MODELING GROWTH OF CHLORELLA SOROKINIANA BY CHANGING CO_2 NUTRIENT CONCENTRATION

by

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As members of the Master's Committee, we certify that we have read the thesis prepared by Armeen Robert Pajouyan titled Modeling Growth of *Chlorella sorokiniana* by Changing CO_2 Nutrient Concentration and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Abstract

Algae research has gained significant attention due to its potential in carbon capture and biofuel production. Understanding the role of CO_2 in algae growth is critical to optimize these processes. This study investigates the impact of CO_2 on the growth of *Chlorella sorokiniana* under various experimental conditions. All experiments were conducted at a temperature of 35°C, a light intensity of 234 μmol m^{-2} s^{-1}, and a 12-hour light window. For the main set of experiments, CO_2 flow percentage was changed from 5% to only air flow to see the effect on growth rate. The results showed that excess CO_2 doesn't significantly affect growth, however when it is no longer in excess as concentration drops the growth rate decreases. A one-parameter model was applied to the data, providing values that accurately represent the data. In another experiment, the effects of CO_2 flow being on always was compared with CO_2 flow only being on during the light phase. The results supported the hypothesis that CO_2 flow at night has a negligible impact on growth. In the final experiment, the flowrate of air was lowered while CO_2 flow remained the same across both runs. This would be so that one run would have a total flow of 2 LPM and the other a total flow of 1 LMP. This is because although air flow is needed for oxygen content, excess can strip the CO_2 from the reactor reducing efficiency. The results confirmed this as the 1 LPM experiment had a lower pH, meaning that there was more dissolved CO_2 in solution.

1. Introduction:

With all the world's social and economic issues, global warming is becoming an increasingly important issue that must be addressed. Global warming is attributed mainly due to greenhouse gas emissions, the most common of those gases being carbon dioxide (CO_2). Greenhouse gases like CO_2 trap heat warming up the planet (1). Many may assume global warming is only about the environment, however it has been shown to affect other things such as population health and the economy (2)(3). These effects of global warming have resulted in a large push to find solutions that can help combat the problem.

The most well-known and currently practiced way in combating this issue is switching to non-CO_2 emitting energy sources such as solar, wind, hydro, etc. Currently, only 14% of the world's energy comes from renewable sources although it is projected by 2040 that the number will rise to 50% (4). The petrol industry will never fade away fully as other things such as plastics and medicine are petrol based. Thus, there needs to be other solutions that can directly combat emissions coming from petrol such as stack gas.

The University of Arizona, Tucson Electric Power, Lawrence Livermore NL, Sandia NL, and Southwest Technologies are working on a collaborative project that involves the usage of algae to capture CO_2. Algae are microorganisms with many different species found in both fresh and salt water that can convert CO_2 into biomass. When CO_2 is dissolved in water it turns into bicarbonate which is what the algae uses for energy production (5). The algae can be used for various things ranging from animal food to its usage in biofuels. This means this form of carbon capture not only reduces emissions but also provides a source of renewable energy (6). The project involves the usage of a porous material that absorbs CO_2 from stack gas which can then be placed in water where the CO_2 will leach off. With the CO_2 dissolved in the water it turns into bicarbonate where the algae can then convert it into biomass. The UA is specifically tasked with running growth experiments and fitting them into a model.

Previous work done at UA explored the effects of light and temperature on algae growth (7). In this work, we explore the effect of varying CO_2 concentrations on algae growth and apply a single parameter model to the data. The idea behind the experiments is that at higher concentrations of CO_2 there is higher growth until it is no longer a limiting reactant where additional CO_2 will have little effect on the growth rate. The research was done using a Bio115

reactor which allowed air and CO_2 to be mixed and injected into the reactor at different ratios. Algae needs oxygen to survive which is why air is mixed with CO_2 (8). Dissolved oxygen, pH, temperature, and cell concentration calculated from optical density measurements were recorded during the experiments. The algae species selected was *Chlorella sorokiniana* because it has been shown to have high light use efficiency and the media of choice was Pecos media (9).

A couple separate experiments were done on top of the main tasks. The first experiment was to see the effect of having CO_2 flow always on versus only having CO_2 flow be on during the light phase. It was hypothesized this would not greatly affect the data as during the dark phase there is not any light for the algae to convert the CO_2 into biomass. The second experiment was to explore the effects of lowering air flow while maintaining the same CO_2 flow. The reason behind this is air is needed but the exact amount is unknown. It is hypothesized excess air will lower the absorption of CO_2 in the system.

2. Literature Review:

2.1 Algae growth

Microalgae are a group of microorganisms like plants that use photosynthesis as a means of growth. Due to the resistance microalgae has to harsh conditions and its fast growth rate, there has been a large interest for its usages in industry. Different species can be found in environments ranging from fresh and salt water to land ecosystems (10). During the day, the algae uses both dissolved CO_2 and carbonate as its fuel source. Dissolved CO_2 is what chemically turns into carbonate and carbonic acid in the water. Because of this, CO_2 can greatly affect the water pH depending on the buffering capacity of the solution (11). During the night, the algae goes into respiration mode and loses some mass. This is because of a few reasons including usages of carbohydrate stores, some cell death, and sensitivity to light. One study found cell diameter reached its maximum around noon and its minimum at night (12).

$$CO_2\ (aq) + H_2O \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$

Starting from an inoculum, algae follow a growth pattern where there is no growth (lag phase), exponential growth, declining rate growth, stationary phase, and eventually death as seen in Figure 2.1. Usually there is a lag phase because the algae need time to adjust to the new conditions. If there is fresh media and similar conditions to its previous setup, the length of the lag phase is reduced. Once the algae start growing it will grow exponentially until there are limiting factors such as light, CO_2, or nutrients. This will cause the rate to slow down until there is no longer any growth. If eventually the nutrients deplete or other factors the algae population will crash (13).

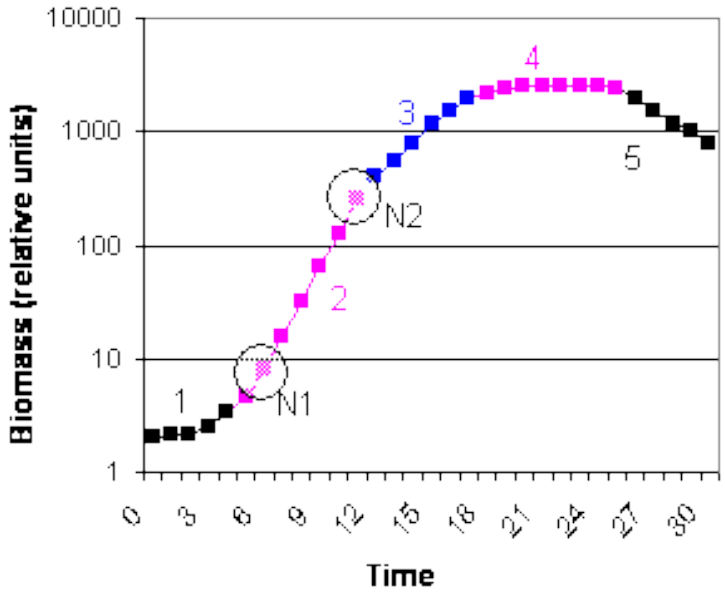

Figure 2.1: General graph of algae growth and its separate growth phases. Number 1 being lag phase, 2 exponential growth, 3 declining rate growth, 4 stationary phase, and 5 death (13).

2.2 Temperature and Light Intensity Growth Models

There are many difficulties when it comes to making and fitting an accurate model of algae growth (14). There are many variables that affect growth, making it difficult to create a model that accounts for everything. Even algae species variance can play a significant role. These variables include dissolved CO_2, water hardness, light intensity, temperature, nutrients, and more. Some variables also change others when they increase or decrease. For example, higher light intensity often means higher temperature. Higher levels of CO_2 means more food for

the algae but can also lower the pH to where the water becomes toxic if it increased too much (15).

To address this, certain models can be used in specific cases where some variables are in great excess, so they are not growth limiting. For example, if the growth of algae is limited by nutrients, a nutrient based model may be appropriate. Complications arise when multiple factors can be limiting growth, which would require a more complex model. The algae can even be limited by different things in different stages of its growth. This further shows the complexity that can be put into designing a model.

When microalgae are under conditions where nutrients are in abundance, light and temperature play a critical role in growth. Insufficient light, or non-ideal temperatures can severely limit growth (16). The algae require a specific amount of light for maximum growth. If light intensity is too low, growth will be light limited and the algae will fail to grow well. If light intensity is too high, there may be photooxidation decreasing growth rate (17).

Experiments also using a BioFlo115 reactor were conducted to observe the effect of light intensity change. Experiments were run with 12 hours of light, 2 LPM, and with enough CO_2 to keep the pH at 8 controlled by a P&I controller. BG-11 was used as media. Three experiments were conducted with light intensities of 108, 134, and 234 μmol m^{-2} sec^{-1} at 25℃. The plot of this can be seen in Figure 2.2. The results were that 108 μmol m^{-2} sec^{-1} saw a significant decrease in growth while 134 and 234 μmol m^{-2} sec^{-1} light intensities did not. It was noted that there was a possibility of photooxidation for the 234 μmol m^{-2} sec^{-1} run as clumping and settling of algae in excess was observed. (7).

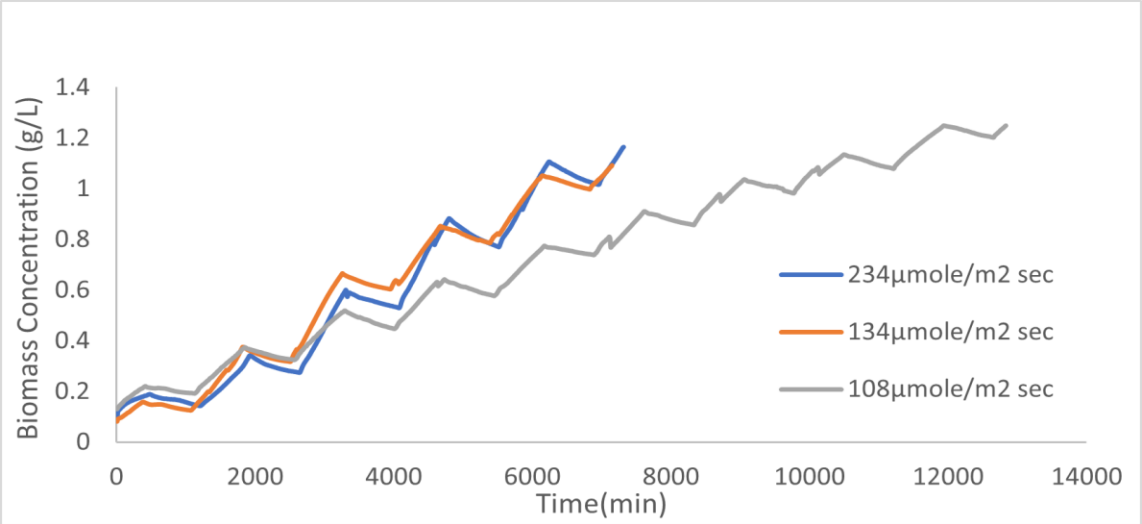

Figure 2.2: Effect of light intensity on the growth of Chlorella sorokiniana grown at 25 ℃ and a flowrate of 2 LPM while maintaining a pH of 8 (7).

The next set of experiments done involved variance of temperature ranging from 20-40℃ at 134 µmol m^{-2} sec^{-1} as seen in Figure 2.3. It was found that the best growth rates were at 25 and 32℃. Interestingly, the 38℃ run isn't far below that of 25 and 32℃. But only 2℃ more at 40℃ there is a tremendous drop in growth rate. This shows the extreme sensitivity of the algae (7).

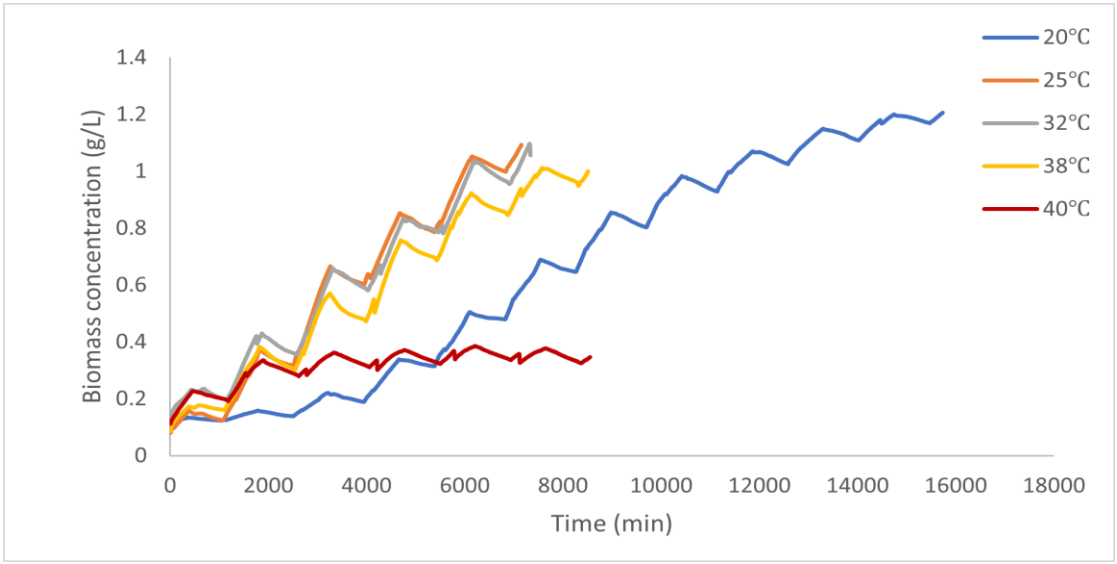

Figure 2.3: Effect of temperature on the growth pattern of Chlorella sorokiniana grown at 134 µmol m^{-2} sec^{-1} and 2 LPM while maintaining a pH of 8 (7).

Another study done explored the effects of both temperature and light intensity on species *Asterarcys quadricellulare* and *Chlorella sorokiniana (18).* The researchers used Bolds Basal medium for these experiments. Under ambient CO_2 and at the optimum temperature of 37°C, light intensity was changed from 50-300 Ein m^{-2} s^{-1} for the first batch of experiments. It was found that at 200 μmol photons m^{-2} s^{-1}, growth rate was maximum as seen in Figure 2.4. As the light intensity increased past that, growth rate decreased. *C. sorokiniana* grew better at a lower light intensity than *A. quadricellulare* while the opposite was true for the highest light intensity.

The same experiments were done but instead of varying light intensity temperature was changed from 23.5-43°C at the most optimal light intensity of 200 μmol photons m^{-2} s^{-1}. The variation in temperature had a much greater impact on growth. It was found the optimal growth for both species was around 37°C as seen in Figure 2.5. The *C. sorokiniana* was able to have some growth at the lowest temperature of 23.5°C and no growth at 43°C. The opposite was true for *A. quadricellulare* which didn't grow at 23.5°C but grew at 43°C (18).

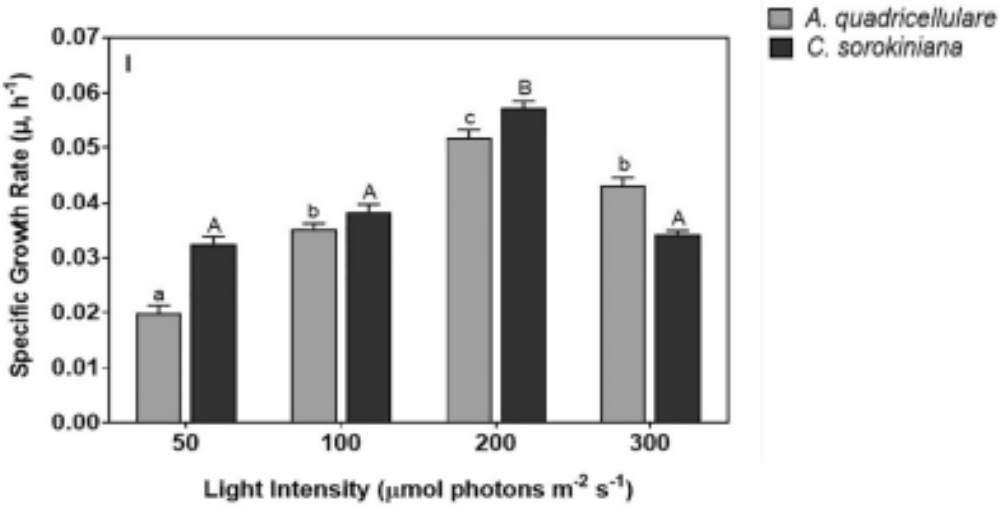

Figure 2.4: Growth rate of A. quadricellulare and C. sorokiniana as a function of light intensity. Experiments were done at ambient CO_2 and a temperature of 37°C (18).

