temperature (C)	23	23	23	23	23	23	23
Relative Humidity (%)	100	100	100	100	100	100	100
Atmospheric CO_2	400 ppm	400 ppm	400 ppm	400 ppm	400 ppm	400 ppm	400 ppm
Flow Rate of Humidicers (ml/min)	300	300	300	300	300	300	300

Table 3. The following chart depicts the tests conducted during the daily sampling of the experiments.

Lists of Tests Conducted:	
Name	**Description**
Optical Density	Measured absorbance of 200 microliters of each cell at 750 nanometers
Nile Red	Stained 1 mL of sample with 1 nanometer of 1000x NR stock Nile Red Stain and then measured fluorescence using the NR microscope
Growth Curve	Used hemocytometers filled with 10 nanometers of sample, then manually counted each cell under a microscope. Used the following equation: [1000(Average ÷ .4) + .1(1000(Average ÷ .4))] * Dilution Factor= cells/mL
pH	Test the sample daily to ensure the pH doesn't go below 6
Pulse Amplitude Modulation (PAM)	Measures photosynthetic efficiency, or the "healthiness" of the sample. Used 1 mL of the sample and pippetted inside a cuvette, then used the aqua pen to conduct the measurement. A healthy range for PAM is greater than .5 Fv/Fm.
Phosphates	Centrifuged 1 mL of the sample for 4 minutes at 4.5 RCF to spin down the Chlamy. Obtained the media that the microalgae was in, and then froze it for future testing. Unfortunately, the samples retrieved were not tested.

Experimental Data

Optical Density

Table 4. The tables below depict the Optical Density for the triplicates of each sample. The data is recorded at 750 nm and contains the average and standard deviation.

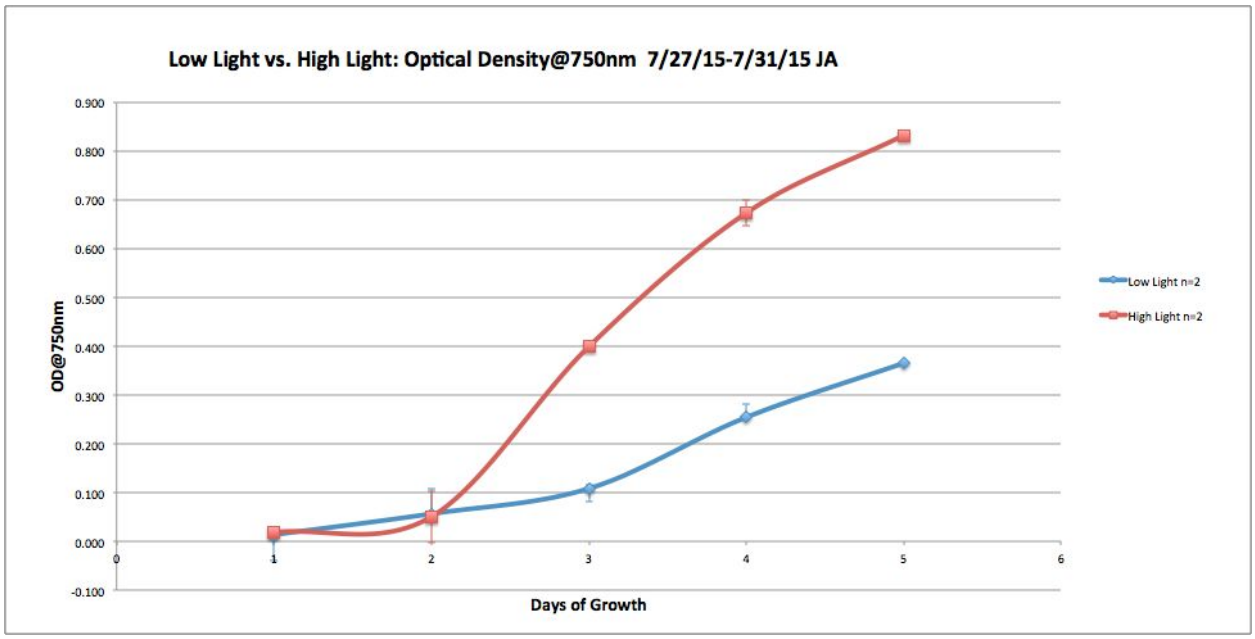
Photoautotrophic.

	Day	1	2	3	4	5
Parameters	**Trial**	**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
OD	**1 Low Light**	0.013	0.057	0.108	0.177	0.241
	2 Low Light	0.014	0.057	0.108	0.332	0.490
mean		0.014	0.057	0.108	0.254	0.365
std.dev		0.001	0.000	0.000	0.110	0.177
	3 High Light	0.015	0.088	0.394	0.692	0.834
	4 High Light	0.023	0.013	0.405	0.654	0.831
mean		0.019	0.050	0.399	0.673	0.833
std.dev		0.005	0.053	0.008	0.027	0.002

Mixotrophic.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
OD	**1 Low Light**		0.001	0.004	0.024	0.148	0.446	0.436
	2 Low Light		0.001	0.006	0.045	0.269	0.528	0.614
mean			0.001	0.005	0.034	0.208	0.487	0.525
std.dev			0.000	0.001	0.015	0.085	0.058	0.126
	3 High Light		0.004	0.024	0.125	0.398	0.592	0.795
	4 High Light		0.001	0.022	0.128	0.376	0.554	0.659
mean			0.003	0.023	0.127	0.387	0.573	0.727
std.dev			0.002	0.001	0.002	0.016	0.027	0.096

Photoautotrophic.



Mixotrophic.

