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EVALUATING ALGAL GROWTH AT DIFFERENT TEMPERATURES

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EVALUATING ALGAL GROWTH AT DIFFERENT TEMPERATURES

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Biosystems and Agricultural Engineering in
the College of Engineering at the University of Kentucky

By

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Lexington, Kentucky

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ABSTRACT OF THESIS

EVALUATING ALGAL GROWTH AT DIFFERENT TEMPERATURES

In recent years, there has been a concern for the amount of carbon dioxide released into the atmosphere and how it will be captured. One way to capture carbon dioxide is with algae. In this study, algae's growth was measured at different temperatures. The first part of the study was to grow *Scenedesmus* and *Chlorella* with M8 or urea growth media at a temperature of 25, 30 or 35ºC. It was found that 30ºC had the best growth rates for both algae. The second part studied Scenedesmus growth with urea, more in-depth, and found the optimum growth temperature to be 27.5ºC with a growth rate of 0.29 1/hr. The last part of the study was a heat transfer model which predicted the temperature of a greenhouse and an outdoor unit. The model could also predict the growth rate of the algae and the temperature if flue gas is mixed in with the algae.

KEYWORDS: algae, CO_2 mitigation, *Chlorella vulgaris*, *Scenedesmus*, temperature

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EVALUATING ALGAL GROWTH AT DIFFERENT TEMPERATURES

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To Team Algae:

(Dr. Czarena Crofcheck, Aubrey Shea, Sarah Short, Tabitha Graham,
Ian Colten, and Maya Bentley)

Without them algae would just be pond scum.

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CHAPTER 1 : INTRODUCTION

For centuries, flue gas from coal-fired power plants has been a problem for our atmosphere and our water supply (i.e. acid rain). At one point in our history, toxic chemicals in flue gas were allowed to leak into the environment without regulations. As people took notice of the pollution, SOx scrubbers, precipitators, low NOx burners, and other regulator devices were put in place to control pollutants. Today, there is an elevated concern for the addition of carbon dioxide into the environment and how the additional carbon dioxide might affect the environment.

There have been numerous studies that claim carbon dioxide is the cause of global warming (Goreau, 1990; Rogers, 1990). In an effort to reduce this effect, several carbon mitigation strategies have been considered (Herzog, 2009). One of the most promising strategies is using algae to mitigate the amount of carbon dioxide emitted to the atmosphere. Algae can achieve carbon fixation at a faster rate than most other plants and can be used for several products, such as biofuels, pharmaceuticals, as well as in agriculture. In addition, algae have the ability to tolerate flue gas pollutants such as sulfur oxide and nitrogen oxide.

Before algae cultivation systems can be used for CO_2 mitigation, several obstacles need to be overcome. One challenge will be keeping the algae cultivation system at a constant temperature that optimizes growth and CO_2 mitigation rate. By closely evaluating the relationship between the temperature of the incoming flue gas (with or without pre-conditioning), the optional greenhouse, and the photobioreactor (PBR) to the growth of the algae, important heat and mass transfer decisions can be made about the best way to run the integrated system.

1.1 FLUE GAS

Flue gas is a mixture of gases at approximately 140°F (60°C) released from coal-fired power plant smoke stacks. The combustion of coal is mainly used for the generation of steam, which then can produce electricity or heat. In Kentucky, coal is used for both heat and electricity. About 93% of Kentucky's electricity is generated from coal (American Coalition for Clean Coal Electricity, 2010).

Typical flue gas derived from coal combustion is comprised of nitrogen oxides (NOx), sulfur oxides (SOx), particulates, carbon monoxide, hydrochloric acid, water, and carbon dioxide (CO_2). NOx are primarily nitric oxide (NO), which oxidizes into nitrogen dioxide (NO_2) when introduced into the environment resulting in acid rain (National Energy Technology Laboratory, 2009). To avoid acid rain, low NOx burner and catalytic reduction are used. After treatment the typical amount of NOx released from coal is approximately 40-100 ppmv (Chen et al., 2010).

The sulfur content of flue gas is made up of sulfur dioxide (SO_2) and sulfur trioxide (SO_3). If sulfur dioxide combines with water vapor, it will form dilute acid (National Energy Technology Laboratory, 2009), resulting in acid rain. To prevent acid rain, scrubbers are used to capture about 90% of sulfur dioxide (National Energy Technology Laboratory, 2009).

During the combustion of coal, particulate matter and ash can be found in the flue gas. The particulate matter composition will depend on the composition of the coal; the bulk of coal is made up of sulfur, sodium, and potassium (Chen et al., 2010). There is a range of ways of collecting the particulate matter and ash, but if the particular matter escapes it will cause health problems such as lung disease and esophageal cancer (Finkelman et al., 2002).

Typically carbon dioxide levels are 10-15% of the flue gas released from the combustion of coal (Lee et al., 2000). While there are currently no statutory regulations limiting carbon dioxide emissions, many companies are seeking for methods to voluntarily reduce CO_2 emissions in order to meet corporate environmental performance goals.

1.2 Current Research on Capturing CO_2

There are several proposed strategies for CO_2 capture, including monoethanolamine (MEA) absorptions process, underground carbon dioxide storage, and capturing carbon dioxide with algae. Using MEA scrubbers is proven technology, but the use of these scrubbers on a large scale is cost prohibitive. The underground storage and algae strategies are still being researched (Keller et al., 2008). Some possibilities show

potential of success; however, it is unknown what the long term effects and the cost will be of these methods (Keller et al., 2008).

A scrubber or amine-based scrubber with monoethanolamine (MEA) absorption process can be used to capture carbon dioxide. It has the ability to capture 96% of CO_2 from coal combustion. The system works by first removing carbon dioxide by a unit using MEA from the other flue gas products such as water and NOx. The carbon dioxide is absorbed by MEA and sent to the stripper. The stripper will heat the MEA solution to release the CO_2. The lean MEA solution will then be recycled. The carbon dioxide will be dehydrated and stored (Herzog, 2009). The disadvantages with this system are the power needed for compressing the carbon dioxide and once the carbon dioxide is compressed it is a waste product that needs to be properly disposed (Haslbeck, 2002).

Underground carbon dioxide storage is an option to decrease carbon dioxide emissions. Carbon dioxide is stored in the pores of oil mined land. Currently, this strategy has not been tested on a large scale but there are plans to implement underground storage on a large scale. There are uncertainties of the cost and what effect it might have on the environment (Rankin, 2009).

Another sustainable option is capturing carbon dioxide with algae (Kurano et al., 1995; Ogbonna et al., 1997). Algae use carbon dioxide as a carbon source and the energy from the sun to produce biomass and oxygen. Along with producing biomass and oxygen, it also produces complex organic compounds from simple inorganic compounds, such as urea. This type of research has not been implicated on a large scale but procedures have been established (although not optimized) for processing, recycling, and disposing of algae once it converts the carbon dioxide.

1.3 Selection of Algae

There are many types of algae: blue green, green, and red. Algae can be both harmful and helpful. Algae can be grown in different environments, high or low temperatures, with or without light, in fresh water or in salt water. In order to find the best way to cultivate algae and maximize carbon dioxide consumption, several parameters need to be

considered, including algae strain, light requirements, temperature requirements, and media requirements.

1.3.1 Algae Strains

In Kentucky, the majority of the water is fresh; if there is salt then it is considered a contamination (Waller, 2005). There are about 40,598 square miles of ground and surface water that make up Kentucky's water resource and about 4 billion gallons are used every day (Dinger, 1997). For a large-scale algal system in Kentucky, the algae should be fresh water, for example *Chlorella* or *Scenedesmus.*

Chlorella vulgaris shows great potential for capturing carbon dioxide. It will grow at a fast rate (0.6 g/L day) and tolerate 10-15% carbon dioxide (Lee et al., 2000). *Chlorella vulgaris* can also grow in extreme environments, high temperatures of 30-35°C (Converti et al., 2009) and acidic environments such as a pH of 3 (Mayo, 1997). When it comes to flue gas, it can tolerate up to 200 ppm of NOx and 50 ppm of SOx (Lee et al., 2000). Once the algae is used for carbon dioxide consumption, it can be used in a secondary process or product such as animal feed. For secondary processes, *Chlorella vulgaris* has a high percent of proteins, minerals, and vitamins (Lee et al., 2001).

In sewage treatment plants, *Scenedesmus* takes up CO_2 and provides oxygen to bacteria as it breaks down organic matter (Encyclopedia Britannica Online, 2010). Hence, *Scenedesmus* is an attractive candidate for CO_2 mitigation with flue gas because it can tolerate being grown in wastewater. The rate of daily carbon dioxide consumption is 28.08% at a 6% carbon dioxide level (de Morais and Costa, 2007). The temperature in which *Scenedesmus* will grow ranges from 10 to 40°C (Christov et al., 2001).

Currently, *Spirulina* is widely used in food applications and has the potential to consume carbon dioxide. Its carbon fixation rate is 318.6 mg_{CO2}/ L day at 5% CO_2 (Sydney et al., 2010). It has the ability grow in temperatures ranging from 20 to 40°C, but the temperature will affect the protein and carbohydrate levels (Oliveira et al., 1999). The composition of *Spirulina* is mostly protein (Sydney et al., 2010). It also has the potential to grow on manure, capture carbon dioxide and produce biogas (Shelef et al., 1980). In

this study, *Spirulina* will not be considered due to the scope of the current project, but it may be considered in future work.

The overarching algae CO_2 mitigation project focuses on *Chlorella* and *Scenedesmus;* this specific work will focus on the same two algal species. *Chlorella vulgaris* was selected because it is used often in research and has been shown to grow rapidly and easily, even in presences of elevated CO_2 levels (Lv et al., 2010). *Scenedesmus sp*. has also been shown to grow easily and rapidly; this particular species was collected locally, illustrating the fact that it grows well in the Kentucky climate. It is unknown how *Scenedesmus sp.* will react at different temperatures, due to conflicting studies. One study says its optimum temperature is 37°C (Martinez et al., 1999) and other study says that it does well at 30°C (Christov et al., 2001).

1.3.2 Autotrophic versus Heterotrophic

Algae can grow either heterotrophically (without light) or autotrophically (with light). Autotrophic growth uses simple inorganic compounds and light energy to produce complex organic compounds, including biomass. With autotrophic growth an increase in light intensity can influence the overall growth rate. In one experiment, the light was increased from 163 µmol/m^2 s to 310 µmol/m^2 s and the growth rate increased from 2 g/L d to 4 g/L d (Ogbonna et al., 1997). More importantly, autotrophic growth results in the removal of carbon dioxide from the environment.

Heterotrophic growth is used in fermentation processes to produces nutraceutical or health food (Apt and Behrens, 1999). This type of growth uses organic carbon (i.e., carbohydrates) to produce carbon dioxide, a simple inorganic compound, and does not require energy from the sun. Heterotrophic and autrophic growth can be combined into one system to increase the production of biomass. In one study, *Chlorella* was used to grow heterotrophically and autrophically to increase the biomass and the carbon dioxide produced from the heterotrophic phase was used in the autotrophic phase. (Ogbonna et al., 1997). The disadvantages associated with heterotrophic growth are that carbon dioxide is produced, nutrient media cost more due to the addition of a carbon source, and

there is a higher risk of a bacterial contamination, since bacteria can also grow in the presence of a carbohydrate carbon source (Feofilova et al., 2010).

1.3.3 Light Requirements

Light is an essential energy source in autotrophic growth and is required for photosynthetic activity. Studies have shown that green algae grow better in blue and red light because they contain chlorophyll *a* and *b* which are major light harvesting pigments that are sensitive to these wavelengths. In one study, for several cell concentrations of *Chlorella vulgaris* the absorption of light was in the 400-500 nm (blue) and 625-675 nm (red) range while the rest of the light was scattered amongst the cells (Yun and Park, 2001). In another study, it was found that in red light (625-675 nm) *Scenedesmus obliquus* increased significantly in cell volume and the division of nuclei occurred earlier (Cepak et al., 2006).

