

**Low Carbon vs. High Carbon
Photoautotrophic Growth vs. Mixotrophic Growth
Submitted by: Meena Reddy
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Dates of Experiment:
Photoautotrophic: 7/27/15 – 7/31/15
Mixotrophic: 8/9/15 – 8/17/15**

List of Tests Conducted:

Name	Description
OD750	Measured absorbance of 200 microliters of each strain/trial at 750 nanometers
pH	Tested pH of each sample daily
Cell Count	[(Average ÷ .4) + .1(Average ÷ .4)] * Dilution Factor= cells/mL; use hemocytometers to manually count cells in a sample, use above equation to average cells/mL
Relative Lipid Fluorescence	Stained 1000 microliters of culture with 1 microliters of Nile Red stain, and then measured the fluorescence using fluorescent microscopy

Data and Charts: Photoautotrophic Experiment

Table 2. The table below depicts the Optical Density for the two replicates of each CO₂ concentration of *C. reinhardtii* at 750 nm, and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

Carbon Type	Trial	7/27/15	7/28/15	7/29/15	7/30/15	7/31/15
Low Carbon	1	0.017	0.028	0.059	0.081	0.109
	2	0.009	0.022	0.047	0.072	0.099
	Mean	0.013	0.025	0.053	0.077	0.104
	Std. Dev	0.006	0.004	0.009	0.007	0.007
High Carbon	3	0.015	0.091	0.198	0.033	0.412
	4	0.010	0.071	0.214	0.029	0.412
	Mean	0.012	0.081	0.206	0.031	0.412
	Std. Dev	0.003	0.014	0.012	0.002	0.000

Table 3. The table below depicts the pH for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

Carbon Type	Reactor	7/27/15	7/28/15	7/29/15	7/30/15	7/31/15
Low Carbon	1	7.41	7.85	7.96	7.98	7.98
	2	7.4	7.93	7.97	7.99	7.99
	Mean	7.405	7.89	7.965	7.985	7.985
	Std. Dev	0.00707107	0.05656854	0.00707107	0.00707107	0.00707107
High Carbon	3	6.99	7.52	7.65	7.55	7.55
	4	7	7.57	7.64	7.66	7.66
	Mean	6.995	7.545	7.645	7.605	7.605
	Std. Dev	0.00707107	0.03535534	0.00707107	0.07778175	0.07778175

Table 4. The table below depicts the cell count (cells/mL) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

Carbon Type	Trial	7/27/2015	7/28/15	7/29/15	7/30/15	7/31/15
Low Carbon	1	-	8.36E+05	1.05E+06	1.36E+06	1.50E+06
	2	6.88E+04	4.21E+05	1.11E+06	2.70E+06	1.33E+06
	Mean	6.88E+04	6.29E+05	1.08E+06	2.03E+06	1.42E+06
	Std. Dev		2.93E+05	4.86E+04	9.43E+05	1.17E+05
High Carbon	3	C	8.33E+05	1.73E+06	3.67E+06	5.39E+06
	4	7.98E+04	8.44E+05	2.29E+06	3.97E+06	4.13E+06
	Mean	7.98E+04	8.39E+05	2.01E+06	3.82E+06	4.76E+06
	Std. Dev		7.78E+03	3.94E+05	2.13E+05	8.94E+05

Table 5. The table below depicts the lipid measure (Au) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

Carbon Type	Trial	7/27/2015	7/28/15	7/29/15	7/30/15	7/31/15
Low Carbon	1	111	1751	2012	884	1099
	2	248	3909	759	791	1450
	Mean	179	2830	1386	838	1275
	Std. Dev	97	1526	886	66	248
High Carbon	3	238	1960	3208	4756	12885
	4	205	1569	4091	4889	11946
	Mean	221	1765	3649	4823	12415
	Std. Dev	23	276	624	94	664

Table 6. The table below depicts the lipids per cell (Au/cell) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

Carbon Type	Trial	7/27/2015	7/28/15	7/29/15	7/30/15	7/31/15
Low Carbon	1	-	2.09	1.93	0.65	0.73
	2	247.50	9.29	0.68	0.29	1.09
	Mean	247.50	5.69	1.30	0.47	0.91
	Std. Dev	0.00	5.08	0.88	0.25	0.25
High Carbon	3	237.67	2.35	1.85	1.30	2.39
	4	204.50	1.86	1.79	1.23	2.90
	Mean	221.08	2.11	1.82	1.26	2.64
	Std. Dev	23.45	0.35	0.05	0.05	0.36

Table 7. The table below depicts the photosynthetic efficiency (PAM) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for days 4 and 5 of the photoautotrophic experiment.

Carbon Type	Trial	7/30/15	7/31/15
Low Carbon	1	0.72	0.68
	2	0.74	0.66
	Mean	0.73	0.67
	Std. Dev	0.014142136	0.014142136
High Carbon	3	0.47	0.29
	4	0.45	0.34
	Mean	0.46	0.315
	Std. Dev	0.01414214	0.035355339

