Optimization of an Atmospheric Carbon Source for Extremophile Cyanobacteria

by

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ABSTRACT

This thesis examines the use of the moisture swing resin materials employed at the Center for Negative Carbon Emissions (CNCE) in order to provide carbon dioxide from ambient air to photobioreactors containing extremophile cyanobacteria cultured at the Arizona Center for Algae Technology and Innovation (AzCATI). For this purpose, a carbon dioxide feeding device was designed, built, and tested. The results indicate how much resin should be used with a given volume of algae medium: approximately 500 grams of resin can feed 1% CO_2 at about three liters per minute to a ten liter medium of the *Galdieria sulphuraria* 5587.1 strain for one hour (equivalent to about 0.1 grams of carbon dioxide per hour per seven grams of algae). Using the resin device, the algae grew within their normal growth range: 0.096 grams of ash-free dry weight per liter over a six hour period. Future applications in which the resin-to-algae process can be utilized are discussed.

Dedicated

To

My family,

who has supported me in so many ways.

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I would like to start off by thanking God for enabling me to get to where I am today.

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CHAPTER 1: INTRODUCTION

This project has been performed through resources from the Center for Negative Carbon Emissions (CNCE), in partnership with the Arizona Center for Algae Technology and Innovation (AzCATI). The algae story is first presented as an introduction, from the different methods of growing algae to what happens after they are grown. The story later meets up with the carbon capture team, to assess the advantages of the two groups working together.

Growing Algae from Ambient Air:

The photobioreactors for growing algae have been developed as a result of open outdoor algae systems struggling with productivity (Lee, Gillis, & Hwang, 2003). This is especially true for extremophile algae, which for the purposes of this project are defined as algae that thrive in high temperature, high acidity environments. Acidophilic algae do not grow as well from ambient air because algae growth requires dissolved carbon dioxide (Cheng et al., 2006). Ambient air works better for algae that grow in neutral waters because the higher pH will allow for more bicarbonates and carbonates to form with the alkalinity (the ability to buffer pH changes) that is present (Cheng et al., 2006; Lackner, 2002). Additionally, a reduction in the solubility of carbon dioxide relative to oxygen is theorized to be partially responsible for reduced photosynthesis with ambient air when accompanied with an increase in temperature (which is useful information if the rate limiting step is different for waters of different pH) (Ku & Edwards, 1977). This

data indicates that growing the extremophile algae in ambient conditions is generally not ideal for biological production, other than that which occurs at the hot springs where they are originally found (which provide dissolved carbon from underground) (P. Lammers, personal communication, April 15, 2016).

Growing Algae from Tanks:

AzCATI currently has three recently isolated strains of cyanobacteria (a type of algae) that grow very well in high temperatures (over 40^{o} C) and high acidity environments (P. Lammers, personal communication, July, 1, 2015; Stanier et al., 1978). The strains can thrive at low pH levels because they have evolved to reduce the proton influx and increase proton pump efficiency (Gross, 2000). But the absence of a bicarbonate pool due to the reduced pH causes a limited CO_2 supply and limits photosynthesis (Gross, 2000). Therefore, an external carbon source is required to support cellular energy needs via respiration (Henkanatte-Gedera et al., 2015). If an external CO_2 source is not employed when the algae are taken away from their natural hot spring source, respiratory energy generation will consume internal carbon reserves which will decrease biomass productivity (P. Lammers, personal communication, April 15, 2016; Henkanatte-Gedera et al., 2015). Closed photobioreactors (PBRs), such as those used by AzCATI, have been shown to have more productivity and less environmental problems because of how the systems allow for control of environmental conditions such as temperature, oxygen, nutrients, and CO_2 (Lee, Gillis, & Hwang, 2003).

In PBRs, carbon dioxide is distributed for algae consumption via a concentration-controlled CO_2 tank that delivers carbon dioxide at a specified flow rate that allows for equal distribution by way of algae mixing. The amount and flow rate of carbon dioxide needed depends on the size of the reactors, but are typically targeted with 3-10 liters per minute (Lpm or L/min) of CO_2 concentrations anywhere from 1-5% CO_2 (N. Csakan, personal communication, March 2, 2016). The flow rate is in the 3-10 L/min range because cultures are run at 0.3 VVM (0.3 liters of air per liter of culture per minute). Steps are being taken in an attempt to lower the VVM as much as possible because life cycle assessments have indicated a need to be closer to 0.005 VVM for appropriate cost and energy consumption (N. Csakan, personal communication, March 7, 2016).

The general time period for algae growth within these closed systems depends on the goals of the culture. Two to three weeks is typical for most cultivation, but some cycles can last anywhere from 1-6 weeks (N. Csakan, personal communication, March 7, 2016). During these cycles, the algae only need CO_2 during the day, or when they are next to a light source (which is all of the time for the indoor algae testing). The photobioreactors used outside have both horizontal and vertical columns to assess the differences in the two mixing systems, but vertical systems are expected to have better light and mixing kinetics (N. Csakan, personal communication, November 20, 2015).

Utilizing Algae:

A photobioreactor system minimizes evaporative water loss and retains oxygen and CO_2, which leads to the oxidation of organic carbon for conversion to biomass with

fewer inputs (Henkanatte-Gedera et al., 2015). The dry weight of a culture can be used to estimate its biomass production (Lee, Gillis, & Hwang, 2003). The production can be put to a variety of uses, such as urban wastewater treatment and biofuel synthesis. Converting most of the carbon in wastewater to biomass enables a high energy recovery, enabling multipurpose algae to be subsequently used for the biofuel process (Lee, Gillis, & Hwang, 2003). These algae therefore incorporate carbon into their structure that does not come from photosynthesis. The algae are to be utilized in wastewater treatment during growth, and in fuels after they have been fully developed.

For the wastewater portion, the pH conditions that the extremophile strains are found in (typically ranging from 1-4) are able to rapidly inactivate wastewater pathogens (Henkanatte-Gedera et al., 2015). Algae metabolism can then remove nitrogen and phosphorus to meet the required effluent standards (see Appendices A and B; Henkanatte-Gedera et al., 2015). Algae can also help remove the biochemical oxygen demand (BOD) as long as they have adequate CO_2 feeding, otherwise the oxygen would develop to toxic levels. Additionally, photosynthesis corrects the C:N:P ratio with urban wastewater in one simple step (in which no oxygenation is needed for conservation of BOD removal), instead of two energy-intensive ones (Henkanatte-Gedera et al., 2015). If BOD, nitrogen, and phosphorus removal can be achieved in one step, there will be more energy rich biomass (Henkanatte-Gedera et al., 2015). The carbon to nitrogen ratio in urban wastewater is closer to that of algal biomass than to bacteria (which are distinct from the algae that are termed cyanobacteria) (Henkanatte-Gedera et al., 2015; Stanier et al., 1978).

Nutrients can then be recycled to increase biomass productivity. Hydrothermal liquefaction (HTL) is used to extract energy content with nutrient content (Henkanatte-Gedera et al., 2015). HTL is a process in which the algae are decomposed and converted in hot compressed water, leaving biocrude oil as the product (see Appendix C; Barreiro et al., 2013). Algae can be used to make biodiesel fuels because they are made up of up to 50% oil (see Appendix D; Barreiro et al., 2013). The product fuel is beneficial in that it has low sulfur emissions and low ash content which allows for reduced particulate emissions (Barreiro et al., 2013).

Valuable uses for algae will flourish if there are ideal scalability conditions. However, acidophilic algae may be difficult to scale up because although there are many acidic sites on earth, they are generally sites that are small in size (Gross, 2000). Therefore, many of these algal populations have different genotypes from those found at other sites (Gross, 2000). Limited genotypes can affect colonization because they can cause reproductive limitations.

Improving Impact/Use of Source:

The Center for Negative Carbon Emissions had the desire to collaborate with AzCATI because of CNCE's mission to take increasingly harmful carbon dioxide out of the atmosphere, and use it to provide carbon for a variety of different sectors. The use or storage of captured carbon dioxide can cancel out excessive CO_2 emissions. The capture process at CNCE starts with polypropylene sheets that are embedded with fine resin powder, which is then used as a sorbent material that binds CO_2 (see Appendix E;

Lackner, 2009). The nanoporous sorbent absorbs carbon dioxide when dry and releases it when wet (see Appendix F). Wetting the material when it is loaded with CO_2 raises the equilibrium partial pressure over the resin roughly five hundred fold. It is therefore possible to collect carbon dioxide and amplify its partial pressure by about two orders of magnitude. The water-driven reactions are characterized by rapid kinetics, allowing for easy regeneration (Wang, Lackner, & Wright, 2011). In fact, there is an improvement on the performance when the material is exercised between wet and dry cycles of the resin. This material is revolutionary compared to other sorbent materials for its regeneration capabilities and its moisture swing process (as opposed to thermal or pressure swing processes) (Wang, Lackner, & Wright, 2011). Not only that, but unlike other materials, the resin also captures CO_2 from *ambient* air rather than other more concentrated sources like power plants (Choi et al., 2011). Because the material is made to capture carbon dioxide from ambient air, the mixing rate of the atmosphere is the rate limiting step for the resin (K. Lackner, personal communication, November 18, 2015). Regardless, the resin can capture carbon approximately 1000 times faster than a tree of the same size (So, 2015).

The abilities of the resin material made CNCE an ideal candidate for testing its function in an algae facility like the Laboratory for Algae Research and Biotechnology at AzCATI. A series of designs were subsequently drawn up, and the materials were put together to implement them.

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CHAPTER 2: MATERIALS AND METHODS

Material Specifications:

The following is a list of all of the materials used to create and employ the resin feeding device, along with what each component was used for and some of their distinguishing specifications:

- *80/20® Struts: T-slotted Aluminum Profiles*
    - Used to create the general framework for the device
        - Frame pieces were cut to size with a band saw
- *Angle & Straight Iron Fittings with Button or Flathead Screws*
    - Used to hold parts together or in place
        - A magnetic rod was used to help put in screws
- *Plastic Tubing (cut to different sizes)*
    - Used for air and water flow
    - ¼" outer diameter, ⅛" inner diameter
    - ⅜" outer diameter, ¼" inner diameter
    - Overall device had a total of about 50 feet of tubing
- *John Guest® Plastic Union Tees and Connectors*
    - Used for tubing connections and changing flow direction
    - Different sizes:
        - ¼" outer diameter
        - ⅜" outer diameter
        - ¼" to ⅜" outer diameter

- *Snoop ® Liquid Leak Detector*
    - Used to find air leaks that needed to be fixed
- *Zip Ties*
    - Used to keep tubing in place and out of the way
    - Different sizes
        - 0.0625” wide, 4” long
        - ⅛” wide, 12” long
- *Wire Stripper*
    - Used to strip red (positive) and black (negative) encased wire tips in order to solder wires together
- *Soldering Gun*
    - Used to weld electrical connections
- *Heat Shrink Tubing and Heat Gun*
    - Used to cover soldered connections and prevent them from short circuiting
- *Minerallac® Pipe Clamps and Push-in Conduit Clamps with Screws*
    - Used to hold heavier pieces into place on the device
        - Pipe clamps hold tighter
- *Polyvinyl Chloride (PVC) Pipes*
    - Used to hold the resin material in canisters (four at one foot long each) and to reduce pulsation (6” long)
    - 3” inner diameter, 3 ½ ” outer diameter
    - Metal grates with squares ¼” by ¼” super glued to the bottom ends to better hold the resin in place