1.3.4 Temperature Requirements

Temperature is an important element for growing algae. It strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates for every species of algae. It is know that the growth rate will increase with the increase in temperature up to its optimum and once it reaches its optimum, growth rate will decrease drastically with the increase in temperature.

For *Chlorella vulgaris*, the optimum temperature ranges from 25 to 30°C. Chinnasamy et al. (2009) reported an increase in biomass content and in chlorophyll content at elevated carbon dioxide (6%) and optimum temperature (30°C). Converti et al. (2009) reported that lipids would increase from 5.9 to 14.7% when the temperature decreased from 30°C to 25°C; at temperatures over 38°C oleic acid, a monounsaturated omega-9 fatty acid, production increased. Bajguz (2009) noted that under heat stress or heat shock the algal protein content will decrease and will produce abscisic acid (ABA), a stress hormone. If the stress hormone is produced, it is considered a key factor in controlling downstream responses such as growth and gene expressions. Mayo (1997) found that when raising the temperature above 40^{o}C, *Chlorella vulgaris* was less resistant to acidic pH than when it was grown at 35^{o}C or lower temperatures.

The optimum temperature to grow *Scenedesmus sp.* is between 20-40°C (Sanchez et al., 2008). Christov et al. (2001) studied *Scenedesmus sp.* at temperatures of 15 to 36°C and found at lower temperatures the chlorophyll and protein levels were reduced, while levels of carotenoids, saccharides, and lipid were increased. They also observed an increase of 30% of the sugars and lipids at extreme temperatures (36°C). Powell et al. (2008) studied how temperature affected the phosphorus content of wastewater using algae; they found phosphorus content in biomass is higher at higher temperatures (25°C) than at lower temperatures. Demon et al. (1989) observed the effect of temperature (0- 22°C) on the uptake of arsenic, cadmium, copper, lanthanum, tungsten, and zinc; they noticed an increase in arsenic, tungsten, zinc and cadmium uptake as the temperature increased.

1.4 PAST USES OF ALGAE

For large-scale production, wastewater treatment has been using algae for years (de la Noue et al., 1992). The algae are used to remove nitrogen and phosphorus, while providing oxygen to bacteria (Rai and Gaur, 2001). Algae can also remove heavy metals such as cadmium zinc, nickel, and lead (Mehta and Gaur, 2005). This process is considered environmentally sound, recycles nutrients more efficiently, does not lead to and secondary pollution, produces biomass that can be harvested (unless the algae removes heavy metals), and produces oxygen (de la Noue et al., 1992).

Another large-scale production of algae is food production. It can be used for human consumption or animal consumption. Algae can contain high amounts of protein β-carotin, and omega-3 (Varfolomeev and Wasserman, 2010). Algae improved immune reaction and reproduction of animals, since they are a good source of vitamins, minerals and fatty acids (Varfolomeev and Wasserman, 2010). It can also help with stomach ulcers and wounds. Algae show great potential for diabetes, cancer and AIDS treatment (Holdt and Kraan, 2010).

1.5 RESEARCH OBJECTIVES

Kentucky derives more than 90% of its electricity from traditional coal-fired power plants. Due to this high dependence on coal, the Commonwealth has a large economic exposure should federal regulations seek to limit carbon dioxide emissions into the

atmosphere. The commonwealth is actively pursuing technologies to reduce carbon dioxide emissions from energy production. The overarching project objective is to determine the feasibility of using algae to mitigate carbon dioxide emissions effectively and efficiently. The objectives for this specific project focus on the role of temperature in the overall process. The project objectives are to:

- Determine how temperature (25, 30, and 35^{o}C) affects the growth of *Scenedesmus* and *Chlorella*. Along with testing the temperature, two different media formulas (M8 and urea) were tested to determine which one enhances the growth rate. Each experiment was done over 5 days, where sampling was taken every 24 hours (n=3). At each sampling, dry weight and pH were measured and the resulting growth rates were calculated for statistical comparison.

- Develop a model to relate the temperature of the greenhouse (or outside of a greenhouse) and to incorporate into the model the temperature of the PBR system with the addition of flue gas to the temperature of the algae photobioreactors. This model could then be used to determine a temperature control strategy involving either injecting the pre-conditioned flue gas directly into the algae culture system or using the heat from flue gas to heat up the greenhouse.

CHAPTER 2 : MATERIALS AND METHODS

2.1 ALGAE CULTURE AND MEDIA PREPARATION

The two algae strains selected were *Chlorella vulgaris* and *Scenedesmus* spp. *Chlorella vulgaris* was purchased from Carolina Biological Supply Company, Burlington, NC. *Scenedesmus* spp. was purchased from UTEX The Culture Collection of Algae (#72, Austin, TX). The pre-cultures were grown at 3% CO_2, room temperature and with a 16 hour light/8 hour dark cycle (Sylvania cool white, model no. FO32/735/ECO). Each of the algae starter cultures was grown on different media, *Chlorella vulgaris* was grown on M8 media (Table 2.1) and *Scenedesmus* was grown on urea media (Table 2.1) at room temperature, about 25°C.

The flask cultures were inoculated with a concentration of about 0.01 g/L. Three sets of flasks were grown at a cool (ranging from 15 to 32.5°C), medium (ranging from 20 to 35°C), or hot (ranging from 22.5 to 37.5°C) temperatures. The light source was four warm fluorescent bulbs (32W Philips) and two cool fluorescent bulbs (30 Sylvania), which were on a 16 hour light/ 8 hour night cycle. The cultures were supplied with an air mixture of 98% house air and 2% carbon dioxide.

Table 2.1. Growth medium composition.

	Concentration (g/L)	
Compound	M8	Urea
Urea		0.55
KNO_3	0.75	
KH_2PO_4	0.185	0.1185
$NaHPO_4$	0.065	
$CaCl_2.2H_2O$	0.00325	0.055
$FeSO_4.7H_2O$	0.0325	
$MgSO_4.7H_2O$	0.1	0.109
Na.EDTA.Fe	0.01	0.02

2.2 EXPERIMENTAL SET UP

In order to test three different temperatures, a thermogradient table was used. The table was constructed out of a 27 in x 27 in x 0.5 in (68.58 cm x 68.58 cm x 1.27 cm) aluminum sheet with a series of aluminum tubing welded underneath. Holes were drilled

on all sides to connect the aluminum tubing to plastic hose barbs. The temperature was controlled with two water baths (Model RTE 10, Neslab Instruments, Newington, NH or Model RTE 211, Neslab Instruments, Newington, NH), connected to the table via vinyl tubing (ID = 0.38, 0.96 cm).

Initially, the thermogradient table tolerance was tested. The water baths controlled the temperature of the water in the reservoir, but once it left the water bath the temperature would change before entering the thermogradient table. Tests were done to determine the relationship between the temperature settings on the water baths and the resulting temperatures in flasks placed on the thermogradient table (Figure 2.1).

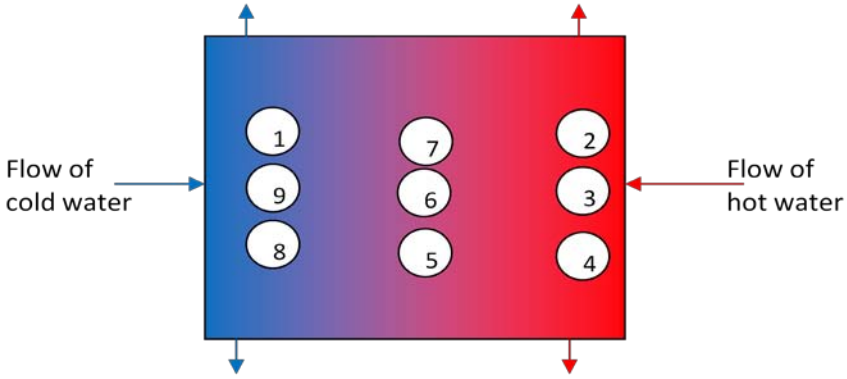


Figure 2.1. Flask placement on the thermogradient table.

With a cold water bath setting of 20°C and a warm water bath temperature of 55°C, the resulting flask temperatures are shown in Figure 2.2. Various cold and warm bath temperatures were tested to determine the correct settings to achieve the desired temperature levels for subsequent experiments.

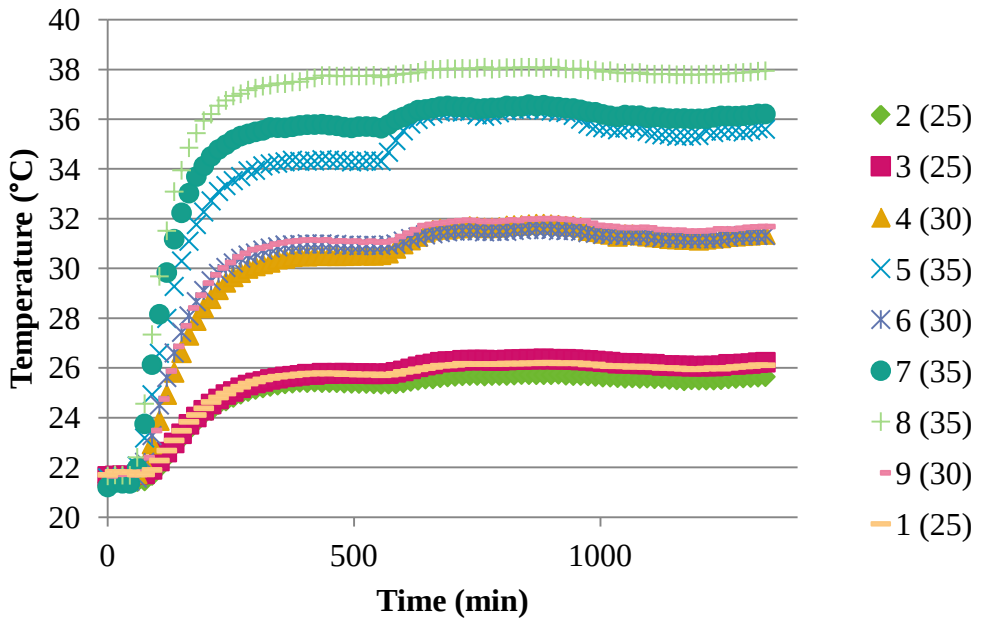


Figure 2.2.Temperature versus time for nine flasks placed on the thermogradient table with a cold water bath temperature of 20°C and a warm water bath temperature of 55°C.

In order to ensure the temperature was as constant as possible and not substantially affected by the ambient temperature of the lab, a chamber made up of 0.25 in (0.64 cm) thick polycarbonate enclosed the thermogradient table. The chamber also supported the gas manifold with one input on one side and nine outputs on the opposite side. The input was the gas mixture and the nine outputs were connected to tubing that supplied air to nine 500 mL Erlenmeyer flask. A schematic diagram and a picture of the experimental set up are shown in Figure 2.3 and Figure 2.4.

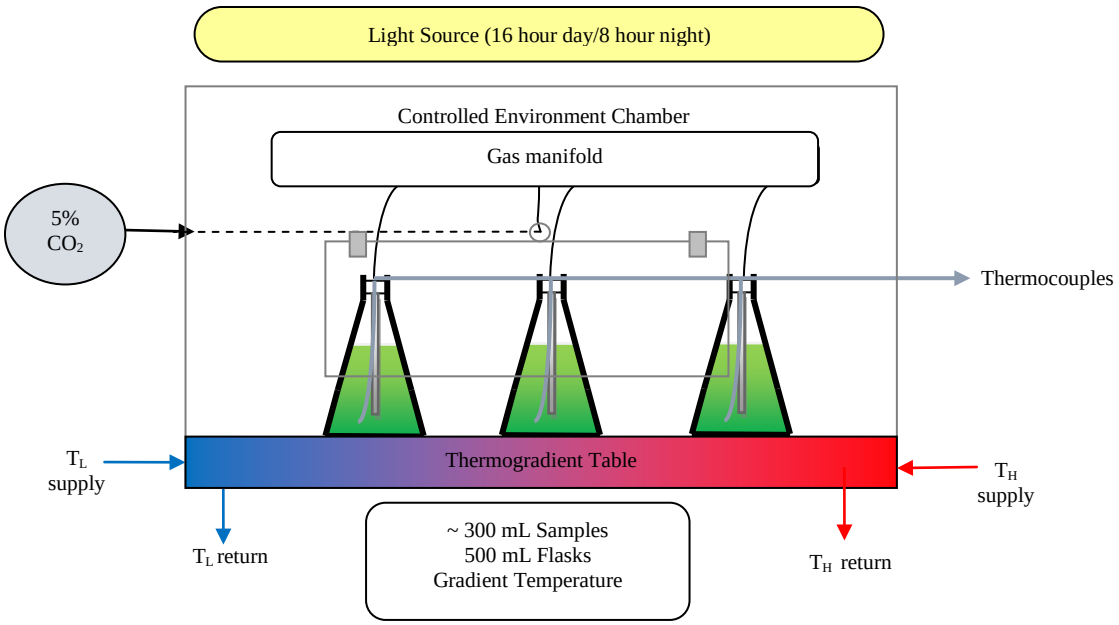


Figure 2.3. Schematic of the experimental set up, including the thermogradient table, chamber, manifold, and nine 500 mL flasks.