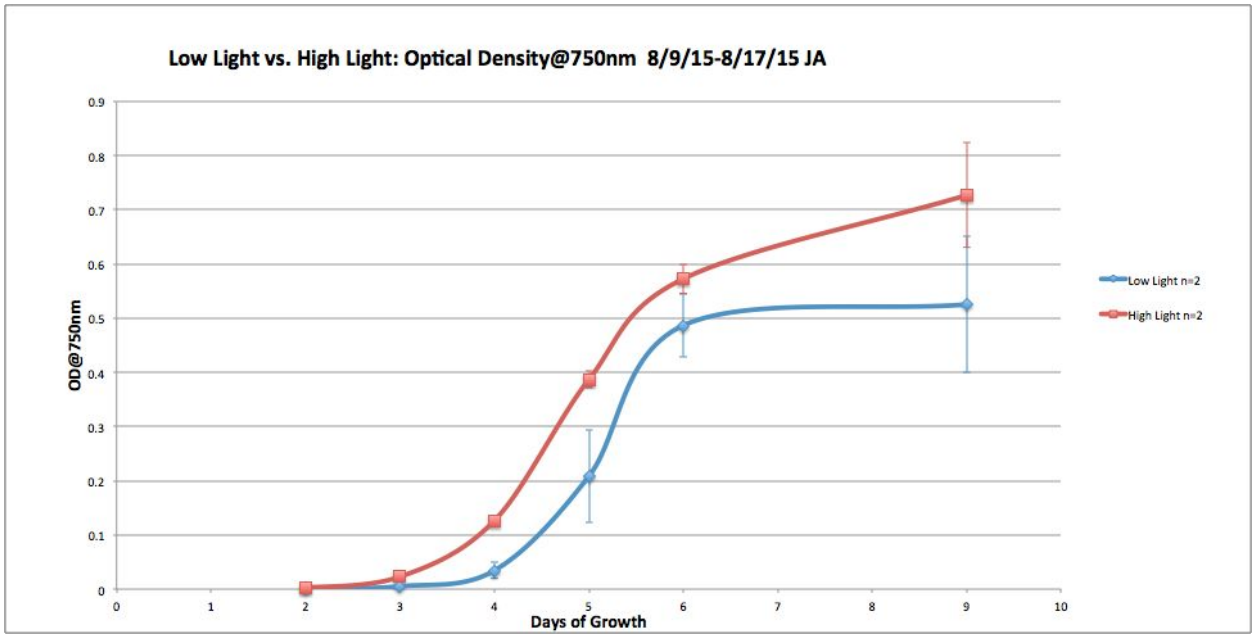


Figure 1. The graphs above depict the average of the data acquired from the two replicates. Included is the the standard deviations of the replicates. Optical density can be used to acquire a baseline for understanding cell growth, but it should not be substituted for cell count data.

Nile Red

Table 5. The table below depicts the lipid fluorescence for the triplicates of each sample. The data, average and standard deviation is recorded. For the mixotrophic experiment, Specific Fluorescence data for day 3 is unavailable due to inaccurate results.

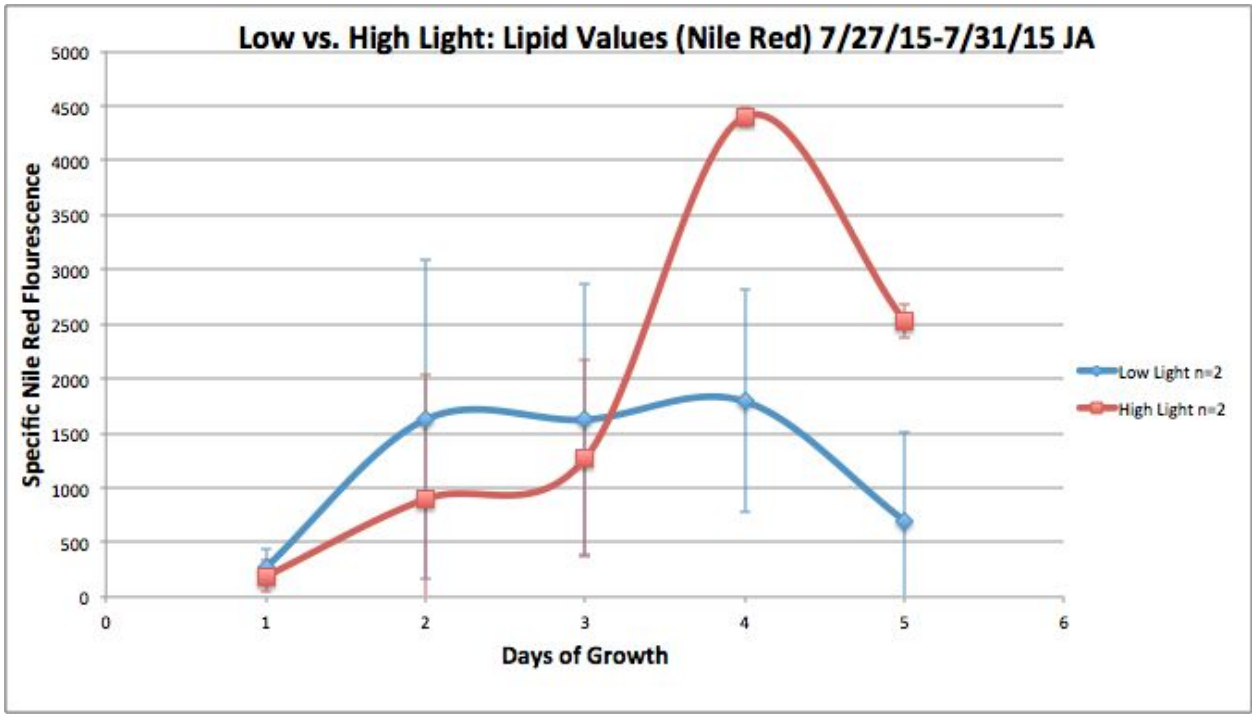
Photoautotrophic.