        - A sand belt was used to get dry glue off
    - Canisters were numbered 1-4
- *Resin Material in a Wavy, Spiral Shape*
    - Canisters labeled 1 and 3 had four resin spirals of approximately the same size, while canisters 2 and 4 had about one inch more space at the top because each one had two spirals of the same size, plus a vertically longer spiral of resin that was about 1.5 times more mass than that of the smaller sizes
    - Each resin spiral was weighed
        - #1 spirals = 51.7 g + 66.4 g + 68.3 g + 68.9 g = 255.3 g total
        - #2 spirals = 67.3 g + 67.8 g + 109.4 g = 244.5 g total
        - #3 spirals = 62 g + 67.6 g + 67.7 g + 66.7 g = 264 g total
        - #4 spirals = 69.7 g + 66 g + 107.1 g = 242.8 g total
- *Fernco Inc.® Flexible Pipe Connectors*
    - Used to close off the PVC pipes and connect them to the system flows
    - Model Number: 1056-33
    - Each comes with two iron couplings for tightening
- *Solid Plastic Cylinders*
    - Used to block air flow through the middle of the resin spirals in order to direct the air through the waves in the resin material
    - Different sizes
        - Diameters: ¾ ” ; 1” ; 1 ½”
        - Heights: 2 ½ ” ; 5”

- *2 Infrared Gas Analyzers (IRGAs)*
    - Used to read the carbon dioxide concentrations to and from the photobioreactors (outlet and inlet, respectively)
    - Model Number: 400
        - Can read up to 20% CO_2 concentrations
- *2 One-Micrometer Filters*
    - Used to keep the IRGAs safe from harmful chemicals and water intrusion
    - Model Number: F1AA17384
- *60 mL Nalgene Plastic Bottle*
    - Used as a water trap to protect the outlet IRGA from water leakage when switching the system from water to air flow
    - Corked with two ¼" diameter holes for the tubing
        - Inlet tubing pushed all the way to the bottom, outlet toward the top
- *5 Gallon Home Depot® Bucket*
    - Used as a cold trap to reduce hot water vapor content in the air stream through the IRGAs
    - Filled about a third of the way with water
    - 7 holes of ¼ " diameter drilled in the lid for different tubing connections
        - 7 labels: upper elbow, lower elbow, return, water pump, drain, flow controller, and air pump
- *2 250 mL Graduated Cylinders with Corks*
    - Used to trap condensate by allowing air flow to go through an area of cooler temperature before going into the IRGAs

    - Corks also had two holes each of ¼ ” diameter for tubing
- *Teledyne Analytical Instruments® Portable Oxygen Analyzer*
    - Used to measure oxygen levels
        - To use: the cap is taken off and plugged into a port on the photobioreactor
    - Model Number: 320 PD, class A – group 1
- *Cole Parmer® Flow Meter (calibrated for CO_2)*
    - Used to make sure carbon dioxide was moving through the system
        - Units in standard cubic feet per hour (SCFH)
    - Model Number: ACRY-010184
- *ThermoScientific® Air Cadet 663U Pump*
    - Used to create air flow to go from the photobioreactor to the resin feeder
    - Model Number: 420 -1901
    - Diaphragm part inside the pump had a tear
        - Silicone glue used because original part had better performance (least differential pressure) compared to newer replacement
- *EcoAir® Commercial Air Pump*
    - Used to create air flow to go from the photobioreactor to the resin feeder (replacement for Air Cadet)
    - Glue was used to close flow relief openings
    - Teflon tape was added to the screws on the bottom to prevent air from escaping
    - Pressure: Up to 2.9 psi

    - Flow rate: Up to 40 Lpm
- *2 VR Series Brass Pressure Relief Valves*
    - Used to provide a release when the system was over or under pressurized
    - Ball inside one of them was flipped so that it would release on negative pressure instead of positive
    - Vacuum setting: 0.4 psi (~11" water); Pressure setting: 1-2 psi
        - Indicated when reached by air blowing through the hole at the top
        - Teflon-laden screws adjusted to settings by tightening a certain amount
    - Pressure: 0-30" Hg
- *Adjustable Proximity® Series Miniature Pressure Regulator*
    - Used to adjust the overall pressure running through the feeding system
    - Maximum value: 5 psi
- *1 Dwyer® and 3 VWR® Flow Meters*
    - Used to control the air flow going through the system
    - Two up to 1 Lpm air (VWR)
    - Two up to 5 Lpm air (1 VWR & 1 Dwyer)
- *300 mL Swagelok Pressure Cylinder*
    - Used to reduce pulsations from the air flow caused by the big air pump
    - Model Number: 304L-HDF4-300
- *12 Volt Karlsson Robotics® Vacuum Pump*
    - Used to direct air from the bucket to the outlet IRGA

        - Creates a pressure differential to force air to go in a direction it would not otherwise go
    - Model Number: ROB-10398A
- *Power Source with 5 Sockets & ON/OFF Switch*
    - 4 of the sockets were used to supply power to different parts
        - One each for the water pump and the small air pump
            - Both contained heat shrink tubing connections
        - One for the large air pump
        - One shared by the two IRGA readers
            - Where the soldering connected the positives together as well as the negatives
- *15 L Plastic Bag*
    - Sample-space port, an inlet port and outlet port for the feeding device, and an additional port that was used for several tasks throughout different trials (i.e. testing the oxygen levels, using a manometer, or just plugging it up)
    - Adjusted so that the fittings would not be on a folded section of the bag (abating leaks)
    - 23” long by 29” tall by 2 ½ ” wide
    - Hard piping connected into a square for structure inside the bag
        - 20” long by 20” tall by 1” wide
        - Put in to allow relief valves to function as intended (because designed to work with a rigid container)

- *The Algae Strain: Galdieria sulphuraria 5587.1*
- *The Algae Medium (about 10 L total):*

Chemical	Concentration (g/L)
$NaCO_3$	0.02
NaCl	5
$NaNO_3$	0.595
$MgSO_4*H_2O$	0.012
$NH_4H_2PO_4$	0.035
KCl	0.175
$FeCl_3*6H_2O$	0.0035
EDTA	0.00436
Trace metals	--
Vitamin B_{12}	--

Table 1. Ingredients found in the algae medium and the corresponding concentrations. Sulfuric acid is sometimes added to bring the pH down, but the algae usually bring it down themselves as long as the initial pH is somewhere below 6.5-7. Additionally, the bright color of the algae comes from the utilization of photocyanine, which is a result of the strains being a type of cyanobacteria as opposed to other algae types.

Design and Adjustments:

Lengths and parts were minimized as much as possible. The reason, in part, was because longer pieces of tubing create more resistance, and so do all of the other parts (i.e. the air controllers, tube connectors, etc.). Minimization was employed with the resin

spirals as well, seeing as some of them were more rigid than others. For instance, the smallest plastic cylinder (¾ ” diameter) was put inside the 68.3 gram resin piece, rather than the smallest one of 51.7 grams, because the material fit more rigidly around that specific piece. The other reason for minimizing lengths was for ease of access and visibility. The air volume of the device totaled about seven liters.

DraftSight is a free CAD (computer-aided design) program that was used to initiate the design drawings. Considered in the design were gravity and different positioning tactics to get the water to flow correctly without any concerns about collection or trapping. The resin off-gassing of CO_2, for instance, can be lost to other canisters through absorption if they are placed in series, which is why they were set up in parallel. The final design was presented in a trial of AutoCAD 2014, frequently referring to the Autodesk Knowledge Network for tips and tricks of the software. Figure 1 shows the experimental design of the feeding device, followed by a more detailed description of some of the parts in the legend of Figure 2.

The basic procedures involved in the use of the device started with a resin wetting cycle by turning on the water pump, which was then turned off and followed by a switch from pump to drain. The air pump was subsequently turned on after there was a switch from water to air flow, and the flow rates and pressure readings were adjusted.

System Design:

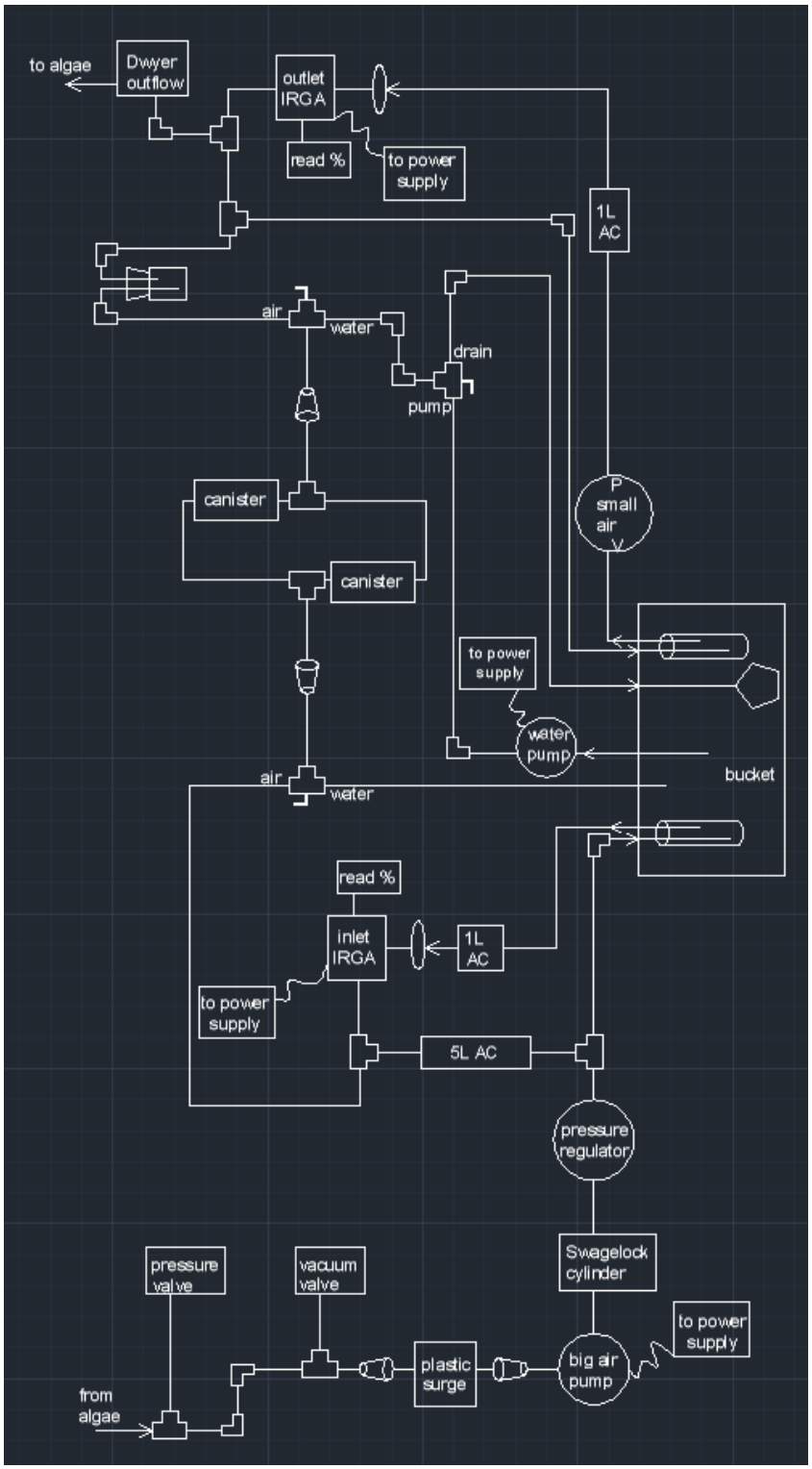

Figure 1. The top of the figure starts with the part of the feeding device that connects to the algae in the photobioreactor, and ends at the bottom of the figure where the algae feed

returns to the device. The lines in between each of the parts indicate tubing, and the arrows show flow direction. Additional labeling can be found in Figure 2.