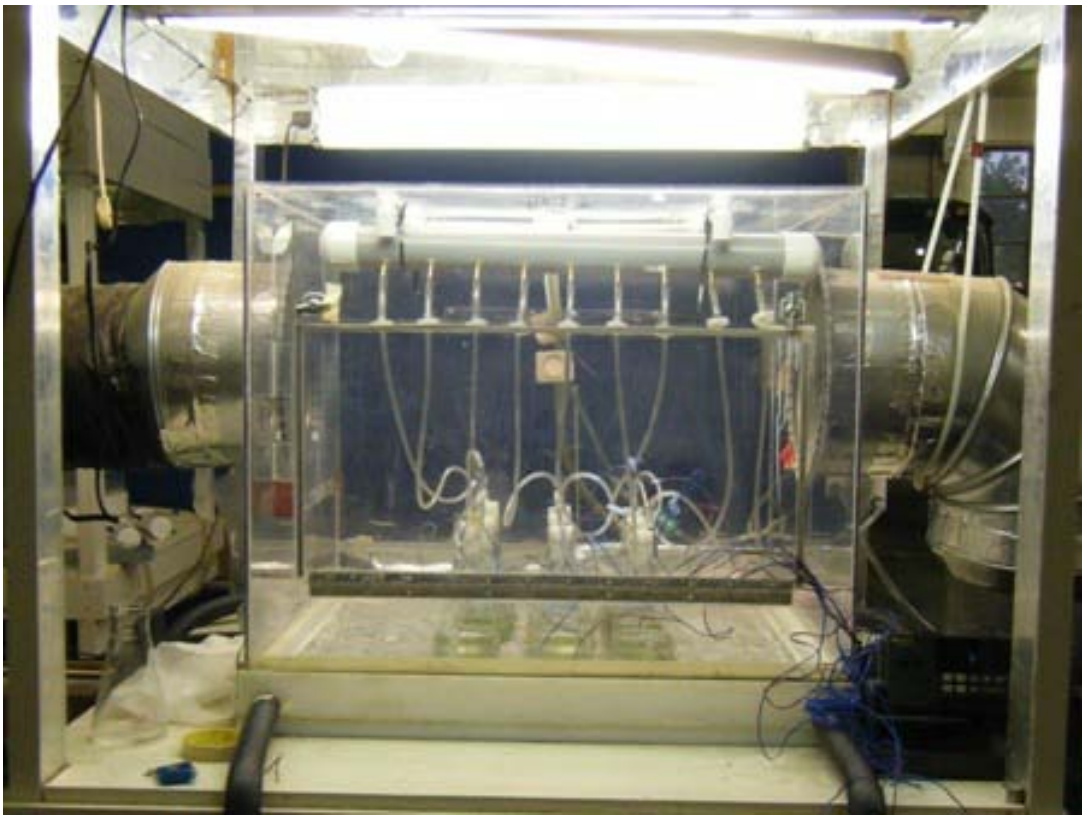


Figure 2.4. Photo of the experimental set up.

The gas mixture flow rate to the manifold was 5.3 L/min, controlled with a rotameter (Riteflow, Sciencewares, Pequannock, NJ) to make sure each culture got the proper amount of mixing and gas of 97% of house air and 3% of carbon dioxide. The carbon dioxide level of 3% was selected based on previous experiments, which showed that with the 500 mL flasks CO_2 concentration in the liquid phase was saturated at 3%. A type K thermocouple, connected to a data acquisition (Fluke hydraseries II, Everett, WA), was also placed in each flask; temperature measurements were recorded every 15 min throughout the experiment.

Once the pre-cultures reached exponential phase growth, a 5 to 15 mL aliquot was taken and placed in a 500 mL Erlenmeyer flask, containing 300 mL of urea or M-8 medium (Table 2.1). Once the samples were prepared, the flasks were randomly placed in the experimental set-up chamber for five days.

2.3 Analytical Methods

During the 5 day culture experiment, a ten milliliter sample was taken at hour 0, 24, 48, 72, and 96. At each sampling, pH (Figure 2.5) and dry weight were measured (NREL, 2008). The pH was measured with a Model AR15 pH meter (Fisher Scientific, Pittsburgh, PA), shown in Figure 2.5. The dry weight was found by placing the sample in a dry (105^{o}C for 24 hours) tared crucible with a 1.5µm pore size, 24 mm diameter glass microfiber filter (Whatman, UK), rinsed with distilled water, and dried at 105°C for 24 hours. The change in the weight of the crucibles with the addition of the rinsed algae after drying off all of the water was considered the dry weight. An example of the samples in crucibles can be seen in Figure 2.6

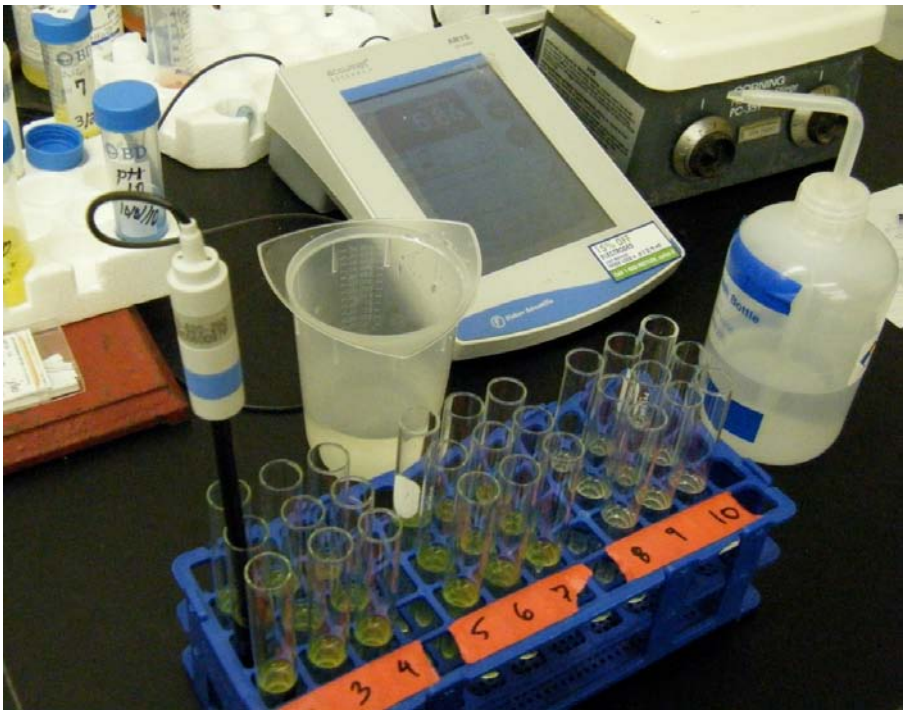


Figure 2.5. Photo of the pH measurements.


Figure 2.6. Photo of the crucibles containing algae samples and filters after drying.

Before experimentation, the light intensity was measured with a light sensor (Spectroradiometer, Apogee, Logan, UT). The light was measured above the chamber

and inside the chamber to see how much light the algae will receive, shown in Figure 2.7. From this graph it appears that the layer of plexiglass between the light source and the algae culture flasks is absorbing some of the light intensity, but is not shifting the wavelengths of the light.

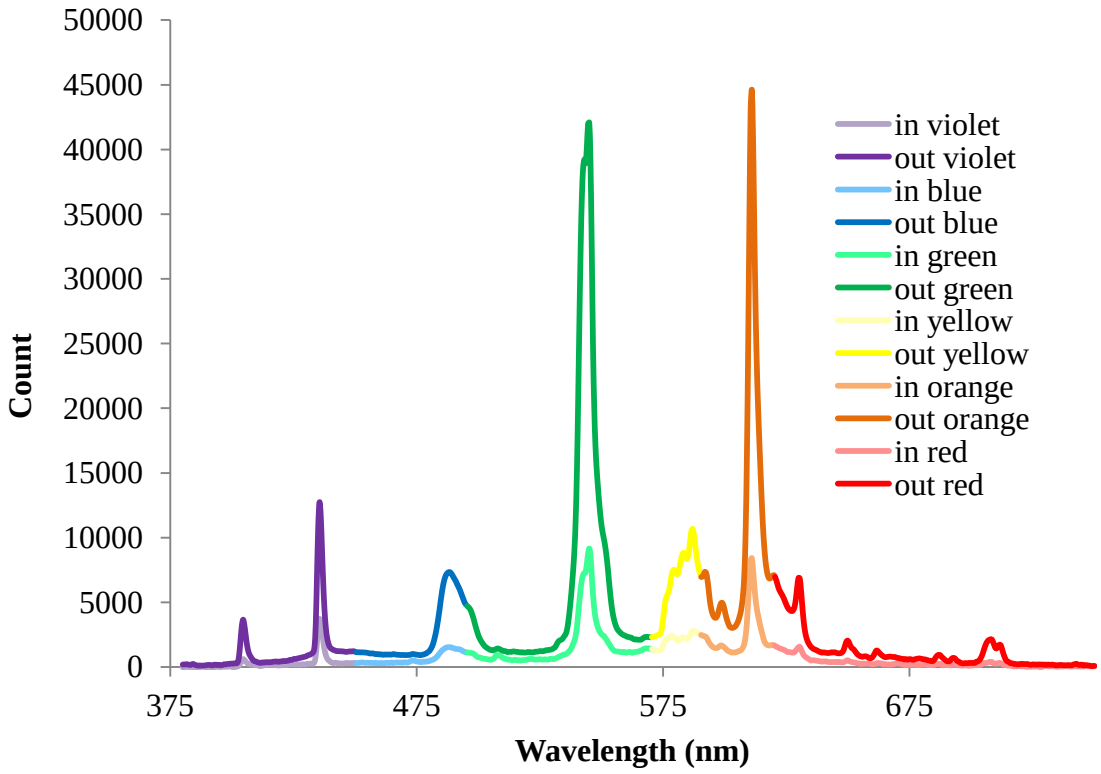


Figure 2.7. The light intensities for the experiment on the thermogradient table, where the lighter colors are inside the chamber and the darker colors are on top of the chamber

2.4 Growth Rate

The dry weights were used to calculate the growth rate of algae. The specific growth rate, μ, is defined as (Shuler and Kargi, 2002):

$$\mu = \frac{1}{x}\frac{dx}{dt} \tag{2.1}$$

where x is the concentration of algae (g/L) and t is the culture time. Hence, from concentration over time data, the specific growth rate can be determined by:

$$\mu = \frac{\ln\left(\frac{x}{x_0}\right)}{t} \tag{2.2}$$

Additional step by step procedures for preparing media, inoculating the samples, running the temperature experiments, and finding the dry weight, can be found in Appendix B.

CHAPTER 3 : TEMPERATURE EXPERIMENTS

3.1 *Varying Strains and Media Results*

Typical growth curves for *Chlorella* and *Scenedesmu,s* with the two different media types, grown at room temperature (25°C) are shown in Figure 3.1. The *Chlorella vulgaris* with the M-8 media showed the highest overall growth rate, but did not perform as well in the urea media. *Scenedesmus* appeared to thrive in the urea initially, but thrive in M-8 after 4 days of culture.