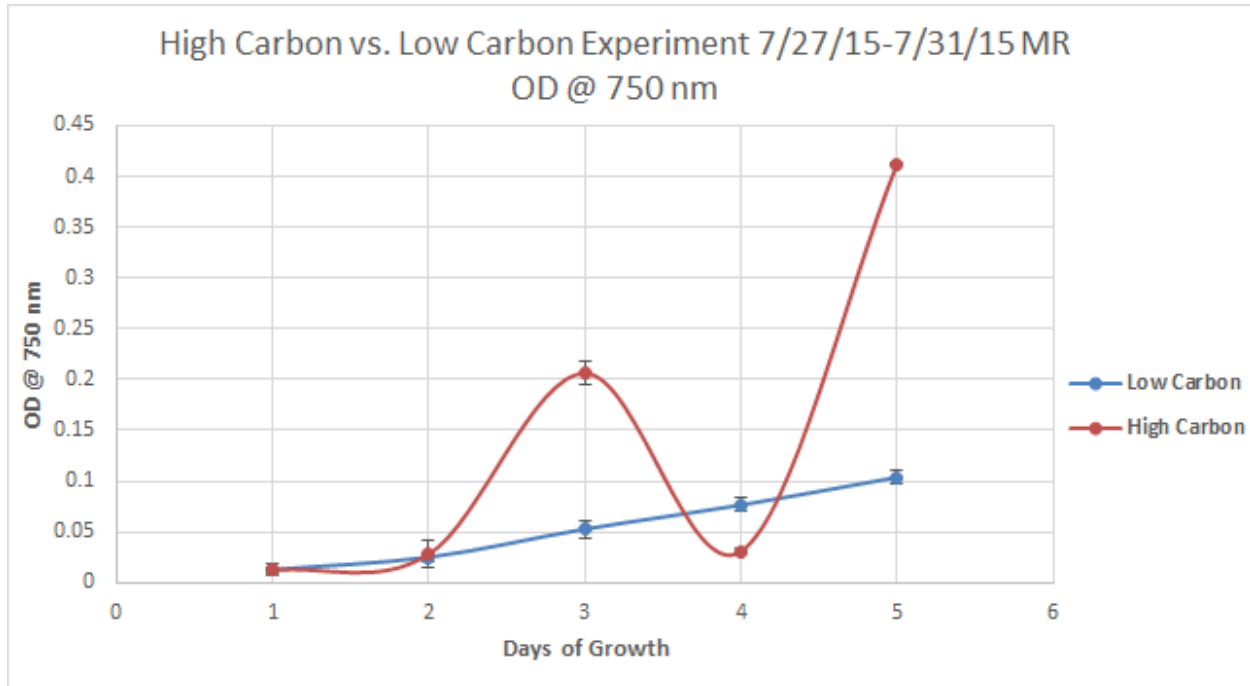


Figure 1. The graph above depicts the line of best fit and the standard error for the averages of the Optical Density for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

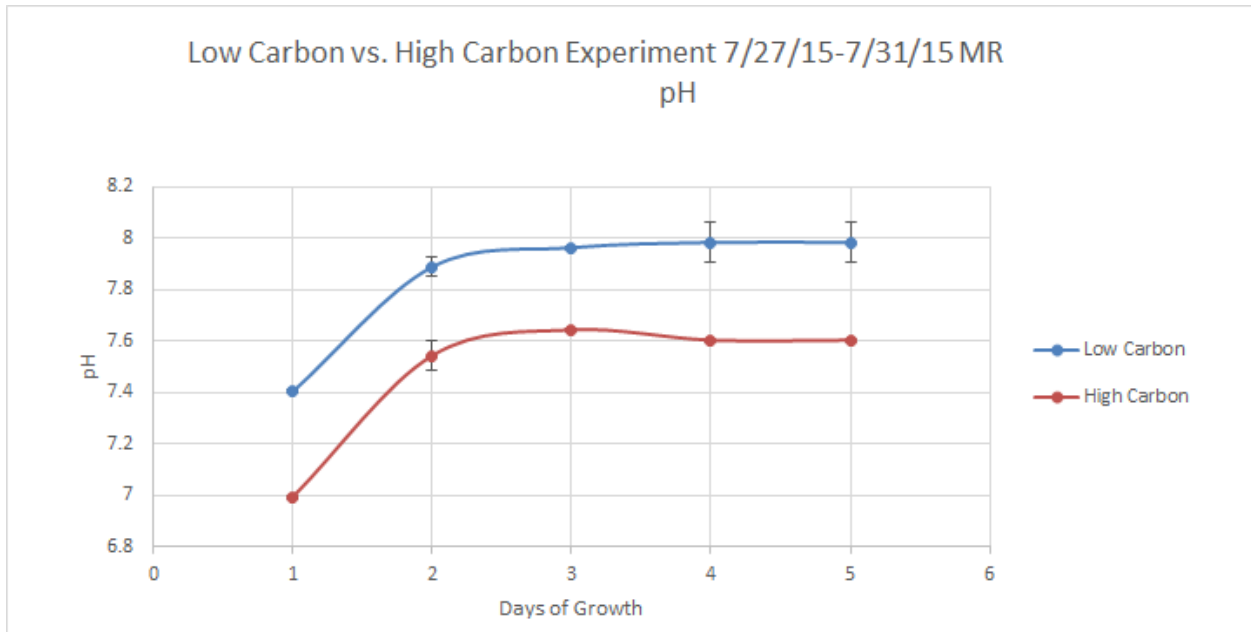


Figure 2. The graph above depicts the line of best fit and the standard error for the averages of the pH for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

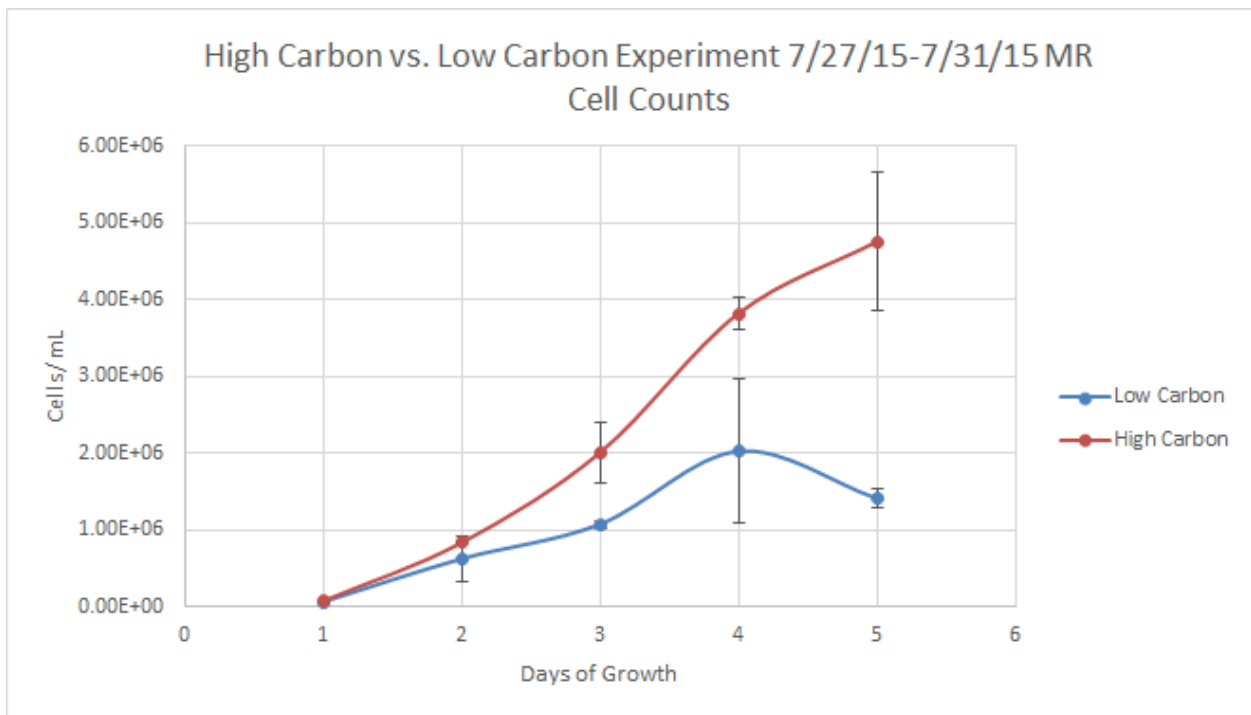


Figure 3. The graph above depicts the line of best fit and the standard error for the averages of the cell counts for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