System Design Key:

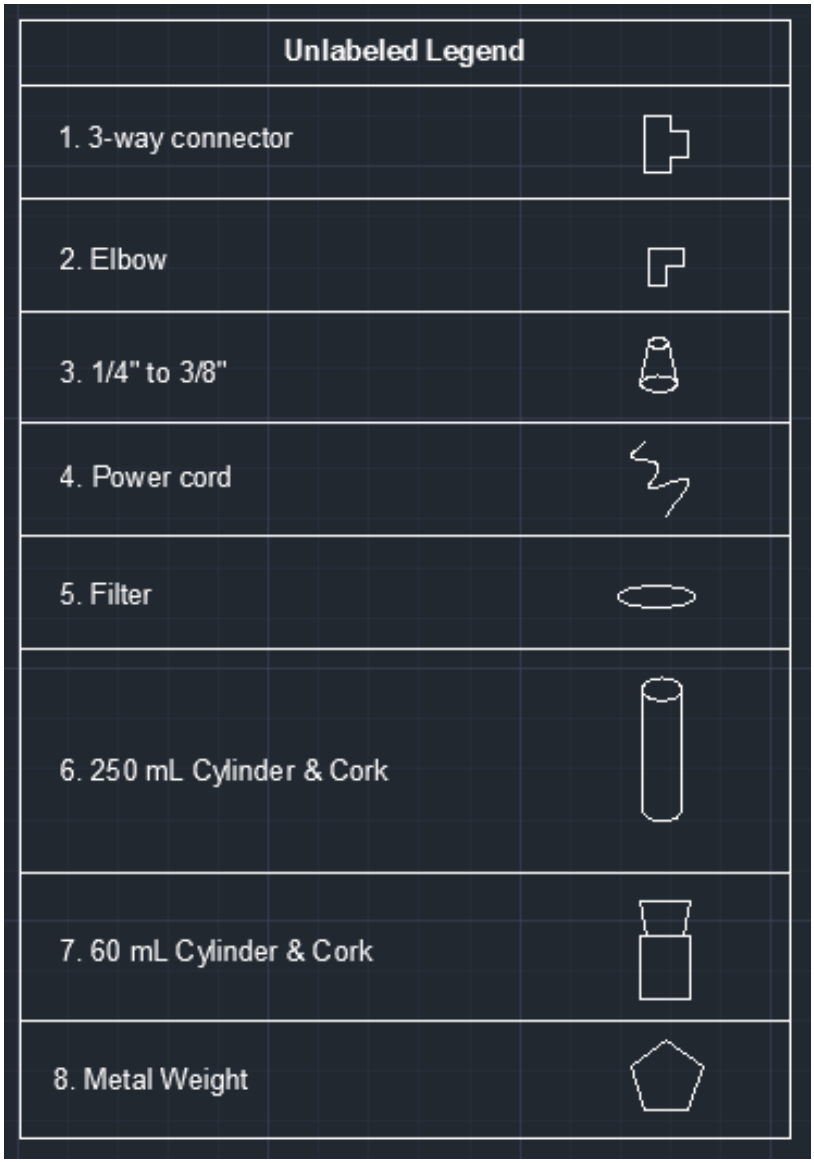

Figure 2. The legend specifying parts in the Figure 1 design that were too small to be labeled visibly.

CHAPTER 3: RESULTS

Preliminary Results and Projections:

The feeding device was tested before starting trials with the algae. First, a 3% CO_2 tank was hooked up to the system with the large flow controller set at 1 L/min and the small ones at 0.2 L/min. The inlet IRGA read 3.01%, while the outlet read about 2.85% (each with ± 0.01% oscillation). The outlet IRGA should have taken longer to reach the correct percentage since the stream had to go through the entire system. However, the outlet IRGA was still not reading as high as it should have been after waiting an appropriate amount of time (about five minutes), so steps were followed from the user manual to calibrate the 400 model on the outlet. Subsequently, the inlet IRGA was reading 3.02 ± 0.01%, while the outlet read 3.01 ± 0.01%. A 0.3% CO_2 tank stream was then run through the system, after which the inlet IRGA read 0.33% and the outlet read 0.25%. The IRGAs were calibrated again, reading 0.32 ± 0.01% on the inlet and 0.31 ± 0.01% on the outlet. This test showed that there can be an approximate difference of 0.07 - 0.16% between the inlet and outlet readings, unless the IRGAs are calibrated to the expected percentages (which is not always constant with this type of process).

After the test, the Air Cadet was turned on, which made the inlet IRGA read about 0.27%, while the outlet read 0.19%. To minimize this margin, leaks were checked for by pinching the tubing and seeing if the flow controllers went to zero as they should. If not, parts were tightened, adjusted, or replaced (such as the replacement of overly stiff or overly loose tubing). It is also worth keeping in mind that the CO_2 given off of the resin will be diluted throughout tubing and other air spaces in the system.

The device was then put through a wetting cycle in order to prepare the resin to give off its stored CO_2 for another test. The water pump was turned on for eight minutes, followed by eight minutes of draining. The resin was observed to be not thoroughly soaked when it was set out to dry after this test, which could either be because of the amount of time an air stream had been blowing through it, or because it should have been soaked for a longer period of time. The optimal time for resin soaking in order to achieve maximum unloading potential was investigated with different trial tests. The subsequent test took five minutes to circulate the water through the system, after which the water pump stayed on for another five minutes until it was drained for ten minutes.

After each water cycle, air was cycled through to look at how much CO_2 the resin could add to the stream. Each of the canisters used totaled about 250 grams of resin. Figures 3 and 4 show the results of the preliminary tests with the resin before testing with the algae. In each case, the inlet IRGA started out at about 0.06% higher than the outlet. On the first trial, the higher input only gave an extra 0.23% CO_2 on the outlet at most, but the lower input ranged from an extra 0.43 - 1.72% CO_2 over a period of about 65 minutes. The dip in outlet concentration from minute 12 to minute 15 of Trial 1 was due to the switch over between tanks that allowed additional air into the system. When testing another canister using only a 0.3% tank for Trial 2, the results showed a range of 0.09 - 0.22% extra CO_2 from the resin over 30 minutes.

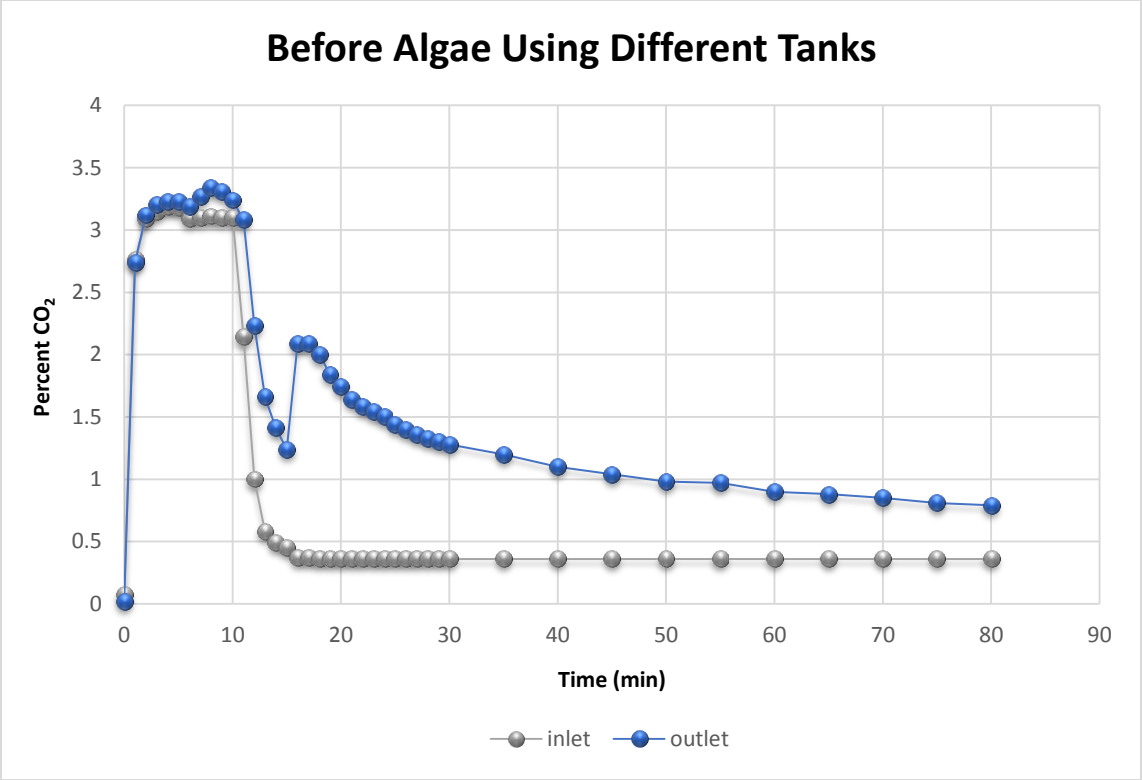

Figure 3. The device was supplied with a tank of 3% CO_2 concentration at t=0 minutes in place of the photobioreactor, and used two canisters of resin. The flow rate started at 5.4 L/min, and was adjusted to 1.4 L/min at t=5 minutes. The supply was switched to a tank of 0.3% CO_2 concentration at t=10 minutes. Inlet concentration (shown in gray), outlet concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

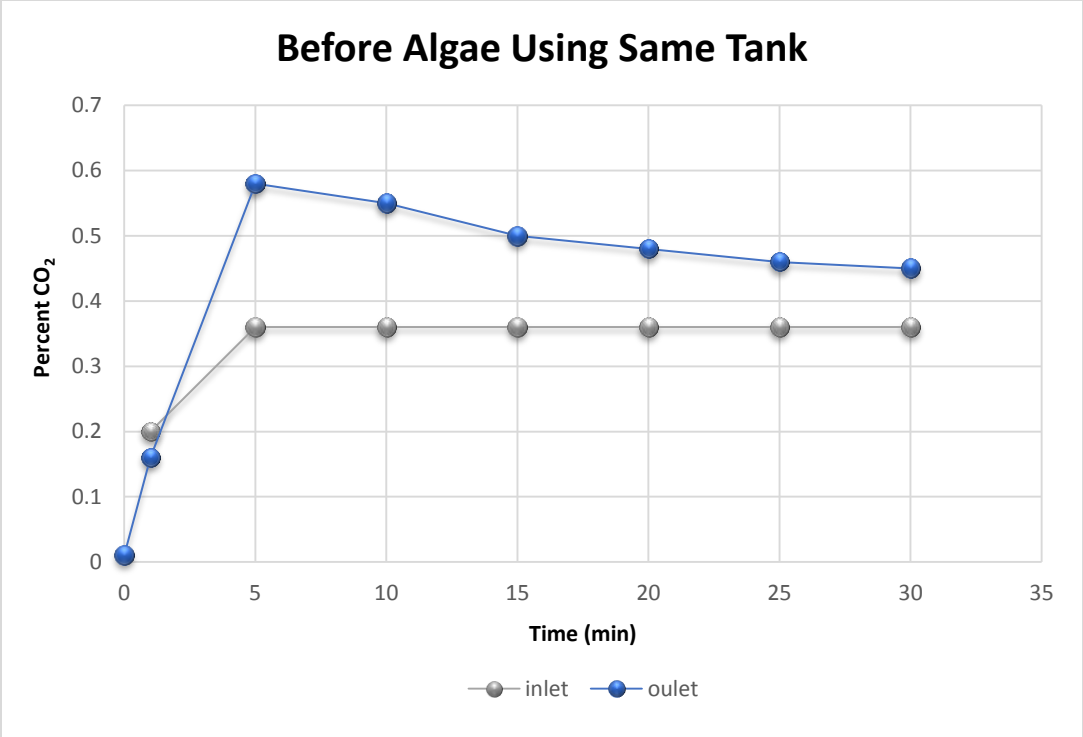

Figure 4. The device was supplied with a tank of 0.3% CO_2 concentration at t=0 minutes in place of the photobioreactor, and used one canister of resin. The flow rate stayed at 1.4 L/min. Inlet concentration (shown in gray), outlet concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

In the following algae trials, the experiments were performed in a room of about 95^{o} F. The heat and humidity from the algae system was a concern for the IRGAs, so the bucket of water had ice added to it to act as a cold trap. Five liters of ice was used (2 - 3 cm^3 per cube), which was enough to have ice water in the bucket for up to two hours. In order to limit the parts needed for the water supply and for the water to be mixed well through the pump, the bucket was chilled minimally.