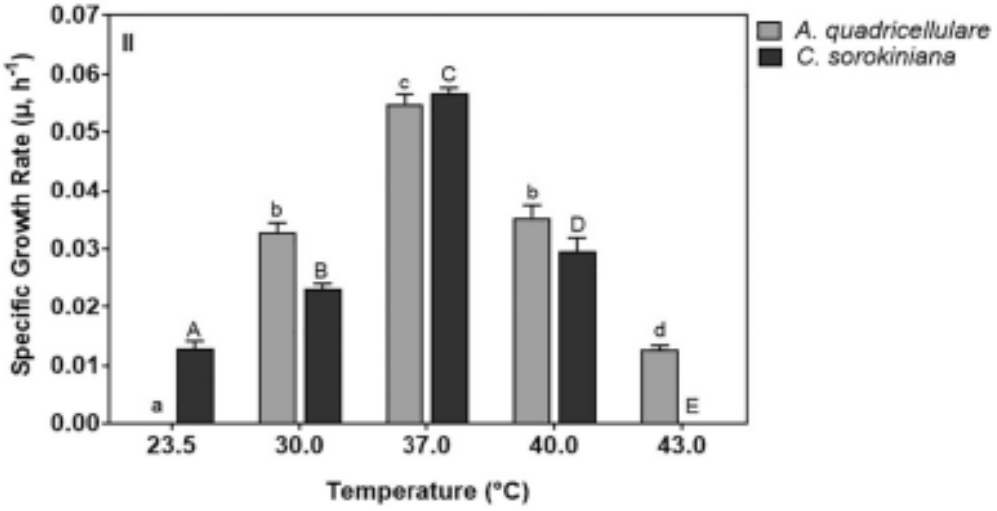

Figure 2.5: Growth rate of A. quadricellulare and C. sorokiniana as a function of temperature. Experiments were done at ambient CO_2 and a light intensity of 200 μmol photons m^{-2} s^{-1} (18).

Comparing and contrasting the results from both papers some takeaway points can be made. While both papers used *Chlorella sorokiniana,* there was some variation in optimal light intensities and temperatures. This is due to the different constraints used when running these experiments. Optimum temperatures and light intensities change when other constraints like the media or total daylight change. Another important take away is the differences in behavior from different species of algae. This provides insight that different species can be used in different industrial settings where some conditions may favor some species over another. Once experimental data is concluded, a model can be applied.

There are many ways a light-based model has been attempted. A review highlighted three different groups of light-based models (19). Group I focuses on light-limited conditions for individual cells, typically used in lab settings. Group II accounts for light attenuation in dense cultures, typically used for industry. And Group III incorporates both light limitation and photoinhibition, typically used for industry in outdoor settings. Each group is better used in certain conditions.

Group I models describe growth under light-limited conditions assuming algae cells receive equal incident light with minimal self-shading. These models are simple using two or three parameters and are widely applied in lab-scale studies with low to moderate algae densities.

For example, the Tamiya model (20) relates growth rate (μ) to light intensity through parameters like the maximum specific growth rate (μ_{max}) and the light saturation constant (K_I). This model has been successfully applied to the growth of *Euglena gracilis (21)*. The model parameters were μ_{max}=0.06 and K_I=178 μmol photons m^{-2} s^{-1}.

Tamiya Model

$$\mu = \mu_{\max} \frac{I}{K_I + I}$$

(1)

Group II models account for light attenuation caused by dense algae cultures where light distribution is uneven due to high cell concentrations in the media. Because of this, these models replace incident light with average or absorbed light intensity. A modified Tamiya model called the Grima model introduces an exponent (n) to account for light heterogeneity in dense algae cultures. The model also adds a photon flux density term (I_k), a light absorption coefficient (K_a), concentration of algae denoted (X), and a productivity constant (p) (22). While effective for optimizing photobioreactors it is noted that average light intensity might oversimplify light distribution across cells (23).

Grima Model

$$\mu = \mu_{\max} \frac{{I_{av}}^n}{I_k^n + {I_{av}}^n}$$
$$I_{av} = \frac{I}{K_a\ p\ X}\left[1 - e(-K_a\ p\ X)\right]$$

(2)

Group III models incorporate both light limitation and photoinhibition which occurs at high light intensities common in outdoor systems. The Steele model (24) combines light-limitation and photoinhibition using an exponential term ($\exp(1-I/I_{opt})$) where (I) is general light intensity and (I_{max}) is the light intensity that produces a maximum photosynthesis rate. This helps make the model useful for predicting growth in fluctuating outdoor conditions. For example, it

has been applied to describe photosynthesis rates under high midday irradiance where photoinhibition impacts growth.

Steele Model

$$\mu = \mu_{\max} \frac{I}{I_{opt}} e\left(1 - \frac{I}{I_{opt}}\right)$$

(3)

Light-based models provide valuable tools for predicting algae growth under various conditions. The three groups of models organized provide great insight into how models can change greatly based on experimental/environmental conditions (19). Group I models being great for settings with low algae density and Group II being great for dense cultures. Group III models incorporating both light limitation and photoinhibition making them for instances with variance in light.

2.3 Nutrient Based Models

When in a light saturated environment and at ideal temperatures, the growth of the algae is dependent on nutrients nitrogen (N), phosphorus, (P), and carbon (C) (19). This is taken advantage of in industrial applications such as wastewater treatment where nitrogen and phosphorus need to be removed from the water. Normally these processes are expensive, which is why there is great interest in research for algae-based removal processes (25). Research has shown that when grown under similar conditions to that of wastewater, lipid production in the algae is quite high (26,27). Lipids from the algae have the potential to be harvested and used in biofuel (28). The species of micro algae chosen can have a big impact on results. *Chlorella sorokiniana* has demonstrated to be good at nutrient take up, which is related to growth (29). It has also been reported to have good lipid accumulation (30).

Some research has investigated the effects of varying both nitrogen and phosphorus on *Chlorella sorokiniana* (31). BG-11 media was used while maintaining a 16-hour light window. Light intensity was maintained at 80 μmol m^{-2} sec^{-1} and the shakers had a rpm of 80. Algae cells were depleted of nutrients so they could be used in the experiments. Different experiments were done where sodium nitrate and potassium phosphate are added at different levels changing the

nitrogen and phosphorus levels. Five experiments were done using high N and high P, medium N and high P, low N and high P, high N and medium P, and finally high N and low P. Figure 2.6 below show the results to the high phosphorus runs while Figure 2.7 shows the results for the high Nitrogen runs.

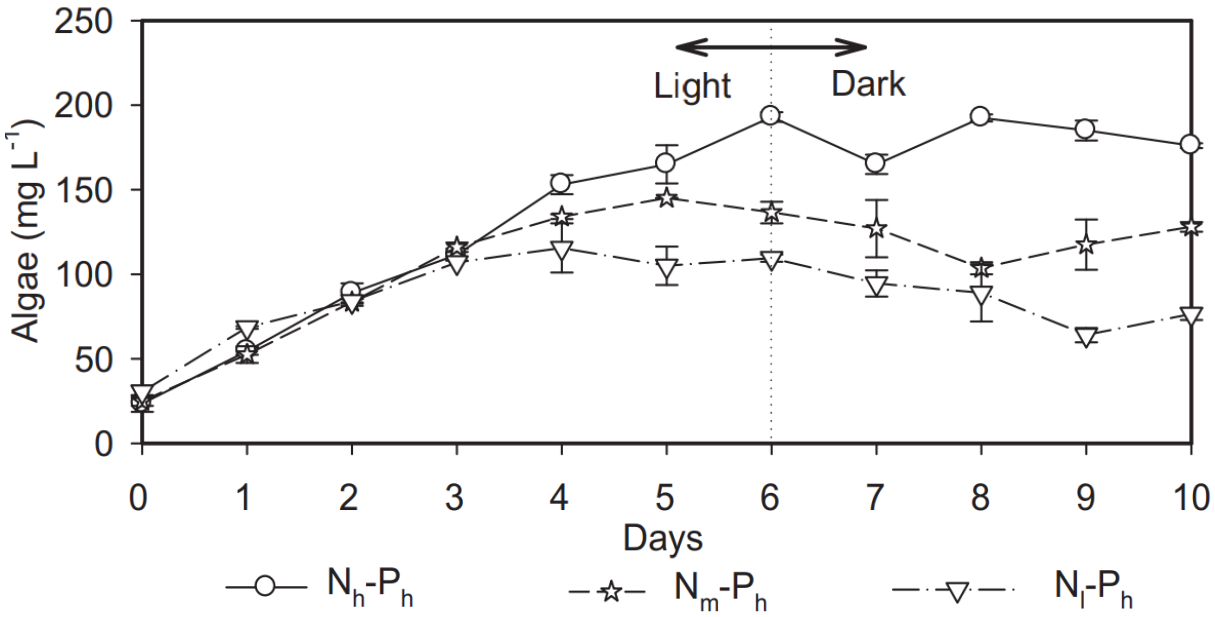

Figure 2.6: Growth of Algae when phosphorus levels are high, and nitrogen levels are varied. A light intensity of at 80 µmol m^{-2} sec^{-1} is maintained and temperature is ambient (31).

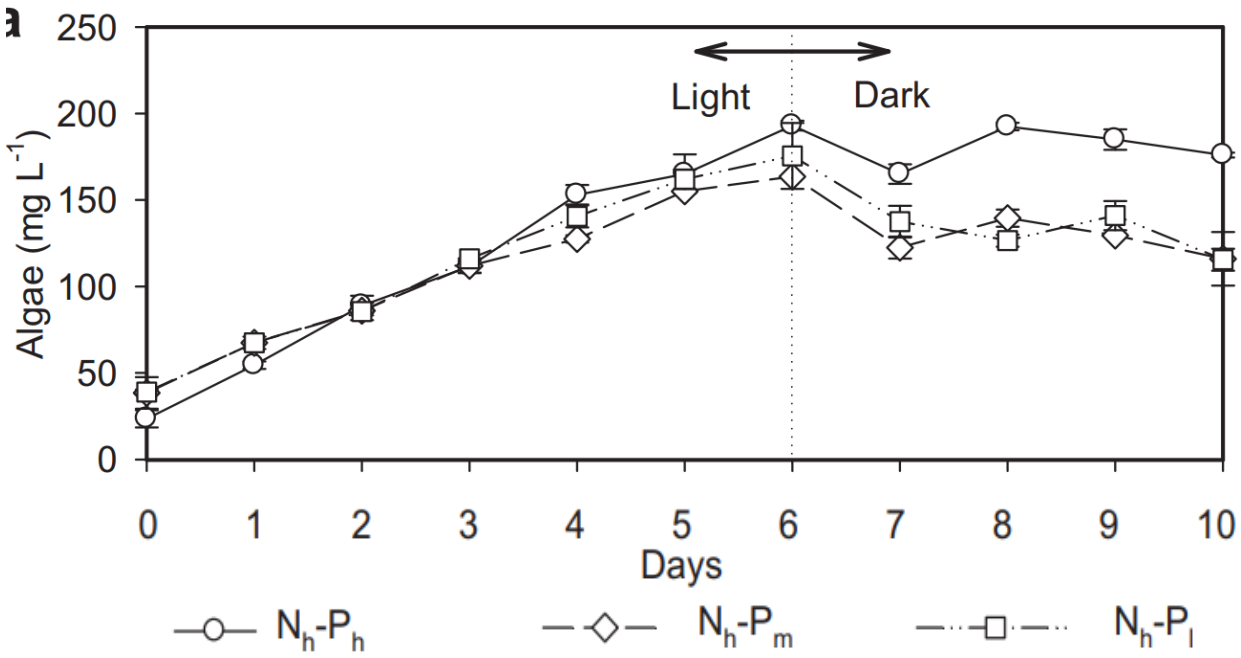

Figure 2.7: Growth of Algae when nitrogen levels are high, and phosphorus levels are varied. A light intensity of at 80 µmol m^{-2} sec^{-1} is maintained and temperature is ambient (31).

In Figure 2.6 it can be seen that with increased nitrogen concentration cell growth increases proportionally. This is not the same case as in Figure 2.7 where it can be seen while cell cultures with high concentrations of phosphorus have the largest growth, cultures with medium and low phosphorus concentrations have similar growth. Another interesting thing to note is a drop in nitrogen created a much larger drop in growth than a drop in phosphorus did. The end cell concentration difference between runs in the varying nitrogen experiment was 80% as compared to 30% for the varying phosphorus experiment. These experiments highlight that while both nitrogen and phosphorus are needed, Nitrogen is what the algae use up the most.

Building on this, nutrient-based models provide a framework for predicting how algae respond to varying nutrient levels. Two different groups of nutrient based models can be highlighted (19). The first being Group I where the models focus on external nutrients. The second being Group II where the models focus on internal nutrients.

Group I models assume growth is limited by nutrient concentrations in the media. The most common way this is done is defining growth as a function of a single nutrient often done using the Monod model where (S) is substrate concentration and (K_s) is half-saturation constant

(32). The Monod model was successfully used describe the growth of *Scenedesmus sp.* under different nitrogen and phosphorus concentrations (33). The problem with the Monod model is that at higher concentrations the Monod model struggles to maintain accuracy due to nutrient toxicity. The Andrews model (34) introduces an inhibition constant (K_I) to account for these effects.

Monod Model

$$\mu = \mu_{\max} \frac{S}{K_s + S}$$

(4)

Andrews Model

$$\mu = \mu_{\max} \frac{S}{K_S + S + \frac{S^2}{K_i}}$$

(5)

Group II models account for internal nutrient storage, that being nutrients in the cells. These models consider how algae cells can use nutrients stored within themselves to grow in absence of external nutrients. The Droop Model is a key example of this where (Q) is the nutrient cell quota and ($\mu'_{max)}$ is the hypothetical maximum growth rate at infinite (Q) (35). The minimum nutrient quota (Q_{min}) is the minimum amount of nutrient a cell needs to survive. The Droop model has been applied to phosphorus-limited growth in *Chlorella sp.* and *Scenedesmus sp*., showing that it better captured growth behavior compared to external-nutrient models (36). The only problem with the Droop Model is it faces inaccuracies at higher growth rates. By integrating the Michaelis-Menten function into the Droop Model this is addressed. This is known as the Flynn model which introduces the maximum nutrient quota (Q_{max}) which is the maximum amount of nutrient a cell can store and a constant (K_q) (37).

Droop Model

$$\mu = \mu'_{\max}\left(1 - \frac{Q_{\min}}{Q}\right)$$

(6)

Flynn Model

$$\mu = \mu'_{\max} \frac{(1+K_q)(Q-Q_{\min})}{(Q-Q_{\min})+K_q(Q_{\max}-Q_{\min})}$$

(7)

Nutrient based models compliment light-based models offering insight on how growth is affected by specific nutrients. While Group I models such as the Monod Model work well for simple systems, Group II models better utilize the complexities of nutrient storage and utilization. Both methods need specific tailoring based on experimental/environmental conditions.

2.4 Review Conclusion

Micro algae growth is influenced by a variety of factors including light, temperature, and nutrients. All of these factors greatly affect the dynamic growth in natural and industrial settings. Light and temperature are driving factors when nutrients are in excess. Previous studies highlighted the sensitivity of *Chlorella sorokiniana* and other species to both light intensity and temperature. Both papers also demonstrate how ideal temperatures and light are dependent on other factors surrounding the algae. This further drives the importance of tailoring growth parameters to specific conditions for enhanced model accuracy. The light-based models organized in groups Group I, Group II, and Group III provide valuable insight on how models need to change based on environmental conditions. Group I models are effective at lower algae densities while Group II and Group III are better for dense algae with Group III adding a photoinhibition factor (19).

Nutrients play an important role in algae growth and cultivation. It is important to note that different nutrients are needed at different levels for algae to thrive. The research done that found that when varying levels of nitrogen and phosphorus, experiments higher in nitrogen saw higher growth for *Chlorella sorokiniana (31)*. This highlights the high importance nitrogen has in the growth of microalgae.

Nutrient-based models complement the light-based models by addressing the roles of important nutrients such as nitrogen, phosphorus, and carbon. Group I for the nutrient models is meant for conditions which consider nutrient concentrations in the media. This is normally done

by basing the model off of one specific nutrient. Group II models provide valuable insights into the behavior of internal storage in the cells. Together, light and nutrient-based models offer a comprehensive framework for understanding microalgae growth behaviors. This enables more accurate usage and cultivation for wastewater treatment, biofuel production, and other applications. By integrating these models into experimental data, researchers can better optimize and understand algae growth for a variety of industrial applications.