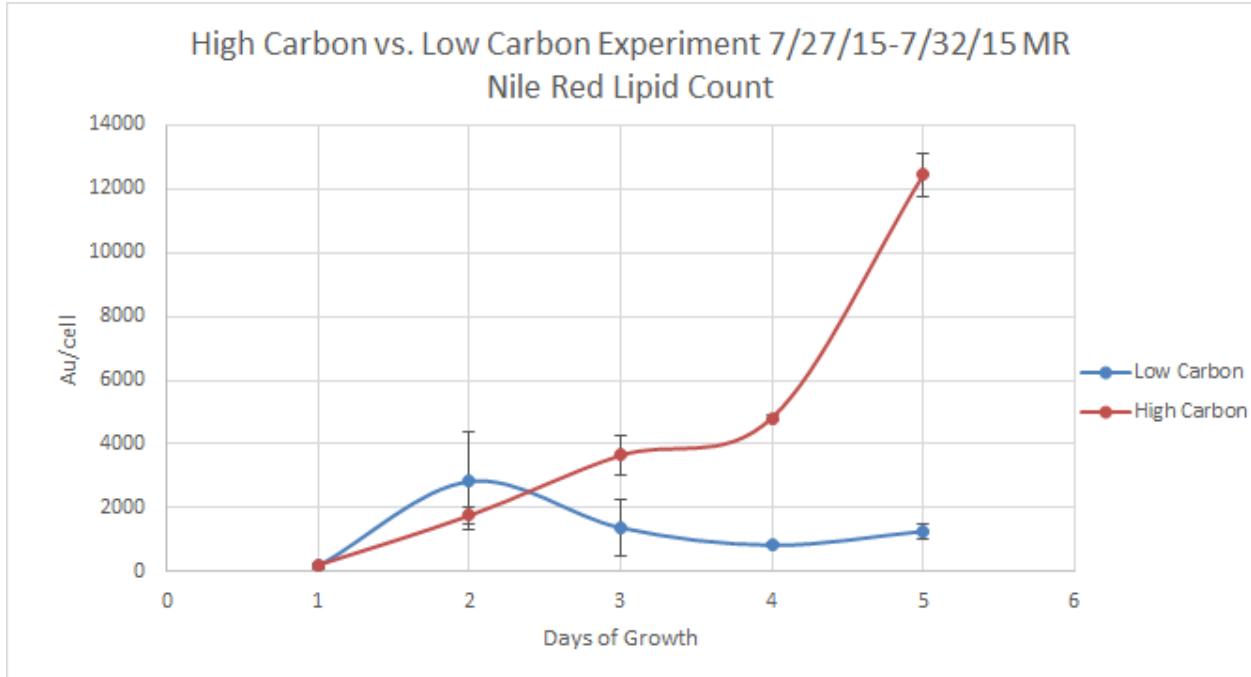


Figure 4. The graph above depicts the line of best fit and the standard error for the averages of the lipids for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

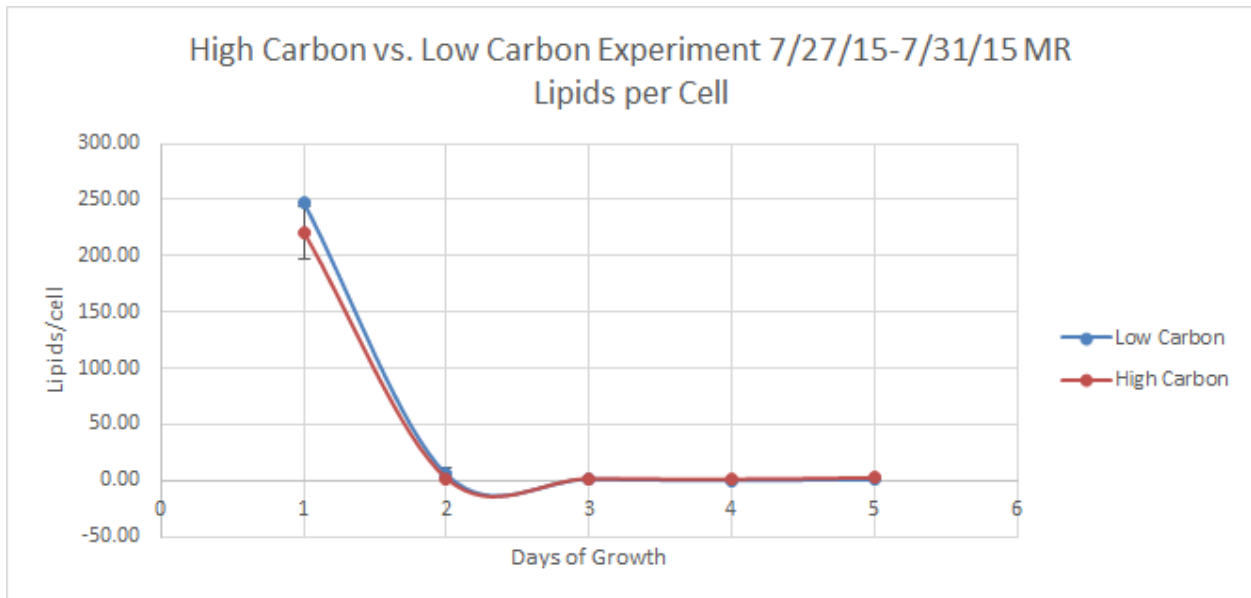


Figure 5. The graph above depicts the line of best fit and the standard error for the averages of the lipids/cell for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