Another concern was that there would be too much of an increase in oxygen levels (which can be toxic to the algae) if the resin's CO_2 supply were to get too low. There is broad consensus that oxygen inhibition of photosynthesis rises with increasing temperature, but that inhibition is substantially reduced when CO_2 concentrations are increased to the 1 - 2% range (P. Lammers, personal communication, October 19, 2015). Therefore, an oxygen analyzer was used to observe oxygen levels. The toxic oxygen amount is unknown, but the expectation was that the system could handle up to 30 - 40% (P. Lammers, personal communication, October 19, 2015). Even though the system ran at under 1% CO_2 for 25 minutes, the oxygen levels did not go past 20%, which revealed that the oxygen build up would not be too much of a concern based on the durations and deliverables of the rest of the trials.

Figures 5 and 6 show the results of the first algae trials (Trials 3 and 4, respectively), one of which used a plastic surge protector to dampen pulsations from the Air Cadet and one that bypassed the surge protector after four minutes. During Trial 3, the outlet CO_2 concentration ranged from 0.74 - 1.8% (an extra 0.1 - 1.56% higher than the inlet) for 45 minutes. The sharp change in difference between concentrations from minute 13 to minute 19 came as a result of the surge protector popping open, allowing an influx of air before it was closed back up. Trial 4 ranged from 1.02 - 2.33% CO_2 (an extra 0.39 - 2.3% beyond inlet concentrations) for 55 minutes. The higher difference between inlet and outlet concentrations came from before the surge protector was bypassed, and was likely more than Trial 3 because of a slight difference in canister resin weights (487.3 grams total versus 519.3 grams). After the surge protector was bypassed, the system eventually pulled a slight vacuum (raising the algae medium level because of

decreased headspace), so air was again let into the system at minute 15 by opening and closing the tubing connection from the algae. Based on the differences between Trials 3 and 4, a more robust surge protector (the Swagelok cylinder) was ordered to improve the next set of results. After which, the original surge tank served as a buffer on the vacuum end.

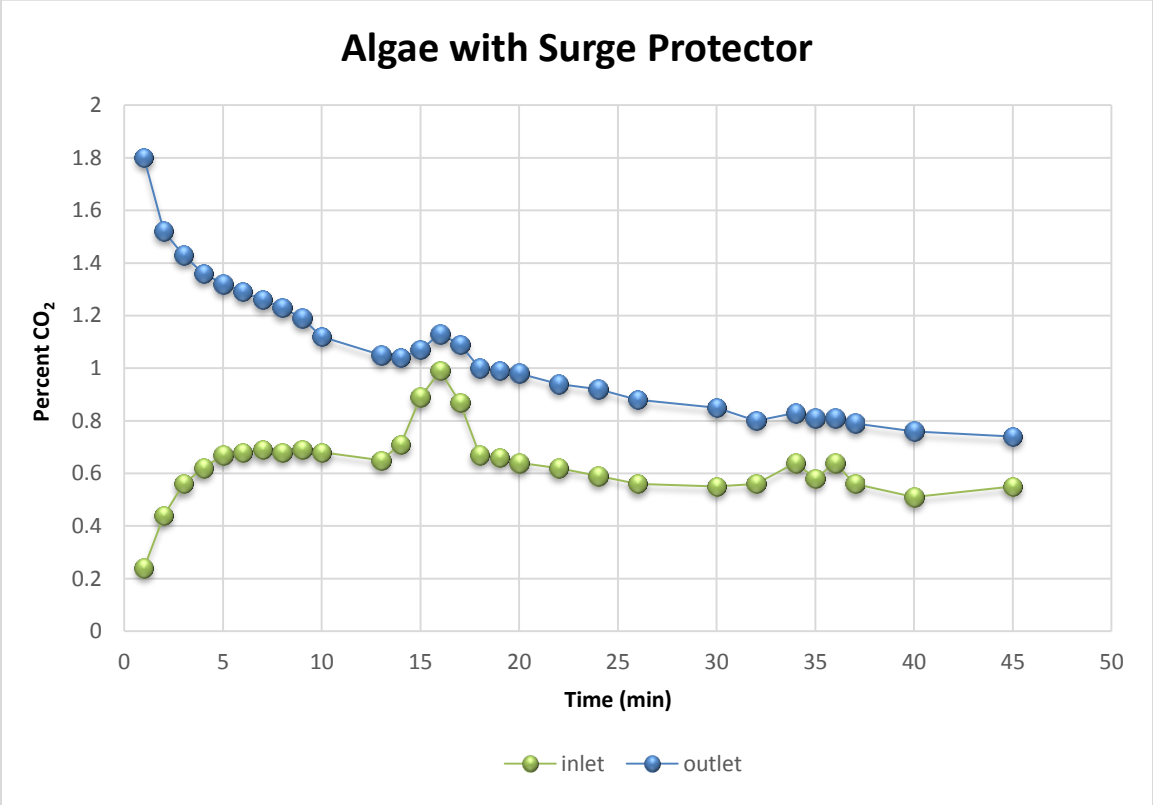

Figure 5. The device was connected to the photobioreactor with two canisters. The flow rate stayed at 2.65 L/min. At t=13 minutes, the surge protector popped open and then was put back together. Oxygen content was also measured: 19.4% at t=1 minute, 19.5% at t=24 minutes, and 19.6% at t=35 minutes. Inlet CO_2 concentration (shown in green), outlet CO_2 concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

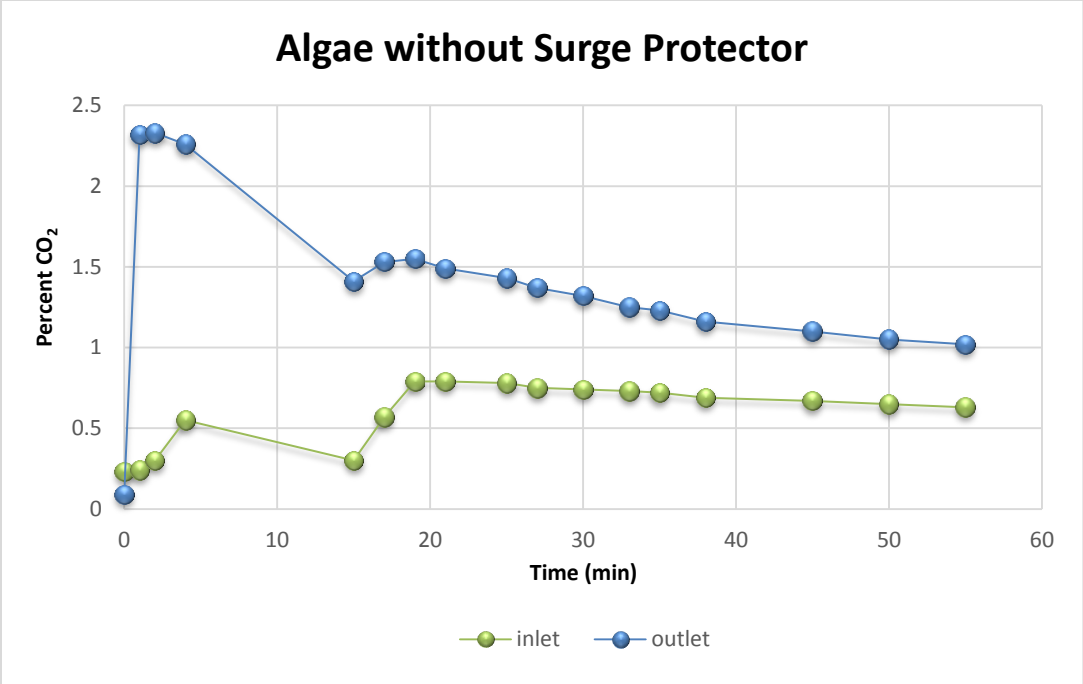

Figure 6. The device was connected to the photobioreactor with two canisters. The flow rate started at 2.65 L/min, then was adjusted to 3.15 L/min at t=8 minutes to assess the difference that an increased flow rate would have. The system bypassed the surge protector at t=4 minutes. Inlet CO_2 concentration (shown in green), outlet CO_2 concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

Results after Improvements:

Because there had been some issues with water getting near the outlet IRGA when the device was switched from water flow to air flow, tubing was repositioned and the plastic bottle was added. The IRGAs were recalibrated right before the new trials with a tank of 30,100 ppm CO_2 ± 2%, after which the inlet and outlet IRGAs read 3.01% ± 0.01% CO_2 and resumed normal functioning.

The algae bioreactor had been running at 0.92% CO_2 before the next set of trails started. They were taken off the CO_2 feed for about an hour while the experiment was set up in an attempt to establish that the culture was not using stored carbon dioxide during the experiment. The bucket was filled about a third full with deionized (DI) water, plus five liters of ice. For the second run with the other set of canisters, the bucket reached about ¾ of the way full after another five liters of ice was put in. The resin was soaked in the DI water for 5.5 minutes past the time of full circulation of the water cycle, after which the resin proved to be virtually fully soaked when viewed at the end of the experiment.

On Trial 5 (Figure 7), the pressure valve was adjusted to open around the time of the system reaching one pound per square inch. When the valve was opened and the ball taken out, the system read about 1.6 psi, which was adjusted with screws to 1.2 psi and the ball was placed back in. After which, the bag reinflated slightly and then stayed inflated for a while. Pressure was later released from the bag after 31 minutes by briefly opening and then closing the extra algae port. The bag was still inflating though, so a version of a manometer (a device that diverts pressure via a column of water) was inserted on the algae side at minute 33. This allowed for the possibility of a small CO_2 release outside of the algae bag throughout the rest of the experiment. This set of experiments used the EcoAir pump instead of the Air Cadet, which enabled the resin of Trial 5 to supply 0.95 - 2.25% CO_2 (an average of about a 0.5% increase on the inlet) for 90 minutes.