3. Methodology:

3.1 Piping and Instrumentation Diagram (PID)

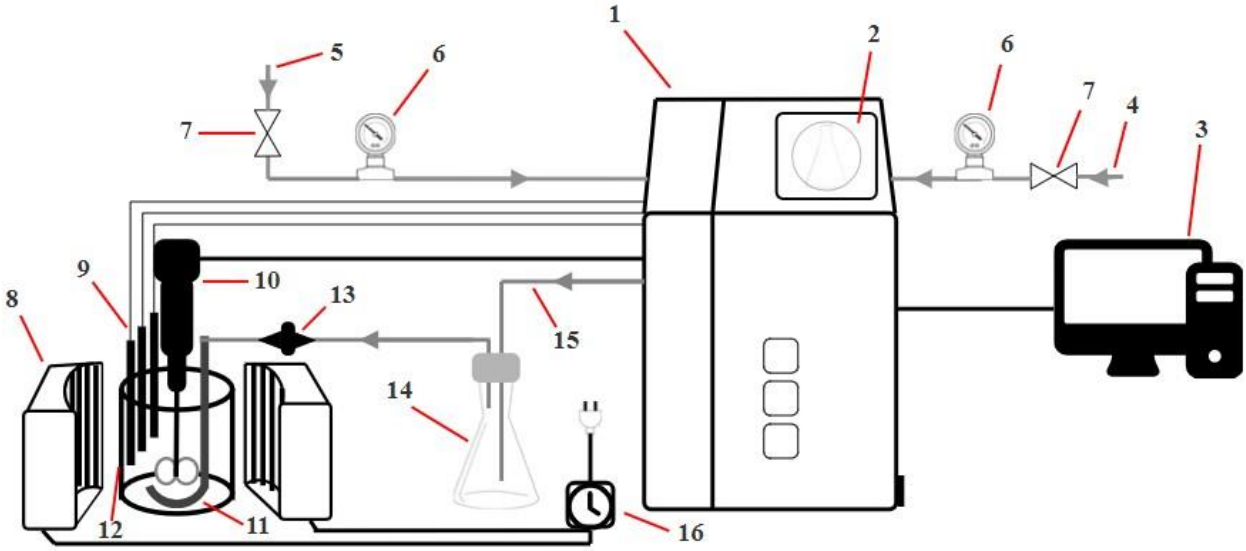

Figure 3.1: PID of full reactor setup

1	Bioflo 115 controller/computer
2	Bioflo interface
3	Desktop PC with Bioflo program and data storer
4	Air gas line
5	CO_2 gas line
6	Pressure gauge/regulator
7	Gas on/off valve
8	Light units

9	DO, pH, and temperature probes
10	Agitation motor
11	Gas bubble diffusor
12	Batch reactor
13	Gas particle filter
14	1L Erlenmeyer flask filled with 500ml water
15	Mixed gas reactor inlet
16	Lights timer

3.2 Experimental Parameters

The percentages of CO_2 that were varied for the main experiments were 5%, 3.6%, 1.8%, 0.8% by volume and a control run with just air. These runs all had a total flowrate of 2 LPM, 12 hours of light, and continuous CO_2 flow. There is an agitation of 125 RPM and temperature around 35°C for all experiments done. The experiment where CO_2 flow was only kept on during the light phase was set at 2 LPM and 5% CO_2. This would be compared to the 5% CO_2 experiment with continuous flow. The experiment where air flow would be lowered was set to 1 LPM at 10% CO_2. This gives a total flow of 0.1 LPM CO_2 and 0.9 LPM air. This can also be compared to the 5% continuous flow run which gives 0.1 LPM CO_2 and 1.9 LPM air.

3.3 Reactor Setup/Takedown

Setting up and dismantling the reactor before and after each run requires careful handling. Many components are delicate and have been known to break during the project. A teardown is necessary after each run to sterilize all equipment using an autoclave.

The first step in taking down the system is to turn off all functions such as gas flow and agitation. Next, move the two light fixtures away from the reactor to create working space. Carefully remove the pH, dissolved oxygen (DO), and temperature probes from the reactor. Pay special attention to the fragile glass construction of the pH and DO probes. Disconnect the probes from the Bioflo computer. Note that if the DO probe is disconnected for more than 60 seconds, it must be reconnected and left for 6 hours to repolarize. If contamination is suspected, autoclave the DO probe. Under normal circumstances, clean the DO and pH probes with soapy

water and rinse with autoclaved milli-q water. Store the pH probe tip in pH 7 stock solution. The temperature probe, which does not contact algae, does not require cleaning.

Next, disconnect the mixed gas line and remove the agitation motor from the reactor, ensuring both are off. Once everything is disconnected, wash the reactor thoroughly with soap and water in a sink. After cleaning, autoclave the entire reactor. Ideally, autoclave with two 1-liter bottles filled with milli-q water required for the media. This process is typically done overnight for the equipment to cool by morning. Cooling is crucial to prevent algae death; it takes around 3 hours for the water to cool sufficiently.

Move the reactor to the chemical shelf area to prepare the media inside it using instructions from the media tab in procedures. Return the vessel to the experimental area and reconnect all equipment. Place the light stations around the reactor, set experimental parameters (gas flow, CO_2 percentage, agitation) in the Bioflo computer. Agitation is typically set to 125 rpm for each run to keep algae suspended, while other values vary. pH, DO, and temperature are measured values, not set; the Bioflo computer controls and records these values, which are stored and graphed on a desktop PC.

Proceed by starting the run on the computer as per the desktop PC section under procedures. Introduce algae into the reactor using 10 ml disposable pipets powered by a pipet gun. These longer pipets are preferred for introducing algae and sampling due to their reach into reactor ports. The algae is sourced from an Erlenmeyer flask on a shaker stand. Generally, 45 mL of algae is sufficient for the reactor size, but variations may require more. This is detailed in the algae shaker section. To determine concentration, take a reactor algae sample and conduct an optical density (OD) test, as described in the Manual OD section under methods. The target OD for startup is 0.12-0.20 for both wavelengths; readings below 0.10 are inaccurate.

3.4 Media

The media chosen for the reactor is the Pecos media. To make the media, start by pouring 1 liter of autoclaved milli-q water into the autoclaved reactor. Using the recipe found in Table 3.1 and 3.2, pipet the listed chemical amounts into the reactor. After all chemicals have been added, fill the reactor with milli-q water till the level reaches the 2L mark on the reactor glass.

Table 3.1-Pecos Media

Pecos media on a 2-liter total basis

Chemical	Final concentration (g/L)	Stock Solution Concentration (g/L)	Volume (mL)
(NH2)2CO (urea)	0.1	20	10
MgSO4*7H2O	0.012	12	2
NH4H2PO4	0.025	25	2
Potash	0.075	37.5	4
FeCl3	0.00315	3.15	2
Na2CO3	0.02	20	2
EDTA	0.00436	1.09	8
Allens solution	/	/	2

Table 3.2-Allens solution

Per 1 liter basis

Chemical	Amount (g/L)	Final Concentration (µM)
H3BO3	2.86	46
MnCl2*4H2O	1.81	9
ZnSO4*7H2O	0.22	0.77
Na2MoO4*2H2O	0.39	1.6
CuSO4*5H2O	0.079	0.3
Co(NO3)2*6H2O	49.4	0.17

3.5 Desktop PC

The BioFlo 115 computer is connected to a desktop pc with the BioFlo program on it. The program is where all the data is stored. There are also graphs one can access that displays and compares any data wanted.

To start a run, begin by launching the program. Make sure you do not close the program as this will stop data logging. If another run is currently on, go to batch settings and end batch. To start a run, select the new run button, for culture mode select cell culture start the run. Culture mode allows for preset conditions if that's wanted.

3.6 Manual OD

Manual OD readings are conducted using a spectrophotometer. This device works by passing various light wavelengths through a 1 ml cuvette and measuring its transparency. These readings can be converted to actual algae mass by correlating the OD reading with ash-free dry weight, see in the methods section. The spectrophotometer is set to measure light wavelengths of 650 nm and 780 nm. This is because at 650 nm chlorophyll is absorbed and at 780 nm the color green is absorbed (38).

The procedure begins by turning on the machine and allowing the bulb to warm up for 15 minutes. Around the 10-minute mark, a 5-10 ml sample of algae is extracted from the reactor using a long pipette. At the OD station, 1 ml of water is pipetted into a cuvette to serve as the blank. Following this, 1 ml of algae from the reactor is piped into three separate cuvettes. This is done to obtain an average of three readings for increased accuracy. With that being said, standard deviation is low as shown in Figure 3.2.

It's important to note that the equipment's accuracy ranges between 0.1 and 0.9 OD units. If the readings exceed 0.9 for any wavelength as the algae concentration increases, dilution is necessary. This should be done on a 1:9 basis, that is 0.9 ml water and 0.1 ml algae. If again if OD exceeds 0.9 as it grows more, use 0.95 ml of water and 0.05 ml of algae. Ensure thorough mixing of the mixture in the cuvette, either by adding algae first followed by water, or by mixing with the pipette tip. Use a 1 ml pipette for transfers between 0.9 and 1 ml, and a 0.1 ml pipette for transfers between 0.05 and 0.1 ml to enhance accuracy. Record the readings over time and enter them into an Excel sheet for analysis.

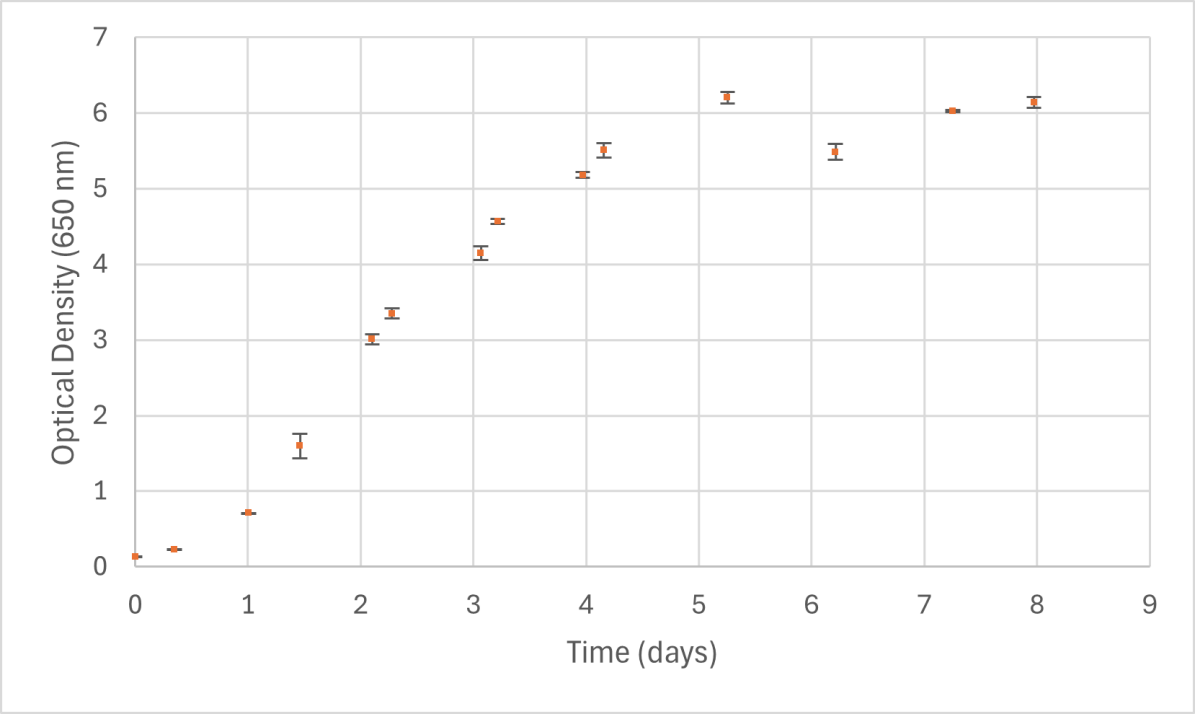

Figure 3.2: Error bars based on standard deviation for the 5% CO_2 run with continuous CO_2 flow and a total flowrate of 2 LPM.

3.7 General Maintenance

Maintaining the reactor requires constant upkeep. The CO_2 gas cylinder should be replaced every 2-3 weeks to when the gas runs out to ensure a consistent supply. Monitoring the pressure on the main line helps anticipate when the cylinder needs changing. The pressure going to the reactor should not exceed 10 psi. This can be adjusted using the pressure regulator on the CO_2 line.

If the CO_2 supply is out for 4 hours or more during the light cycle, one should consider resetting the run to maintain model integrity. Four hours is a third of the light time the algae has in one day to grow, thus it is significant. To mitigate the chances of this happening, someone should come in at the very least within an hour of the start and end of the light cycle to check on the system. This is usually done at the same time as when the OD readings are taken.

Another critical aspect is managing water absorption within the reactor. As the mixed gas flows up the reactor, it saturates with water causing the level to drop. A steady water level is essential to ensure accurate readings and constant mass-transfer kinetics. To mitigate water loss,

the gas passes through an Erlenmeyer flask filled with water, saturating it before entering the reactor. This only reduces the daily level drop and does not get rid of the need to fill both the flask and the reactor with water daily. The flask should be kept to the 500 ml mark and the reactor at the 2 L mark to ensure an accurate model.

3.8 Calibration of Probes and Sensors

Many probes and sensors operate by converting voltage signals into readable values such as pH, dissolved oxygen (DO), etc. Over time, these voltage signals can drift or become disconnected from the actual values they represent. Therefore, calibration is essential to reset the voltage signal and align it accurately with the corresponding physical value.

Calibration involves comparing the voltage signal output by the sensor with known standard values (such as pH 4, pH 7, pH 10 solutions for pH probes) and adjusting the sensor's internal settings to ensure accurate measurement readings. This process helps maintain the reliability and precision of measurements over time, ensuring that the sensor continues to provide accurate data for scientific or industrial applications.

Regular calibration, typically performed at regular intervals or when significant changes in measurement accuracy are observed, helps mitigate inaccuracies caused by environmental factors, sensor aging, or other variables. By calibrating sensors periodically, users can trust the accuracy of the readings and make informed decisions based on reliable data.

3.9 Calibration of pH probe

To begin the pH probe calibration using the BioFlo computer, start by rinsing the probe thoroughly with milli-q water. This initial rinsing step is crucial to remove any residues or contaminants that could affect the accuracy of the calibration. It's important to repeat this rinsing step before switching to each new pH standard solution to maintain the integrity of the calibration process. Once rinsed, secure the pH probe in place using a clamp or similar mechanism. Ensuring the probe is securely fixed prevents any movement during calibration, which can impact the accuracy of the readings.

The calibration process for the pH probe in the BioFlo computer involves a 2-point calibration. For a wider measurement range, calibrate the probe in two stages: pH 4-7 and pH 7-

10. After securing the probe, immerse it carefully into the pH 4 standard solution. Ensure the probe is fully suspended in the solution and not touching the walls of the container, as contact with the container walls can interfere with the calibration process. Navigate to the calibration tab in the BioFlo computer software and select the pH calibration.

Allow the pH probe to stabilize in the pH 4 solution, which typically takes between 5 to 20 minutes depending on the probe's previous calibration history and stability. Once the voltage reading stabilizes, enter the known pH value (pH 4) into the "Set Zero" slot on the computer interface and click the corresponding "Set Zero" button to lock in this calibration point. After completing the calibration at pH 4, rinse the pH probe again with milli-q water to remove any traces of the pH 4 solution. Then, proceed to immerse the probe in the pH 7 standard solution. Wait for the voltage reading to stabilize once more.

For the second point of calibration, enter the pH 7 value into the "Set Span" slot on the BioFlo computer and click the "Set Span" button to confirm this calibration point. This 2-point calibration process ensures accurate pH measurements across the desired range. If a broader pH range or higher accuracy is required, repeat the calibration process using pH 7-10 standard solutions in the same manner.

By following these steps systematically and ensuring proper rinsing and secure fixation of the pH probe, you can maintain accurate and reliable pH measurements using the BioFlo computer system for your experiments or industrial processes.

3.10 Calibration of DO Probe

To calibrate the DO probe in the reactor using the BioFlo computer, calibrate with algae media instead of water for a more accurate calibration. Access the calibration tab on the BioFlo computer interface and navigate to the DO calibration section. This was calibration was done at room temperature.

The calibration process starts with setting the zero point, which can be done in two ways. The first method involves flushing the reactor with nitrogen gas to eliminate any dissolved oxygen. Allow the voltage reading from the probe to stabilize after flushing. Once stable, input "0" into the "Set Zero" slot on the computer interface and click the corresponding "Set Zero" button to lock in this calibration point.

Alternatively, you can perform the zero calibration by briefly disconnecting the DO probe from the BioFlo computer. Again, input "0" into the "Set Zero" slot on the computer interface and click the corresponding "Set Zero" button to lock in this calibration point. Then promptly reconnect the probe to the computer. It's crucial not to leave the probe disconnected for more than one minute to avoid sensor polarization issues. If the probe remains disconnected for an extended period, approximately six hours of reconnection will be necessary for repolarization.

After setting the zero point, proceed to calibrate the span by flushing the reactor with air at a flow rate between 5-10 liters per minute (LPM). Allow the voltage reading to stabilize once again which can take up to 30 minutes. Enter "100" into the "Set Span" slot on the BioFlo computer and click the "Set Span" button to confirm this calibration point. Once these steps are completed, the DO probe calibration is finished.

By following these calibration procedures systematically and ensuring proper handling of the probe throughout, you can maintain the accuracy and integrity of dissolved oxygen measurements using the BioFlo computer system in your reactor setup.

3.11 Algae Shaker

The source of algae for all runs done in the lab comes from 500 mL Erlenmeyer flasks filled with algae and media on a shaker stand. The media from these shakers are supposed to be changed every month or 2. Because of the large amount of algae taken for the experiments done with the Bioflo (roughly 50 mL or more) as compared to other experiments in the lab that use 10 mL, the level will drop significantly in the flask. This means that when the media is changed and level is back to 500 mL, the algae will be slightly diluted. This is why there is variation in how much algae are added to the reactor at the beginning of each run.

To change the media, the algae need to be centrifuged out of the old media and then placed into clean flasks with fresh media. The first step is to autoclave the same number of Erlenmeyer flasks that are being changed. While the autoclave is running, make the media which is 500 mL per flask. The media currently used in the shaker flasks is BG11, the formula for that can be found in Table 3.3. The next step is to turn off the shaker stand and remove the flasks. Be sure to label the new sterile flasks and the 500 ml centrifuge bottles with the same corresponding algae species name as listed on each of the old tags. Pour the algae from the old flasks into the

correctly labeled bottles. Using a balance, put one of the bottles of algae on one side and an empty bottle on the other. The caps for each bottle need to be on the balance when evening out the weight. Fill the empty bottle with water until it matches the weight of the bottle with the algae in it. Using a 1 ml pipette can help with fine adjustments. Repeat this process with all of the algae bottles. Once done, place the bottles in the centrifuge making sure each algae bottle and its corresponding bottle of water are across from each other in the centrifuge. Then, turn on the centrifuge for 15 minutes at 7000 RPM. Wait for the centrifuge to stop fully. Once done, carefully remove the bottles from the centrifuge. You will notice all the algae settled into the bottom of the bottle. Open the bottle, and carefully drain the old media from it. Then, add 500 mL of the new media, close the bottle, and shake it till it is all resuspended in solution. The algae and media can then be transferred to the new flasks and put back onto the shaker stand.