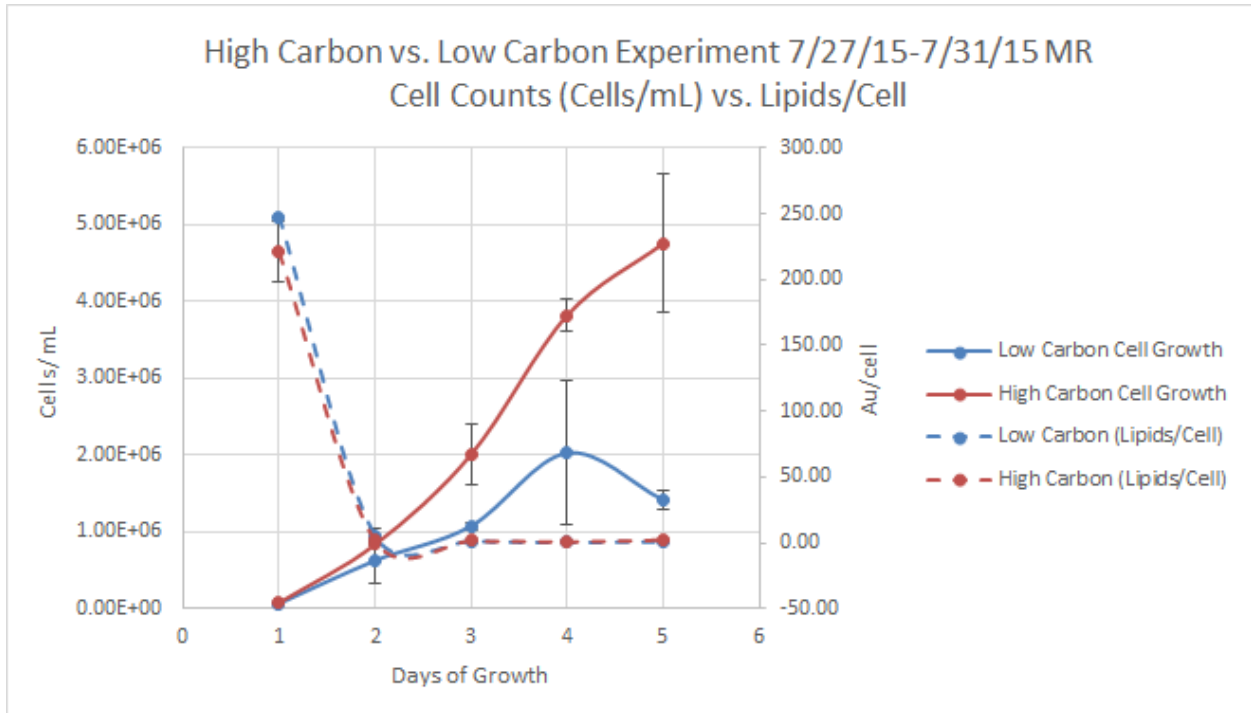


Figure 6. The graph above depicts the line of best fit and the standard error for the averages of the lipids/cell compared to the line of best fit and the standard error for the averages of the cell counts for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

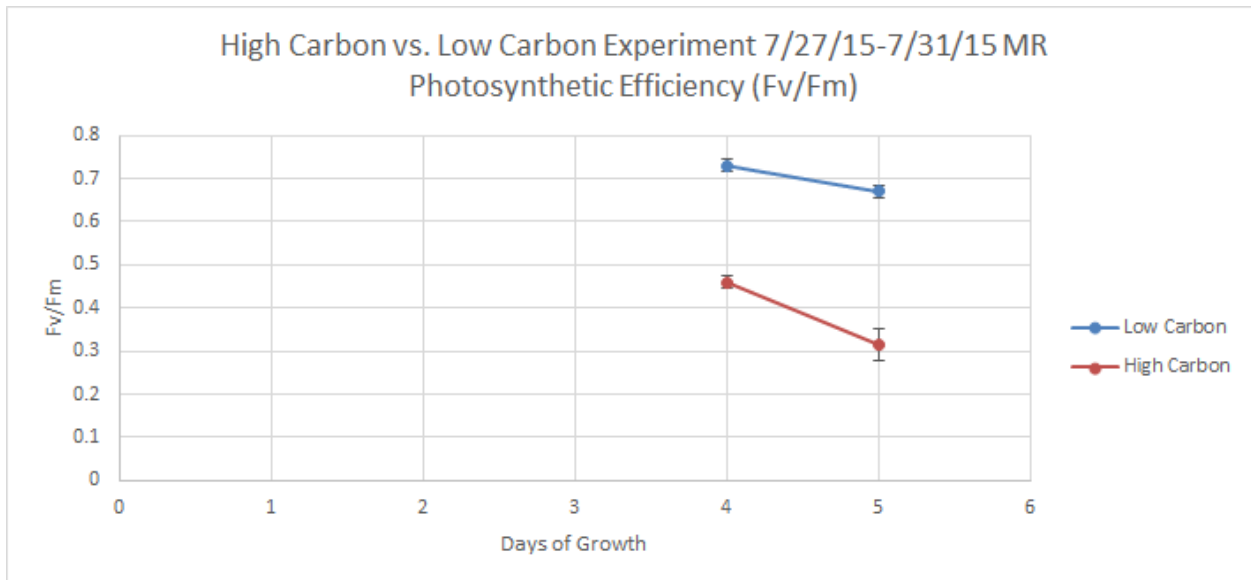


Figure 7. The graph above depicts the line of best fit for the photosynthetic efficiency for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for days 4 and 5 of the photoautotrophic experiment. The “healthy” range for photosynthetic efficiency is 5-7.5 (Fv/Fm)

Data and Charts: Mixotrophic Experiment

Table 8. The table below depicts the Optical Density for the two replicates of each CO₂ concentration of *C. reinhardtii* at 750 nm, and the average, standard deviation, and standard error for the duration of the mixotrophic experiment.

	Day	1	2	3	4	5	6	9
Carbon Type	Reactor	8/9/15	8/10/15	8/11/15	8/12/15	8/13/15	8/14/15	8/15/15
Low Carbon	1		0.001	0.005	0.036	0.141	0.643	0.817
	2		0.001	0.013	0.043	0.183	0.647	0.737
	Mean		0.001	0.009	0.039	0.162	0.645	0.777
	Std. Dev		0.000	0.005	0.005	0.030	0.002	0.056
High Carbon	3		0.001	0.011	0.091	0.266	0.613	0.803
	4		0.001	0.009	0.129	0.522	0.668	0.889
	Mean		0.001	0.010	0.110	0.394	0.641	0.846
	Std. Dev		0.000	0.001	0.027	0.181	0.039	0.061

Table 9. The table below depicts the pH for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the mixotrophic experiment.