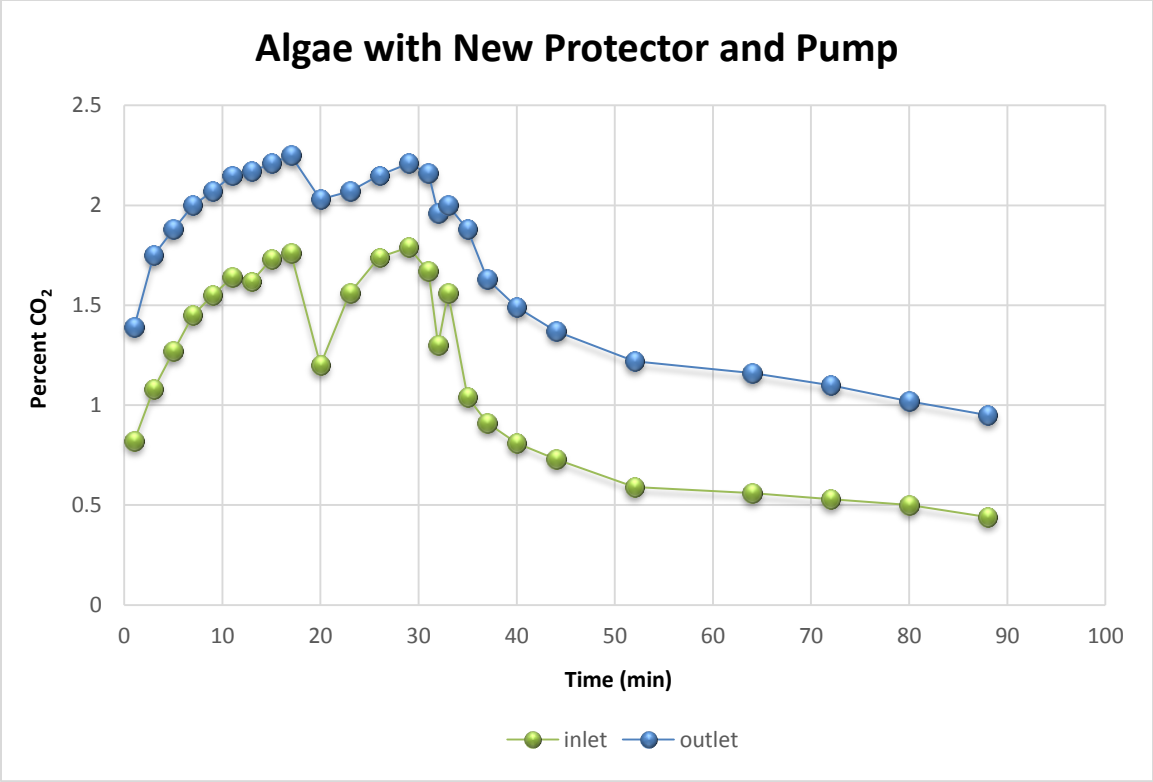

Figure 7. The device was connected to the photobioreactor with two canisters. The flow going to the PBR was 2.9 L/min, while coming from it was 2.4 L/min. Pressure had reached 1.5 psi by t=23 minutes. Inlet CO_2 concentration (shown in green), outlet CO_2 concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

The CO_2 meter (which was initially used instead of the outlet flow meter) read about eight standard cubic feet per hour (SCFH) when the feeding device was running. The small air controller coming out of the small air pump measures the flow into both IRGAs, whereas the other small air controller and the large inflow air controller are additive of the whole input flow through the system. The small air controller from the small air pump was at a constant 0.4 L/min for Trials 5 and 6, oscillating more than the

large air controller. Trial 6 had a constant 2% CO_2 stream inserted at 1.5 L/min (seeing as the air flow on the feeding device was going add to up to around 1.5 L/min, which would give a total of about 3 L/min air flow through the whole system). Although the outdoor algae systems can run at higher flow rates, the indoor system flow rates typically run at 3 L/min (sometimes less). It is important to note that some of the flow meters on the device are made by different companies, one that uses steel balls (VWR) and one that uses plastic balls (Dwyer). Because of this, the readings may differ slightly. Before testing with the algae, the output flow showed a 0.04 L/min rate less than the input. During the algae testing, lower flow rates caused bigger gaps between inflow and outflow, which was likely coming from somewhere on the algae side since the feeding device was tested for leaks.

The difference between inflow and outflow was measured throughout the constant stream and resin combination of Trial 6 (Figures 8 and 9). While transitioning into the combination trial, the algae were flooded with the constant 2% CO_2 stream at 1.5 L/min, and the resins were soaked for 4.5 minutes past full circulation. Changing the flow rate to reach a 2.5% CO_2 target was successful for about two hours. The resin consistently supplied more than half a percent of carbon dioxide beyond the inlet stream during the entire experiment. Most of the time, output flow rates from the resin device ranged from 1 - 1.5 L/min. As seen in Table 2, there was a clear correlation between flow rates and pressure levels in the system. Namely, higher flow rates allowed for lower pressure readings, which could potentially be related to the properties of the flow meters.

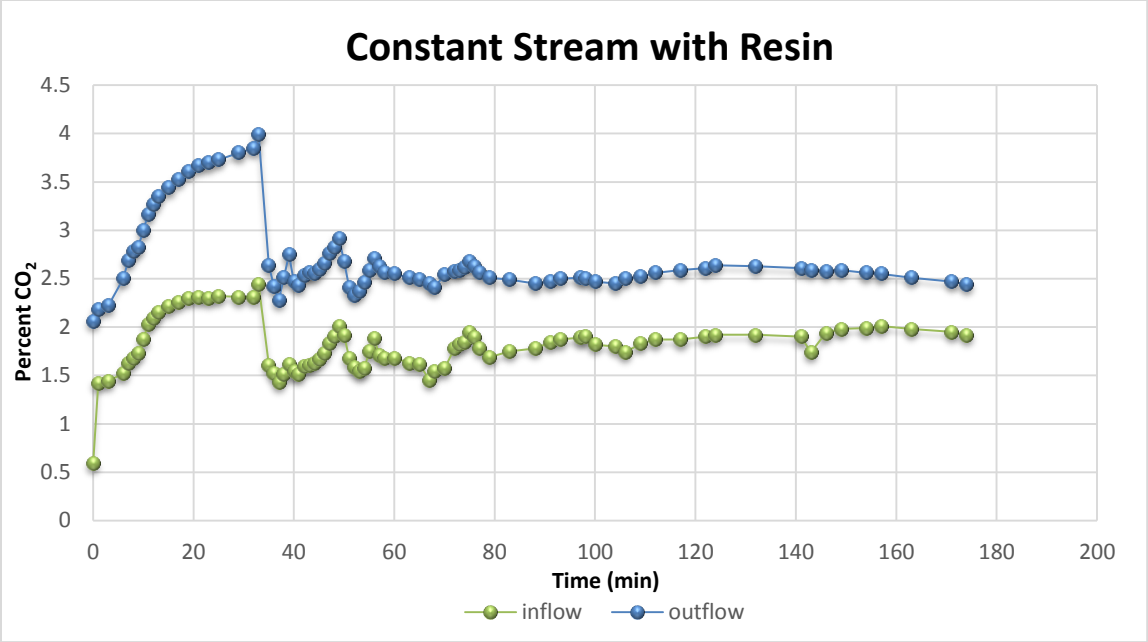

Figure 8. The device was connected to the photobioreactor with two canisters. The flow rate was continuously adjusted (as seen in Figure 9) in attempts to keep a 2.5% CO_2 outlet stream. Inlet CO_2 concentration (shown in green), outlet CO_2 concentration (in blue), and time were recorded manually by watching the IRGAs and a stopwatch, and writing down the information.

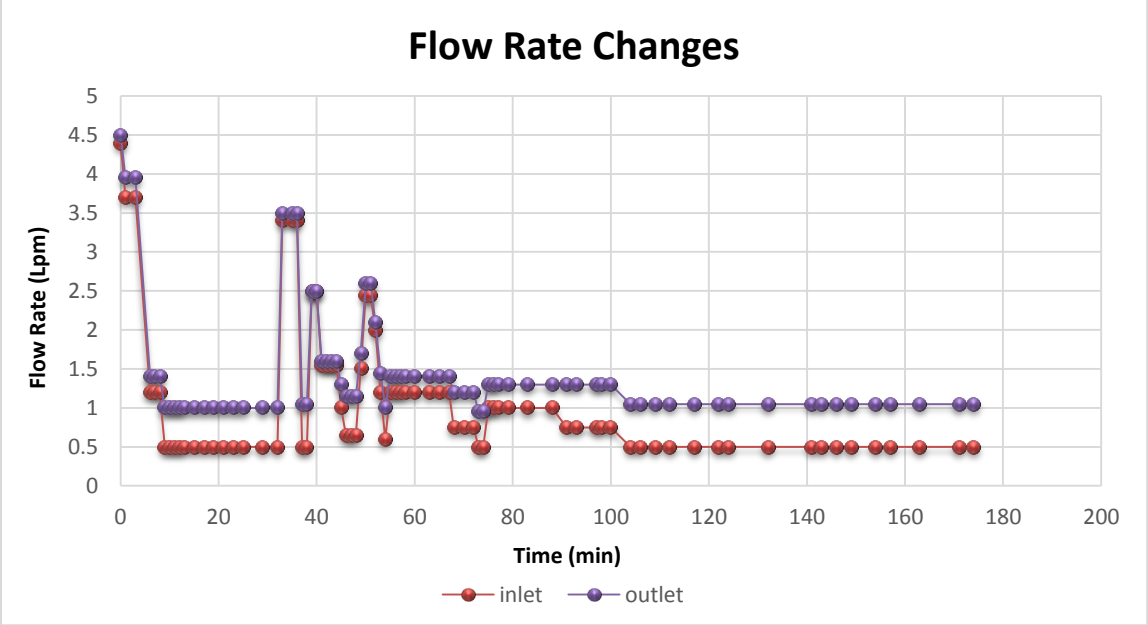

Figure 9. The device was connected to the photobioreactor with two canisters. The flow rate was continuously adjusted in attempts to keep a 2.5% CO_2 outlet stream during the

trial shown in Figure 8. Inlet flow from the PBR (shown in red), outlet flow to the PBR (in purple), and time were recorded manually by watching the flow controllers and a stopwatch, and writing down the information.

Flow Pressure Correlation:

Outflow (L/min)	**Pressure (psi)**
0.95	2.45
1	2.4
1.05	2.4
1.2	2.35
1.3	2.25
1.4	2.25
2.6	1.85
3.5	1.55
3.95	1.5

Table 2. The outflow (in L/min) in order of increasing rates corresponds to the pressure (in psi) in order of decreasing values.

The bioactivity was measured to verify how well the carbon dioxide that the resin device provided was being consumed during the trials. Optical densities are typically used to measure productivity, but if the duration of the experiment is too short (less than six hours), the growth measurements may be unreliable (N. Csakan, personal

communication, February 26, 2016). Culture data shows that growth is similar for most strains: 0.1 - 0.16 grams of ash-free dry weight per liter per day (P. Lammers, personal communication, February 25, 2016). Optical density measurements correlate to ash-free dry weight production. The end sample was taken after the air flow was increased slightly because there was some settling of the algae toward the end of Trial 6 with the lower flow rates. The input flow into the algae container should be high enough to pump through the bottom of the water column and circulate the medium.

Approximately 15 mL of the medium was pulled out with a syringe before running the system with the device and used as the first sample. The first optical density (OD) sample produced a measurement of 0.712 g/L. Another sample of the medium was taken between the canister switch over (between Trials 5 and 6). The second optical density set produced a measurement of 0.824 g/L. The difference in densities here, 0.112g/L, matches the typical growth range, but was taken at only about half of the necessary time for reliable data. The final sample of medium was taken at the end of the entire experiment (after Trial 6). The last optical density set produced a measurement of 0.808 g/L. The decrease from the second to the third measurement could be due to the manometer that was required and let off some of the air. Overall, the experiment resulted in a 0.096 g/L growth over a six hour period. When rounded to the hundredth decimal place, this measurement is on par with the standard growth of a culture.

A rough estimate of the amount of carbon dioxide delivered by the resin in each trial was calculated (Table 3). Table 3 was created as a result of the Excel tables shown in Appendix G. The time columns were created by putting the time between each data set as one interval, with the final interval of each trial being one minute. The

concentrations for each trial were determined by calculating the difference between the inlet and outlet CO_2 concentrations of each data point. The concentrations did not stay constant during the given time intervals, which is why the data in Table 3 only provide an estimate. The total amount of carbon dioxide provided by the resin (in grams) was calculated for each interval (and summed at the end) by multiplying the following: the difference in concentration (% CO_2) * 10,000 [to convert % to ppm] * the molar volume (22.4 L * 1.13 *1/1.14 to adjust for the 13% temperature increase and the 14% pressure increase from standard temperature and pressure) over the molecular weight (44.01 g/mol) [to convert ppm into micrograms/L] * 1 gram over 10^6 micrograms * the flow rate (L/min) * the time interval (min). The average rate that can be given by 500 grams of resin in a given trial is about 0.01 g/min (extrapolating that the 250 gram trial is multiplied by two). The results of Table 3 indicate that 500 grams of resin can provide seven grams of algae (the product of the 0.7 g/L starting OD sample and the 10 L medium volume) with about 0.054 grams of carbon dioxide over 84.67 minutes.