	Day	1	2	3	4	5
Parameters	**Trial**	**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
Lipids	**1 Low Light**	156	594	750	1072	1269
	2 Low Light	397	2658	2500	2512	127
mean		277	1626	1625	1792	698
std.dev		170	1459	1237	1019	807
	3 High Light	90	86	634	4342	2642
	4 High Light	293	1705	1903	4461	2423
mean		192	896	1268	4401	2532
std. dev		144	1145	898	84	155

Mixotrophic.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
Lipids	**1 Low Light**		189		1157	1129	1972	2182
	2 Low Light		89		131	1972	2790	580
mean			139		644	1551	2381	1381
std.dev			71		726	596	578	1133
	3 High Light		35		1939	7286	13589	4855
	4 High Light		55		2213	6092	13571	5961
mean			45		2076	6689	13580	5408
std. dev			14		194	845	13	782

Photoautotrophic.



Mixotrophic.

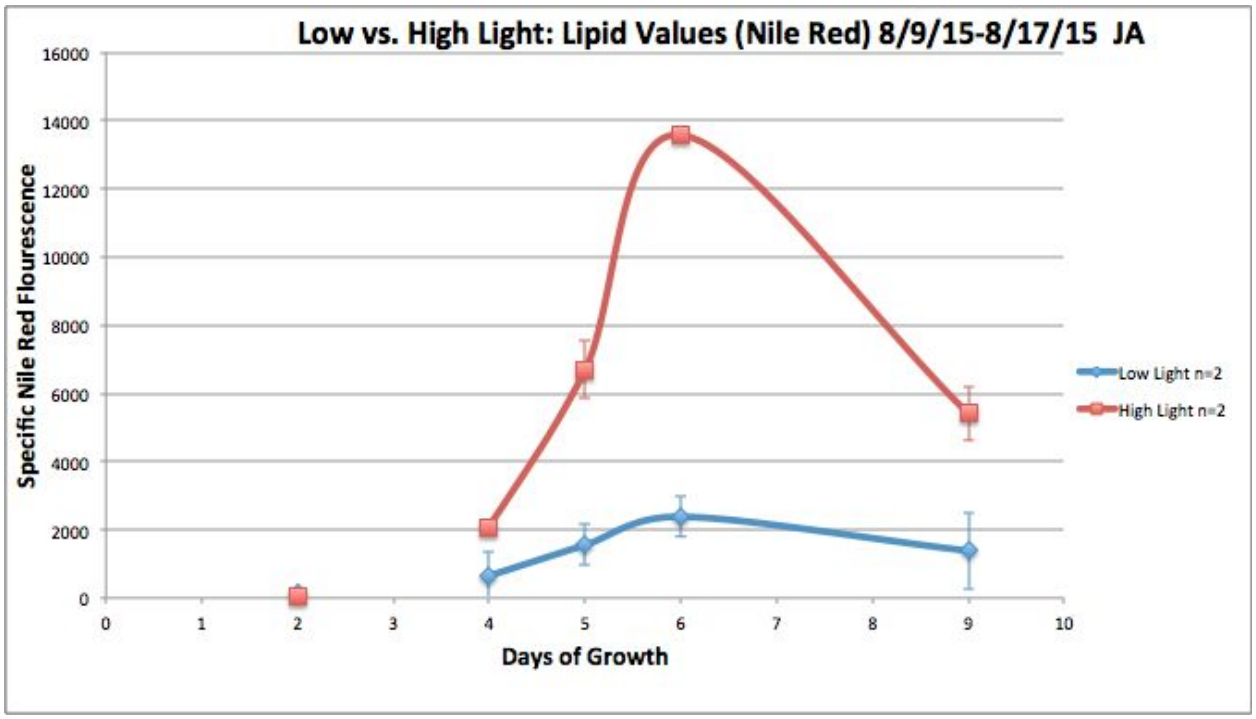


Figure 2. The graphs above depict the average data acquired for the two biological replicates of the Specific Fluorescence. Nile Red can be used to determine lipid accumulation; however, it should be supplemented with a cell count to find the lipids/cell to identify any lipid accumulation.

Cell Count

Table 6. The following tables depict the acquired average data for the growth curve of the two biological replicates for low and high light. Derived from hemocytometers, the data includes its averages and standard deviation.

Photoautotrophic.

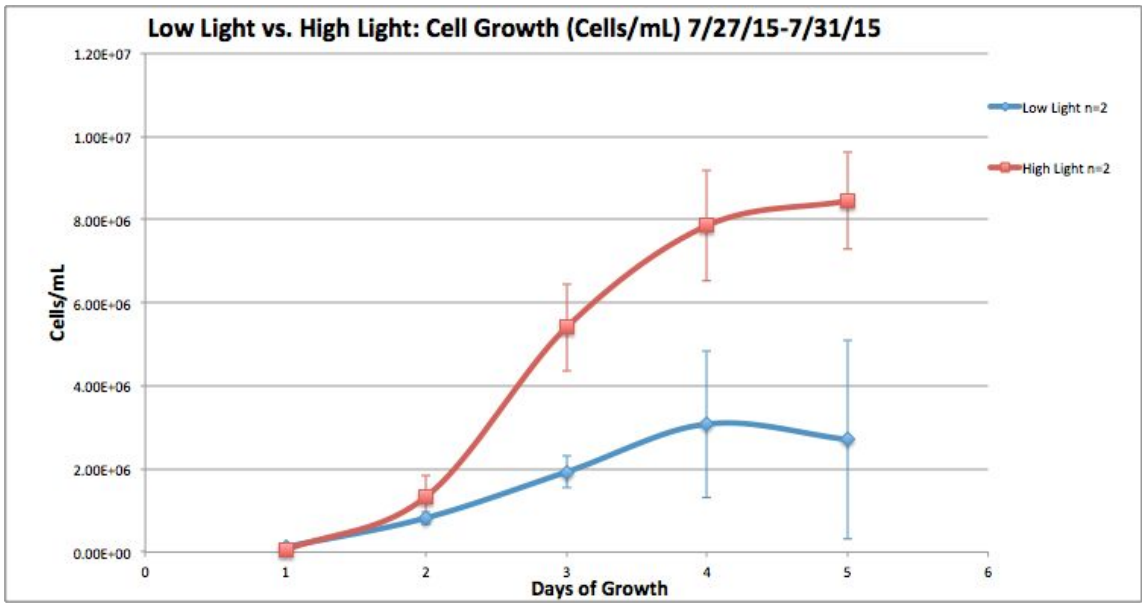
	Day	1	2	3	4	5
Parameters	**Trial**	**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
Cell Count	**1 Low Light**	9.35E+04	712250	2193125	1828750	1017500
	2 Low Light	1.35E+05	935000	1663750	4331250	4400000
mean		1.14E+05	8.24E+05	1.93E+06	3.08E+06	2.71E+06
std dev.		29168.1547	157508.035	374324.652	1769534.72	2391788.68
	3 High Light	4.68E+04	962500	4663750	8795000	9267500
	4 High Light	6.60E+04	1705000	6153125	6916250	7631250
mean		5.64E+04	1.33E+06	5.41E+06	7.86E+06	8.45E+06
std dev.		13611.8055	525026.785	1053147.16	1328476.86	1157003.47