Table 3.3-BG-11

BG-11 media on a 500 ml total basis

Chemical	Amount (ml)	Stock Solution Concentration (g/200 ml)	Final Concentration mM
NaNo3	5	30	17.6
K2HPO4	5	0.8	0.22
MgSO4*7H2O	5	1.5	0.03
CaCl2*2H2O	5	0.72	0.2
Citric Acid*H2O	5	0.12	0.03
Ammonium Ferric Citrate	5	0.12	0.02
Na2EDTA*2H2O	5	0.02	0.002
Na2CO3	5	0.04	0.18
Allens solution	0.5	0.04	0.18

3.12 Ash Free Dry Weight

Ash free dry weight is a procedure used to match the OD readings to an actual mass of algae. The first thing to do is to get filters and pre-ash them. This can be done by putting water through the filter utilizing the vacuum system and then using the muffle furnace at 540°C for four hours. After this step is complete, it is required to weigh the filter so there is a baseline weight. Once this is complete, the optical density of the algae is taken, and 20 mL of the allergist solution is put through the filter utilizing the vacuum system. Once the liquid is done draining from the system, the filter with the algae is placed into an oven at 105°C for two days in order to fully dry the algae.

Once the algae are dried at the end of the two days, the filter is weighed again to determine how much weight can be attributed to the algae. After waiting, the filter is put again into the muffle furnace at 540°C for four hours. Lastly, the filter is again weighed for the ash content of the 20 mL of algae. From this, it can be determined what the amount of organic algae was in the 20 mL sample. This can then be graphed against optical density to determine a curve. Once the line of best fit is created, all data from the experiments can be transformed from OD to ash free dry weight values.

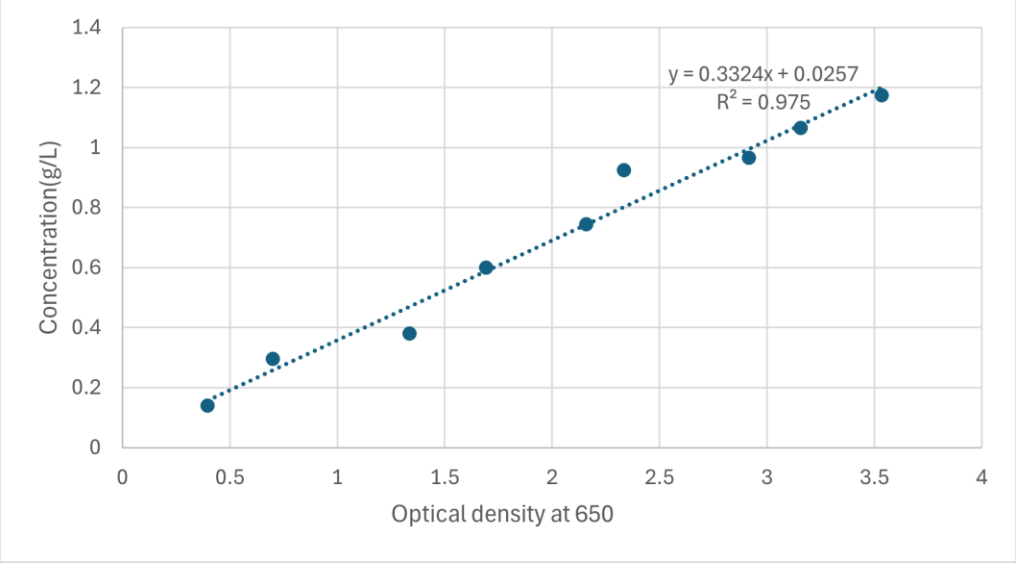

Figure 3.3: Ash free dry weight fit to wavelength 650nm

4. Results and Discussion:

Experimental data and modeling of that data provided valuable insights into how varying CO_2 concentrations influence algae growth and behavior. The experimental data demonstrate the impact of varying CO_2 input percentages on growth, pH, and DO. The experimental data are fitted by a growth model with a single adjustable parameter. Through reviewing and analyzing the experimental and modeling data, patterns can be seen that further provide a better understanding of algae growth and behavior.

4.1 Experimental Results and Discussion

4.1.1 Difference in Growth Between Constant CO_2 Versus Light-Phase-Only CO_2

The original plan was to run the experiments with CO_2 only flowing during the light phase. However, this quickly became difficult to manage with the current setup. The flow had to be manually turned on and off each day at specific times, which was challenging to coordinate, especially since we also had to take manual midday readings. To simplify the process, we decided to keep the CO_2 flow on continuously throughout the entire experiment.

We hypothesized that leaving the CO_2 on during the dark phase wouldn't significantly affect growth, if at all. This is because there is little to no light that is needed to convert CO_2 into biomass. Looking at the growth results in Figure 4.1, this appears to hold true. Although the CO_2 always-on run showed slightly higher growth, the difference wasn't significant. The small difference is likely attributed to other factors. One observation was seen in the dissolved oxygen (DO) data in Figure 4.2. Oxygen is released during photosynthesis, because of this algae growth and DO can directly be related (39). Both runs had similar DO levels during the night which reinforces the idea the continuous flow of CO_2 at night didn't facilitate extra growth. However, during the light phase, the CO_2 always-on run consistently showed higher DO.

It is unclear why DO is a bit higher during the light phase here. There is a slightly higher temperature, as seen in Figure 4.3. Solubility of oxygen decreases as temperature increases, meaning the algae are releasing enough oxygen to offset that and even increase levels at high temperature (40). Because temperature can influence growth rates, even small differences may contribute to higher growth rate and thus DO production. However, the difference in temperature

is so small it is likely not the main contributing factor. Another potential cause is the differences in pH as seen in Figure 4.4.

There is a computer error around day 4 for the CO_2 light-phase-only run, and the missing data should have resembled the peaks observed on other days. Beyond this error, notable trends emerge. In the CO_2 always-on run, pH increased during the light phase and decreased during the dark phase. The higher pH during the light phase is likely due to the algae's consumption of carbonate, which is acidic. By contrast, in the light-phase-only CO_2 run, pH was lower during the light phase and higher during the dark phase. This is because the added CO_2 is acidifying and without it during the night pH rises. The slightly lower DO in the light-phase-only run could be due to stress the high pH fluctuations cause on the algae cells (41). Further research needs to be conducted to explore this. These findings highlight how CO_2 delivery timing affects the system's pH dynamics, even though it has minimal impact on overall growth.

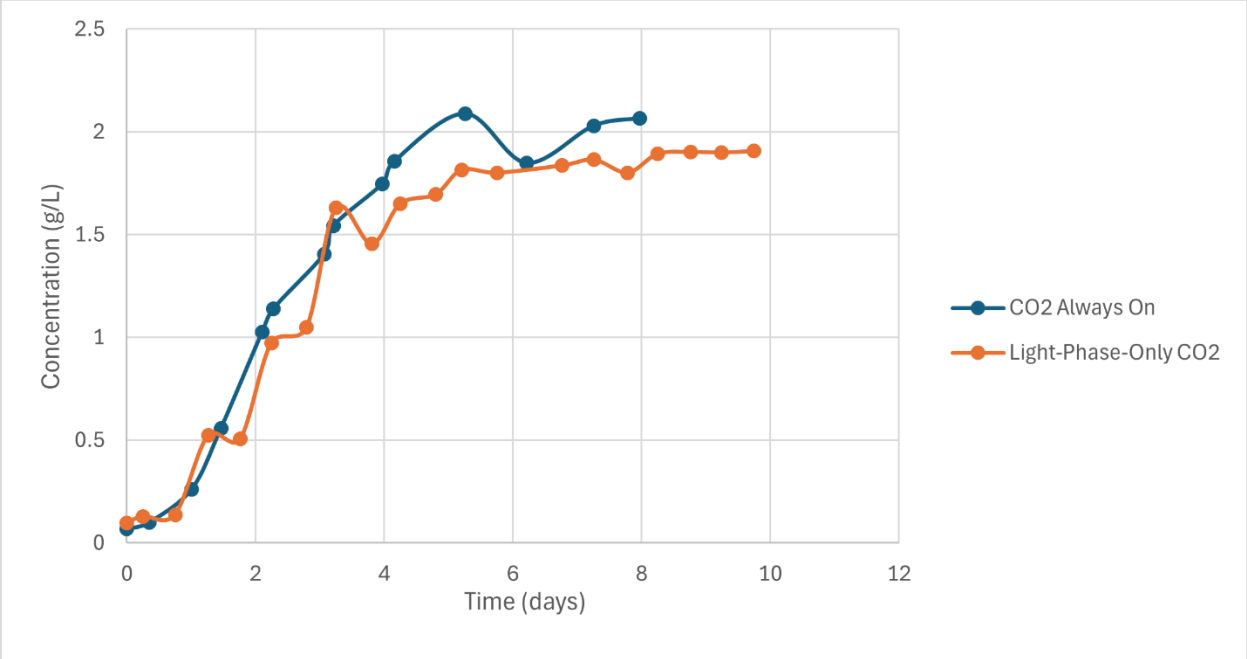

Figure 4.1: Growth of CO_2 always on vs CO_2 on only during light-phase. Experiments done at 5% CO_2 and a total flowrate of 2 LPM.

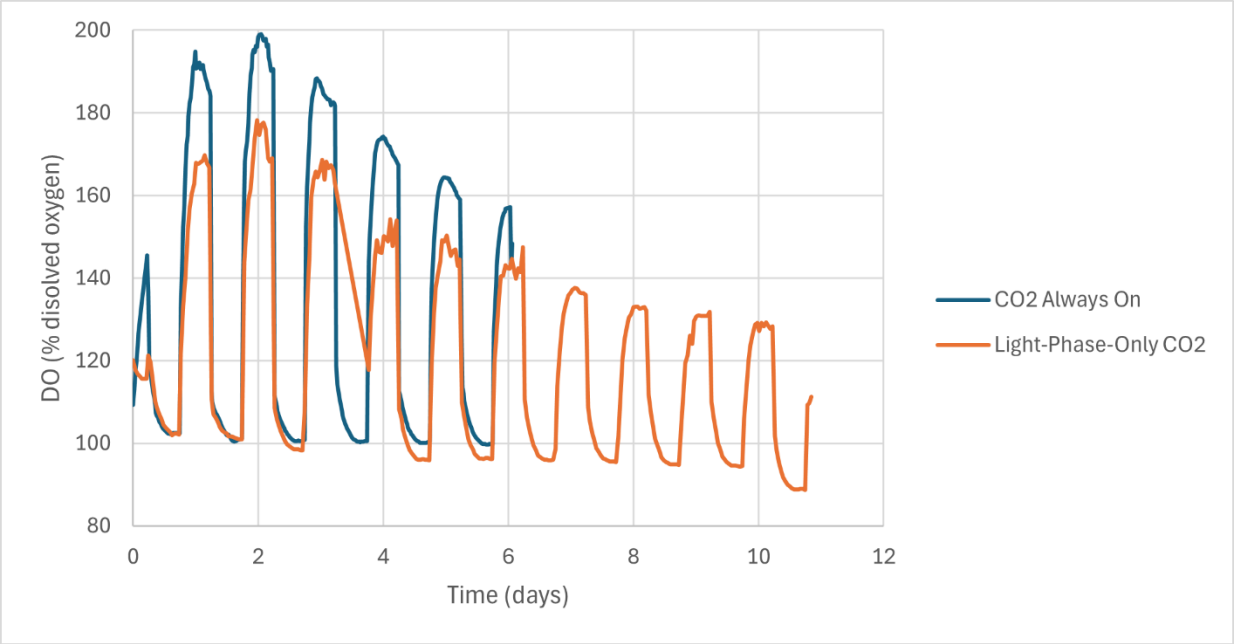

Figure 4.2: DO of CO_2 always on vs CO_2 on only during light-phase. Experiments done at 5% CO_2 and a total flowrate of 2 LPM.

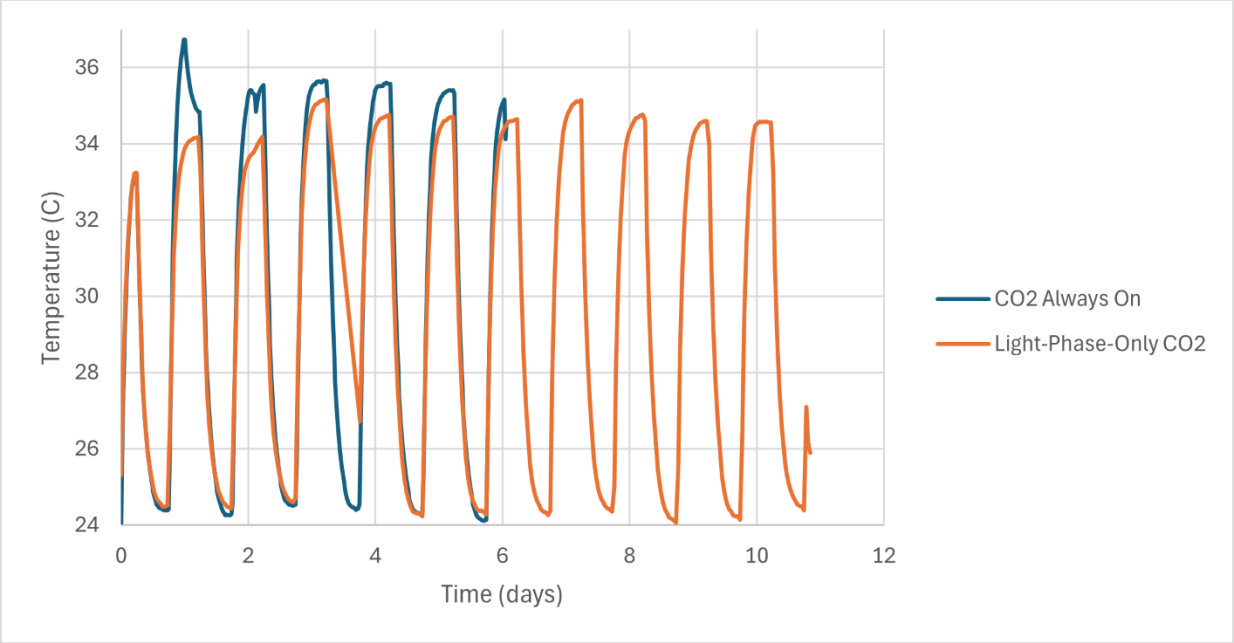

Figure 4.3: Temperature of CO_2 always on vs CO_2 on only during light-phase. Experiments done at 5% CO_2 and a total flowrate of 2 LPM.

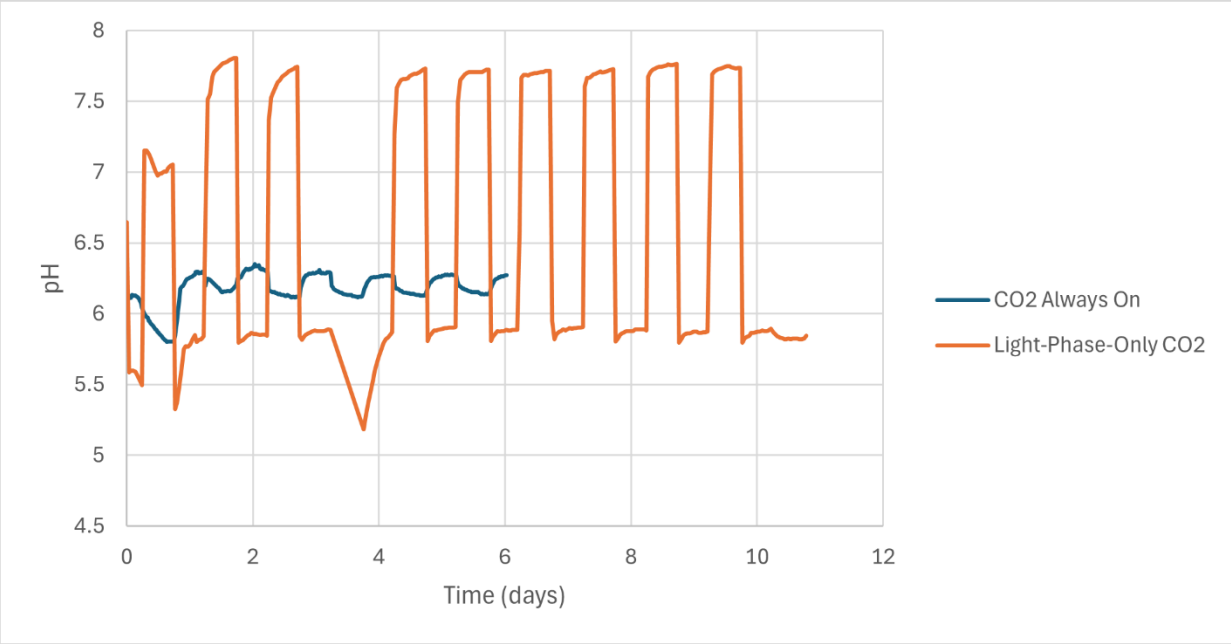

Figure 4.4: pH of CO_2 always on vs CO_2 on only during light-phase. Experiments done at 5% CO_2 and a total flowrate of 2 LPM.