	Day	1	2	3	4	5	6	9
Carbon Type	Reactor	8/9/15	8/10/15	8/11/15	8/12/15	8/13/15	8/14/15	8/17/15
Low Carbon	1		7.34	7.59	7.71	7.94	8.47	8.66
	2		7.16	7.45	7.59	7.89	8.41	8.56
	Mean		7.25	7.52	7.65	7.915	8.44	8.61
	Std. Dev		0.1272792 206	0.09899494 937	0.084852813 74	0.035355339 06	0.042426406 87	0.070710678 12
High Carbon	3		7.02	7.33	7.56	7.93	8.15	8.04
	4		7.04	7.34	7.58	8.02	8.17	8.01
	Mean		7.03	7.335	7.57	7.975	8.16	8.025
	Std. Dev		0.0141421 3562	0.00707106 7812	0.014142135 62	0.063639610 31	0.014142135 62	0.021213203 44

Table 10. The table below depicts the cell count (cells/mL) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the mixotrophic experiment.

	Day	1	2	3	4	5	6	9
	Trial	8/9/15	8/10/15	8/11/15	8/12/15	8/13/15	8/14/15	8/17/15
Low Carbon	1		1.77E+04	1.39E+05	1.11E+06	3.30E+06	7.26E+06	9.35E+06
	2		1.65E+04	2.60E+05	1.56E+06	4.43E+06	5.53E+06	6.19E+06
	Mean		1.71E+04	1.99E+05	1.33E+06	3.86E+06	6.39E+06	7.77E+06
	Std. Dev		8.31E+02	8.56E+04	3.25E+05	7.97E+05	1.23E+06	2.24E+06
High Carbon	3		1.10E+04	1.95E+05	2.04E+06	7.99E+06	1.20E+07	1.50E+07
	4		1.24E+04	1.75E+05	1.95E+06	7.21E+06	1.04E+07	1.82E+07
	Mean		1.17E+04	1.85E+05	2.00E+06	7.60E+06	1.12E+07	1.66E+07
	Std. Dev		9.72E+02	1.46E+04	6.03E+04	5.54E+05	1.13E+06	2.26E+06

Table 11. The table below depicts the lipid measure (Au) for the two replicates of each CO₂ concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the mixotrophic experiment.

	Day	1	2	3	5	6	9
Carbon Type	Reactor	8/9/15	8/10/15	8/11/15	8/13/15	8/14/15	8/17/15
Low Carbon	1		99	1751	1134	4616	5903
	2		22	3909	3638	11483	7768
	Mean		61	2830	2386	8050	6835
	Std. Dev		54	1526	1771	4856	1319
High Carbon	3		33	1960	5928	6032	6452
	4		141	1569	8513	8596	7160
	Mean		87	1765	7221	7314	6806
	Std. Dev		77	276	1828	1813	500

Table 12. The table below depicts the lipids per cell (Au/cell) for the two replicates of each CO2 concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the photoautotrophic experiment.

	Day	1	2	3	5	6	9
Carbon Type	Reactor	8/9/15	8/10/15	8/11/15	8/13/15	8/14/15	8/17/15
Low Carbon	1		5.620	12.611	0.344	0.636	0.631
	2		1.354	15.043	0.822	2.077	1.255
	Mean		3.487	13.827	0.583	1.357	0.943
	Std. Dev		3.017	1.720	0.338	1.019	0.441
High Carbon	3		3.00	10.04	0.74	0.50	0.43
	4		8.99	8.99	1.18	0.82	0.39
	Mean		5.99	9.51	0.96	0.66	0.41
	Std. Dev		4.23	0.74	0.31	0.23	0.03

Table 13. The table below depicts the photosynthetic efficiency (PAM) for the two replicates of each CO2 concentration of *C. reinhardtii* and the average, standard deviation, and standard error for the duration of the mixotrophic experiment.

	Day	1	2	3	4	5	6	9
Carbon Type	Reactor	8/9/15	8/10/15	8/11/15	8/12/15	8/13/15	8/14/15	8/17/15
Low Carbon	1				0.65	0.65	0.65	0.61
	2			0.6	0.65	0.62	0.57	0.47
	Mean			0.6	0.65	0.635	0.61	0.54
	Std. Dev			0	0	0.0212132034	0.0565685424	0.0989949493
High	3			0.41	0.68	0.45	0.53	0.5

Carbon								
	4			0.51	0.7	0.62	0.62	0.56
	Mean			0.46	0.69	0.535	0.575	0.53
	Std. Dev			0.0707106	0.0141421		0.0636396103	0.0424264068
				7812	3562	0.1202081528	1	7

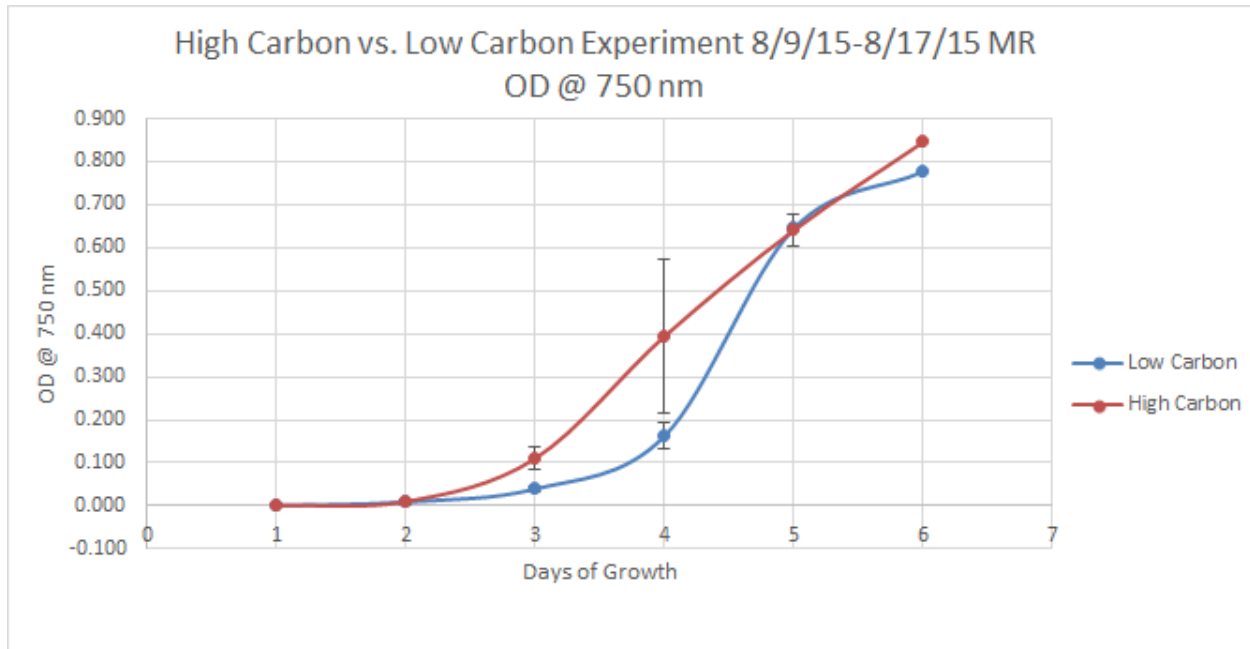


Figure 8. The graph above depicts the line of best fit and the standard error for the averages of the Optical Density for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the mixotrophic experiment.

