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PHOTOBIOREACTOR CONTROL ALGORITHMS IN LABVIEW UTILIZING ALGAE BAG REACTORS

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ABSTRACT

A LabVIEW program was successfully coded to control algae growth in a thin, vertical short light path length photobioreactor with low level CO_2 carbonic acid buffering. Two photobioreactors were run for seven days, one automated by LabVIEW and the other manually controlled. It was found during the experiment that the purging of accumulated nitrogen and oxygen from the silicone CO_2 diffusion delivery line had a much greater effect than previously anticipated and needs to be dealt with more readily in future experiments. The automated bag grew to an $OD_{550} = 3.56$, 11.6% more than the manual bag which grew to an $OD_{550} = 3.19$. Looking at the linear light limited growth phase above $OD_{550} = 0.60$, the automated bag grew 12.7% faster than the manually controlled bag. Although the trend was for higher performance growth with automation, there is no conclusive evidence that either bag grew significantly better than the other. Further studies need to be executed to revise the program so that it can control a continuous reactor and for outdoor operation. Environmental factors, including temperature and outdoor light levels need to be considered as additional inputs that moderate system and controller response. For a continuous system, the culture cell density is also an important variable that needs to be considered.

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Chapter 1: Introduction

The traditional aim of bioreactor operational strategy is to achieve high productivity based on effective process control. Usually one of the easiest process variables to control is the pH of the system which is achieved either by carrying out the biological process in a buffered media, by simple basic acid-base additions to the reactor in response to pH fluctuations, or by specific media formulation. Another technique is to grow the culture at only low density which makes pH control of the system easier and only minimally affected by the metabolism of the culture; however this approach does not lead to productive cultures. From studies executed by Robert Hendrix in the Curtis Lab group, the pH of the algae photobioreactor can be buffered by carbonic acid equilibrium which is accomplished through low level CO_2 delivery by diffusion through silicone tubing (Curtis Lab Unpublished). Equation **1-1** shows the carbonic acid equilibrium when CO_2 is dissolved in water. The relative amounts of inorganic species are

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (1\text{-}1)$$

dependent on the pH of the system and the partial pressure of CO_2. Dissolved CO_2 predominates at low pH, HCO_3^- at neutral pH, and CO_3^{2-} at high pH. The amount of buffering capacity depends how readily the carbonic acid dissociates in solution which is dependent on two things, the pH of the system and Henry's law for dissolved gases for how much CO_2 can be dissolved in the solution. If the partial pressure of CO_2 fed is decreased, the buffering capacity also decreases creating a greater need for pH control. Also in the absence of media carbonates, the pH fluctuations are more dependent on the other media components.

The purpose of the experiment is to run an algae bioreactor automated by LabVIEW by controlling the pH and keeping the media balanced. The reactors are run under batch conditions or what a continuous reactor would experience at startup. The main focus of this experiment is to test the LabVIEW program and demonstrate that it is able to maintain a stable culture by

maintaining a stable pH. The reactor is designed to create a thin film of algae to decrease light path length so that the culture does not become light-limited except at higher culture densities thus increasing the productivity of the culture. Future experimentation can then revise the program to control a continuous reactor system, the goal for high productivity algae systems. Where the pH control logic evolved from is prior experimentation in the Curtis Lab Group and most recently the trickle film reactor study (Grady, L.). The program is going to eventually be used to run a large scale trickle film reactor.

LabVIEW was used because of the flexibility that the program has for instrumentation and control. LabVIEW will be used to control the pH based on the metabolism of NH_4NO_3, KNO_3, and NH_4Cl. There will also be traditional pH control built within the program which will add KOH or HCl where necessary. The full logic behind each addition to the reactor system is explained in the Methods and Materials, section **2.1**.

Chapter 2: Methods and Materials

The experimental test bed included two 15 L plastic bag reactors in a temperature and humidity controlled Conviron BDW120, one for LabVIEW control and the other for manual control for the growth of *Chlorella vulgaris* algae species. The Convrion was kept at 28°C during the day, 9:00 a.m. to 1:00 a.m. when the lights were on, and 25°C at night. It was kept at a RH of 35% 24 hours a day and the lighting would dim by 1/3 during the first and last hours of the lighted period.