Mixotrophic.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
Cell Count	**1 Low Light**		15125	100375	632500	2392500	6682500	10477500
	2 Low Light		9625	115500	965250	3478750	8882500	13227500
mean			1.24E+04	1.08E+05	7.99E+05	2.94E+06	7.78E+06	1.19E+07
std dev.			3889.087297	10694.99007	235289.7814	768094.7411	1555634.919	1944543.648

	3 High Light		26125	475750	1724250	4661250	4867500	8112500
	4 High Light		45357	475750	1795750	3245000	3877500	6737500
mean			3.57E+04	4.76E+05	1.76E+06	3.95E+06	4.37E+06	7.43E+06
std dev.			13599.07762	0	50558.13485	1001439.979	700035.7134	972271.8241

Photoautotrophic.



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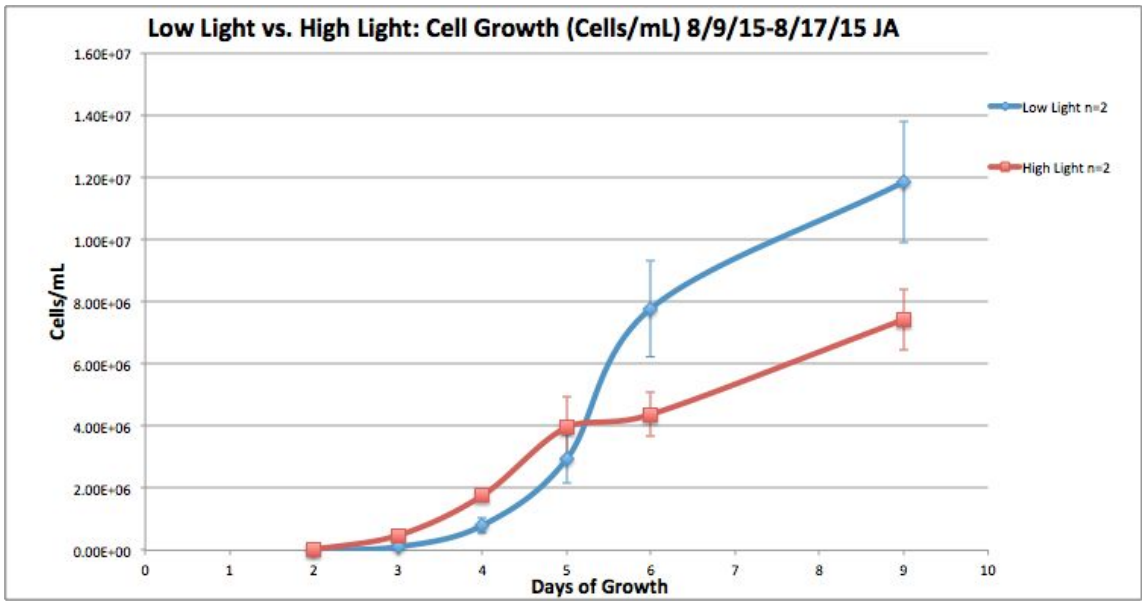


Figure 3. The graphs above depict the average data acquired of the two biological replicates for low and high light, and its standard deviations.

Lipids/Cell

Table 7. The following tables depict the acquired data for the lipids/cell of the two biological replicates for the low and high light. By identifying cells/mL and retrieving the data from nile red, we can identify lipids/cell. For the mixotrophic day 3 data, the lipids/cell is unavailable because of no Nile Red data for that day. The following equation was used to find lipids/cell: 1000(# of Lipids/# of Cells)=Lipids/cell