4.1.2 Effects of lowering air flowrate while keeping CO_2 flow the same

In one experiment, CO_2 flow rate was kept the same and air flow rate was reduced. The idea behind this was that while air is essential for algae growth, excess air might strip CO_2 from the reactor, reducing efficiency. To optimize growth, air flow should ideally be minimized until the oxygen in the air isn't a limiting reactant. CO_2 flow percentage should also be lowered to a minimum in respect to the air flow. This means as air is lowered, CO_2 at the same flow rate will dissolve easier and need to be lowered as well.

It may be argued that the best way to find what minimum air needed is to first find the lowest amount of CO_2 needed in an excess air run. This can be the runs done at 2 LPM. The optimal 2 LPM run, being the run with maximum growth and minimal added CO_2 will provide a pH value. Experiments can then be done where the total flowrate is lowered 1.5, 1, 0.5 LPM and the CO_2 percentages can be changed to match that of the pH in the optimal 2 LPM run. The same pH should mean the same dissolved CO_2 while lower total flow means lower air and lower CO_2 flow. Ideally, one will find that at a certain point the total flow will be lowered to the point to where air is the limiting reactant and that will be the most optimal combination. These

experiments should be done in the media without the algae and will be short. The dissolved air and CO_2 also depend on other things such as bubble size and residence time so this will be different for every setup. Although this wasn't the primary focus of this paper, I decided to briefly explore this concept. This is a possibility for future research.

For this experiment, I compared a 5% CO_2 run at 2 LPM and a 10% CO_2 run at 1 LPM. At 2 LPM this gives 0.1 LPM CO_2 and 1.9 LPM air while for 1 LPM total this gives 0.1 LPM CO_2 and 0.9 LPM air. In Figure 4.5 it can be seen that in the 1 LPM run starts with an increase in growth but then begins to decrease. This is unlike normal growth, where the algae remain in the stationary phase for some time before dying off. The temperature for both runs was similar, as shown in Figure 4.6, so slight temperature differences likely didn't play a major role here.

The cause is likely shown in Figure 4.7, where the pH of the 1 LPM run is significantly lower than that of the 2 LPM run. This agrees with the hypothesis since with higher CO_2 absorption pH should be lower. However, it's possible that the pH became so low that it inhibited algae growth, which could explain the observed decline in growth over time. It is unclear if the lower average DO for the 1 LPM run seen in Figure 4.8 is because lower oxygen production from the algae or lower LPM of air. Perhaps it is a combination of both factors.

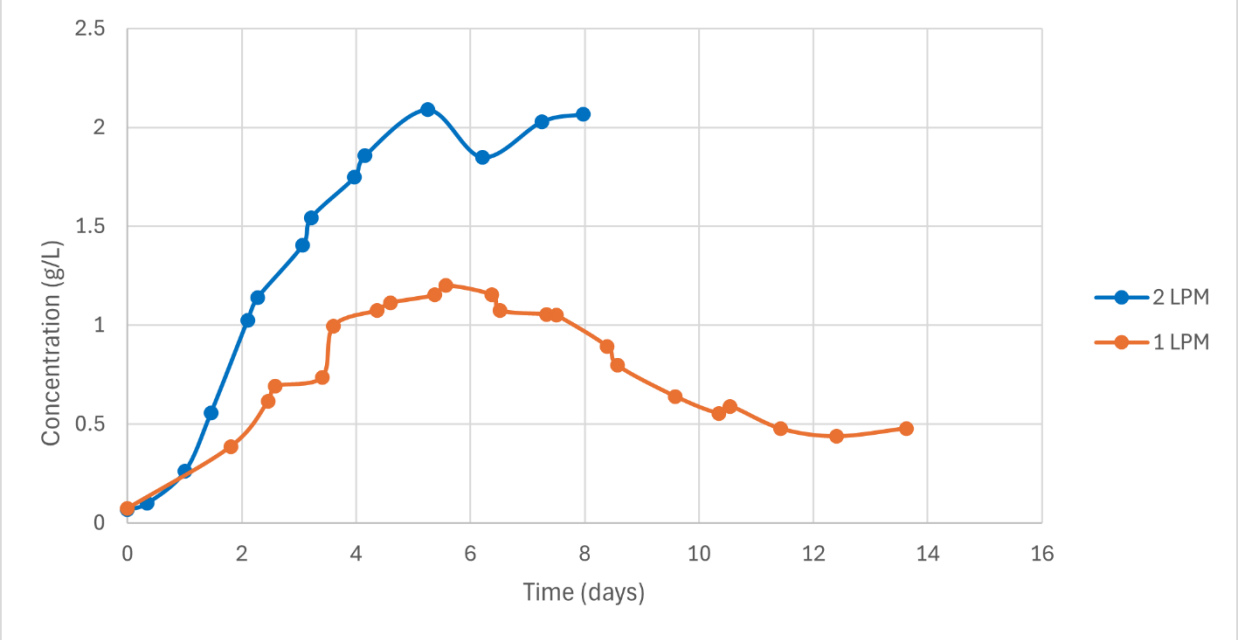

Figure 4.5: Growth of algae at 10% CO_2 with a total flowrate of 1 LPM and 5% CO_2 at a total flowrate of 2 LPM.

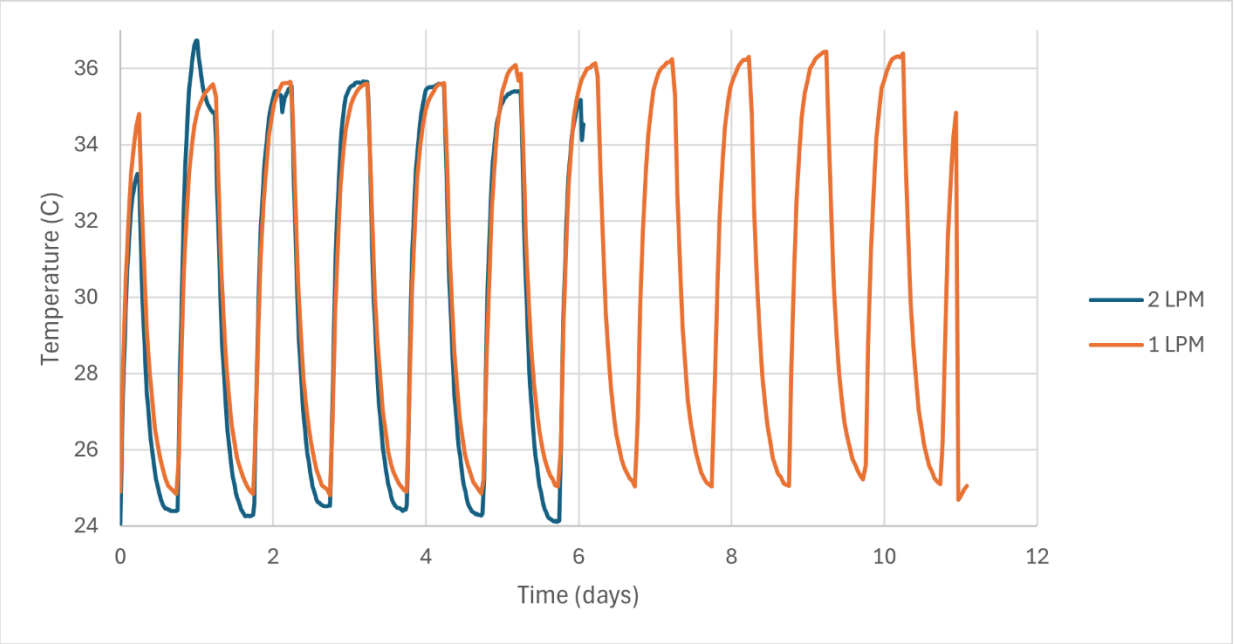

Figure 4.6: Temperature at 10% CO_2 with a total flowrate of 1 LPM and 5% CO_2 at a total flowrate of 2 LPM.

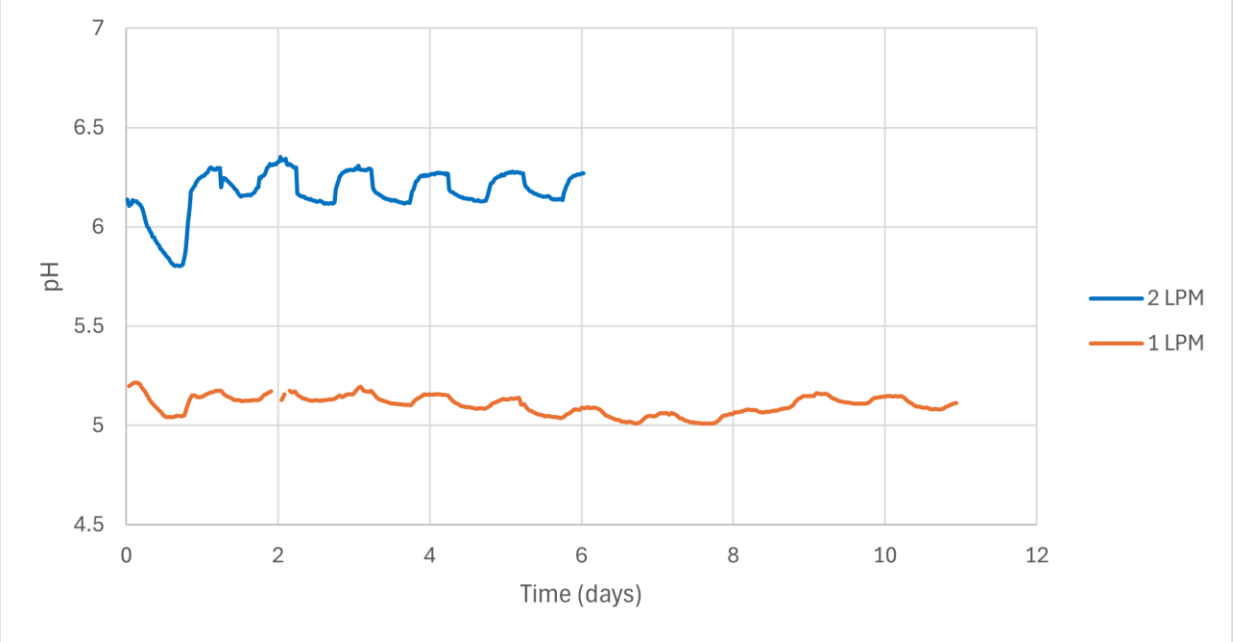

Figure 4.7: pH of 10% CO_2 at 1 LPM and 5% CO_2 at 2 LPM.

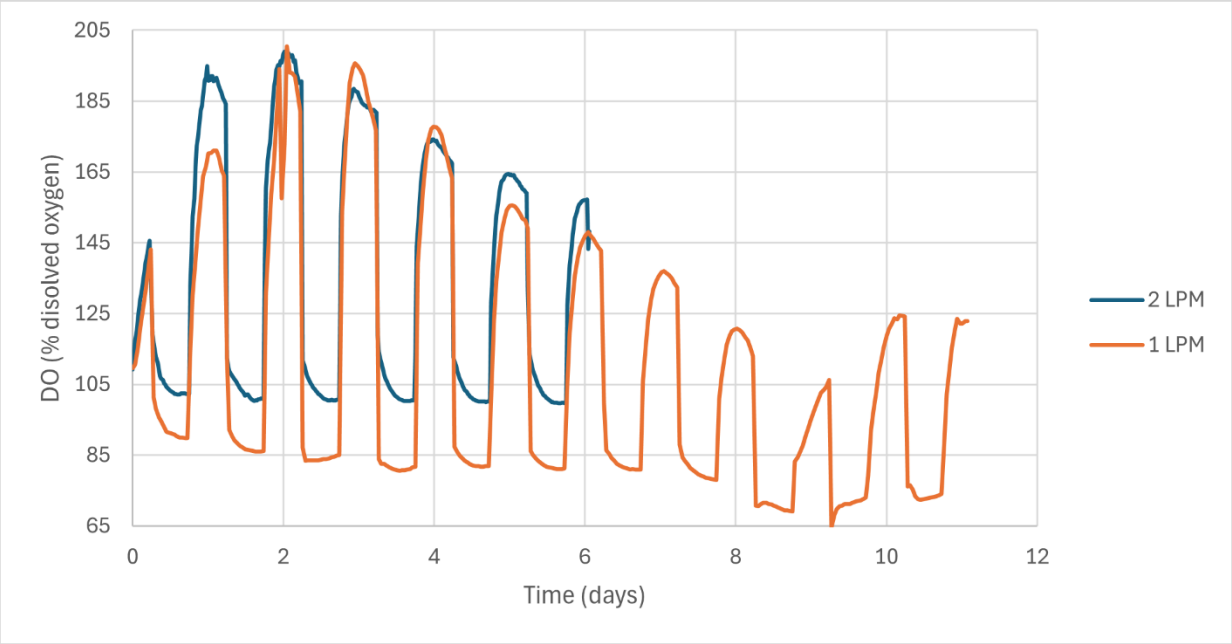

Figure 4.8: DO of 10% CO_2 at 1 LPM and 5% CO_2 at 2 LPM.

4.1.3 Effects of different CO_2 percentages on algae growth

In these experiments CO_2 was added at concentrations of 5%, 3.5%, 1.8%, 0.8%, 0.3% and a control group using only air. Air naturally contains about 0.04% CO_2 which is why the algae still grow. The goal was to vary the percentages of CO_2 to create a gradient of data to help find the lowest amount of CO_2 needed for optimal growth. The results showed that all the runs with added CO_2 had very similar growth rates, although the 0.3% run was slower than the rest as seen in Figure 4.9. It can also be seen that for the control run with only air, the algae grew significantly slower than the rest as shown in Figure 4.10. The slower growth in the 0.3% and air only run is likely due to the lower level of CO_2. At 0.3%, it is likely that CO_2 was no longer in excess. On the other hand, in the other runs where CO_2 was added, there was an excess of CO_2. This means growth was likely limited by another factor, such as light or a different nutrient.

Although Figure 4.9 shows minor differences in growth among the added CO_2 runs, there doesn't appear to be any consistent trend other than 0.3% having the lowest growth. For example, the final biomass concentration, X_m in the 5% CO_2 run was lower than in the other CO_2 runs, excluding 0.3% and air. These differences aren't very significant and are likely due to other things than CO_2. This shows that once CO_2 passes a certain threshold, it doesn't increase growth

and other factors control growth. In Figure 4.11 all excess CO_2 data is merged via a polynomial fit including the 5%, 3.5%, 1.8% and 0.8% runs. This is because they all have a similar growth curve. In Figure 4.12 the merged polynomial fit is compared to the 0.3% and only air runs. This better shows the trend in decrease growth rate as CO_2 concentration drops. Examining variables such as temperature, pH, and dissolved oxygen levels during these runs may provide insight on algae behavior.

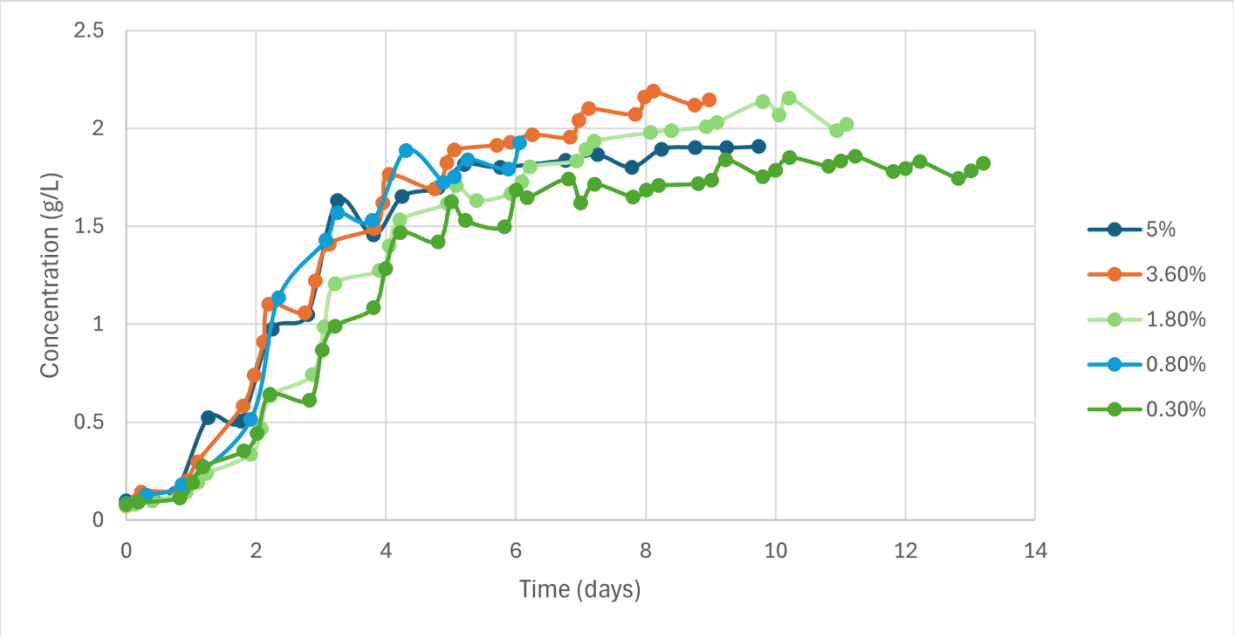

Figure 4.9: Algae growth with 2 LPM gas flow rate at various inlet CO_2 concentrations ranging from 0.3% to 5%. Lines added to reduce confusion due to data density.