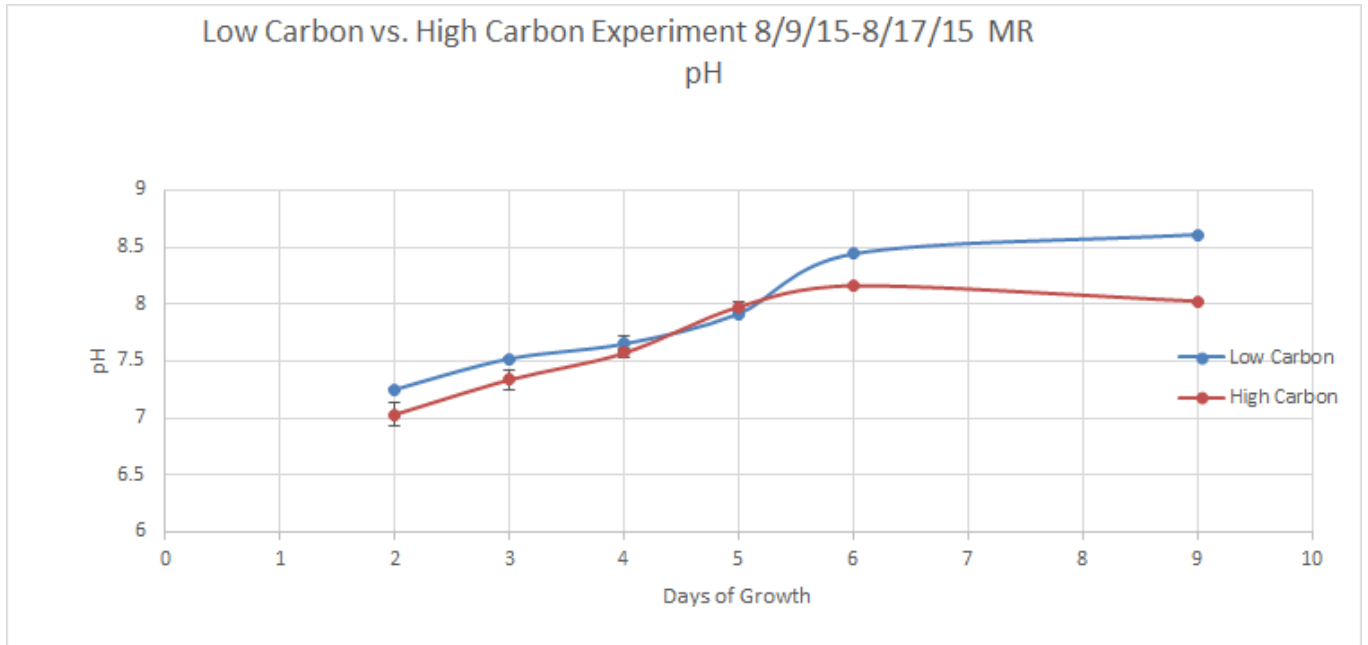


Figure 9. The graph above depicts the line of best fit and the standard error for the averages of the pH for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the mixotrophic experiment.

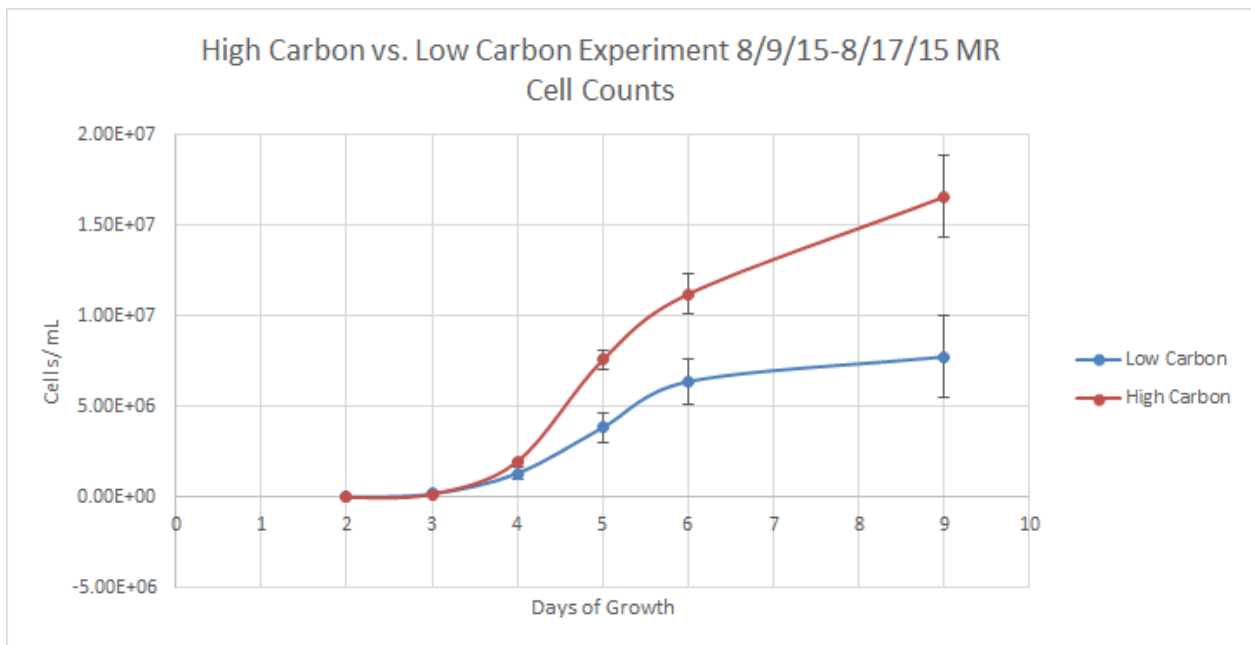


Figure 10. The graph above depicts the line of best fit and the standard error for the averages of the cell counts for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the mixotrophic experiment.