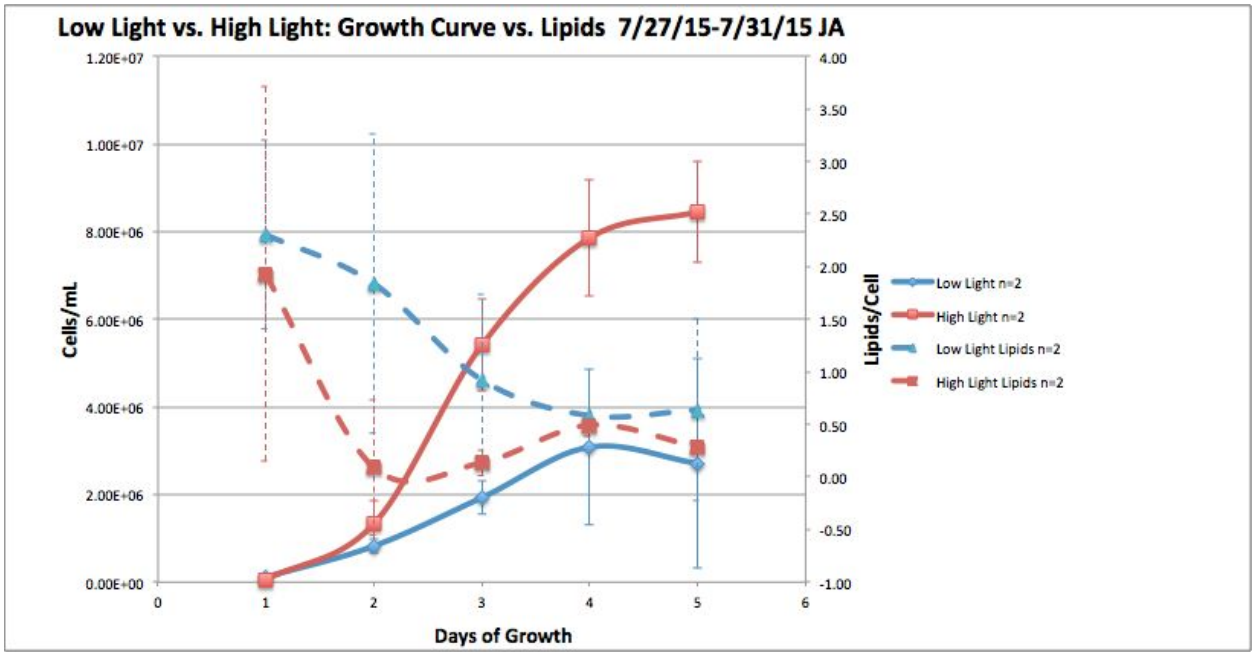
Photoautotrophically.

	Day	1	2	3	4	5
Parameters	**Trial**	**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
Lipids/Cell	**1 Low Light**	1.67	0.83	0.34	0.59	1.25
	2 Low Light	2.94	2.84	1.50	0.58	0.03
mean		2.31	1.84	0.92	0.58	0.64
std dev		0.90	1.42	0.82	0.00	0.86
	3 High Light	1.93	0.09	0.14	0.49	0.29
	4 High Light	4.44	1.00	0.31	0.64	0.32
mean		1.93	0.09	0.14	0.49	0.29
std dev		1.78	0.64	0.12	0.11	0.02

Mixotrophically.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
Lipids/Cell	**1 Low Light**		5.91		1.83	0.47	0.30	0.21
	2 Low Light		9.28		0.14	0.57	0.31	0.04
mean			7.59		0.98	0.52	0.30	0.13
std dev			2.39		1.20	0.07	0.01	0.12
	3 High Light		1.33		1.12	1.56	2.79	0.60
	4 High Light		1.21		1.23	1.88	3.50	0.88
mean			1.33		1.12	1.56	2.79	0.60
std dev			0.09		0.08	0.22	0.50	0.20

Photoautotrophically.



Mixotrophically.

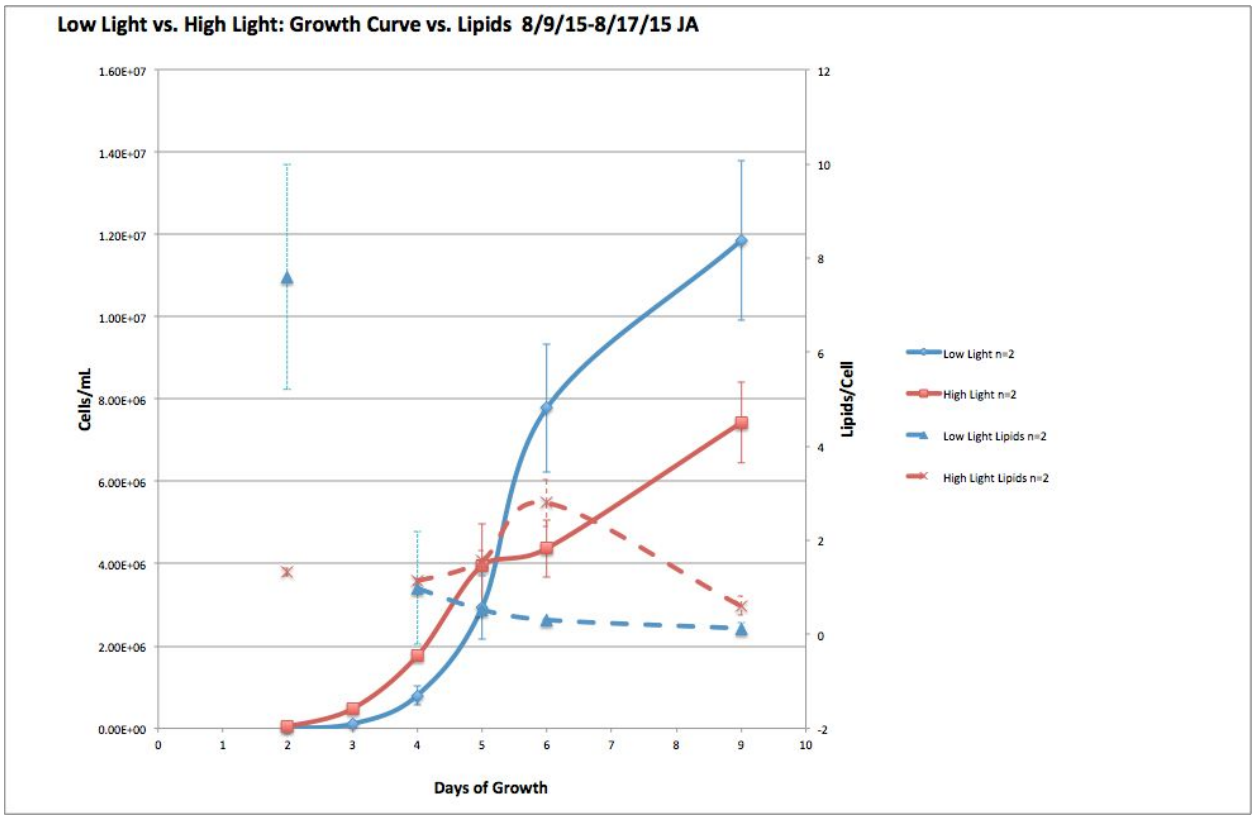


Figure 4. The graphs above contain a left vertical axis representing the growth curve of the average data for the two biological replicates of high and low light. The right vertical axis shows the average data retrieved for lipids/cell for the two biological replicates of high and low light.

pH

Table 8. The tables below depict the data for the pH of the two biological replicates for low and high light, as well as the average and standard deviation.