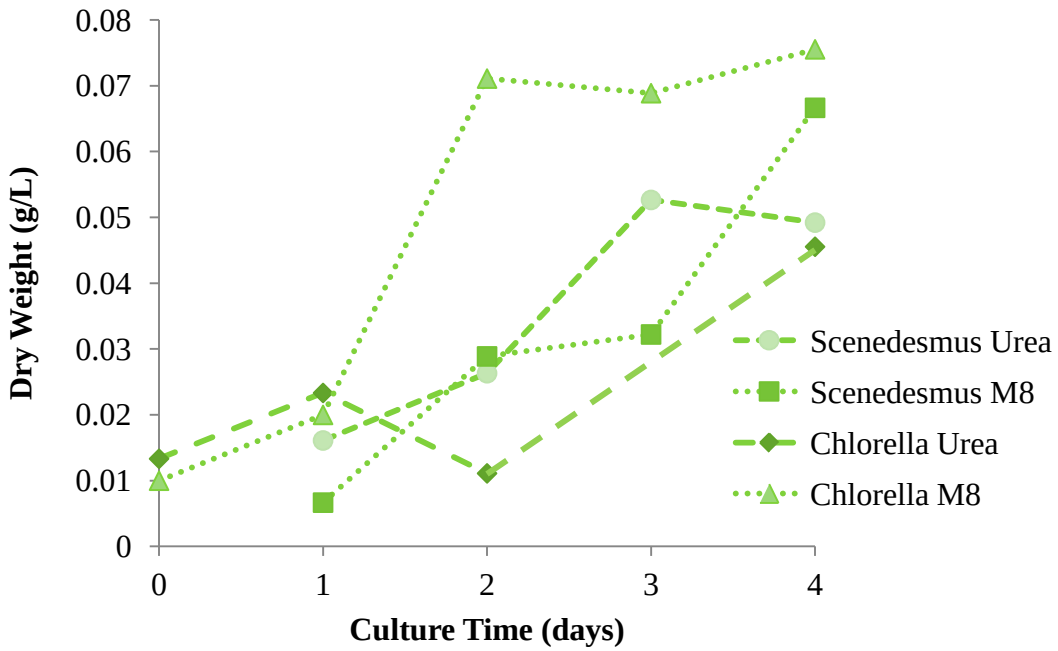


Figure 3.1. Algae dry weight for *Scenedesmus and Chlorella vulgaris* with urea and M-8 media for a four day cultivation time at 25°C.

Initially, both *Chlorella* and *Scenedesmus* were tested at 25, 30, and 35°C using both M-8 and urea growth media. The growth rates were calculated to compare the growth of the two species in the two mediums, using equation (2.2). The resulting growth rates are shown in Figure 3.2 and with standard errors provided in Table 3.1.

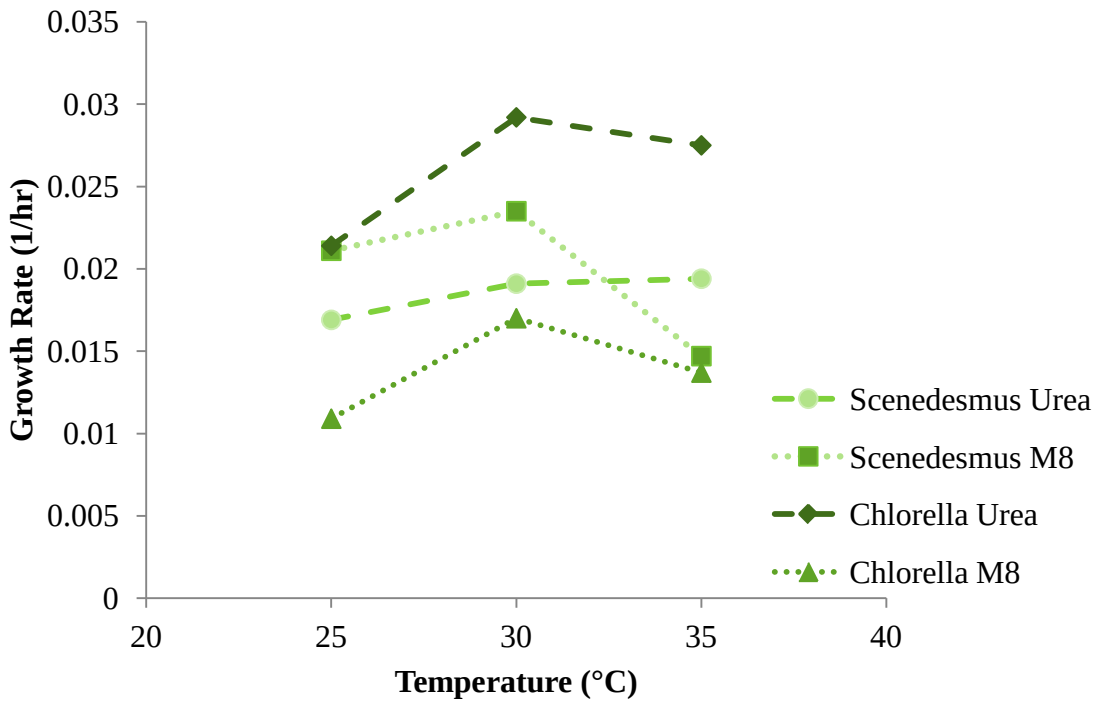


Figure 3.2. The growth rate of *Scenedesmus* and *Chlorella* in M-8 and Urea grown at a variety of temperatures.

Table 3.1. Gradient experiment growth rates (μ) and standard error (Stan Err) with n=3 for both *Scenedesmus* (Sc) and *Chlorella vulgaris* (Ch).

	Sc Urea		Sc M8		Ch Urea		Ch M8	
T(°C)	μ (1/hr)	Stan Err	μ (1/hr)	Stan Err	μ (1/hr)	Stan Err	μ (1/hr)	Stan Err
25	0.0169	0.01683	0.0211	0.02927	0.0214	0.01213	0.0109	0.0157
30	0.0191	0.0206	0.0235	0.02913	0.0292	0.01113	0.017	0.0161
35	0.0194	0.02017	0.0147	0.0235	0.0275	0.01207	0.0137	0.02539

As Sorokin and Krauss (1962) predicted, that algae growth is slow at colder temperatures, reaches an optimum growth temperature and grows slower or not at all at hotter temperatures. *Chlorella vulgaris* had the best growth rates; however, as the literature notes, it will not grow very well over temperatures of 30°C. Converti et al. (2009) said temperatures over 30°C affect the growth and Bajguz (2009) said above 30°C was considered a heat stress. On the other hand, *Scenedesmus* has more consistent growth with favorable temperatures ranging from 20 to 40°C (Sanchez et al., 2008) and 30°C tends to be the optimum (Christov et al., 2001).

The statistical analysis of these results showed that the interactions between strain, media, and temperature were not significantly different (p-value > 0.05). When looking at just the main effects, (Table 3.2), strain and temperature were not significantly different (p-value > 0.05) and media was significantly different (p-value < 0.05).

Table 3.2. ANOVA table of the main effects of *Chlorella* and *Scenedesmus*.

Source	SS.	d.f.	MS.	F	P-value
Strain	0.00069	1	0.00069	0.91	0.3408
Media	0.00309	1	0.00309	4.09	0.0436
Temperature	0.00335	2	0.00168	2.22	0.1095
Error	0.40375	535	0.00075		
Total	0.41088	539			

Along with overall statistical analysis, individual ANOVA tests were performed. *Chlorella* (Table 3.3) showed no significant difference (p-value > 0.05) between the interaction between media and temperature. *Scenedesmus* (Table 3.4) statistical analysis proved there was a significant different (p-value < 0.05) between media and temperature.

Table 3.3. ANOVA for *Chlorella*

Source	SS	d.f.	MS	F	P-value
Media	0.03582	1	0.03582	37.33	0
Temperature	0.00446	2	0.00223	2.32	0.0999
M*T[1]	0.00229	2	0.00115	1.2	0.3042
Error	0.25332	264	0.00096		
Total	0.2959	269			

[1] Interaction between media and temperature

Table 3.4. ANOVA for *Scenedesmus*

Source	SS	d.f.	MS	F	P-value
Media	0.01225	1	0.01225	32.61	0
Temperature	0.00023	2	0.00011	0.3	0.7382
M*T[1]	0.00264	2	0.00132	3.52	0.0311
Error	0.09917	264	0.00038		
Total	0.11429	269			

[1] Interaction between media and temperature

Along with ANOVA test, pairwise t-tests were used to analyze the data for *Chlorella* and *Scenedesmus*. The test showed that there was no significant difference (p-value > 0.05) between any of the growth rates at different temperatures.

Table 3.5. The p-value of difference in temperature for *Scenedesmus* and *Chlorella*.

(°C)/ (°C)	25/30	25/35	30/35
***Scenedesmus*, urea**	0.441	0.339	0.906
***Scenedesmus*, M-8**	0.984	0.482	0.508
***Chlorella*, urea**	0.731	0.981	0.652
***Chlorella*, M-8**	0.907	0.273	0.288

3.2 *Scenedesmus* and Urea Media Results

Based on its robustness and growth potential, *Scenedesmus* was selected for additional temperature testing. Urea growth media was chosen because it was more economical. The results of further temperature studies with urea are shown in Figure 3.3 and with standard errors in Table 3.6. The maximum growth rate was found at a temperature less than 30°C, but the same overall trend to peak in the middle was seen.

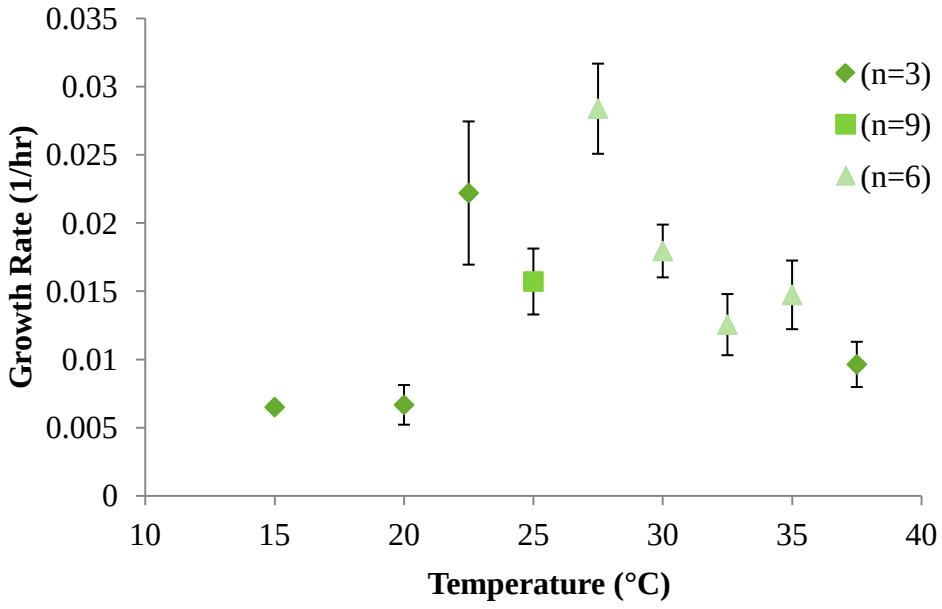


Figure 3.3. Growth rate of *Scenedesmus* at varying temperatures, shown with standard error bars.

Table 3.6. *Scenedesmus* growth rates for various temperature standard errors.