The experiment was run under batch conditions for both bioreactors. For each bag reactor, 54.5ft of dead ended 0.309 OD silicone tubing with pure CO_2 was used to supply a low level of CO_2 for pH buffering. Each bag was also sparged with air via a PETCO AC-9904 5W aquarium pump at approximately 2.25 L/min/bag for circulation and air delivery in each bag as shown in Figure **2-1**. The pH of each bag was recorded continuously with two Cole-Parmer EW-27001-90 in-line pH probes interfaced to two Valley Instruments 1506 MC-L-0-0-P-S-00 pH controllers. The pH of the automated bag was recorded by the National Instruments USB 6008 DAQ and the pH of the manual bag was recorded with a LICOR LI-1400 data logger. There were two different logging methods used because the LabVIEW logging has not be fully tested under continuous experimental conditions and the LICOR logger was previously known to work correctly and therefore can be used as a control for comparison.

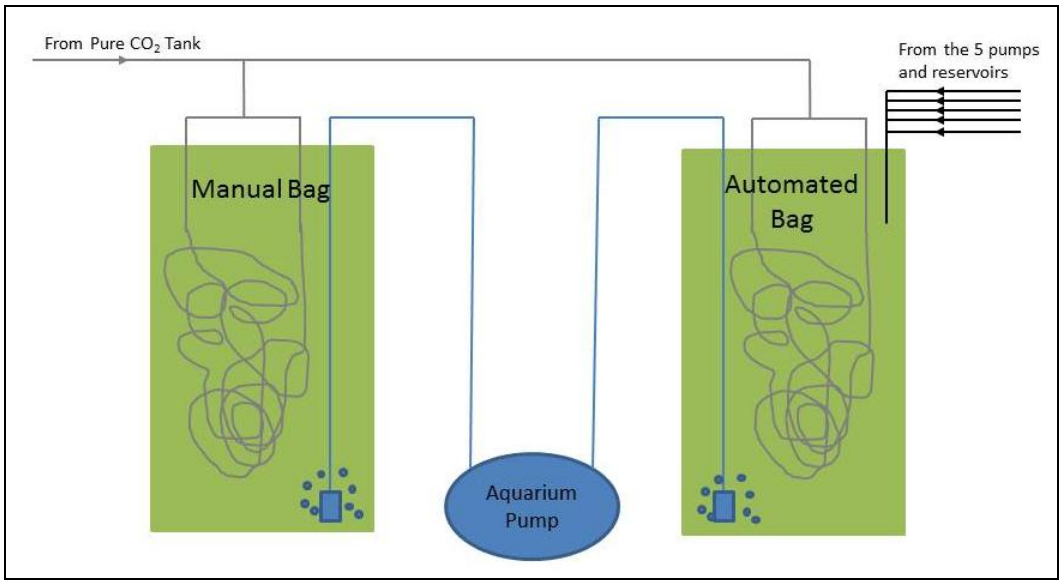

Figure 2-1 The general experimental setup of the bag reactors, silicone CO_2 delivery, and sparging.

The pH was controlled by five solutions; NH_4NO_3, KNO_3, NH_4Cl, KOH, and HCl with concentrations as described in Appendix B. The addition of inorganic nitrogen to a reactor was balanced with the corresponding volume of WFAM 6.0, its formulation is also found in Appendix B. For the automated bag, the solutions were stored in 50 mL test tubes with air filters on each of the air inlets and were added using two Valley Instruments 1520C 1500PTS Support Modules utilizing five different low volume pumps. Each pump line had to be primed with solution prior to operation since all of the pumps had such a low flow rates. The lines were primed using a syringe to force air into the test tube and push solution out of the tubing. Each line was clamped when it was placed in its corresponding peristaltic pump. The reservoirs were then refilled after priming. For the manual bag, all of the solutions were added via pipettes. For both bags the media was added manually by pipettes.