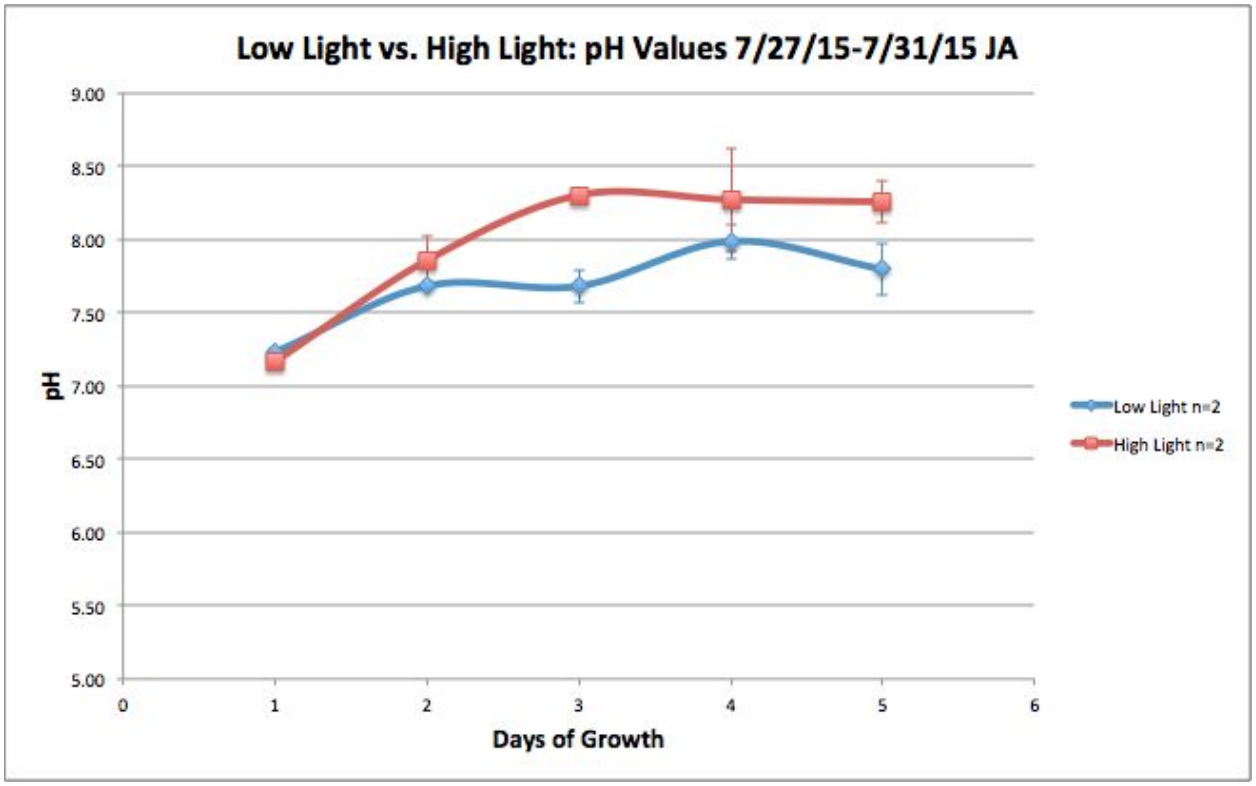
Photoautotrophic.

	Day	1	2	3	4	5
Parameters	**Trial**	**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
pH	**1 Low Light**	7.20	7.67	7.60	7.90	7.67
	2 Low Light	7.25	7.69	7.76	8.07	7.92
mean		7.23	7.68	7.68	7.99	7.80
std dev		0.04	0.01	0.11	0.12	0.18
	3 High Light	7.16	7.74	8.27	8.52	8.15
	4 High Light	7.17	7.97	8.33	8.02	8.36
mean		7.17	7.86	8.30	8.27	8.26
std dev		0.01	0.16	0.04	0.35	0.15

Mixotrophic.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
pH	**1 Low Light**	-	7.26	7.43	7.55	7.89	8.35	8.49
	2 Low Light	-	7.23	7.44	7.58	7.93	8.41	8.52
mean			7.25	7.44	7.57	7.91	8.38	8.51
std dev			0.02	0.01	0.02	0.03	0.04	0.02
	3 High Light	-	7.20	7.47	7.66	7.96	8.31	8.50
	4 High Light	-	7.18	7.46	7.68	7.95	8.23	8.49
mean			7.19	7.47	7.67	7.96	8.27	8.50
std dev			0.01	0.01	0.01	0.01	0.06	0.01

Photoautotrophic.



Mixotrophic.