Temp (°C)	Growth Rate (1/hr)			Average	Standard Error	n
15	0.0064	0.0064	0.0067	0.0065	0.0001	3
20	0.009	0.007	0.004	0.0067	0.0015	3
22.5	0.0117	0.0277	0.0272	0.0222	0.0053	3
25	0.0247	0.0166	0.0257	0.0157	0.0024	9
	0.0084	0.0093	0.0062			
	0.0113	0.018	0.0212			
27.5	0.0385	0.0346	0.029	0.0284	0.0033	6
	0.0178	0.0304	0.02			
30	0.0152	0.0169	0.0138	0.0180	0.0019	6
	0.014	0.024	0.0238			
32.5	0.0126	0.0153	0.0221	0.0126	0.0022	6
	0.0093	0.008	0.008			
35	0.0109	0.0098	0.0072	0.0147	0.0025	6
	0.0221	0.0193	0.0191			
37.5	0.007	0.0092	0.0127	0.0096	0.0017	3

For the *Scenedesmus* grown on urea experiment, a pairwise t-test (Table 3.7) was carried out to compare growth rates at each temperature. The comparison found the optimum temperature (27°C) was significantly different (p-value < 0.05) from all other temperatures except for 22.5°C. This contradicts what other studies have found. Westerhoff et al. (2010) found growth rates do not vary with temperatures ranging from 27-39°C.

Table 3.7. Pairwise comparison for *Scenedesmus* grown on urea. Bold p-values indicate the two treatments are significantly different with $\alpha = 0.05$.

(°C)/(°C)	15	20	22.5	25	27.5	30	32.5	35	37.5
15	-	0.914	**0.040**	0.059	**0.003**	**0.005**	0.108	0.061	0.133
20	-	-	**0.046**	0.066	**0.003**	**0.007**	0.129	0.072	0.250
22.5	-	-	-	0.233	0.332	0.372	0.082	0.182	0.085
25	-	-	-	-	**0.007**	0.519	0.382	0.791	0.198
27.5	-	-	-	-	-	**0.021**	**0.003**	**0.008**	**0.007**
30	-	-	-	-	-	-	0.098	0.335	0.029
32.5	-	-	-	-	-	-	-	0.532	0.427
35	-	-	-	-	-	-	-	-	0.226
37.5	-	-	-	-	-	-	-	-	-

Along with concentration, pH was also measured (Figure 3.4). All the temperatures had similar results, the pH would drop within the first day then gradually rise each day after that. The temperature will affect the solubility of CO_2 in the water phase, which may also contribute to the change in pH and subsequently the growth of the algae.

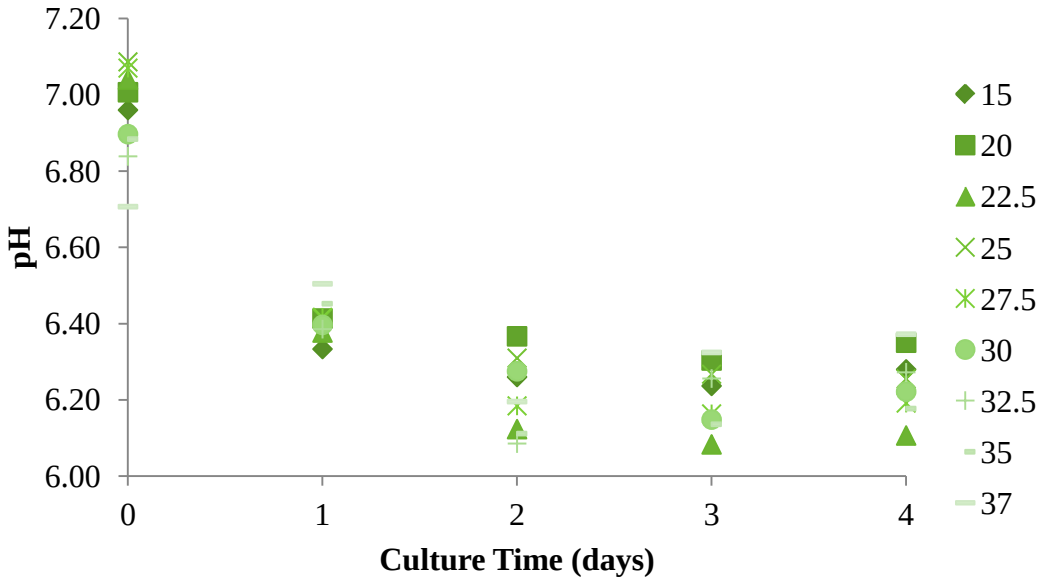


Figure 3.4. pH measurements for *Scenedesmus* grown in urea at various temperatures over a four day cultivation time.

These experiments determined the expected growth rate as a function of temperature. This relationship is used in the model development, discussed in the next chapter, to estimate the algae growth rate at different temperatures in or outside of the greenhouse with varying temperatures of the flue gas.

CHAPTER 4 : THE MODEL

4.1 INTRODUCTION

Temperature is a key growth parameter especially for growing algae at its optimum. Optimum growth means the growth rate is at its maximum as well as having maximum conversion of nutrients, gas, and sunlight. In an ideal environment, temperature would be kept constant and the algae would grow at its optimum. However, in natural environments, this is not possible. In this study, a model was developed to calculate how the algae will react at different temperatures if grown inside a greenhouse.

Keeping the greenhouse at a constant temperature can be a challenge, due to typical temperatures swings during the summer months in Kentucky. During the summer months, Lexington, KY has humidity of about 75% and can reach the upper 90's (30°C). The Center for Applied Energy Research (CAER) greenhouse is equipped with an evaporative cooling pad which is not effective when the humidity is high.

There is also a concern about the heat from the flue gas and how it will be distributed. The flue gas could be added directly into the photobioreactor to heat the algae culture on cold days or excess heat could be recovered from the flue gas when the algae culture is already at the correct temperature.

4.2 THE FLUE GAS ENERGY BALANCE

4.2.1 Introduction

The flue gas will be pumped directly into the bioreactor. If the temperature from flue gas is too hot for the algae, it might have to go through a heat exchanger. Flue gas does not consist of just temperature; it is made up of other components such as ash, carbon dioxide, nitrogen oxides, and sulfur oxides, which tolerate 200 ppm of NOx and 50 ppm of SOx (Lee et al., 2000) and elevated carbon dioxide levels.

For each of the models, a flue gas energy balance was added in order to show how the flue gas will affect the temperature of the bioreactor. The energy balance was developed on the basis of a simple mixture problem (Figure 4.1).

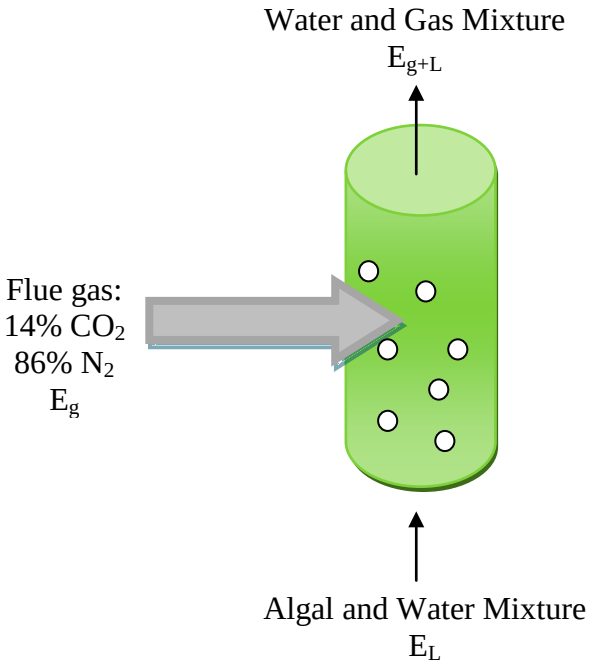


Figure 4.1. Diagram of the flue gas energy balance.

The resulting algae growth rate, as a function of temperature, with or without the addition of flue gas, was determined based on the growth rate versus temperature relationship found in the previous chapter (Figure 4.2).

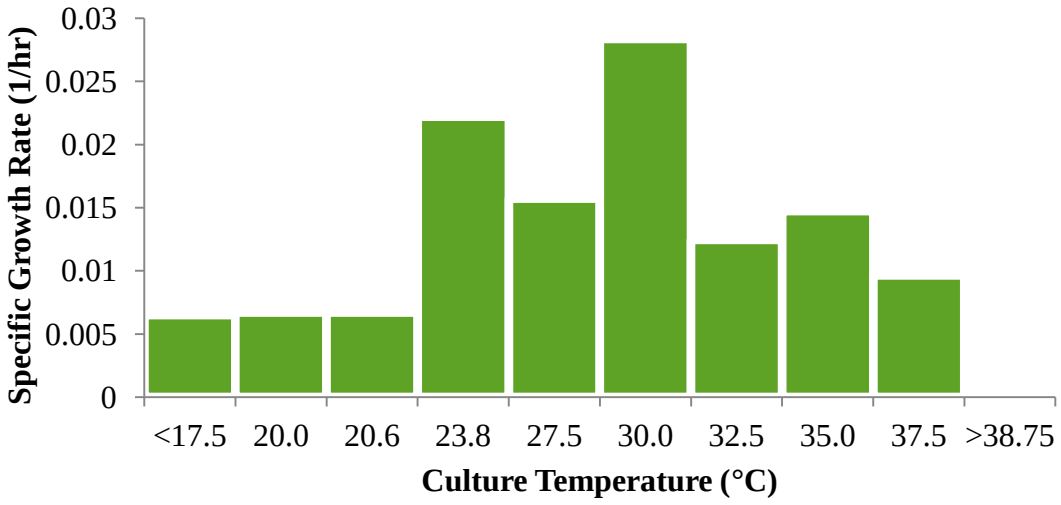


Figure 4.2. Growth rate used in the Greenhouse Model to determine the algae growth rate at various temperatures.

4.2.2 Assumptions

The following assumptions were made for model development purposes:

- The flue gas will be made up of 14% carbon dioxide and 86% nitrogen.
- The algae and water mixture entering and exiting the boundary will be mostly water; therefore, fluid properties will be based on water's properties (i.e., density and specific heat).
- The flue gas temperature is assumed to be the same throughout the day.
- All densities and specific heats are specified at 25°C.

4.2.3 The energy balance

The equation used to calculate the flue gas energy balance is:

$$E_{in} = E_{out} \tag{4.1}$$

$$T_{l,out} = \frac{\left[\left(mc_p\right)_{CO_2} + \left(mc_p\right)_{N_2}\right] T_{g,in} + mc_p T_{l,in}}{\left(mc_p\right)_{H_2o}} \tag{4.2}$$

Where, $T_{l,out}$ is the water mixture temperature exiting the system, m is the mass of the compound, c_p is the specific heat, $T_{g,in}$ is the flue gas temperature entering the system, and $T_{l,in}$ is the water mixture entering the system. In the base case, the temperature of the flue gas is assumed to be at 316°C (600°F) and the flue gas flow rate is assumed to be 10% of the liquid flow rate. The specific heat capacities for nitrogen, carbon dioxide, and water were 1.041, 0.851, and 4.18 J/g°C , respectively (Incropera and DeWitt, 1996).

4.3 THE GREENHOUSE MODEL

4.3.1 Introduction

The Greenhouse Model will be based on a greenhouse used at Center of Applied Energy Research (CAER) at the University of Kentucky, Lexington, KY. The area of the CAER greenhouse is 2700 sq ft (251m^2). The semicircle roof and walls are made up of GE Lexan 8mm Thermoclear Plus (Pittsfield, MA) and the floor is concrete. The building is equipped with a CELdek 7090-15 evaporative cooling pad (Munters, Mason, MI) and

three DCA42 Windmaster fans (ACME, Muskogee, OK) for the summer months and radiant floor heating with an additional propane heater for the winter months. In addition to the fans and cooling pad, misters have been attached to the bioreactor to help cool the algae. Figure 4.3 and Figure 4.4 show the CAER greenhouse.