Carbon Dioxide Delivered:

Trial	Grams	Minutes	Grams/Min
1	0.45	81	0.01
2	0.04	62	0.001
3	0.23	45	0.01
4	0.77	56	0.01
5	0.76	89	0.01
6	1.01	175	0.01
Average	**0.54**	**84.67**	**0.01**

Table 3. The total amount of CO_2 given by the resin in each trial (in grams), along with the overall time of the trials and the average rate delivered (in g/min) using approximately 500 grams of resin each.

CHAPTER 4: DISCUSSION

Conclusions:

Each trial completed with the CO_2 feeding device allowed for a better understanding of the system in which it was tested. The preliminary tests indicated that the resin device worked better at lower flow rates. But because the algae are supposed to be circulated continuously, the total flow rate that best accommodated both systems was around 3 L/min. Percent CO_2 delivered from the resin depends on the flow rate. Therefore, an alternative flow addition that can boost flow after the resin stream would be the most successful. Although the resin performs better at lower flow rates, the pressure of the system proves to be more stable (closer to the atmospheric pressure of 14.7 psi) at higher flow rates. This is useful in constant stream applications because those systems require a higher combined flow regardless. The constant stream data could indicate a superior performance addition to constant stream combinations.

The data found with the new protector and pump was much more promising, in that the replacements granted more consistent improvements on the outlet stream. A constant stream addition permits an extended period of use for the resin, if flow rates are adjusted accordingly. The flow rate does not need to change all that much once flow has occurred for a while. Still, consistency in parts and brands will allow for more precise measurements and functioning. Dry weight measurements are the most important indicator of success, and can make up for errors in calibrations and readings.

In order to achieve maximum resin off-gassing, it is possible that the material needs to be fully soaked. If so, the best resin wetting time was determined to be a six

minute extension past full water circulation. Once wetted, the algae need a minimum of about 1% CO_2 at a 3 L/min flow rate. The data from the latest trial without a constant stream, as shown in Table G5, reveal that two canisters were able to supply the algae with slightly over 2% for about 30 minutes. That means that if the canisters were to be employed one at a time, two canisters could supply 1% for one hour (which given the average of 0.01 g/min from Table 3, would give 0.6 grams of carbon dioxide to the average seven grams of algae). The additional CO_2 beyond the 1% could then be used to add to a constant stream, potentially giving the canisters an extra three hours of functioning each. Therefore, a 12 hour day would require 6-24 canisters of 250 grams of resin per day for a 15 L reactor.

Future Applications:

Several of the parts and processes in the experiments with the feeding device were manually operated. There are several options to automate the system, including the concentration, flow, and time recordings. Also, instead of refilling the bucket with ice every few hours, an automatic chiller could be employed to keep the water at a continuously cooled temperature.

Throughout the trials, the air pump was turned off during the resin wetting phase, which allowed it to cool off as it was frequently running hot when it remained on for an extended period of time. The pump was also occasionally dabbed with a cool, wet paper towel, plus the O-Ring inside the pump was cooled by wiping it with cool water after the fifth trial and placing it back in. A fan facing the pump would be a helpful addition. An

airtight seal was not used in order not to overheat the pump. Although, water was getting through the tubing leading to the large air pump, so a desiccant placed inside the tubing could also be valuable.

The system initially was pulling a partial vacuum, indicated by the pressure gauge going to slightly below zero when the flow controller read about 0.08 L/min from the top of the surge tank. This means that the system was not putting back all of the air it was taking out. A potential fix for the vacuum could be to use outside air, which is what eventually happened when the surge tank and ports were opened and when the manometer was put in. Another option for the vacuum relief would be to add a separate or additional valve specified for vacuum purposes (rather than reversing the parts in a pressure valve).

In the case of over pressurization, there is the option of creating an acrylic, rectangular box to replace the bag that the algae were put in, which could be pressurized to the exact specifications that would be required. If a new algae container were to be made of the harder material, it should ideally be pressurized to at least three pounds per square inch to allow less volume expansion. The box would also need to be leak tested, and a lid compatible with the bag ports would need to be built. The pressurized box is a reasonable possibility because pressure is determined by thermodynamics, even though concentration is not. The feeding device gradually adds carbon dioxide, which builds volume and goes back through the system as O_2 from photosynthesis, so another possible addition is an internal oxygen scrubber.

If the system were to be adjusted to a pressure-specific encasement around the algae, the lower flow rates for better resin performance may be more acceptable as long

as circulation is upheld. However, avoiding pressurization may be a good idea because it could incur unnecessary costs and effort. The system should be as sealed as possible though, so if the pump has leaks when it is not sealed, then it could be installed differently. For example, the pump could be put into a separate container (possibly near the chiller) into which air would flow from the bioreactor, and the pump would be left without seals on it. It is acceptable for the pump to leak slightly if it saves costs in other areas (K. Lackner, personal communication, April 9, 2016).

In order to make the feeding process a cyclical system, a drying system for the resin also needs to be put into place. This would involve having extra sets of canisters to rotate, and having the wet material from the used canisters laid out in front of a fan, wind, and/or the sun. Drying stage variances need to be assessed, as the material takes up to two days in room temperature fan air to completely dry after being soaked. Ideal drying time scenarios would condense to two hours at the most to enable multiple uses of the resin in the same day. The Center for Negative Carbon Emissions is currently working on filter units that could accomplish this.

Another possibility for the feeding system not yet explored is using the free moisture and temperature increase of the algae system to the resin's advantage. Cooling slows down evaporation, so taking out that process could speed up the usefulness of resin applications. The moisture could be used to supply the resin with water to release the carbon dioxide, and the temperature could be used to allow the material to perform better and release more carbon dioxide (Wang, Lackner, & Wright, 2011). A moisture analyzer could be introduced to estimate how much moisture is needed for the best resin-algae advantage. One way the resin device could be put under these optimal conditions is by

bypassing the IRGAs, but the CO_2 levels in and out of the device would then not be able to be measured. Bioactivity data via optical density measurements would need to be monitored in order to assess proper functioning of the material. The IRGAs could also be decoupled from the pump, and measure carbon dioxide in a small slip stream.

It is important to remain a carbon neutral source of carbon dioxide with this system. Currently, there are environmental facilities in Arizona that contribute to local CO_2 needs by collecting what they create and distributing it to tank bottlers (Randazzo, 2007). There is a possibility of being able to supply CO_2 from the resin as a carbon negative source via a tank. But this would require the ability to compress the resin's outlet stream, which would take cost advantage away. It would, however, simplify the design process because compression would be separated from the feeding, which in turn would allow for the ability to go back to the original algae feeding system. Still, because the extremophile algae strains grow best in the same conditions as the resin (high temperature and humidity levels), the design for this particular system is likely better suited for the resin to algae feedback.

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APPENDIX A

NITROGEN/PHOSPHORUS REDUCTION IN WASTEWATER

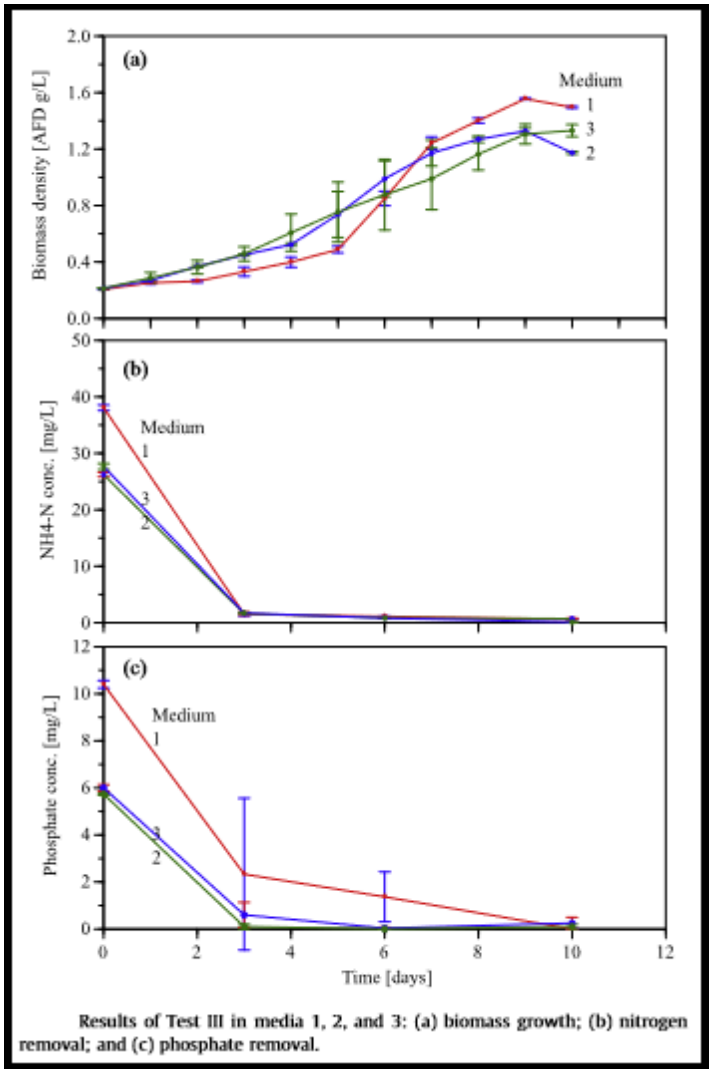

Figure A1. Graphs from Henkanatte-Gedera et al. (2015) showing a decrease in harmful nitrogen and phosphorus compounds as algae biomass increases.

APPENDIX B

NITROGEN/PHOSPHORUS REDUCTION RATES

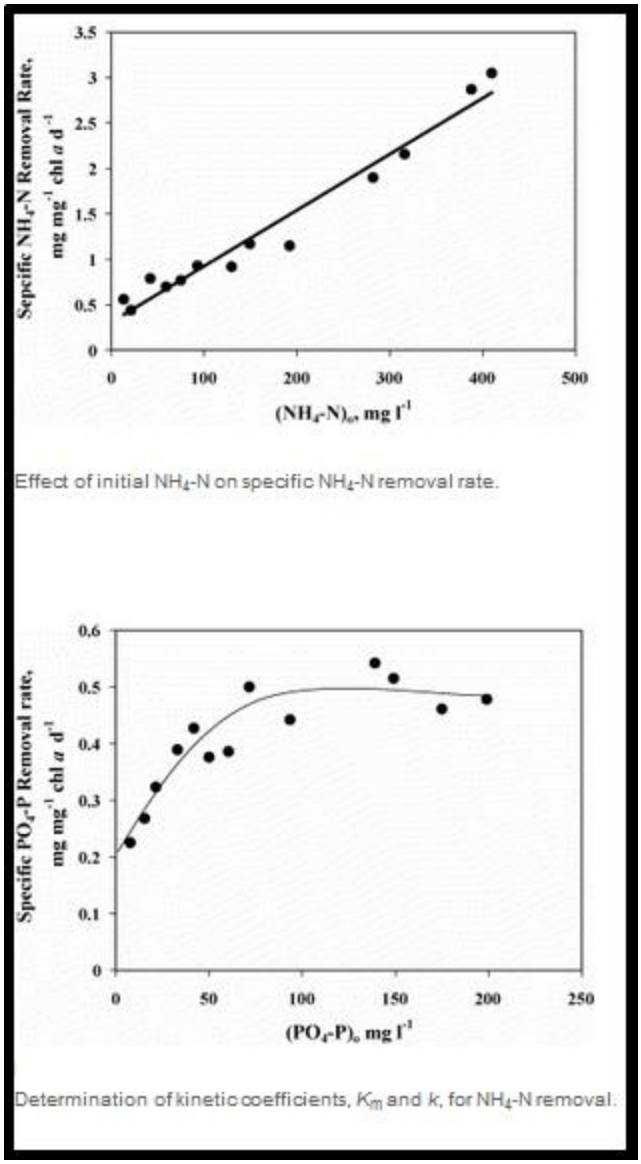

Figure B1. Graphs from Aslan & Kapdan (2006) to demonstrate how removal rates of nitrogen and phosphorus increase as algae concentrations increase.