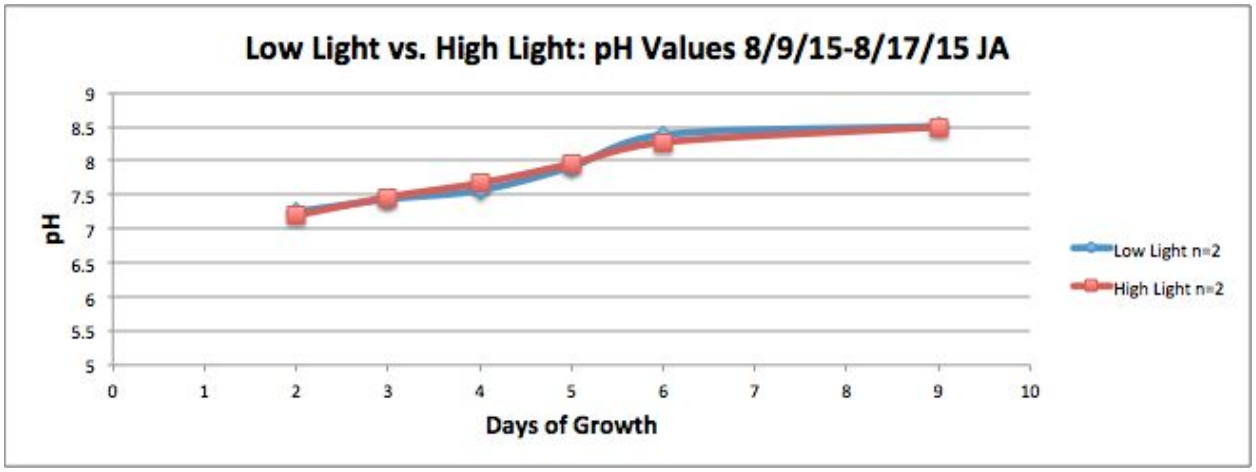


Figure 5. The graphs above represents the pH of the average data of the two biological replicates for the high and low light, as well as the standard deviation.

Pulse Amplitude Modulation (PAM)

Table 9. The tables below depicts the photosynthetic efficiency of the two biological replicates for the high and low light, as well as the average and standard deviation.

Using PAM through the use of an aqua pen, we are able to determine the healthiness of the cell. PAM was used only for the last two days of the experiment during photoautotrophic growth. For the mixotrophic experiment, no data was retrieved on the second day, due to the sample's low concentration of cells.

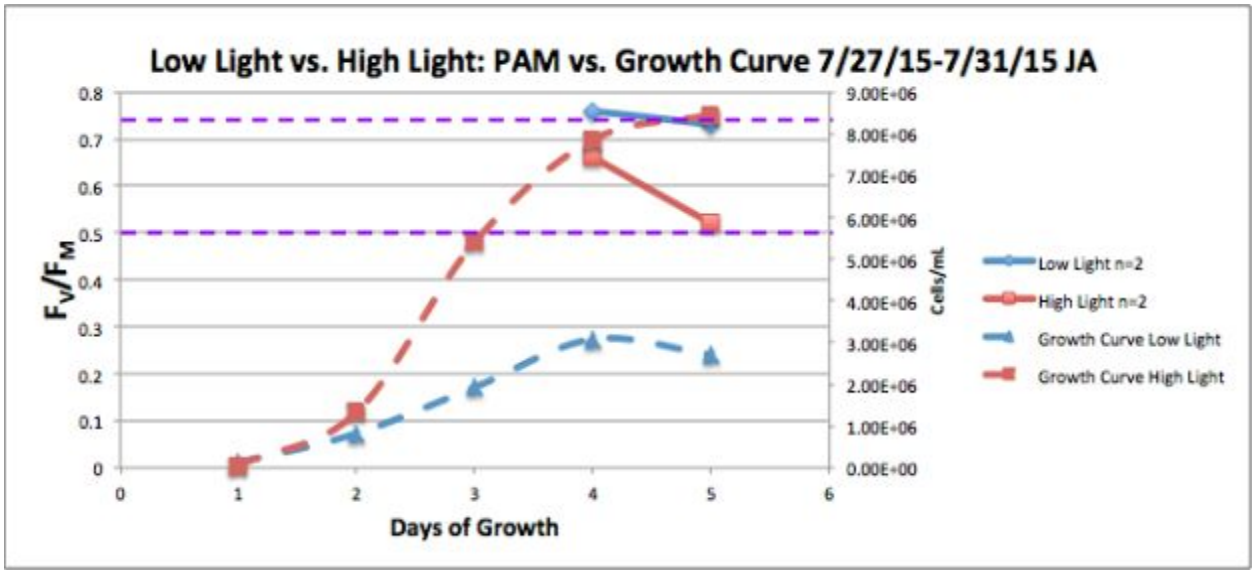
Photoautotrophic.

	Day	1	2	3	4	5
Parameters		**7/27/15**	**7/28/15**	**7/29/15**	**7/30/15**	**7/31/15**
PAM	**1 Low Light**	-	-	-	0.79	0.72
	2 Low Light	-	-	-	0.73	0.74
mean					0.76	0.73
std. dev					0.04	0.01
	3 High Light	-	-	-	0.63	0.53
	4 High Light	-	-	-	0.69	0.51
mean					0.66	0.52
std. dev					0.04	0.01

Mixotrophic.

	Day	1	2	3	4	5	6	9
Parameters	**Trial**	**8/9/15**	**8/10/15**	**8/11/15**	**8/12/15**	**8/13/15**	**8/14/15**	**8/17/15**
PAM	**1 Low Light**	-	-	-	0.72	0.71	0.71	0.69
	2 Low Light	-	-	0.61	0.70	0.68	0.68	0.65
mean				0.61	0.71	0.70	0.70	0.67
std. dev			0.00	0.00	0.01	0.02	0.02	0.03
	3 High Light	-	-	0.65	0.57	0.51	0.51	0.39
	4 High Light	-	-	0.65	0.55	0.43	0.43	0.27
mean				0.65	0.56	0.47	0.47	0.33
std. dev			0.00	0.00	0.01	0.06	0.06	0.08

Photoautotrophic.



Mixotrophic.