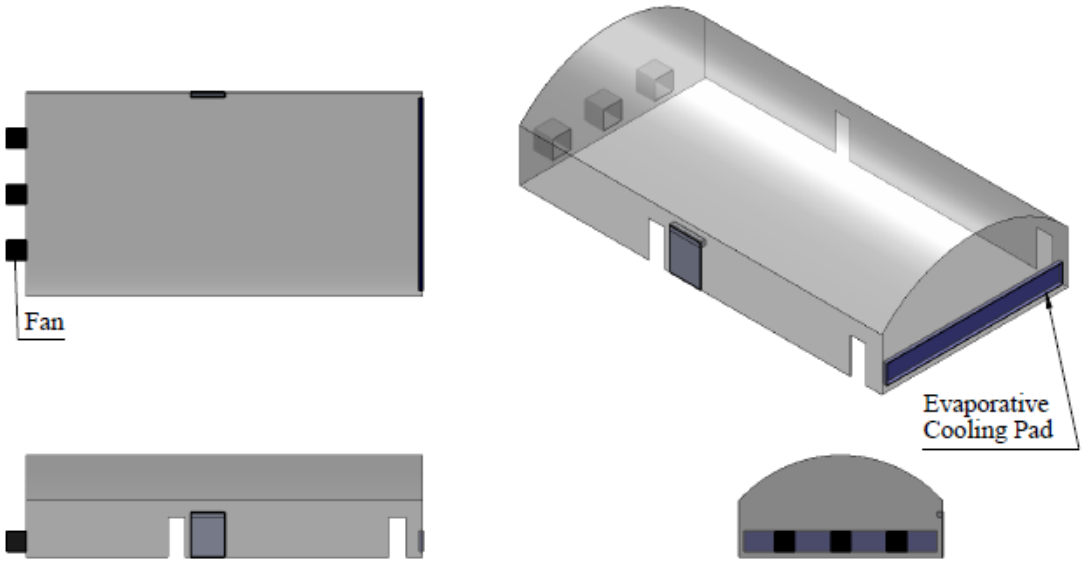


Figure 4.3. Schematic of the CAER greenhouse, top and side views.

Figure 4.4. Photo of the CAER greenhouse.

4.3.2 Assumptions

For model development purposes, the following assumptions were made:

- It is assumed the system is at steady state.

- The temperature of the bioreactor is the same as the temperature of the greenhouse.
- The solar radiation is an average of that hour.

4.3.3 The Greenhouse Model

The Greenhouse Model was formulated based on other studies with similar goals, specifically, Iga et al. (2008) and Perdigones et al. (2008). Iga et al. (2008) looked at effects of air density variations on a greenhouse model, caused by the humidity changes on the air temperature in a greenhouse growing tomatoes in Marin, Mexico. The model also included a fog system, fans, shade cloths, heat transfer through the greenhouse and the soil, heat loss due to evaporation from transpiration, and heat loss due to condensation of water vapor. Perdigones et al. (2008) looked at cooling strategies for a greenhouse growing African daisies in the summer months in Madrid, Spain. The cooling strategies they looked at were a fogging system and a shade screen.

The same greenhouse equation was used in Iga et al. (2008) and Perdigones et al. (2008), but the assumptions and inputs for CAER greenhouse are slightly different. While the Iga et al. (2008) and Perdigones et al. (2008) model looked at fog systems, shade cloths, and heat influences from the crop, the CAER greenhouse model only has to consider fans, evaporative cooling pad and/or the misters and heat transfer through the greenhouse. The resulting equation for the CAER greenhouse is;

$$T_i(next\ period) = T_i + \left\{\frac{\tau bs - U(T_i - T_o)}{C_g}\right\} \times \Delta t \tag{4.3}$$

Where T_i is the greenhouse temperature, τ is the transmittance of the greenhouse, b is the percentage of solar radiation converted into sensible heat, s is the solar radiation, U is the overall heat transfer coefficient, T_o is the temperature outside of the greenhouse C_g, is the greenhouse heat capacity and t is the time. The outdoor temperature and the temperature inside the greenhouse were collected by CAER. The temperature data were automatically acquired using an in-house developed data acquisition system using

LabView hardware and software purchased from National Instruments. The greenhouse heat capacity, C_g, can be calculated by the following equation (Iga et al., 2008):

$$C_g = \rho_{air} c_p \frac{V_g}{A_g} \tag{4.4}$$

The constants used in the model for this equation can be found in Table 4.1. Where ρ_{air} is the density of air, c_p heat capacity of air, V_g is the volume of the greenhouse, and A_g is the area of the greenhouse roof. For these equations, the constants are shown in Table 4.1.

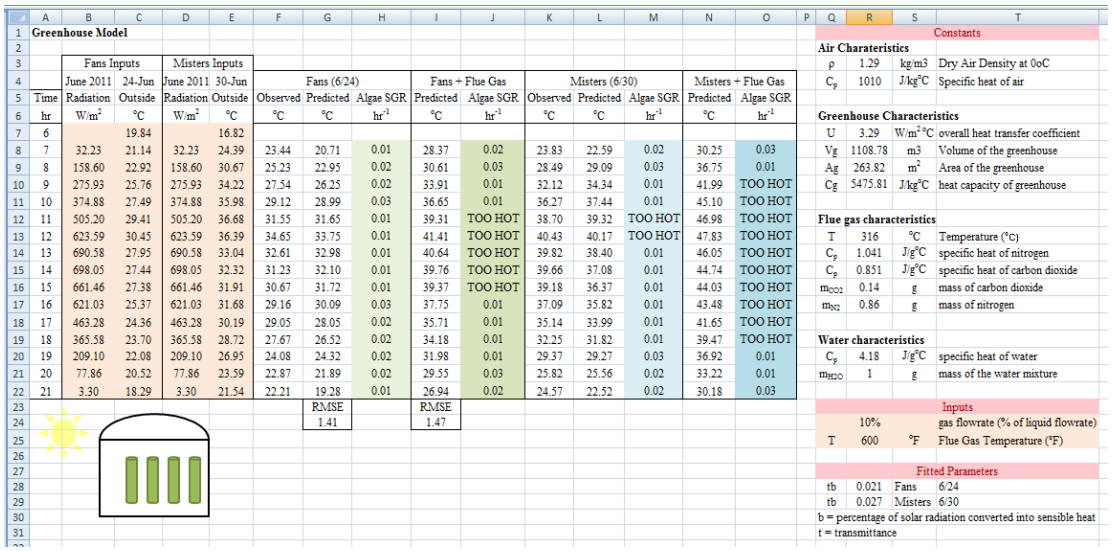
Table 4.1. Constants for the greenhouse model.

Air and Greenhouse Characteristics			
ρ	1.29	kg/m3	dry air density at 0°C[#]
C_p	1010	J/kg°C	specific heat of air[#]
U	3.29	W/m^2 °C	overall heat transfer coefficient[*]
Vg	1108.78	m^3	volume of the greenhouse
Ag	263.82	m^2	area of the greenhouse

(Iga et al., 2008)
*www.structuredproducts.ge.com

4.3.4 Results

The model is formulated in Microsoft Excel (Figure 4.5). The user must supply the hourly outside temperatures and the hourly solar radiation from 6 am to 9 pm, the temperature and the flow rate percentage of the flue gas. The solar radiation can change from day to day, based on whether it is sunny, cloudy, or rainy. Solar radiation is represented in the model using a constant, which is based on historical data. The solar radiation, was taken as an hourly average, was determined using a University of Kentucky resource (The KY Mesonet Hourly Database, 2011) which measured radiation every hour. To minimize error due to cloud cover, the information was then averaged out every hour for every day for each month.



Greenhouse Model

	Fans Inputs		Misters Inputs		Fans (6/24)			Fans + Flue Gas		Misters (6/30)			Misters + Flue Gas	
	June 2011	24-Jun	June 2011	30-Jun										
Time	Radiation	Outside	Radiation	Outside	Observed	Predicted	Algae SGR	Predicted	Algae SGR	Observed	Predicted	Algae SGR	Predicted	Algae SGR
hr	W/m^2	°C	W/m^2	°C	°C	°C	hr^{-1}	°C	hr^{-1}	°C	°C	hr^{-1}	°C	hr^{-1}
6		19.84		16.82										
7	32.23	21.14	32.23	24.39	23.44	20.71	0.01	28.37	0.02	23.83	22.59	0.02	30.25	0.03
8	158.60	22.92	158.60	30.67	25.23	22.95	0.02	30.61	0.03	28.49	29.09	0.03	36.75	0.01
9	275.93	25.76	275.93	34.22	27.54	26.25	0.02	33.91	0.01	32.12	34.34	0.01	41.99	TOO HOT
10	374.88	27.49	374.88	35.98	29.12	28.99	0.03	36.65	0.01	36.27	37.44	0.01	45.10	TOO HOT
11	505.20	29.41	505.20	36.68	31.55	31.65	0.01	39.31	TOO HOT	38.70	39.32	TOO HOT	46.98	TOO HOT
12	623.59	30.45	623.59	36.39	34.65	33.75	0.01	41.41	TOO HOT	40.43	40.17	TOO HOT	47.83	TOO HOT
13	690.58	27.95	690.58	33.04	32.61	32.98	0.01	40.64	TOO HOT	39.82	38.40	0.01	46.05	TOO HOT
14	698.05	27.44	698.05	32.32	31.23	32.10	0.01	39.76	TOO HOT	39.66	37.08	0.01	44.74	TOO HOT
15	661.46	27.38	661.46	31.91	30.67	31.72	0.01	39.37	TOO HOT	39.18	36.37	0.01	44.03	TOO HOT
16	621.03	25.37	621.03	31.68	29.16	30.09	0.03	37.75	0.01	37.09	35.82	0.01	43.48	TOO HOT
17	463.28	24.36	463.28	30.19	29.05	28.05	0.02	35.71	0.01	35.14	33.99	0.01	41.65	TOO HOT
18	365.58	23.70	365.58	28.72	27.67	26.52	0.02	34.18	0.01	32.25	31.82	0.01	39.47	TOO HOT
19	209.10	22.08	209.10	26.95	24.08	24.32	0.02	31.98	0.01	29.37	29.27	0.03	36.92	0.01
20	77.86	20.52	77.86	23.59	22.87	21.89	0.02	29.55	0.03	25.82	25.56	0.02	33.22	0.01
21	3.30	18.29	3.30	21.54	22.21	19.28	0.01	26.94	0.02	24.57	22.52	0.02	30.18	0.03
						RMSE		RMSE						
						1.41		1.47						

Constants

Air Charateristics			
ρ	1.29	kg/m3	Dry Air Density at 0oC
C_p	1010	J/kg°C	Specific heat of air
Greenhouse Characteristics			
U	3.29	W/m^2°C	overall heat transfer coefficient
Vg	1108.78	m3	Volume of the greenhouse
Ag	263.82	m^2	Area of the greenhouse
Cg	5475.81	J/kg°C	heat capacity of greenhouse
Flue gas characteristics			
T	316	°C	Temperature (°C)
C_p	1.041	J/g°C	specific heat of nitrogen
C_p	0.851	J/g°C	specific heat of carbon dioxide
m_{CO2}	0.14	g	mass of carbon dioxide
m_{N2}	0.86	g	mass of nitrogen
Water characteristics			
C_p	4.18	J/g°C	specific heat of water
m_{H2O}	1	g	mass of the water mixture

Inputs

	10%		gas flowrate (% of liquid flowrate)
T	600	°F	Flue Gas Temperature (°F)

Fitted Parameters

τb	0.021	Fans	6/24
τb	0.027	Misters	6/30

b = percentage of solar radiation converted into sensible heat

t = transmittance

Figure 4.5. Excel screenshot of the Greenhouse Model.

The product of the transmittance and the percentage of solar radiation converted to sensible heat, τb, must be found through a calibration step. The output of the model includes the temperature with the fan or the misters, with and without the addition of flue gas and the resulting algae growth rate for all four cases. Data for the fans calibration was taken on June 24, 2011 and for the misters on June 30, 2011. The τb for the fans was 0.021 and was 0.027 for the misters with a RMSE of 1.41 and 1.47, respectively.