As an indirect assessment of growth, the optical density's (OD's) were monitored for both bags a various times during the experiment. The OD's were recorded at 550nm, 600nm, and

680nm using a Beckman DU520 General Purpose UV/VIS Spectrophotometer, wavelengths that correspond to chlorophyll content. Both systems were monitored continuous for seven days during the daylight hours and parameters where changed in the LabVIEW program when necessary.

2.1: LabVIEW Programing

2.1.1: Basic Control Logic

The original logic for the pH control of Algae bag reactor came from the trickle film reactor experiment run by Lisa Grady from which the pH control parameters were used for this experiment as shown in Figure **2.1.1-1** (Grady, L.). The pH range for the program is a range in which the algae grow at during ideal conditions.

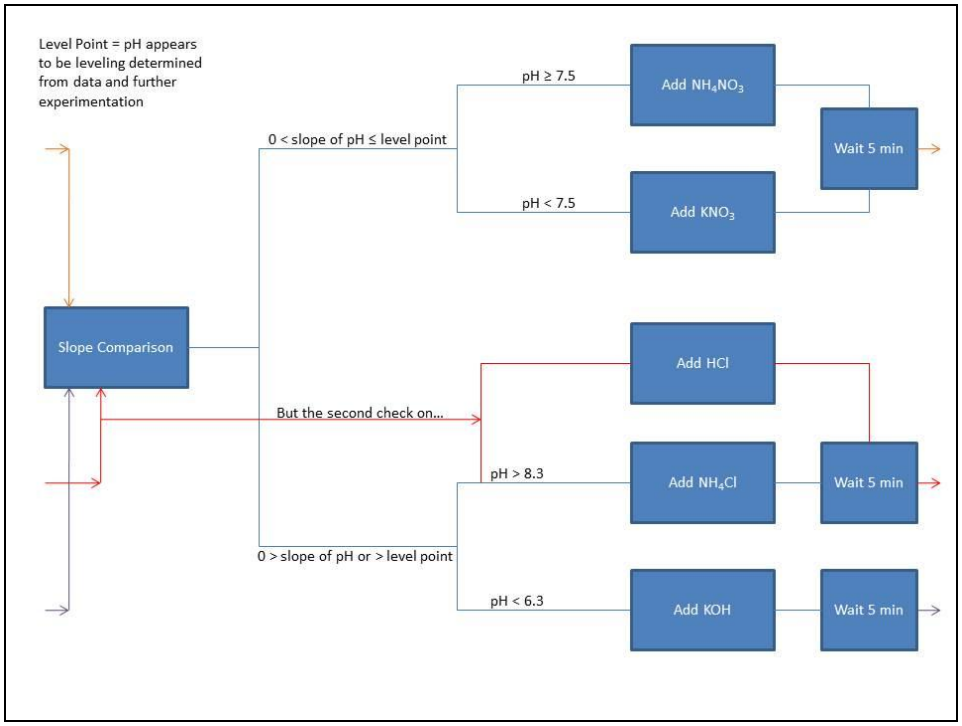

Figure 2.1.1-1 The original logic used to build the LabVIEW program used in this experiment.

Overall, the program was designed to calculate the slope of the pH over a certain amount of time. Once the slope began to level off NH_4NO_3 would be added if the pH $\geq$ 7.5 or KNO_3 if the pH < 7.5. The reasons for the addition when the slope levels out because at this point, the algae are assumed to be out of metabolites and need to be fed. If the pH $\geq$ 7.5, NH_4NO_3 would cause a drop in the pH until the NH_4^+ is metabolized since NH_4^+ is metabolized preferentially over NO_3^-. If the pH < 7.5, the NO_3^- would cause a rise in pH until the NO_3^- is consumed and since there is no NH_4^+ in the reactor the NO_3^- will be consumed. From there the program would wait 5 minutes to recheck the slope to account for any lag in the pH change. If this logic was false, then the program would check if the pH > 8.3 then add NH_4Cl or if the pH < 6.3 then add KOH and for each addition wait 5 minutes. KOH is used so not to add too much nitrogen to the system that is not consumed right away. If the pH was in between the two, it would continue to check and see if the slope was leveling off or not. If NH_4Cl was just added to the reactor and the pH was still greater than 8.3, HCl was to be added to bring down the pH if the NH_4^+ was not metabolized by the algae yet. The addition of HCl provides for the recovery of the situation if the pH becomes too high and the algae are too stressed to recover. NH_4^+ is able to decrease the pH itself but in this instance the HCl is needed to recover the culture. How the logic was going to be implemented was to be determined but the idea was something such as if the pH drop was shallow, the program could add NH_4Cl again and not HCl. This would be determined by the slope.