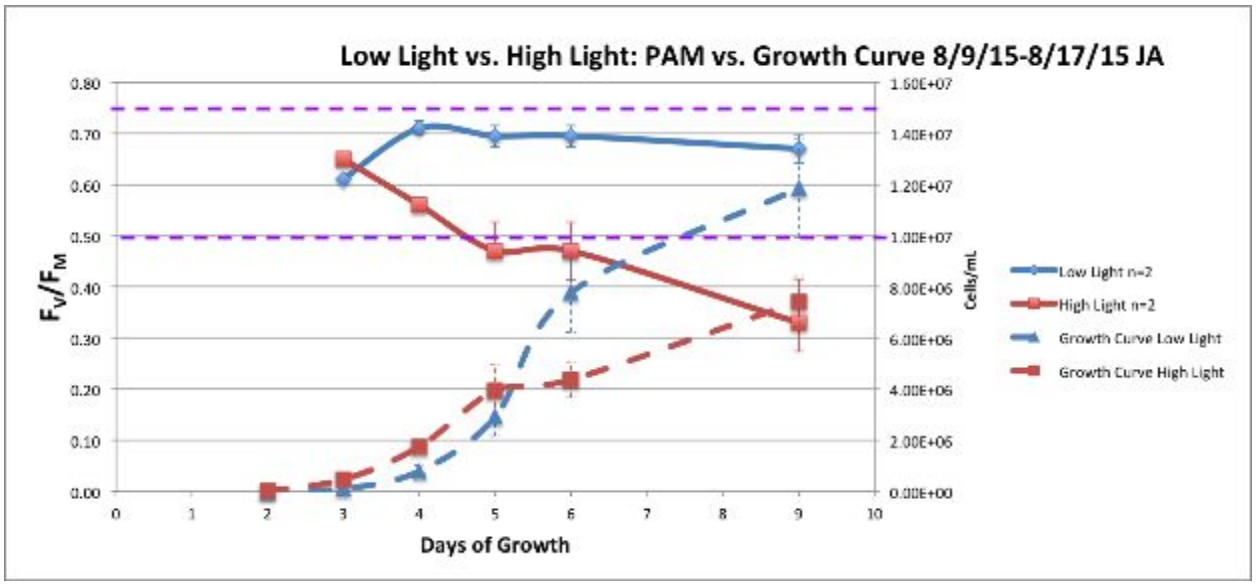


Figure 6. The graphs above contain a left vertical axis representing the average PAM data of the two biological replicates for the low and high light. The right vertical axis shows the growth curve of the average of the two biological replicates for high and low light. The straight lines in purple represent the “healthy” range (.5-.75) of the photosynthetic efficiency.

Comparison of Cell Growth & PAM for All Experiments

Table 10. The table below depicts the results of the average growth curve for both biological replicates for both Joan and Meena's experiments.

	Photoautotrophic				Mixotrophic			
Trial	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
CO_2 (ppm)	5000	5000	5000	400	400	400	5000	400
Light	Low	High	High	High	Low	High	Medium	Medium
Growth	2.71E+06	8.45E+06	4.76E+06	1.42E+06	1.19E+07	7.43E+06	1.66E+07	7.77E+06

Conclusion

During the inoculation of chlamy, the photoautotrophic reactors were given 28 and 32 mL of microalgae culture. However, for mixotrophic growth the inoculation was only 1 mL. Despite inoculating less culture, the mixotrophic cells ended up with an average of more cells than photoautotrophic growth, as shown in table 10. Thus, mixotrophic growth demonstrated a better growth than the photoautrophic conditions.

For the photoautotrophic growth chlamy relied solely on photosynthesis. During this experiment, the samples grew better in high light. This can be seen in table 10 where with a CO_2 level of 5000 ppm, the high lights had a growth of 8.45E+06 and 4.76E+06, compared to the low light of 2.71E+06. However despite using high light, Trial 4 in photoautotrophic growth had the lowest cell count out of all the reactors. Despite the abundance of light, the limiting factor of low carbon greatly affected the reactor's growth. Therefore, during photosynthesis, CO_2 is a limiting factor in growth. More precisely, low CO_2 is more limiting than low light because Trial 4 had a lower cell count than Trial 1, as shown in table 10.

During mixotrophic growth acetic acid was in the media; therefore, providing organic carbon for heterotrophic growth. The bioreactors were also supplemented with light and CO_2 for photoautotrophic growth. The microalgae prefered to utilize heterotrophic growth during mixotrophic conditions. In fact, high light seems to overwhelm the cells, inhibiting the population growth by overloading photosystem II. As shown in table 10, high light for mixotrophic growth had the lowest cell population of 7.43E+06.This can be seen again in trial 4 in which medium light inhibited cell growth. With a CO_2 of 400 ppm and medium light, the growth was 7.77E+06. However, with the same CO_2 conditions and low light, trial 1 had a significant cell growth of 1.19E+07. On the other hand, as shown in table 10, High CO_2 doesn't inhibit cell growth under mixotrophic conditions. In fact it had the greatest cell count of 1.66E+07 out of both experiments.

Out of all the trials for both experiments the results clearly show that, high carbon with medium light under mixotrophic conditions is the best option in maximizing Chlamydomonas reinhardtii populations. Under these conditions, microalgae biofuels can provide a higher biomass yield than before. By understanding the population growth of the microalgae, researchers can become more efficient in growing microalgae for biofuel; therefore, increasing the possibility of algae biofuels being a viable long term resource for the rising population of tomorrow.

Future Steps

The purpose of this experiment was to enhance microalgae biofuel production through the optimization of lipid and biomass yield. However, this experiment was primarily focused on identifying the best conditions to maximize biomass yield. Thus, lipid accumulation was not induced, resulting with no accumulation of lipids. Therefore, to fully achieve our objective, future experiments containing induced lipid accumulation should be done.

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