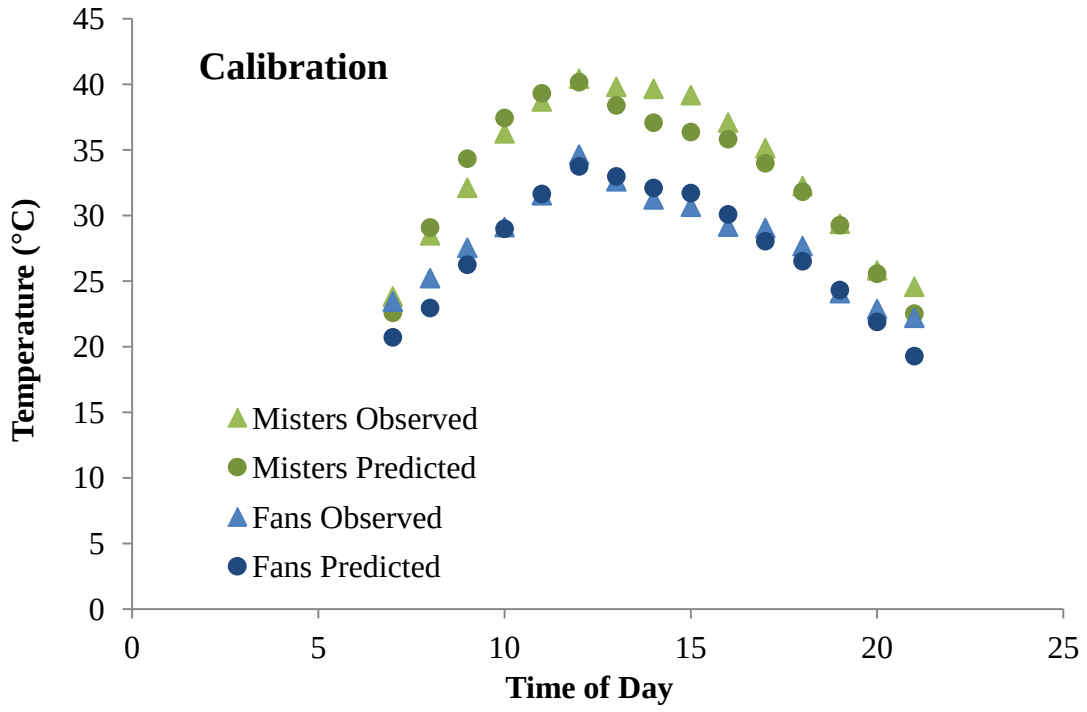


Figure 4.6. Calibration data for determining τb for fans and misters.

Using the calibrated values for τb and validation data for the fans from June 23, 2011 and for the misters on June 29, 2011, Figure 4.7 shows the observed and predicted values used for validation of τb. The RMSE for the fans was 1.65 and for the misters was 1.60. While the validation of the model would be improved with more data from various seasons (currently unavailable), these results show a reasonable agreement between the observed and predicted models. When plotting observed versus predicted values, the confidence interval ($\alpha = 0.05$) for the slopes are $0.88 < \beta < 1.26$ for fans and $0.88 < \beta < 1.24$ for misters. Since unity is a part of both confidence intervals, the agreement between the observed and predicted values can be considered not significantly different.

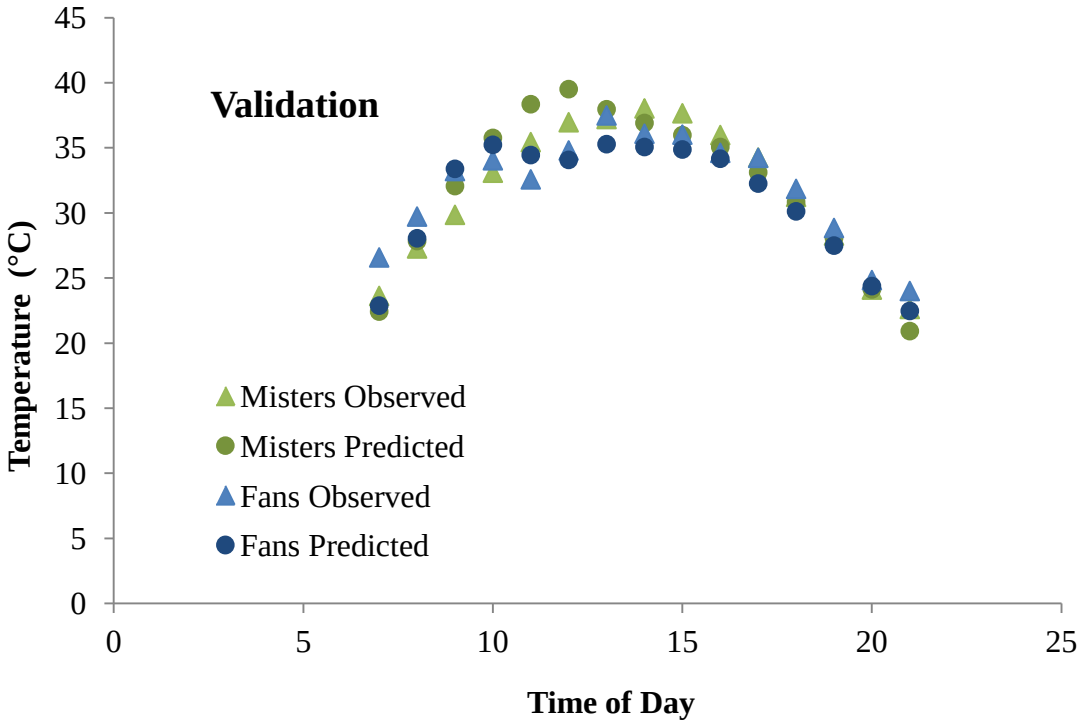


Figure 4.7. Validation data for fans and misters.

Sensitivity to the Solar Radiation Constant

The τb values for the fans and the misters were determined in a calibration step assuming the solar radiation to be an average over the entire month of June 2011. In an effort to identify the sensitivity of the model on the solar radiation value, predicted temperatures based on an average over a month were compared to predicted temperatures based on the same day (using the calibration data). The results are shown in Figure 4.8. For the fans, the RMSE when using the same day solar radiation was 1.64, while it was 1.41 when using the average solar radiation. For the misters the RMSE when using the same day radiation was 1.7, while it was 1.47 when using the average solar radiation. These results suggest that we can use an average solar radiation in the model. However, the length of time over which the solar radiation is averaged should be based on the data over the entire year. It may be taken over a 30 day period or over shorter periods of time. This will be determined when addition temperature data are available.

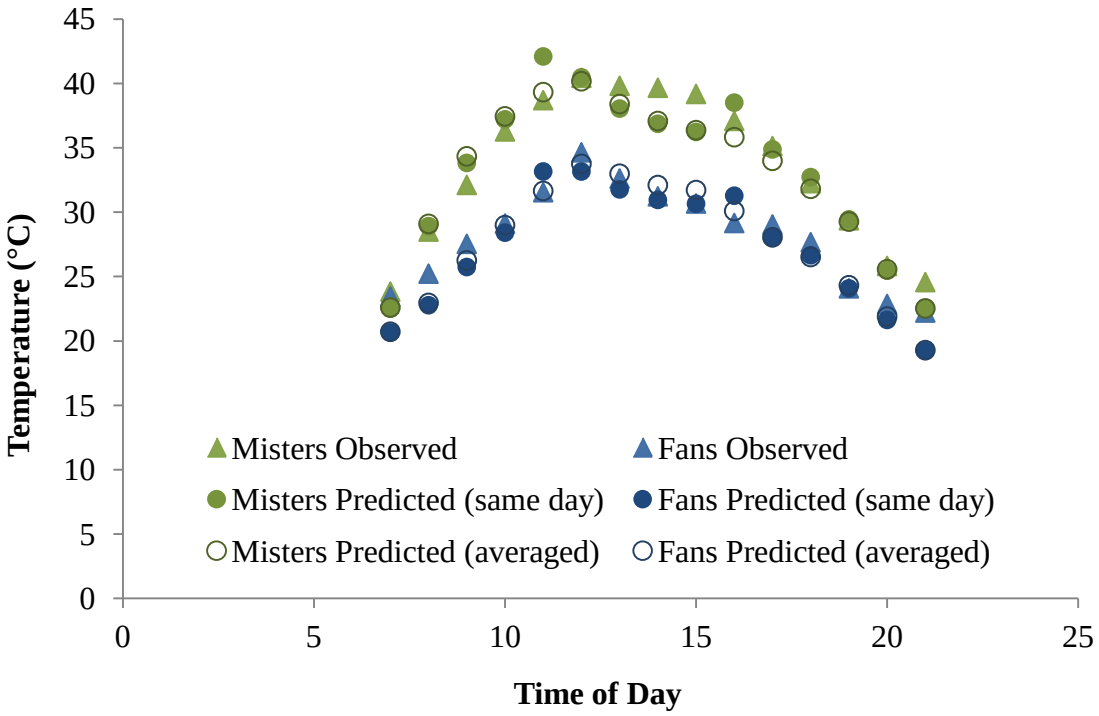


Figure 4.8. Observed and predicted temperatures for fans and misters using solar radiation averaged over June 2011 and using the solar radiation from the same day the observed values were taken.

Influence of Flue Gas

Based on the validation data, the temperature of the PBR with the introduction of the flue gas was determined for the fans' case. The base case was based on the values expected, based on original design plans for the algae based system for CO_2 mitigation at a Kentucky coal-fired plant. The temperature of the flue gas was assumed to be 316°C (600°F), which would be the temperature after moderate cooling measures. The flow rate of the gas inlet was assumed to be 10%. These results are shown in Figure 4.9, where the base case is too hot for nine hours of the day. By varying the inlet temperature of the flue gas and flue gas flow rate, the temperature of the PBRs can be kept under the upper limit of 38.75°C. For example, by using either 10% flue gas at 149°C or 5% flue gas at 316°C, the temperature of the PBRs is below the upper limit.

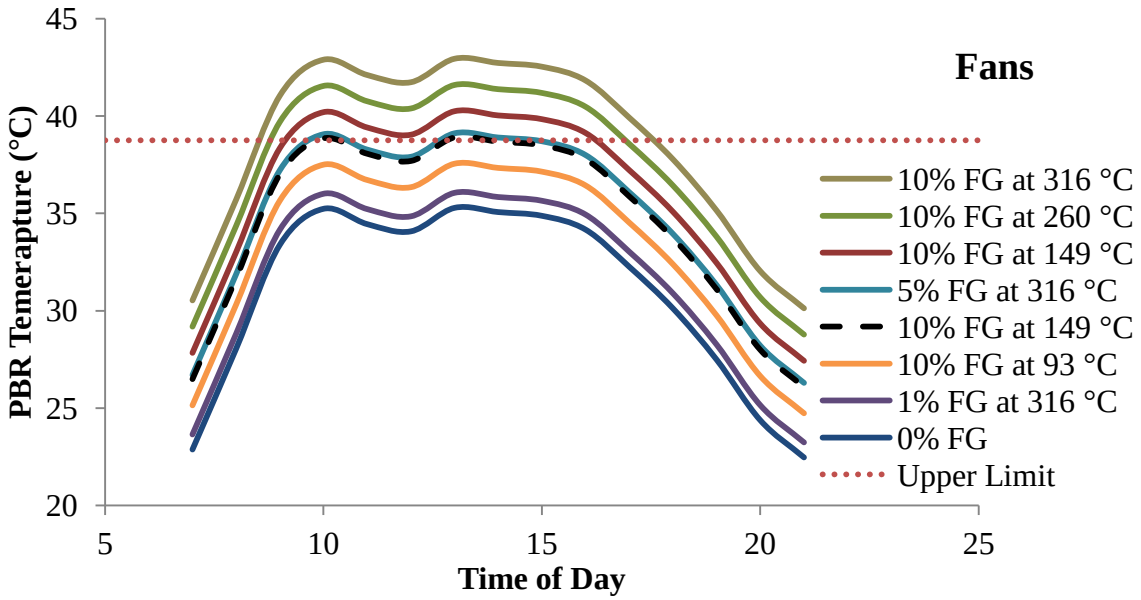


Figure 4.9. Predicted temperatures for the PBR system when flue gas is also introduced, based on the validation data.