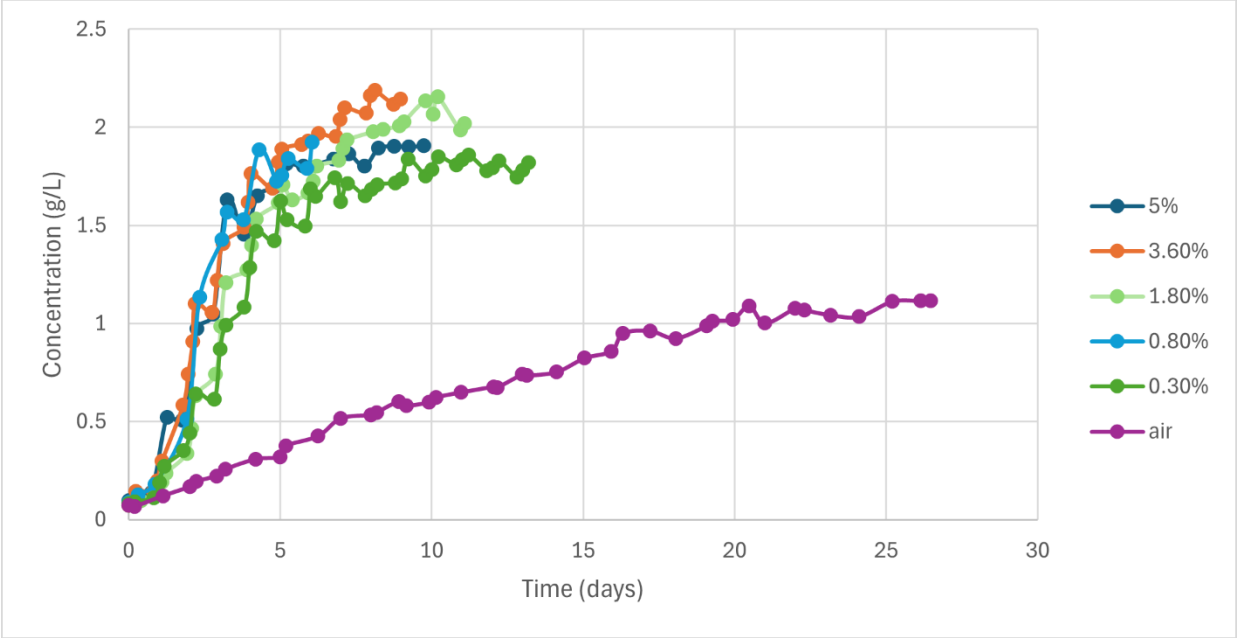

Figure 4.10: Algae growth with 2 LPM gas flow rate at various inlet CO_2 concentrations ranging from 0.3% to 5%. An additional control run is included, growth in pure air (approximately 400 ppm = 0.04 % CO_2). Lines added to reduce confusion due to data density.

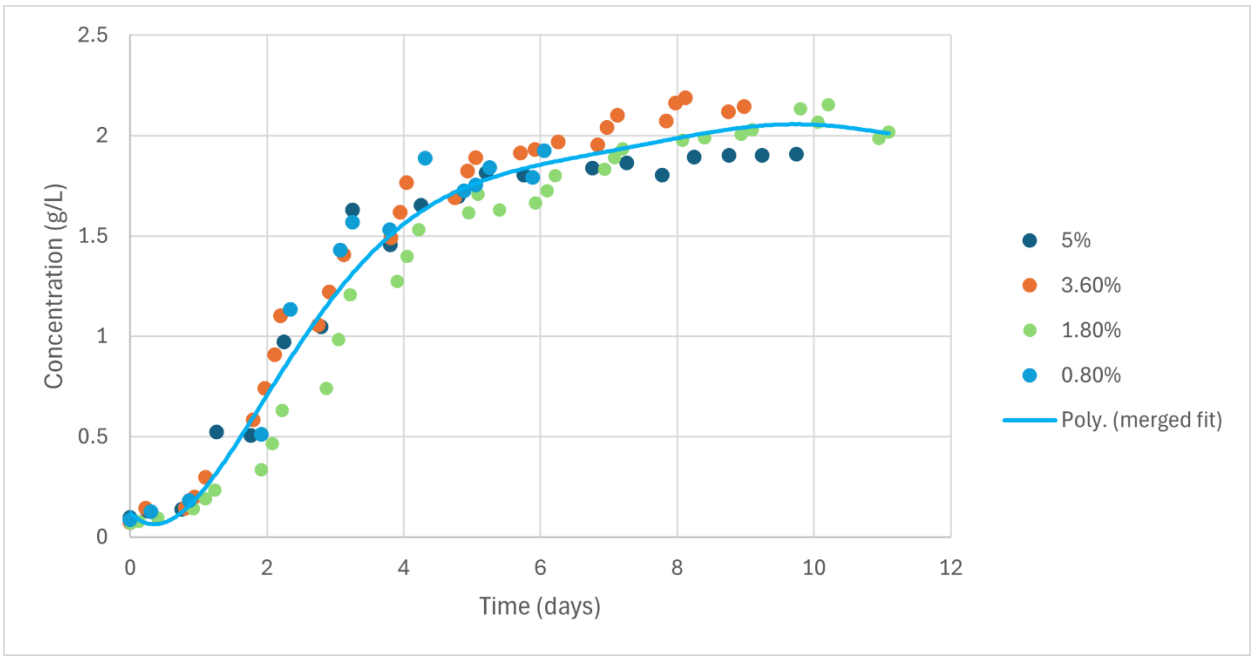

Figure 4.11: Merged growth of excess CO_2 runs including 5%, 3.6%, 1.8%, and 0.8% runs. A 6^{th} order polynomial fit was applied in excel.

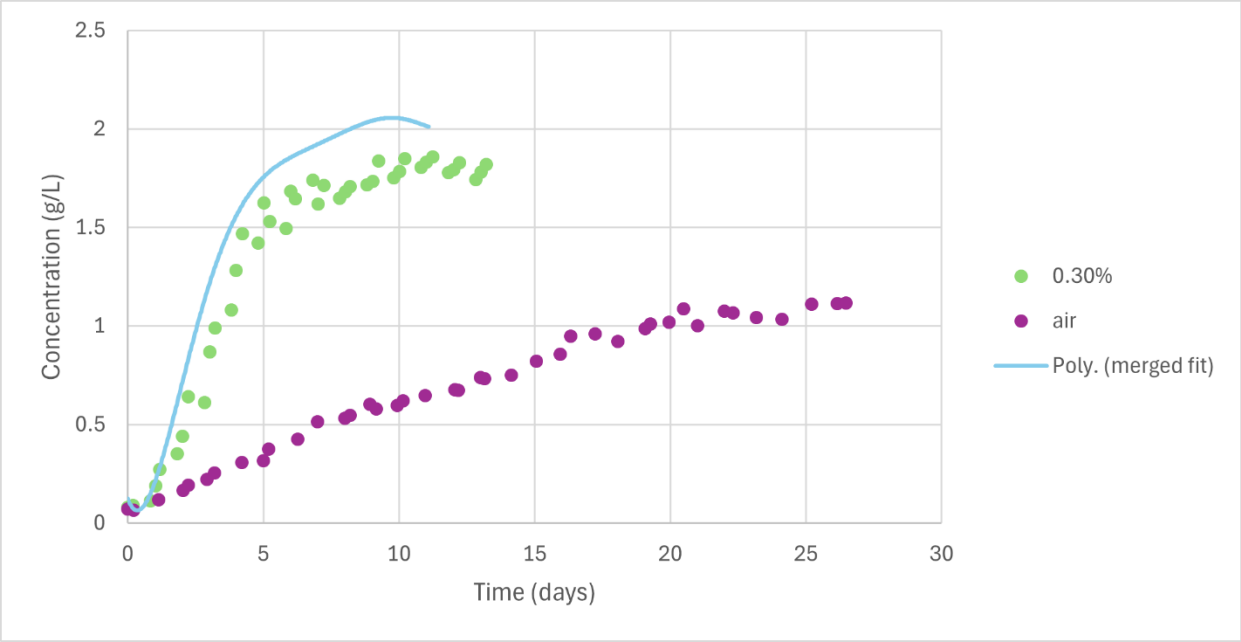

Figure 4.12: Merged growth of excess CO_2 runs with 0.3% and air growth curves.

The pH, dissolved oxygen (DO), and temperature were heavily influenced by whether the lights were on or off. When the lights turned on the temperature, pH, and DO increased. When the lights turn on, the temperature increases due to the heat from the bulbs. Photosynthesis that occurs when the lights turn on increases pH because carbonate is consumed, which is acidic. Oxygen is also released as a product of photosynthesis which is why DO increases as well (13, 39). Looking at the Figures 4.13-4.18 provides insight on how different CO_2 percentages affect these variables.

Although there was some variance in temperature during the experiments it wasn't significant nor was it due to different experimental parameters. This can be seen in Figure 4.13 and Figure 4.14. Controlling temperature to a high level was tricky in these experiments since we relied on the lights to heat the reactor. The temperature regulation device that came with the Bio115 reactor couldn't be used because it blocked light from entering the reactor. On top of that, the gas running through the reactor may have been at different temperatures, which caused small shifts in the reactor's overall temperature. This made it hard to maintain fully consistent

conditions. With that being said, there isn't a large enough difference for this to be greatly significant. The seems to be at most 2-3 degrees Celsius difference between all runs.

Observing the DO data provides some interesting trends. In Figure 4.15, when comparing the CO_2-enriched runs, all of them show a big spike in DO during the first couple of days, followed by a gradual drop. This lines up with the growth data in Figure 4.10, where the steepest slopes in the growth curves happen around day 2, when DO levels were at their highest. Another interesting thing is that the 0.3% CO_2 run has lower average DO than the rest of the CO_2 runs. Also, for the air-only run shown in Figure 4.16, DO production was much lower compared to all of the CO_2-enriched runs. This further supports the idea that higher DO levels are linked to faster growth and lower levels of CO_2 will show decreased DO levels.

The pH seemed to be to have the largest change of the 3 recorded variables. Figure 4.17 show a decent distribution for pH in the CO_2 runs. Like growth, there are small differences but there isn't much correlation other than 0.3% CO_2 having the highest average pH of all the CO_2 runs. These small differences are likely due to experimental error. Looking at Figure 4.18, it shows air run had big differences in pH trends compared to the other runs. First, the air run had a significantly higher pH than the other runs. This isn't surprising as there is relatively no CO_2 to acidify the water. What is surprising is that the pH reaches 10.7. The air-only run also had massive daily swings in pH, changing by 3-2.5 units of pH between night and day. On the other hand, the CO_2-enriched runs had much smaller daily changes, only about 0.1 to 0.2 units of pH. The pH of the runs increased during the day as the algae eats the CO_2. It is likely that in the CO_2-enriched runs the CO_2 is still in decent excess keeping the pH low while in the air run the algae eats all of the little bit of CO_2 available spiking the pH more.

This experiment tested the effect of different CO_2 concentrations on algae growth. The results showed similar growth rates with all CO_2 enriched runs apart from the 0.3% and air run. This suggests that when CO_2 is in excess, other factors such as light or nutrients become the limiting factor. Temperature, dissolved oxygen (DO), and pH varied both daily and between runs. A higher DO was expected for runs with less CO_2 as the growth rate would be slower. This was as both the 0.3% run and the air run had lower DO than the excess CO_2 runs. The pH of the runs was expected to increase as CO_2 was lowered which also held to be true. It is important to note that the lowest percentage setting on the Bioflo115 was 0.3%. Further experimentation needs to

be done to get more of a gradient between the added CO_2 and the air-only run. This can be done by diluting the CO_2 gas that enters the Bioflo115 reactor.

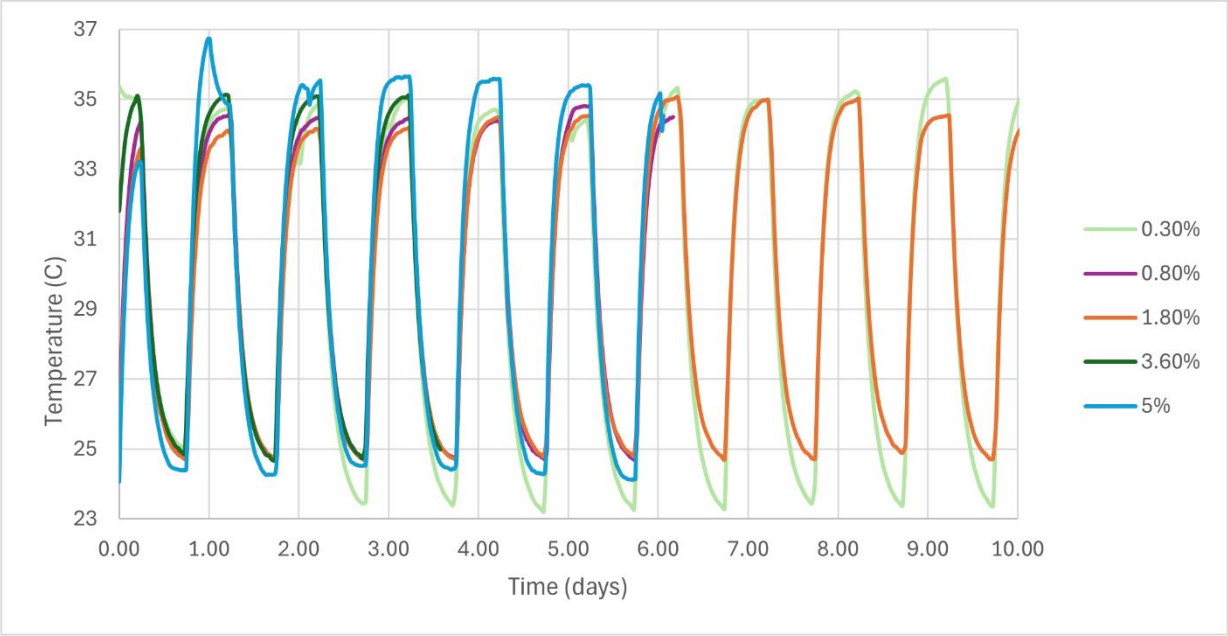

Figure 4.13: Temperature of all runs excluding air, all runs with flowrate of 2 LPM.

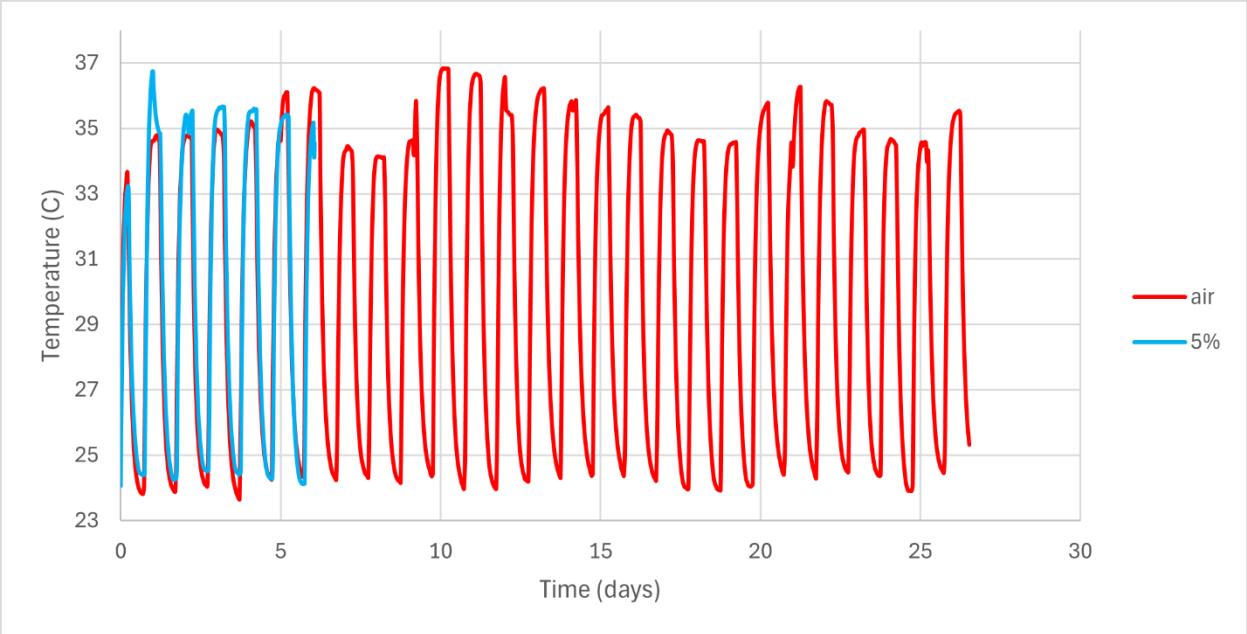

Figure 4.14: Temperature of air and 5% CO_2 run, all runs with flowrate of 2 LPM.