APPENDIX C

HYDROTHERMAL LIQUEFACTION PATHWAYS

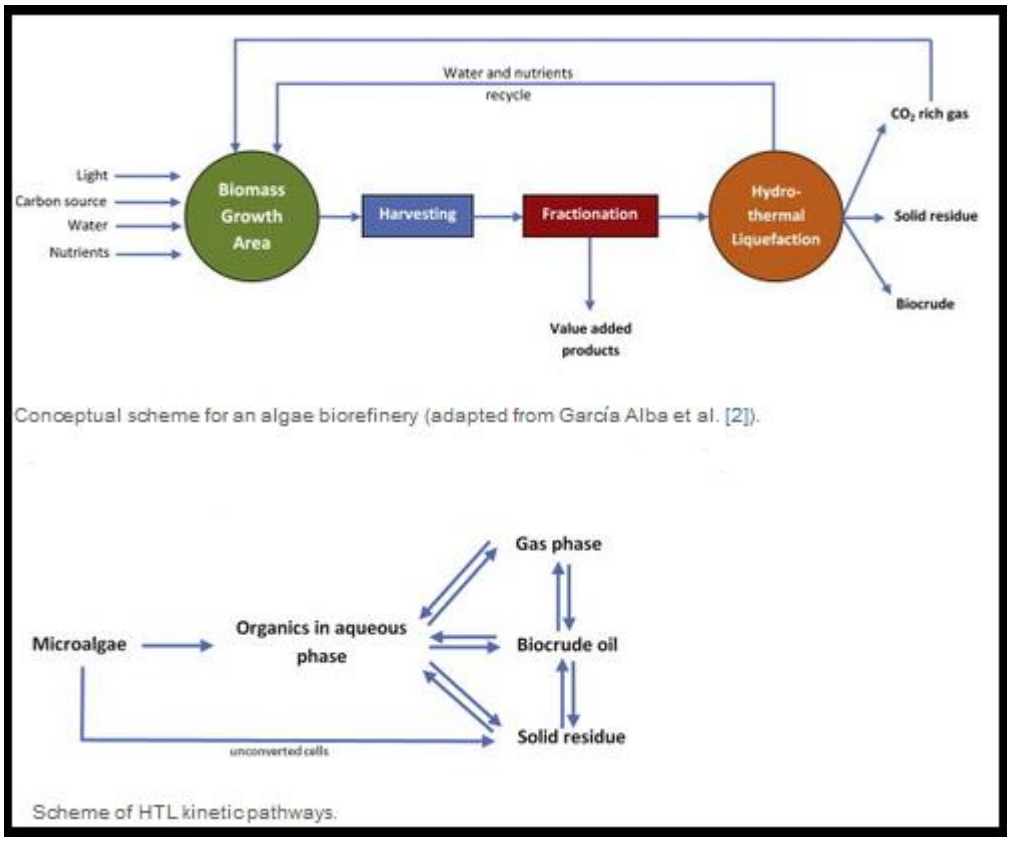

Figure C1. Schematics from Barreiro et al. (2013) showing the pathways involved in hydrothermal liquefaction from algae production.

APPENDIX D

HYDROTHERMAL LIQUEFACTION NUMBERS

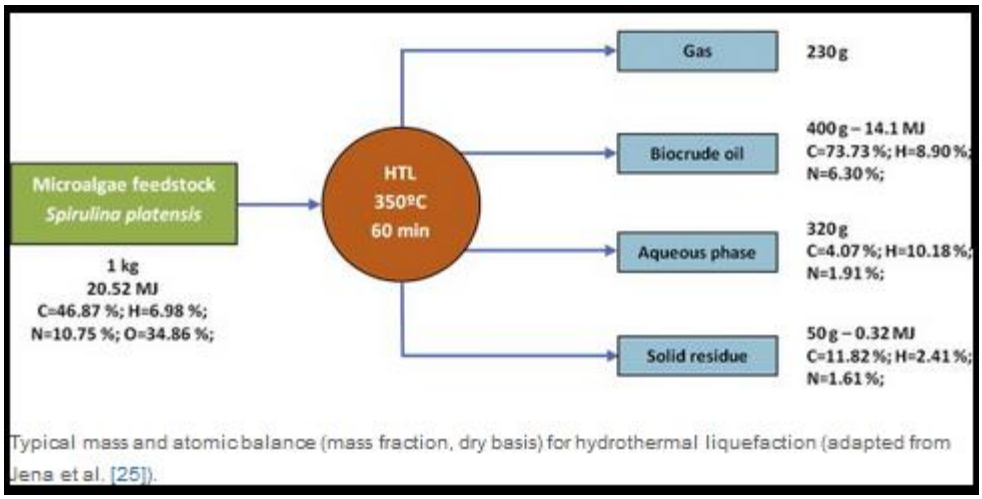

Figure D1. Proportions from Barreiro et al. (2013) involved in the HTL pathways.

APPENDIX E

RESIN AND MATERIAL COMBINATIONS

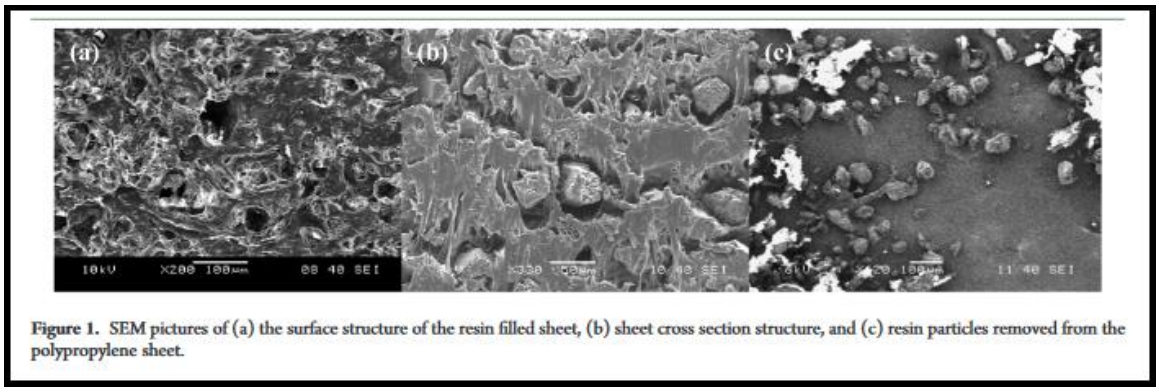

Figure 1. SEM pictures of (a) the surface structure of the resin filled sheet, (b) sheet cross section structure, and (c) resin particles removed from the polypropylene sheet.

Figure E1. Images from Wang, Lackner, and Wright (2011), providing a close-up of the resin material when combined with and removed from the polypropylene material.

APPENDIX F

RESIN CYCLE CHEMISTRY

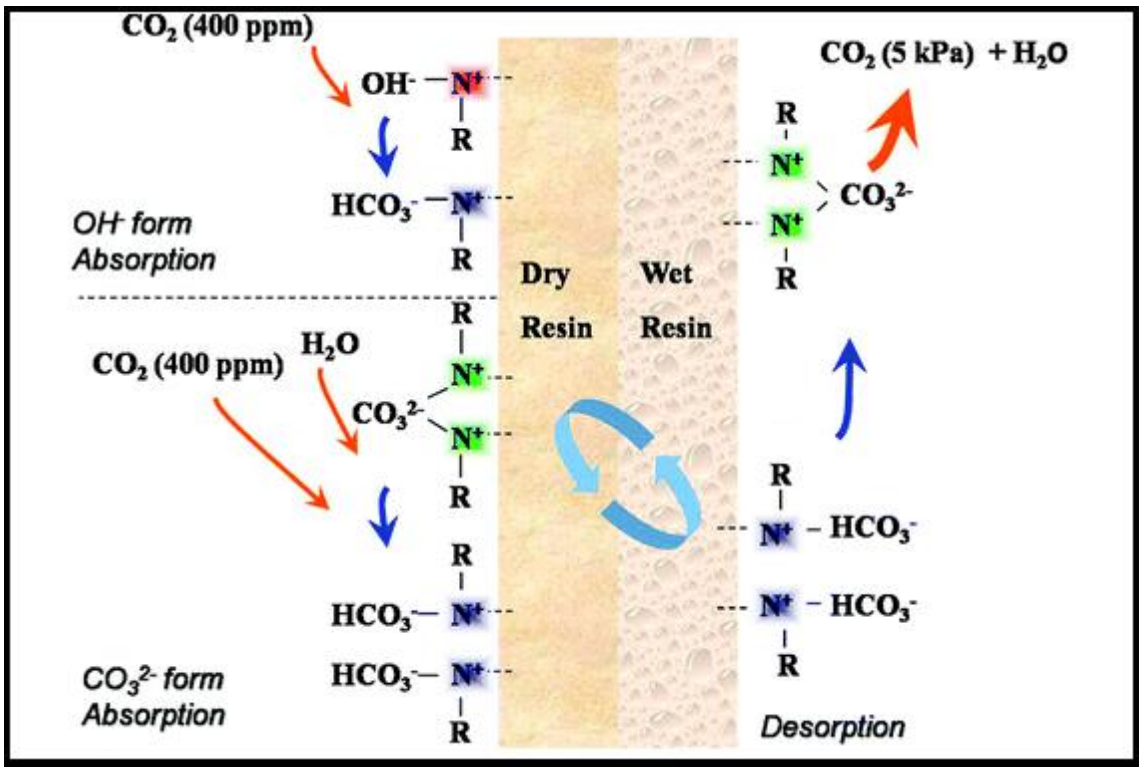

Figure F1. The chemical bonds that cause the resin to absorb carbon dioxide when dry and release it when wet, taken from Wang, Lackner, and Wright (2011).