For the Greenhouse Model, the solar radiation from the same month of interest appeared to result in adequate model predictions. The calibrated values for τb resulted in an agreement between the observed and predicted values for a separate set of validation data. Further tuning of these model constants could be done to further improve the model predictions, when data are available for months other than June.

CHAPTER 5 : CONCLUSIONS

As this study mentioned, carbon dioxide emission might be the cause of global warming, and one way to reduce the emission is by algae. Like all living things, algae needs the correct environment in order for it to perform at its best, and, for this case, capturing carbon dioxide. From this study, the optimum temperature for the algae growth was found and a heat transfer model was developed to see how the temperature of the greenhouse would affect algae growth.

In this study, the growth of algae was measured at different temperatures, showing that as temperature rises the algal growth will increase, reach an optimum, and then decrease. This type of growth pattern was observed for *Chlorella* and *Scenedesmus* grown on M-8 and urea growth media. The temperatures tested were 25, 30, and 35°C, where 30°C was considered as an optimum for both strains. The growth rate was 0.0191 and 0.0235 1/hr for *Scenedesmus* grown on urea and M-8 and 0.0292 and 0.017 1/hr for *Chlorella* grown on urea and M-8. *Chlorella* had the best growth rate of 0.0292 1/hr while grown on urea growth media; however, other studies (Converti et al. (2009) and Bajguz (2009)) have said it will not grow very well with temperatures above 30°C. *Scenedesmus*' growth was more consistent and favors temperatures ranging from 20-40°C. This information and the consistent growth rate led to further testing of *Scenedesmus* grown on urea growth media. The further testing proved *Scenedesmus*' optimum temperature is 27°C with a growth rate of 0.0284 1/hr. The test also proved the growth rate was statistically different from the other temperatures.

A heat transfer model was developed for the flue gas introduction, algae bioreactor, and the greenhouse. The model will predict the inside temperature of the greenhouse using the outdoor temperatures and solar radiation. In addition, the temperature of the PBRs with the introduction of flue gas can be predicted, such that the expected algae growth rate can be determined.

CHAPTER 6 : FUTURE WORK

To the best of our knowledge, the heat transfer model is the first of its kind. There are similar models, but none involve algae photobioreactors in a greenhouse with flue gas being pumped directly into the photobioreactors. To fully validate the model, additional data was required in order to determine the appropriate constants for the varying solar radiation values due to clouds and weather changes during the day. There is also a need for experimental validation of how the flue gas temperature will affect the temperature in the bioreactor.

Other items to consider for the heat transfer models are 1) the effect of evaporation, 2) input of mechanical heat from the pump, 3) the use of a more realistic flue gas composition (e.g., including water), and 4) the inclusion of the changes in algae growth rate as a function of both temperature and light.

Once the model has been adequately validated, it can be used to test various strategies for controlling the temperature using excess heat from the flue gas. With a better understanding of how the climate will affect the growth of algae being used for CO_2 mitigation, the system can be optimized.

APPENDICES

Appendix A: MODEL DEVELOPMENT DATA

Table A.1. Solar radiation data used in the Greenhouse Model.

	Radiation				
Hour	**6/23/2011**	**6/24/2011**	**6/29/2011**	**6/30/2011**	**June 2011**
7	30.10	16.90	0.00	51.30	32.23
8	129.80	120.30	292.60	240.90	158.60
9	181.70	174.00	439.70	433.10	275.93
10	617.00	286.30	621.20	612.60	374.88
11	265.70	903.40	781.20	772.70	505.20
12	315.70	310.20	893.40	882.00	623.59
13	426.70	531.90	957.40	943.60	690.58
14	198.20	511.50	961.90	947.10	698.05
15	963.90	504.40	903.20	900.90	661.46
16	173.10	977.40	792.70	795.50	621.03
17	639.80	299.10	652.70	648.30	463.28
18	499.60	457.50	485.60	472.60	365.58
19	255.20	116.60	304.00	288.90	209.10
20	116.90	55.40	126.00	107.30	77.86
21	5.50	4.50	6.60	8.30	3.30

Table A.2. Temperature values for outside of the CAER greenhouse.

	Outside			
Hour	**6/23/2011**	**6/24/2011**	**6/29/2011**	**6/30/2011**
6	20.04	19.84	16.39	16.82
7	24.74	21.14	23.95	24.39
8	28.95	22.92	28.98	30.67
9	33.57	25.76	31.61	34.22
10	33.05	27.49	34.04	35.98
11	30.76	29.41	35.00	36.68
12	30.08	30.45	34.68	36.39
13	31.48	27.95	31.43	33.04
14	30.29	27.44	30.98	32.32
15	30.68	27.38	30.17	31.91
16	29.84	25.37	29.66	31.68
17	28.49	24.36	28.47	30.19
18	27.03	23.70	27.01	28.72
19	25.13	22.08	25.29	26.95
20	22.72	20.52	21.93	23.59
21	21.93	18.29	19.88	21.54

Table A.3. Temperature values for inside of the CAER greenhouse.

	Inside			
	Fans		Misters	
Hour	**6/23/2011**	**6/24/2011**	**6/29/2011**	**6/30/2011**
6	22.98	22.31	21.83	21.69
7	26.58	23.44	23.62	23.83
8	29.72	25.23	27.27	28.49
9	33.22	27.54	29.86	32.12
10	34.05	29.12	33.09	36.27
11	32.58	31.55	35.44	38.70
12	34.80	34.65	36.97	40.43
13	37.49	32.61	37.22	39.82
14	36.09	31.23	38.03	39.66
15	36.02	30.67	37.66	39.18
16	34.62	29.16	35.99	37.09
17	34.23	29.05	34.25	35.14
18	31.85	27.67	31.24	32.25
19	28.85	24.08	28.27	29.37
20	24.84	22.87	24.11	25.82
21	24.00	22.21	22.62	24.57

Appendix B: TEMPERATURE EXPERIMENT PROTOCOL

Preparing Media

1. Prepare media 24 hours before experimentation.
2. 2, 2L jar with tops are autoclaved.
3. Measure out media (Table 2.1).
4. Mix 2L of tap water, media and 2 pellets of sodium bicarbonate for about 30 min.
5. Filter media through a 0.2 µm nylon membrane filter, 47 mm diameter (Nalgene, Rochester, NY) and pour into filtered media into an autoclaved jar.

Algae Inoculation

1. For this experiment, place aluminum foil on nine flask openings.
2. Autoclave the nine flasks on “labwares” setting.
3. After flasks are cooled, turn on laminar flow hood light and air.
4. Spray methanol all over the inside of the laminar flow hood and gloves.
5. Place flask, media and algae inoculums into the laminar flow hood.
6. Turn on gas and light Bunsen burner.
7. Remove aluminum foil and brush the flask opening over the flame.
8. Pour 400 mL of media into each flask.
9. Brush flask opening over the flame and replace aluminum foil.
10. After all flasks contain media, repeat step 7.
11. Add a sample of 5 to 15mL of pre-culture to each flask. Note: the sample will depend on the growth of the pre-culture.
12. Brush flask opening and place a foam cork containing an ID tube into the flasks opening.
13. Randomly place the flasks in the chamber.
14. Once in the chamber connect flasks to the air supply by the ID tube and place a thermocouple inside the flask. Make sure the thermocouple is clean by wiping each with methanol.

Sampling

1. For this experiment, collect 27 test tubs, 9 pipettes, and 1 pipettor.
2. Shake each of the flasks to make sure the algae are evenly distributed.
3. With one pipette, take three 10 mL samples from flask.
4. Pour each 10 mL sample into a test tube.
5. Repeat for the rest of the flasks making sure to use a new pipette for each flask.
6. After sampling, measure dry weight and pH.
7. pH is measured by a AR15 pH meter (Fisher Scientific, Singapore).
8. Dry weight is then measured using the following procedure.

Measuring Dry Weight

Prepare crucible with filters 24 hours prior to any sampling.
1. Set a crucible on top of the vacuum flask.

2. Place a 1.5µm pore size, 24 mm diameter glass microfibre filter (Whatman, UK) at the bottom of the crucible.
3. Connect the vacuum flask to the vacuum.
4. Turn on vacuum.
5. Pour about 5 mL of distilled water onto the filter.
6. Turn off vacuum and remove crucible from the vacuum flask.
7. The crucibles are placed in a convection oven for 24 hours at 105^{o}C.
8. After 24 hours, remove crucible and place into a desiccator for 2 hours to cool.
9. When crucibles are cool, remove from the desiccator and take initial weight.
10. Store in a desiccator for at least 24 hours before adding the algae samples to the crucibles.
11. Set the crucible onto the vacuum flask.
12. Turn on vacuum.
13. Pour 10mL of sample into the crucible.
14. In order to make sure all of the sample is measured, pour distilled water into test tub and vortex.
15. Pour distilled water mixture into the crucible.
16. Turn off vacuum
17. Remove crucible and put in convection oven for 24 hours at 105^{o}C.
18. After 24 hours, remove crucible and place into a desiccator for 2 hours to cool.
19. Once the crucible is cool, weigh.

Appendix C: CALIBRATION OF EQUIPMENT

Thermocouples

Before the experiments were performed a thermocouple calibration curve was generated. To test the thermocouples, they were place in a water bath for ten minutes at one temperature. After ten minutes, the temperatures of the thermometer and the thermocouples were recorded.

Table C.4. Calibration curve of thermocouples.

Temp.	Thermometer	2	3	4	5	6	7	8	9	10
20	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1	20.1
30	30	30.1	30.1	30.1	30.1	30.1	30.1	30.1	30.1	30.1
40	40	40.2	40.1	40.2	40.2	40.2	40.1	40.2	40.2	40.1
50	49	50.1	50.1	50.1	50	50.1	50	50.1	50.1	50

Carbon Dioxide Flow Meter

A carbon dioxide calibration curve was made to ensure that the right amount of carbon dioxide was being delivered to the algal cultures. A known flow rate was attached to the flow meter and recorded.

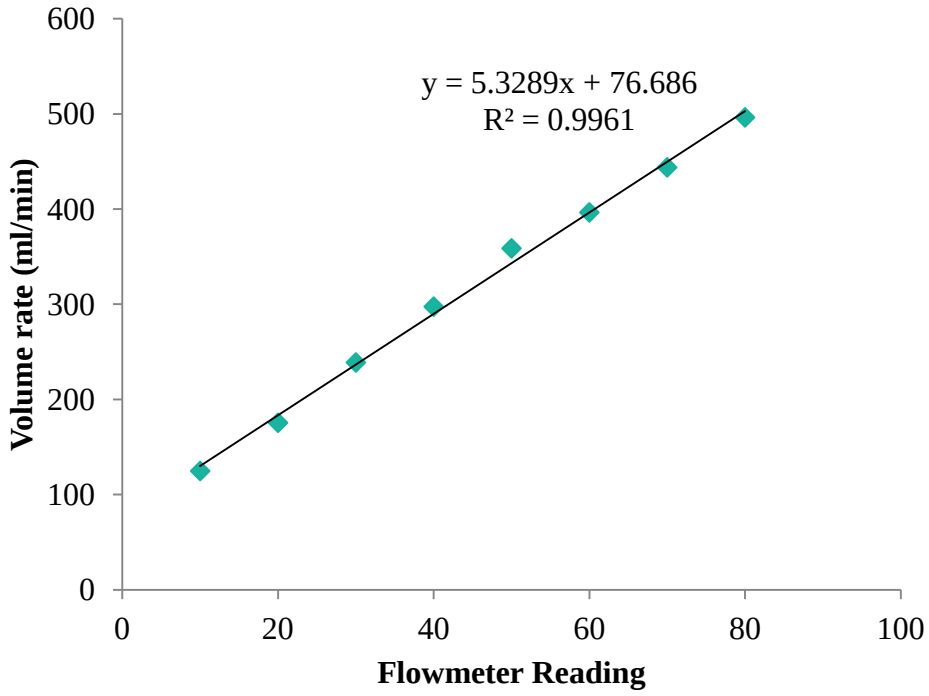


Figure C.1. Carbon dioxide flow meter calibration curve.

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