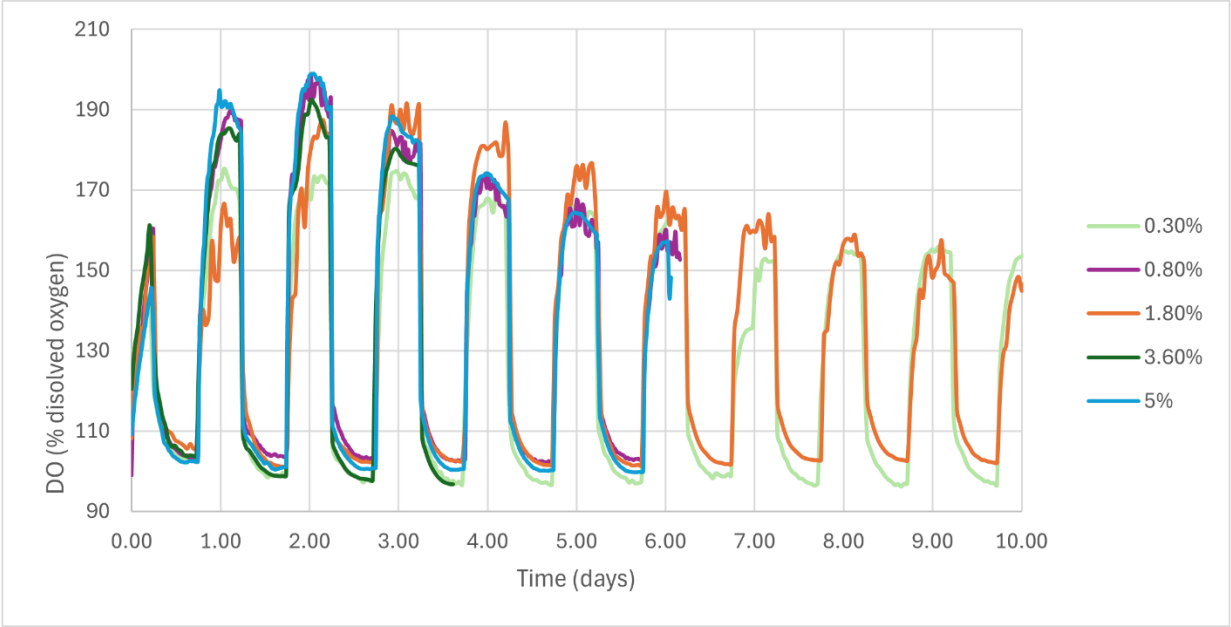

Figure 4.15: DO of all runs excluding air, all runs with flowrate of 2 LPM.

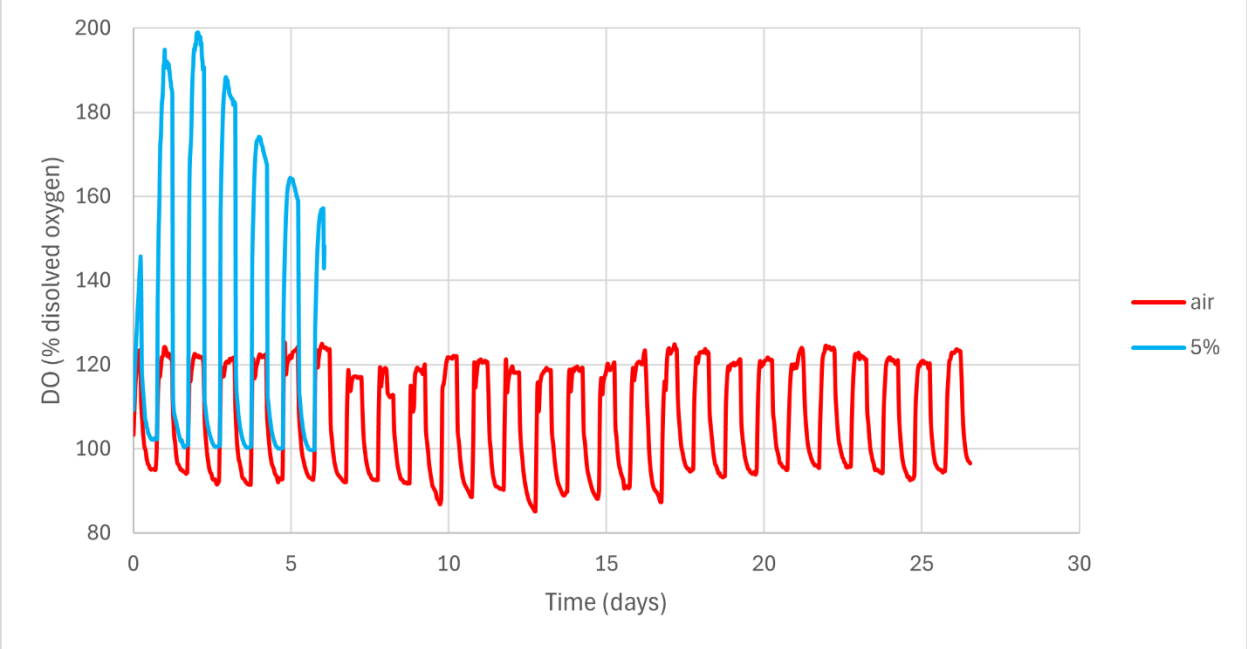

Figure 4.16: DO of air and 5% CO_2 runs, all runs with flowrate of 2 LPM.

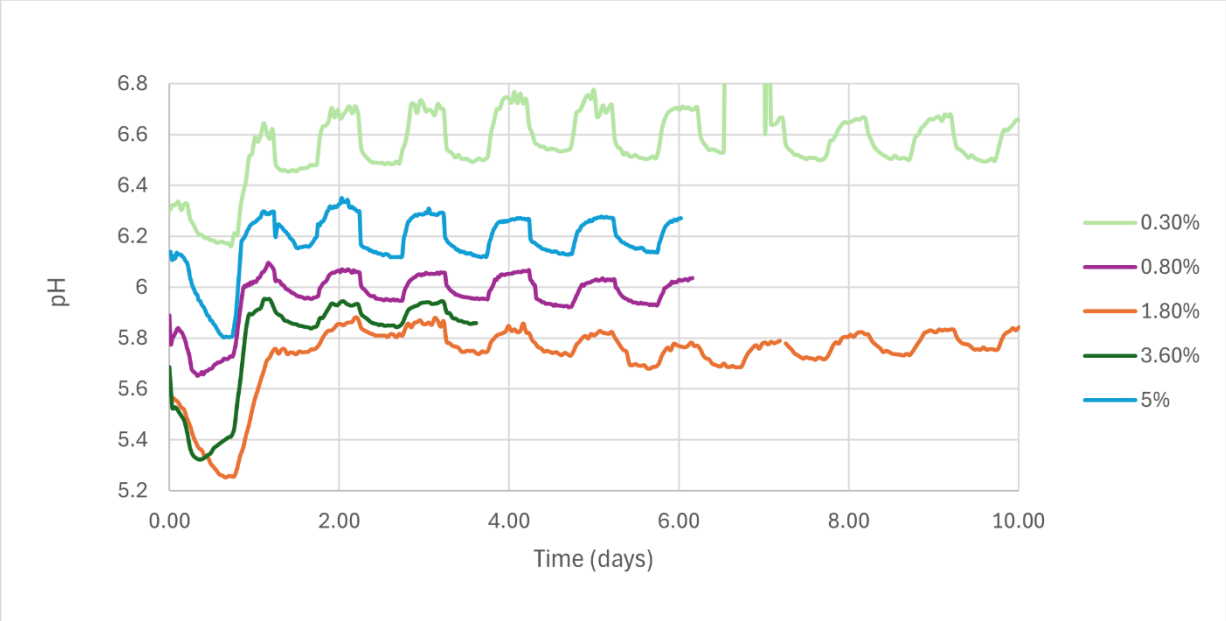

Figure 4.17: pH of all runs excluding air, all runs with flowrate of 2 LPM.

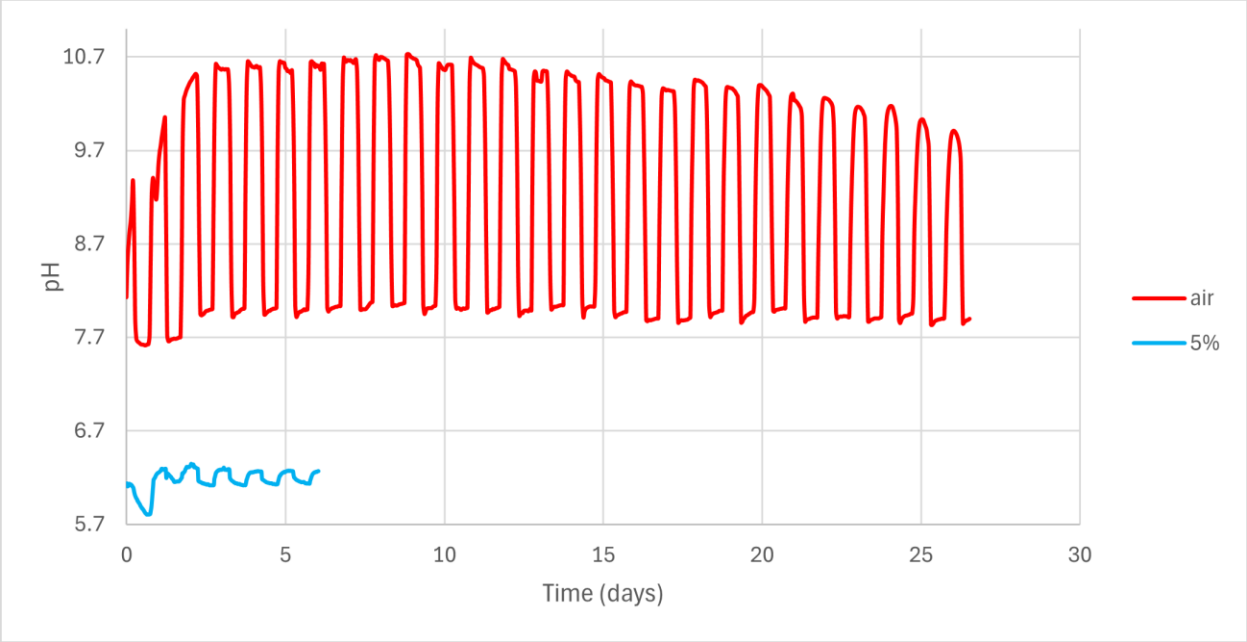

Figure 4.18: pH of air and 5% CO_2 runs, all runs with flowrate of 2 LPM.

4.2 Model Results and Discussion

Modeling for algae growth in the laboratory-scale photobioreactor was done by using Verhulst kinetics, where (X) is algae biomass concentration, (X_m) is the maximum biomass achieved after full growth, and (μ) is the growth rate as seen in equation 8 (19).

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_m}\right)$$

(8)

The growth rate (μ) is a function of all parameters that affect growth: CO_2 concentration, absorbed light intensity, nutrients concentrations, temperature, presence of inhibitors. During a growth period in which the growth rate can be considered constant, integration of the ODE using $X = X_0$ at $t = 0$ leads to equation 9.

$$X = \frac{X_0 X_m e^{\mu t}}{X_m - X_0(1 - e^{\mu t})}$$

(9)

This solution has a sigmoid shape, as shown in Figure 4.19.

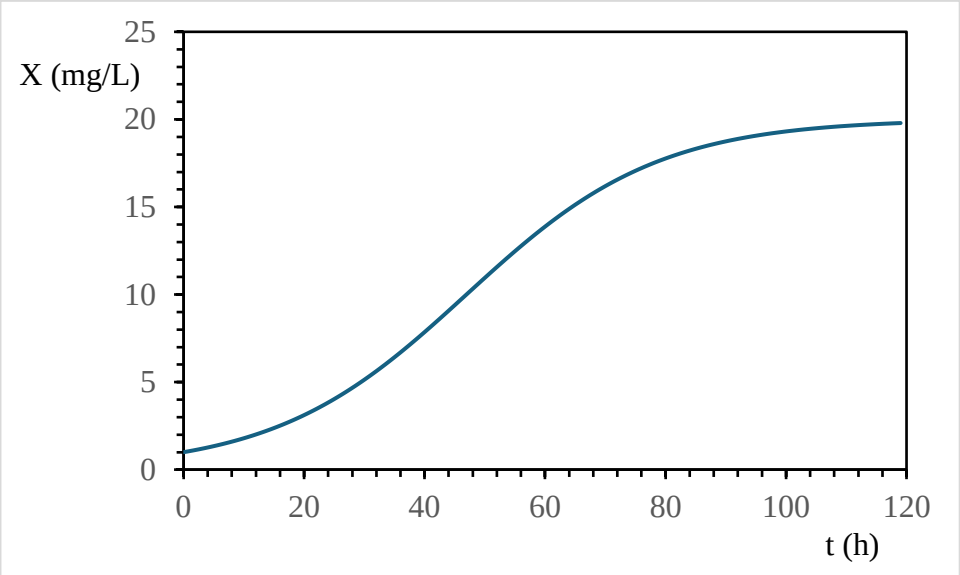

Figure 4.19: Sample plot of equation 9, the derivation of equation 8 using as parameters: $X_0 = 1$ mg/L, $X_m = 20$ mg/L, $m = 0.0627$ h^{-1}.

Growth is not continuous since light is only available for 12 hours. It is assumed with this model that there is neither growth nor death during the dark phase. Equation 9 can be applied for every light period with a starting concentration being that of the previous day's final biomass concentration. In this work, experimental data was fitted to equation 9 by obtaining X_0 and X_m from the experimental growth curves and using μ as a single adjustable parameter.

Using Excel, the time, experimental data, and the model data are paired next to each other. The model equation is only applied during the light phase while the dark phase remains at the last value of the previous light cycle. A least squares fit value is found between the model and the experimental values. By using solver, the least square fit value was minimized by changing the model constant μ. It is important to note the parameter value was found for OD data, then graphs were converted to concentration.

After the data was fit and the parameters values for each experimental run were found, some trends can be seen. All excess CO_2 runs, being 5% 3.6%, 1.8% and 0.8% have roughly the same parameter values seen in Figures 4.20-4.23. The parameter values were all close, ranging from 2.79-2.19. The merged fit plot, being Figure 4.11 containing the average of these values was modeled as seen in Figure 4.24 giving a parameter value of 2.58. The differences truly start to appear when looking at the 0.3% and air-only parameter values being 1.61 and 0.609, respectively as seen in Figures 4.25 and 4.26. This shows that as growth decreases the parameter value will decrease.

Another important note is the general decrease in X_m with lower CO_2 percentage as seen in Table 4.1. This is not surprising as more CO_2 will not only allow for a higher growth rate, but excess will allow for greater cell production compared to cell death when it hits the ceiling, X_m. It can also be seen that air has the largest area difference with a value of 5.06. This means it has the poorest model fit, which can also be seen in Figure 4.26 when compared to the other plots. Overall, the model equation fits the data well where CO_2 not limiting. Although the 0.3% run has some CO_2 limitation which is why it fits well, it is not enough for there to be a large enough difference. In the future if more experiments are done below 0.3%, one should expect the fit quality to decrease more as concentration decreases till it reaches the air only run which had a poor fit.