2.1.2: Revised Control Logic Used for Programing

As programing and testing progressed, there were some revisions to the logic. Figure **2.1.2-1** summarizes the pH control logic used in the final program in this experiment.

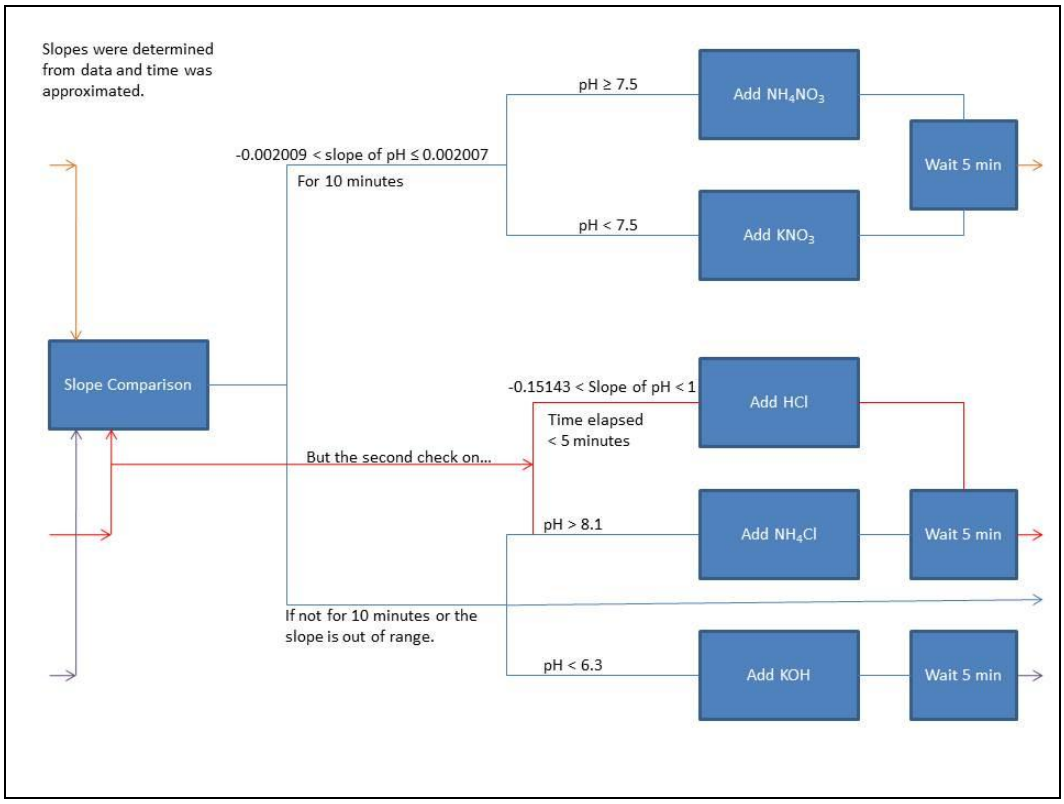

Figure 2.1.2-1 The revised logic that was built into the final LabVIEW program that was used in this experiment.

Comparing Figure **2.1.2-1** to Figure **2.1.1-1**, the main change to the logic was the determination of the slopes to be used in the program along with a wait time to determine whether or not the pH was leveling out or not, where the slope is in range for the specified time. The high slope threshold was estimated from Lisa Grady's semi-steady state trickle film reactor run shown in Figure **2.1.2-2** (Grady, L.).