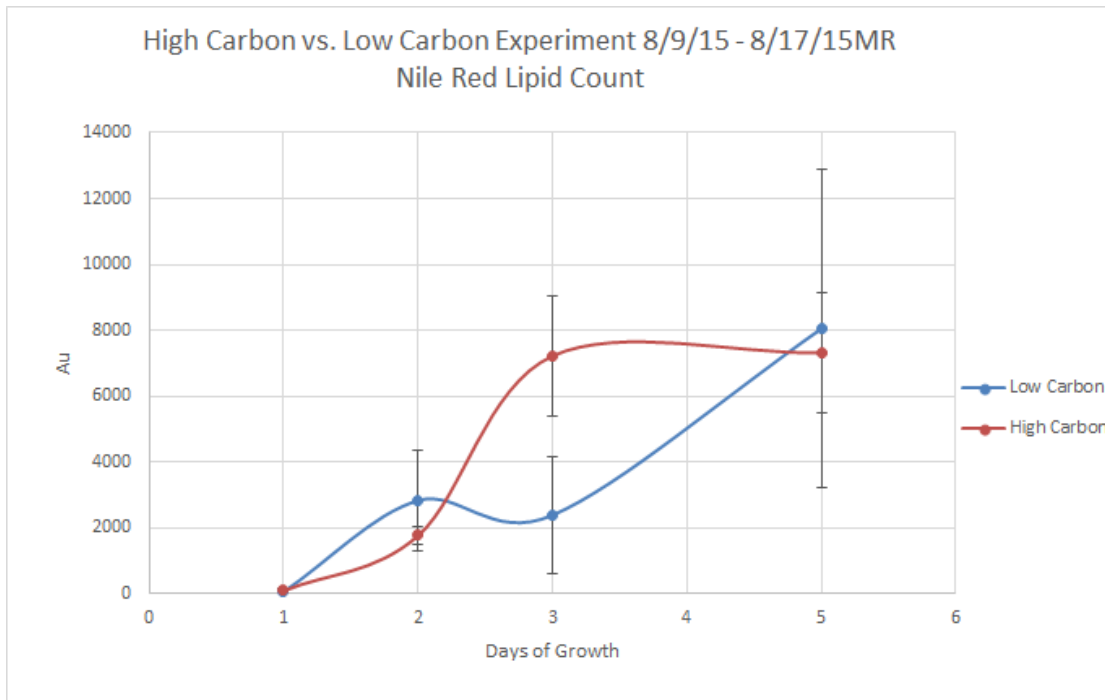


Figure 11. The graph above depicts the line of best fit and the standard error for the averages of the lipids for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the mixotrophic experiment.

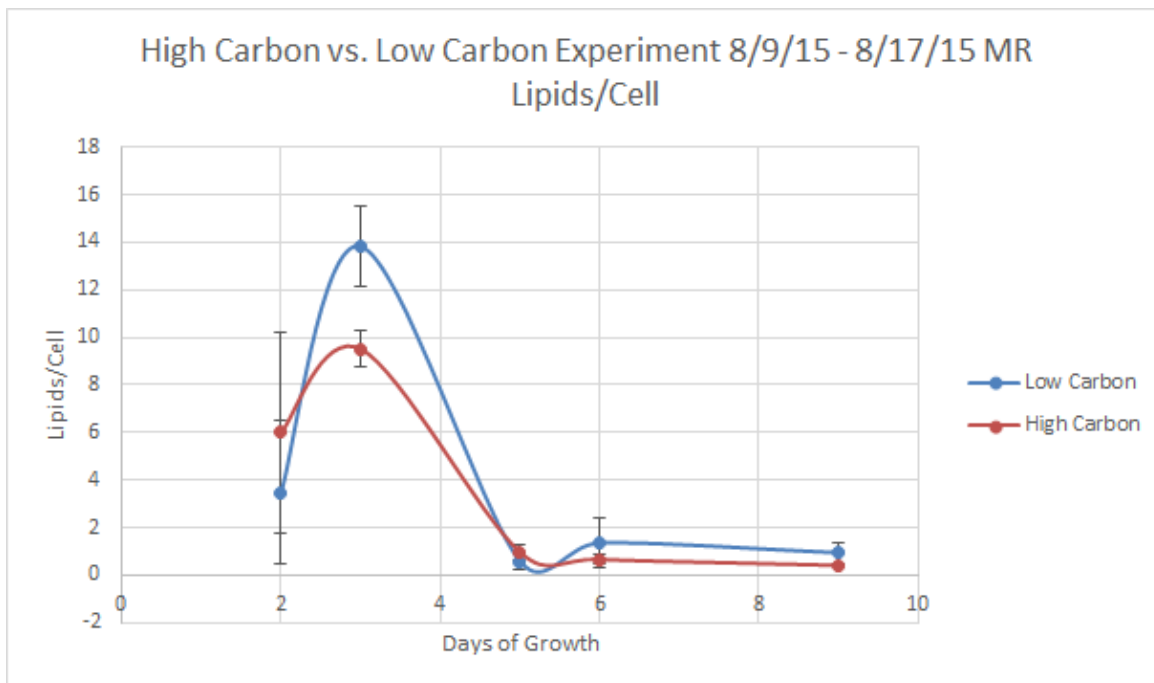


Figure 12. The graph above depicts the line of best fit and the standard error for the averages of the lipids/cell for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