APPENDIX G

CARBON DIOXIDE DELIVERED PRELIMINARIES

	Time (min)	Inlet (% CO2)	Outlet (% CO2)	Concentration Difference	Flow Rate (L/min)	CO2 Given (grams)
	1	0.07	0.02	-0.05	5.4	-0.011
	1	2.76	2.74	-0.02	5.4	-0.004
	1	3.09	3.12	0.03	5.4	0.007
	1	3.15	3.2	0.05	5.4	0.011
	1	3.19	3.23	0.04	5.4	0.009
	1	3.18	3.23	0.05	5.4	0.011
	1	3.09	3.19	0.1	5.4	0.022
	1	3.1	3.27	0.17	5.4	0.005
	1	3.11	3.34	0.23	5.4	0.006
	1	3.1	3.31	0.21	1.4	0.001
	1	3.1	3.24	0.14	1.4	0.001
	1	2.14	3.08	0.94	1.4	0.007
	1	1	2.23	1.23	1.4	0.009
	1	0.58	1.66	1.08	1.4	0.008
	1	0.49	1.41	0.92	1.4	0.006
	1	0.45	1.24	0.79	1.4	0.006
	1	0.37	2.09	1.72	1.4	0.012
	1	0.37	2.09	1.72	1.4	0.012
	1	0.36	2	1.64	1.4	0.012
	1	0.36	1.84	1.48	1.4	0.010
	1	0.36	1.74	1.38	1.4	0.010
	1	0.36	1.64	1.28	1.4	0.009
	1	0.36	1.58	1.22	1.4	0.009
	1	0.36	1.54	1.18	1.4	0.008
	1	0.36	1.5	1.14	1.4	0.008
	1	0.36	1.44	1.08	1.4	0.008
	1	0.36	1.4	1.04	1.4	0.007
	1	0.36	1.36	1	1.4	0.007
	1	0.36	1.33	0.97	1.4	0.007
	1	0.36	1.3	0.94	1.4	0.007
	5	0.36	1.28	0.92	1.4	0.032
	5	0.36	1.2	0.84	1.4	0.030
	5	0.36	1.1	0.74	1.4	0.026
	5	0.36	1.04	0.68	1.4	0.024
	5	0.36	0.98	0.62	1.4	0.022
	5	0.36	0.97	0.61	1.4	0.022

	5	0.36	0.9	0.54	1.4	0.019
	5	0.36	0.88	0.52	1.4	0.018
	5	0.36	0.85	0.49	1.4	0.017
	5	0.36	0.81	0.45	1.4	0.016
	1	0.36	0.79	0.43	1.4	0.003
Total	**81**					**0.45**

Table G1. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 1 for Table 3.

	Time (min)	**Inlet (% CO2)**	**Outlet (% CO2)**	**Concentration Difference**	**Flow Rate (L/min)**	**CO2 Given (grams)**
	1	**0.07**	**0.01**	-0.06	1.2	0.000
	4	0.2	0.16	-0.04	1.2	-0.001
	5	0.36	0.58	0.22	1.2	0.007
	5	0.36	0.55	0.19	1.2	0.006
	5	0.36	0.5	0.14	1.2	0.004
	5	0.36	0.48	0.12	1.2	0.004
	5	0.36	0.46	0.1	1.2	0.003
	1	0.36	0.45	0.09	1.2	0.001
Total	**31**					**0.02**

Table G2. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 2 for Table 3.

	Time (min)	Inlet (% CO_2)	Outlet (% CO_2)	Concentration Difference	Flow Rate (L/min)	CO_2 Given (grams)
	1	0.24	1.8	1.56	2.65	0.021
	1	0.44	1.52	1.08	2.65	0.014
	1	0.56	1.43	0.87	2.65	0.012
	1	0.62	1.36	0.74	2.65	0.010
	1	0.67	1.32	0.65	2.65	0.009
	1	0.68	1.29	0.61	2.65	0.008
	1	0.69	1.26	0.57	2.65	0.008
	1	0.68	1.23	0.55	2.65	0.007
	1	0.69	1.19	0.5	2.65	0.007
	3	0.68	1.12	0.44	2.65	0.018
	1	0.65	1.05	0.4	2.65	0.005
	1	0.71	1.04	0.33	2.65	0.004
	1	0.89	1.07	0.18	2.65	0.002
	1	0.99	1.13	0.14	2.65	0.002
	1	0.87	1.09	0.22	2.65	0.003
	1	0.67	1	0.33	2.65	0.004
	1	0.66	0.99	0.33	2.65	0.004
	2	0.64	0.98	0.34	2.65	0.009
	2	0.62	0.94	0.32	2.65	0.009
	2	0.59	0.92	0.33	2.65	0.009
	4	0.56	0.88	0.32	2.65	0.017
	2	0.55	0.85	0.3	2.65	0.008
	2	0.56	0.8	0.24	2.65	0.006
	1	0.64	0.83	0.19	2.65	0.003
	1	0.58	0.81	0.23	2.65	0.003
	1	0.64	0.81	0.17	2.65	0.002
	3	0.56	0.79	0.23	2.65	0.009
	5	0.51	0.76	0.25	2.65	0.017
	1	0.55	0.74	0.19	2.65	0.003
Total	**45**					**0.23**

Table G3. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 3 for Table 3.

	Time (min)	Inlet (% CO_2)	Outlet (% CO_2)	Concentration Difference	Flow Rate (L/min)	CO_2 Given (grams)
	1	0.23	0.09	-0.14	3.15	-0.002
	1	0.24	2.32	2.08	3.15	0.033
	2	0.3	2.33	2.03	3.15	0.065
	11	0.55	2.26	1.71	3.15	0.299
	2	0.3	1.41	1.11	3.15	0.035
	2	0.57	1.53	0.96	3.15	0.031
	2	0.79	1.55	0.76	3.15	0.024
	4	0.79	1.49	0.7	3.15	0.044
	2	0.78	1.43	0.65	3.15	0.021
	3	0.75	1.37	0.62	3.15	0.030
	3	0.74	1.32	0.58	3.15	0.028
	2	0.73	1.25	0.52	3.15	0.017
	3	0.72	1.23	0.51	3.15	0.024
	7	0.69	1.16	0.47	3.15	0.052
	5	0.67	1.1	0.43	3.15	0.034
	5	0.65	1.05	0.4	3.15	0.032
	1	0.63	1.02	0.39	3.15	0.006
Total	**56**					**0.77**

Table G4. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 4 for Table 3.

	Time (min)	Inlet (% CO_2)	Outlet (% CO_2)	Concentration Difference	Flow Rate (L/min)	CO_2 Given (grams)
	2	0.82	1.39	0.57	2.9	0.017
	2	1.08	1.75	0.67	2.9	0.020
	2	1.27	1.88	0.61	2.9	0.018
	2	1.45	2	0.55	2.9	0.016
	2	1.55	2.07	0.52	2.9	0.015
	2	1.64	2.15	0.51	2.9	0.015
	2	1.62	2.17	0.55	2.9	0.016
	2	1.73	2.21	0.48	2.9	0.014
	3	1.76	2.25	0.49	2.9	0.022
	3	1.2	2.03	0.83	2.9	0.036
	3	1.56	2.07	0.51	2.9	0.022
	3	1.74	2.15	0.41	2.9	0.018
	3	1.79	2.21	0.42	2.9	0.018
	1	1.67	2.16	0.49	2.9	0.007
	1	1.3	1.96	0.66	2.9	0.010
	2	1.56	2	0.44	2.9	0.013
	2	1.04	1.88	0.84	2.9	0.025
	3	0.91	1.63	0.72	2.9	0.032
	4	0.81	1.49	0.68	2.9	0.040
	8	0.73	1.37	0.64	2.9	0.075
	12	0.59	1.22	0.63	2.9	0.111
	8	0.56	1.16	0.6	2.9	0.070
	8	0.53	1.1	0.57	2.9	0.067
	8	0.5	1.02	0.52	2.9	0.061
	1	0.44	0.95	0.51	2.9	0.007
Total	**89**					**0.76**

Table G5. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 5 for Table 3.

	Time (min)	Inlet (% CO2)	Outlet (% CO2)	Concentration Difference	Flow Rate (L/min)	CO2 Given (grams)
	1	0.59	2.06	1.47	4.5	0.033
	2	1.42	2.18	0.76	3.95	0.030
	3	1.44	2.22	0.78	3.95	0.047
	1	1.52	2.5	0.98	1.4	0.007
	1	1.63	2.69	1.06	1.4	0.007
	1	1.68	2.78	1.1	1.4	0.008
	1	1.73	2.83	1.1	1	0.006
	1	1.87	3	1.13	1	0.006
	1	2.03	3.17	1.14	1	0.006
	2	2.09	3.27	1.18	1	0.012
	2	2.15	3.35	1.2	1	0.012
	2	2.21	3.44	1.23	1	0.012
	2	2.26	3.53	1.27	1	0.013
	2	2.3	3.61	1.31	1	0.013
	2	2.31	3.67	1.36	1	0.014
	4	2.3	3.7	1.4	1	0.028
	3	2.32	3.73	1.41	1	0.021
	1	2.31	3.8	1.49	1	0.008
	1	2.31	3.85	1.54	1	0.008
	2	2.44	3.99	1.55	3.5	0.055
	1	1.61	2.64	1.03	3.5	0.018
	1	1.52	2.42	0.9	3.5	0.016
	1	1.43	2.28	0.85	1.05	0.005
	1	1.51	2.51	1	1.05	0.005
	1	1.62	2.75	1.13	2.5	0.014
	1	1.54	2.47	0.93	2.5	0.012
	1	1.51	2.43	0.92	1.6	0.007
	1	1.6	2.53	0.93	1.6	0.008
	1	1.61	2.57	0.96	1.6	0.008
	1	1.63	2.55	0.92	1.6	0.007
	1	1.67	2.6	0.93	1.3	0.006
	1	1.73	2.66	0.93	1.15	0.005
	1	1.83	2.76	0.93	1.15	0.005
	1	1.91	2.83	0.92	1.15	0.005
	1	2.01	2.92	0.91	1.7	0.008
	1	1.92	2.68	0.76	2.6	0.010
	1	1.68	2.41	0.73	2.6	0.010

	1	1.59	2.33	0.74	2.1	0.008
	1	1.54	2.37	0.83	1.45	0.006
	1	1.57	2.46	0.89	1	0.004
	1	1.75	2.59	0.84	1.4	0.006
	1	1.88	2.71	0.83	1.4	0.006
	1	1.71	2.63	0.92	1.4	0.006
	2	1.68	2.57	0.89	1.4	0.013
	3	1.68	2.55	0.87	1.4	0.018
	2	1.63	2.51	0.88	1.4	0.012
	2	1.62	2.49	0.87	1.4	0.012
	1	1.45	2.45	1	1.4	0.007
	2	1.54	2.41	0.87	1.2	0.011
	2	1.57	2.54	0.97	1.2	0.012
	1	1.78	2.58	0.8	1.2	0.005
	1	1.82	2.59	0.77	0.95	0.004
	1	1.84	2.62	0.78	0.95	0.004
	1	1.95	2.68	0.73	1.3	0.005
	1	1.89	2.63	0.74	1.3	0.005
	2	1.78	2.56	0.78	1.3	0.010
	4	1.69	2.51	0.82	1.3	0.022
	5	1.75	2.49	0.74	1.3	0.024
	3	1.78	2.45	0.67	1.3	0.013
	2	1.84	2.47	0.63	1.3	0.008
	4	1.87	2.5	0.63	1.3	0.017
	1	1.89	2.51	0.62	1.3	0.004
	2	1.9	2.5	0.6	1.3	0.008
	4	1.82	2.47	0.65	1.3	0.017
	2	1.8	2.45	0.65	1.05	0.007
	3	1.74	2.5	0.76	1.05	0.012
	3	1.83	2.52	0.69	1.05	0.011
	5	1.87	2.56	0.69	1.05	0.018
	5	1.87	2.59	0.72	1.05	0.019
	2	1.9	2.61	0.71	1.05	0.008
	8	1.92	2.64	0.72	1.05	0.031
	9	1.92	2.63	0.71	1.05	0.034
	2	1.9	2.61	0.71	1.05	0.008
	3	1.74	2.59	0.85	1.05	0.014
	3	1.94	2.58	0.64	1.05	0.010
	5	1.98	2.59	0.61	1.05	0.016

	3	1.99	2.56	0.57	1.05	0.009
	6	2.01	2.55	0.54	1.05	0.017
	8	1.98	2.51	0.53	1.05	0.022
	3	1.95	2.47	0.52	1.05	0.008
	1	1.92	2.44	0.52	1.05	0.003
Total	**175**					**1.01**

Table G6. The table used to calculate the total time and amount of CO_2 given (in grams) in Trial 6 for Table 3.