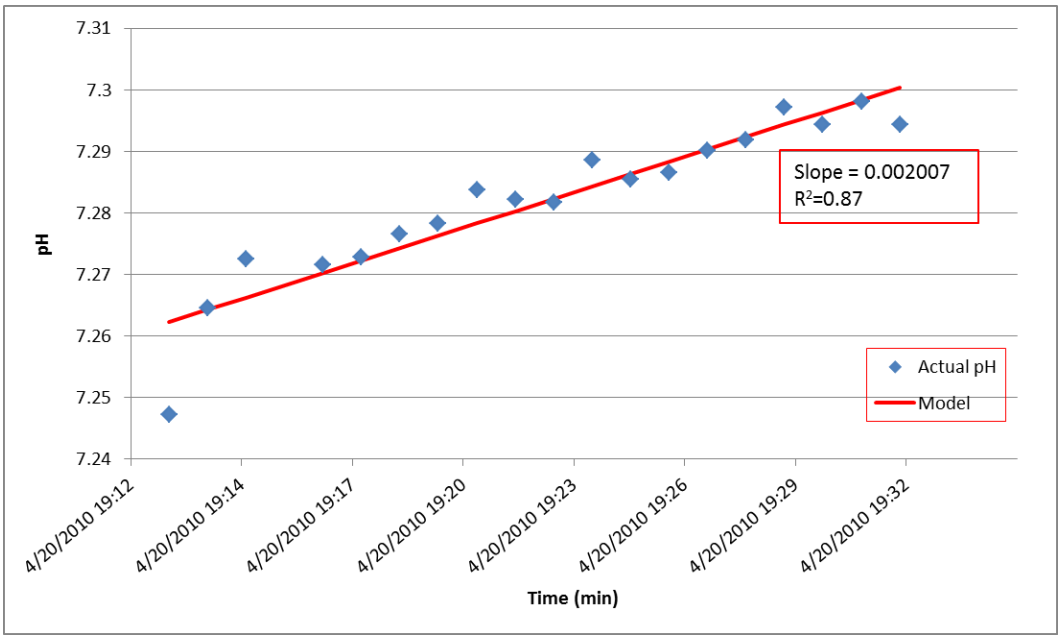

Figure 2.1.2-2 The determination of the slope for a section that the pH was determined to be essentially not changing. This data is from right before NH_4NO_3 was added to the trickle film reactor where the slope was shallow by Lisa Grady.

To determine if the model was a good fit, the R^2 value was determined. Since no multiple regressions were taken to form the model, 0.002 is a good value to use as an estimate for the slope parameter to be used in LabVIEW. This value will be used as a starting point when running the program and changed as needed during the experiment. The level time, or the amount of time that the slope has to be continuously in range between the two slope values, was set at 10 minutes. This value was determined again by Figure **2.1.2-2** and approximating a decent wait time to confirm the pH was leveling out.

If the slope stays within range for the prescribed period of time and there are no other additions to the reactor, NH_4NO_3 would be added if the pH $\geq$ 7.5 or KNO_3 if the pH < 7.5. If the slope is not in range for enough time and falls out of the prescribed range, the second addition logic will occur. With the second logic, if the pH < 8.1 the program will add NH_4Cl or if the pH < 6.3 it will add KOH, but if it is between 8.1 and 6.3 there will be no additions and the program

will continue to see if the pH is leveling out since the pH of the reactor is the range for algae growth and does not need to be fed yet. Any time there is an addition to the reactor, the program will still wait 5 minutes before rechecking the slope as in the original logic to account of any lag in pH change.

The last logic change was to what the program was going to do to determine whether or not to re-add NH_4Cl or HCl instead. The idea is that if NH_4^+ was metabolized there would be a shallow change in the pH and if not there would be little or no change in pH immediately. Once NH_4Cl was added to the reactor, HCl would be the next thing to add if the pH continued to rise and not level off. When the HCl is added, the pH would drop rapidly out of the slope range. This would continue until the NH_4^+ begins to metabolize that should be theoretically shown by the shallow pH drop. The slopes used for the range are unconfirmed and will be tested during the experiment. The "reset time" is so when the HCl pH drop rebounds, it will not reset and added NH_4Cl on the next iteration.