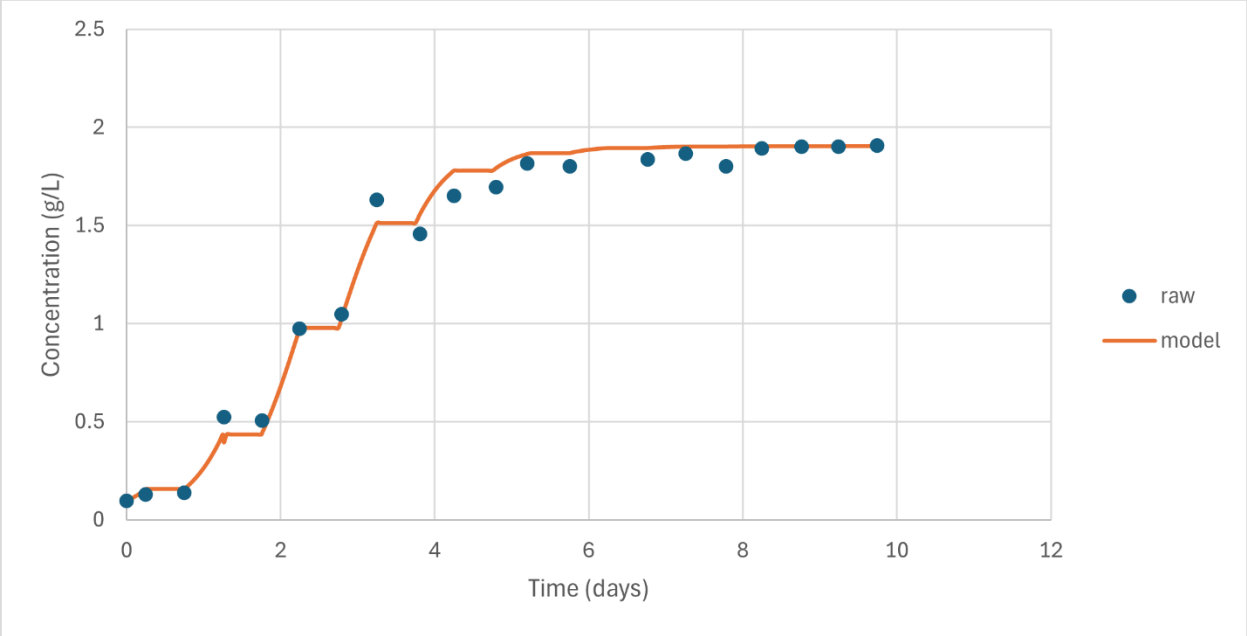

Figure 4.20: 5% CO_2 run fitted to model with constant value of 2.61

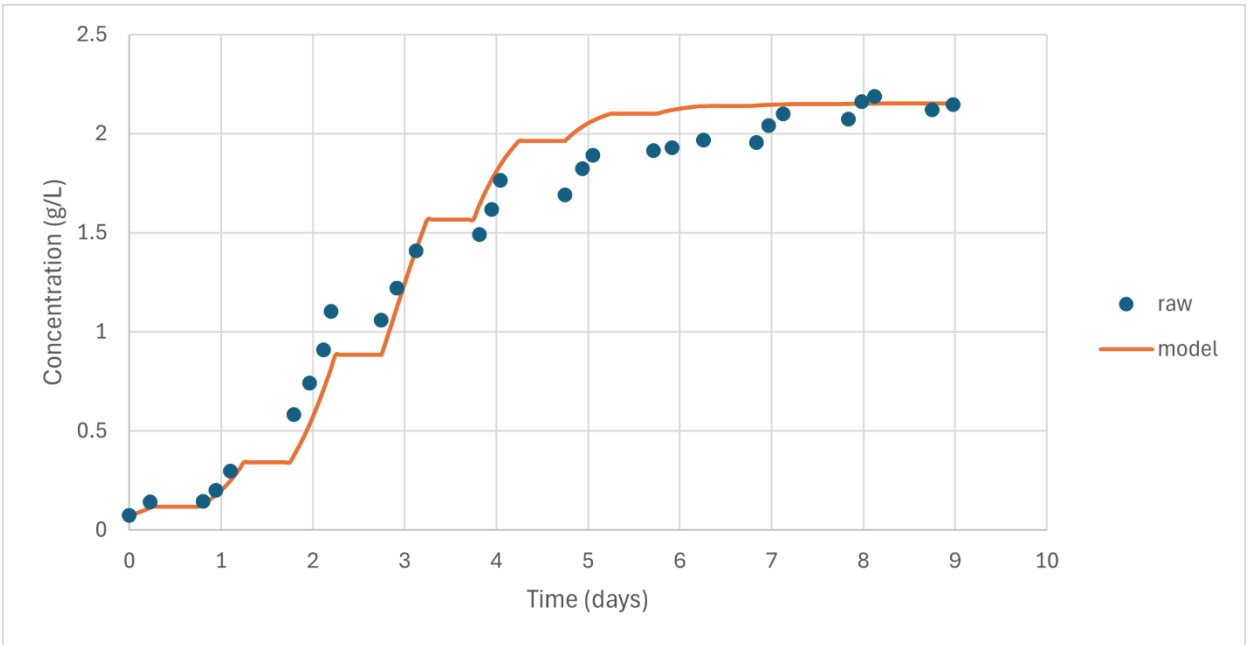

Figure 4.21: 3.6% CO_2 run fitted to model with constant value of 2.71

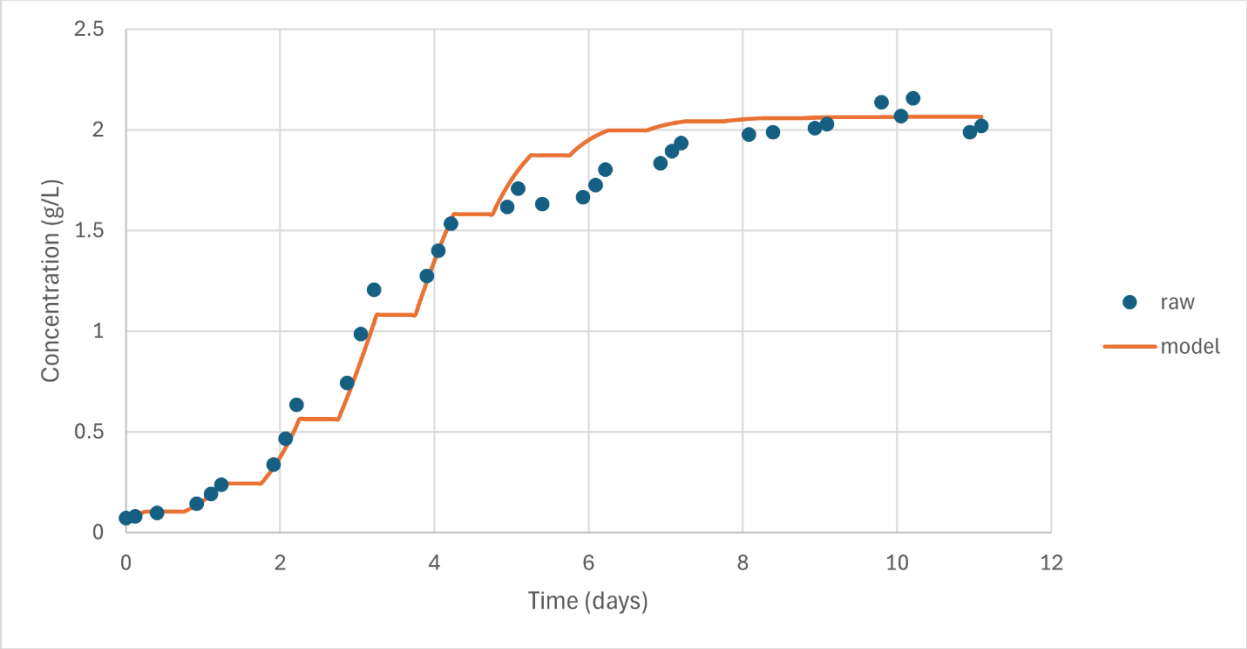

Figure 4.22: 1.8% CO_2 run fitted to model with constant value of 2.19

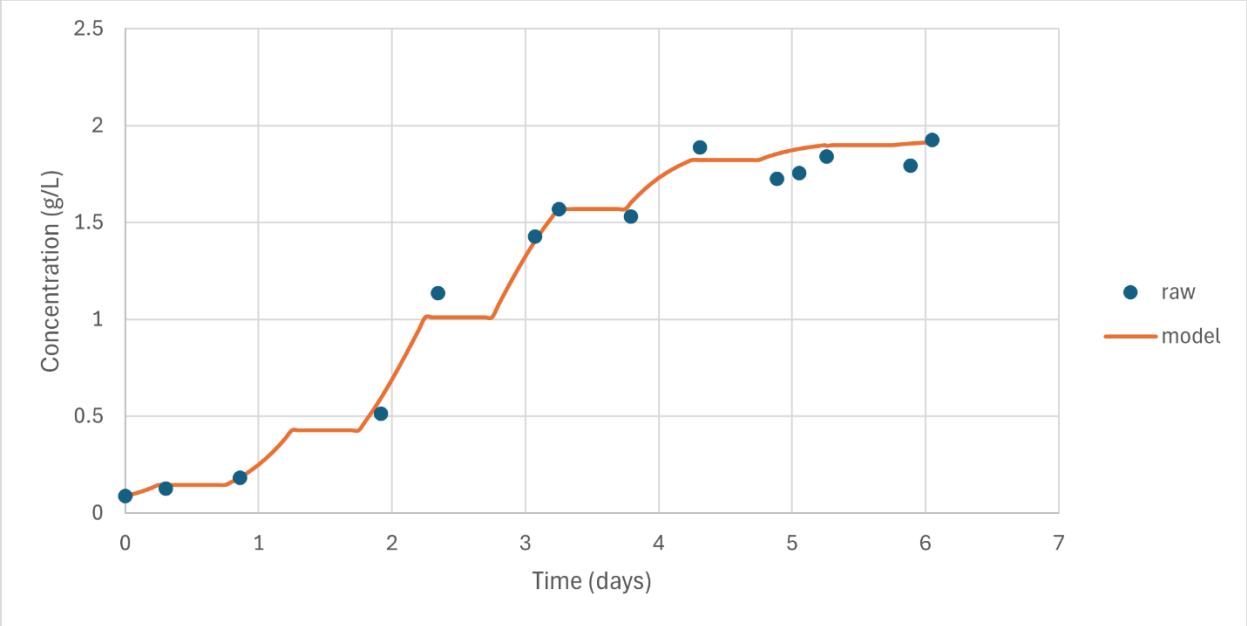

Figure 4.23: 0.8% CO_2 run fitted to model with constant value of 2.78

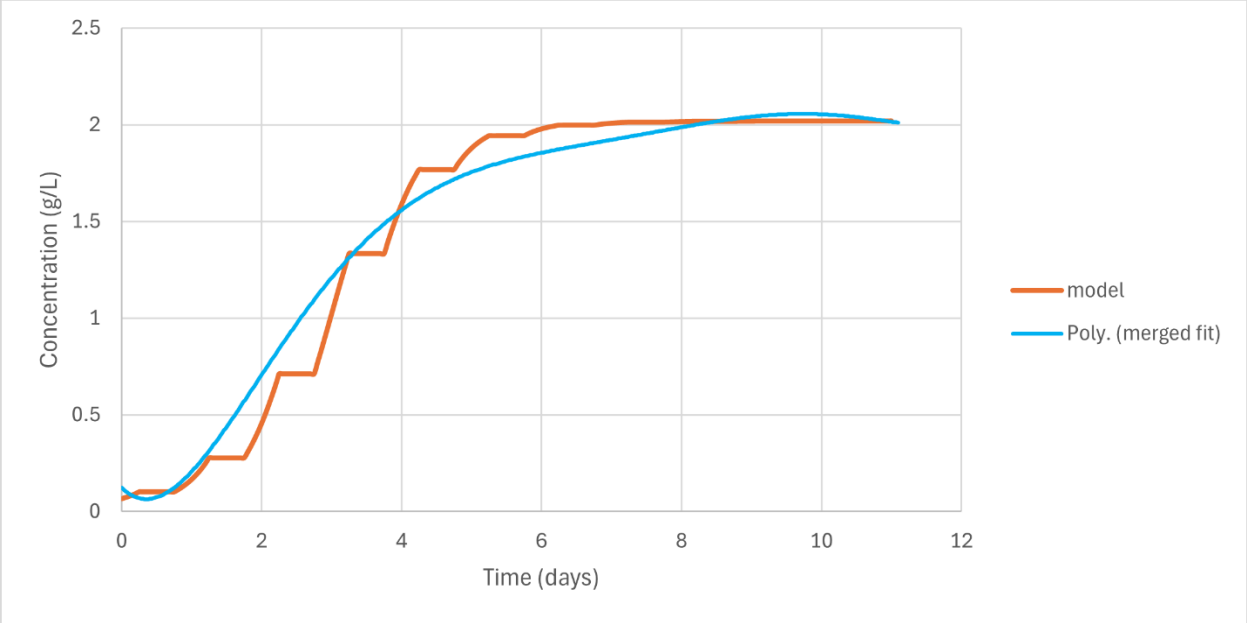

Figure 4.24: Merged fit(5%, 3.6%, 1.8%, 0.8%) fitted to model with constant value of 2.58

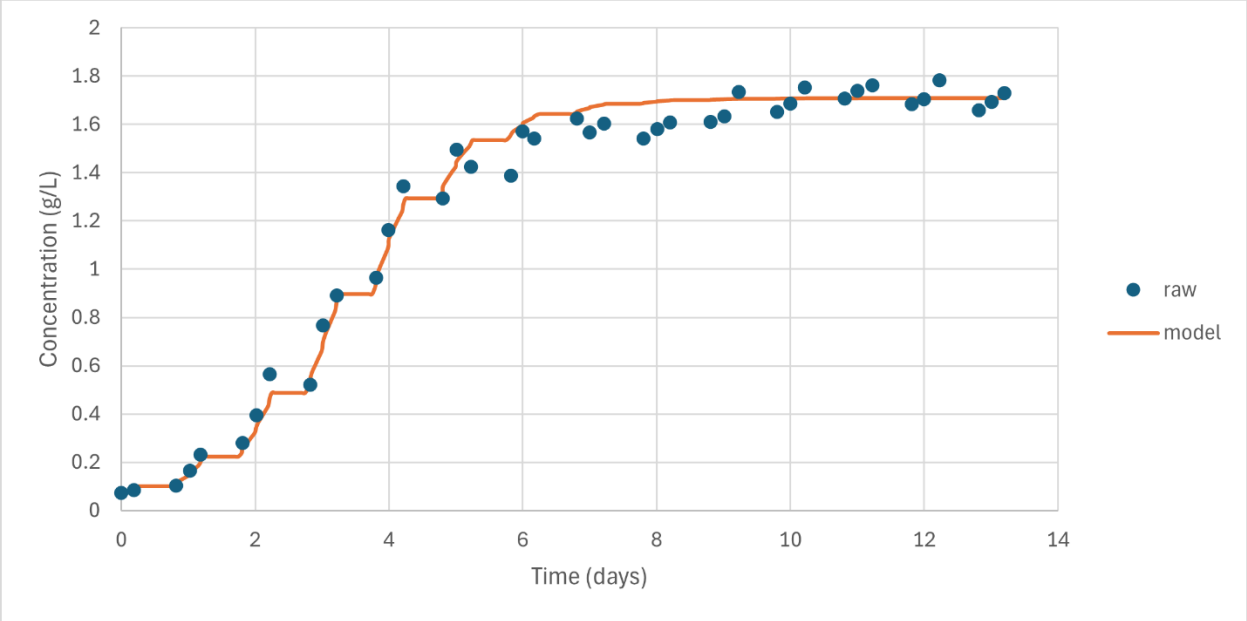

Figure 4.25: 0.3% CO_2 run fitted to model with constant value of 1.61

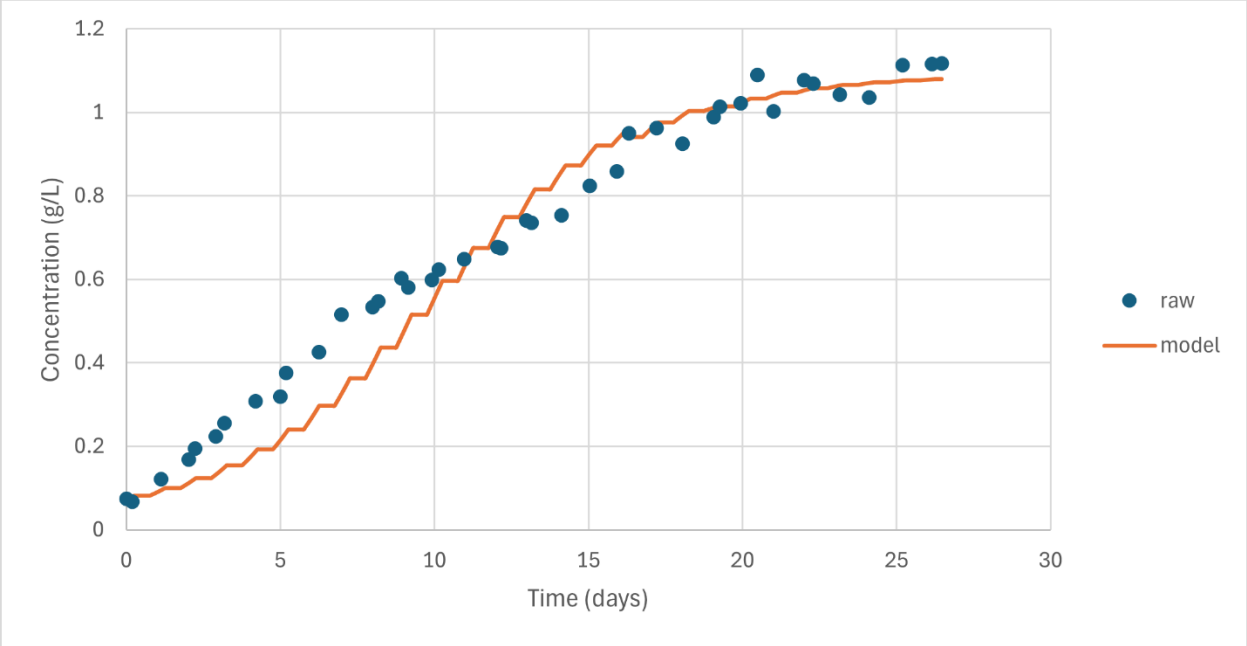

Figure 4.26: Air only run fitted to model with constant value of 0.609

Table 4.1-Modeling data

Percentage CO_2	Parameter Value	Starting OD(Xo)	Final OD(Xmax)	Area difference
5%	2.61	0.213	5.649	1.6108
3.6%	2.71	0.142	6.401	3.2268
1.8%	2.19	0.139	6.137	2.8512
0.8%	2.78	0.183	5.713	1.0771
0.3%	1.61	0.143	5.063	2.1742
air	0.609	0.144	3.200	5.0600
Merged Fit	2.58	0.123	6.0	/

5. Conclusion:

The experiments demonstrated the critical role of CO_2 on algae growth. The comparison between always-on and light-phase-only CO_2 flow showed minimal differences in growth, supporting the hypothesis that CO_2 availability during the dark phase has negligible impact. In the reducing air flow experiment, reducing air flow to 1 LPM improved CO_2 retention but led to lower pH levels, ultimately resulting in declining growth. This suggests that while reducing air flow may enhance CO_2 absorption, it must be balanced to maintain conditions suitable for algae. The comparison between CO_2-enriched runs and the air-only run showed a lack of added CO_2 significantly decreases growth. However, among the runs where CO_2 was in excess such as 5%, 3.5%, 1.8% and 0.8% CO_2, growth rates were similar showing excess CO_2 has little effect on growth. Growth started to decrease in the 0.3% CO_2 run showing that above 0.3% CO_2 is in excess. A one parameter model was fit to the data however, only runs with added CO_2 showed a good fit. In the future, more research needs to be conducted to strengthen these results. Experiments lowering CO_2 percentage while maintaining 2 LPM total flow will allow for a more complete gradient of growth. A more complex model will likely be needed to fit the data as CO_2% drops and the fit becomes worse. Once that is complete, experiments lowering the air flow can be done to find the best combination of air and CO_2 flow. Together, these results emphasize the importance of CO_2 and air flow volume and timing to optimize growth without introducing adverse effects.

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