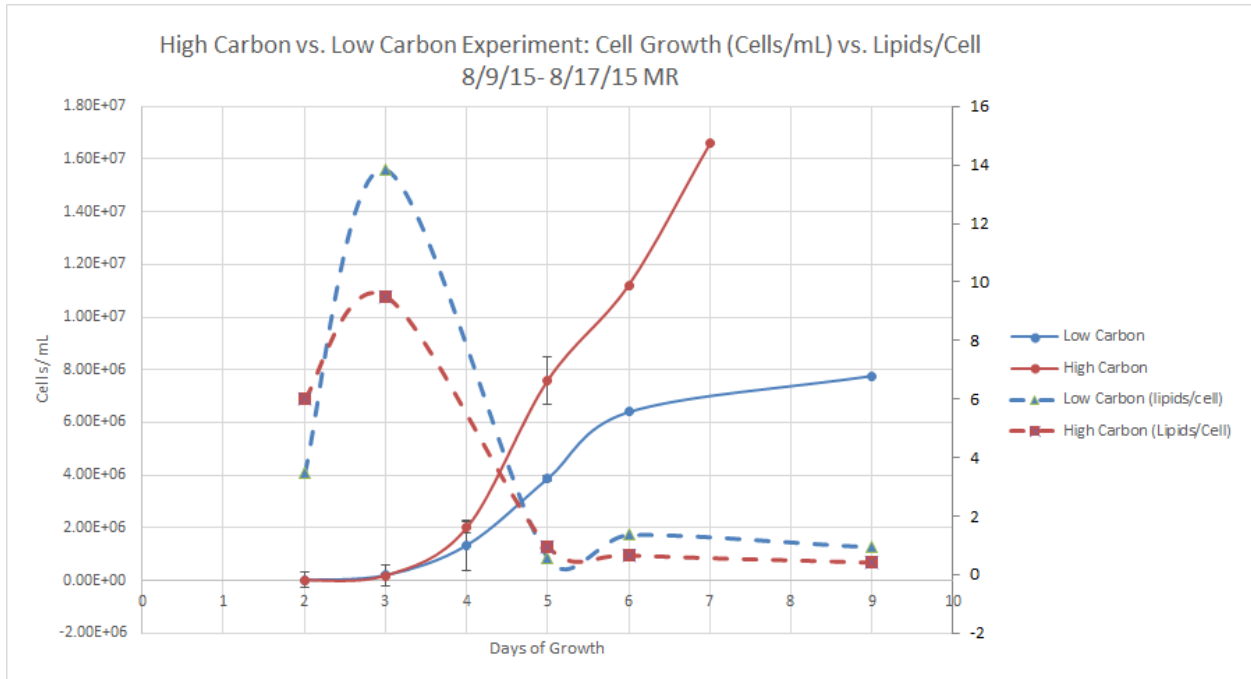


Figure 13. The graph above depicts the line of best fit and the standard error for the averages of the lipids/cell compared to the line of best fit and the standard error for the averages of the cell counts for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for the duration of the photoautotrophic experiment.

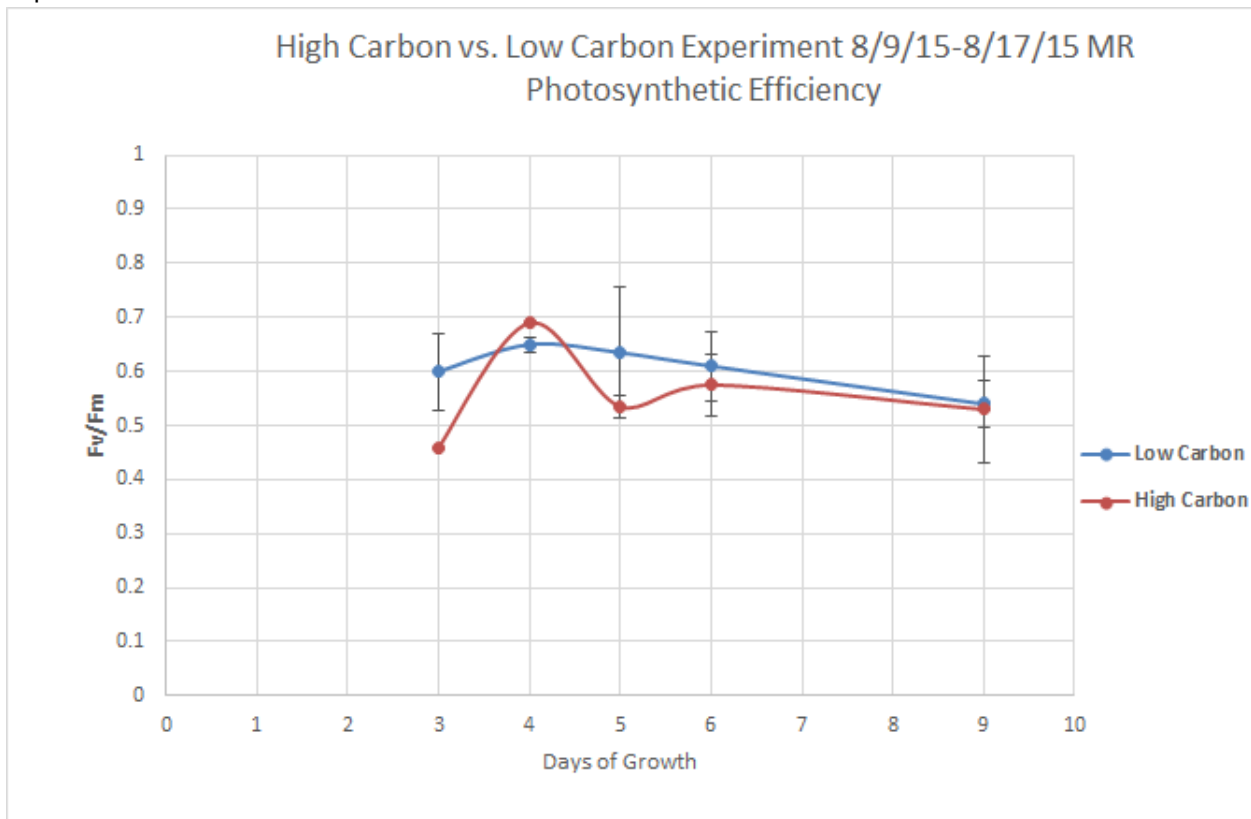


Figure 14. The graph above depicts the line of best fit for the photosynthetic efficiency for the 2 replicates of each CO₂ concentration of *C. reinhardtii* for days 4 and 5 of the photoautotrophic experiment. The “healthy” range for photosynthetic efficiency is 5-7.5 (Fv/Fm)

Conclusions:

When *Chlamydomonas* was grown photoautotrophically, both light and CO₂ were limiting factors in its growth, as evidenced by the fact that it showed less cell growth in low-light and low-CO₂ environments. CO₂ availability also seemed to be more of a limiting factor than light availability when grown photoautotrophically, as the low-CO₂ reactors had fewer cells than low-light reactors. However, when *Chlamydomonas* was grown mixotrophically, with an supplemented organic carbon source of acetate, high light conditions instead overloaded photosystem II and overstressed the cells, leading to lower cell growth. However, high CO₂ conditions did not seem to have the same negative effect as high light conditions, as the cells grew better with medium light and high CO₂ than they did with medium light and low CO₂.