2.1.3: Main LabVIEW Coding

The main program was divided into four primary independent loops; the data acquisition and slope calculation loop, the pH and slope record loop or RECORD loop, the slope check or SLOPE COMPARISON loop, and the CONTROL loop. Each of the loops can be stopped by pressing the "KILL SWITCH DO NOT PRESS" button on the front panel as shown in Figure **2.1.3-1** which is associated by the "Stop All" local variable. When the button is pressed, all of the main loops will end once the sub loops are stopped which are also stopped by the same local variable. For the CONTROL loop, if a solution is being added to the reactor, the program will stop the pump and record the amounted added to the reactor before the program ends.

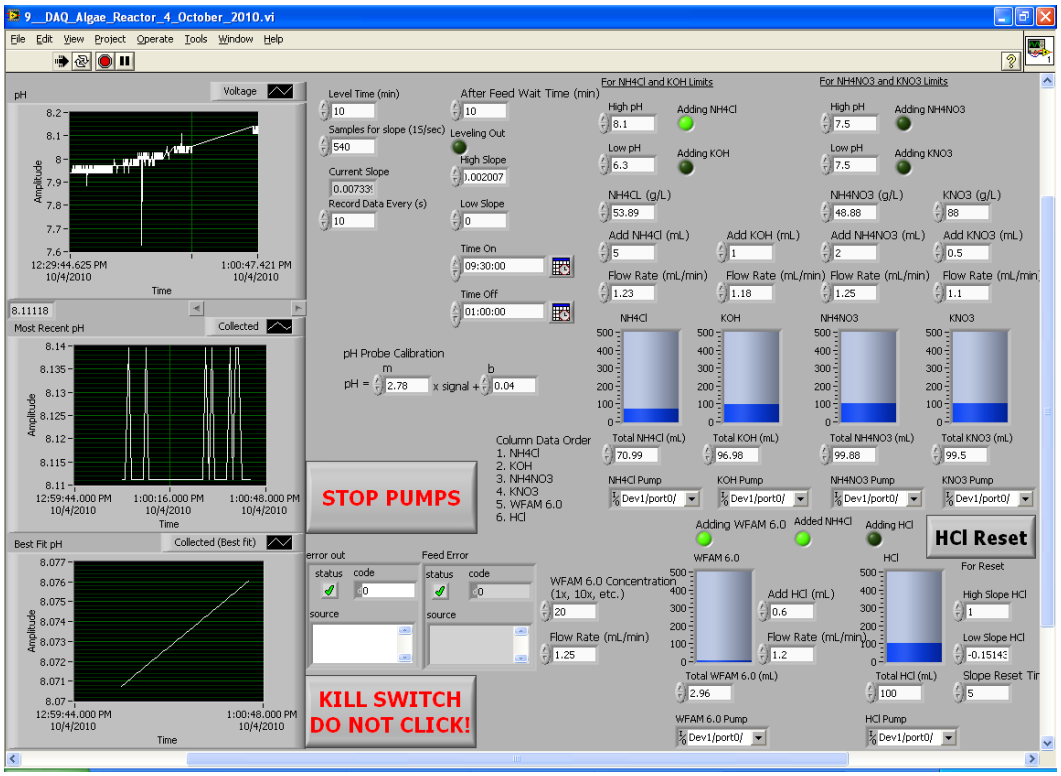

Figure 2.1.3-1 A screen shot of the front panel taken October 4, 2010 at 1:00 pm during the experimental run.

The data acquisition and slope calculation programing is presented in Figure **2.1.3-2**. This loop's only dependence is on the "Stop All" local variable. The "DAQ Assistant.vi" was used to collect the raw pH data though the analog channel "ai0" which was modified in the front panel through a linear calibration of the Valley Instruments pH controller from voltage to pH. The calibration is found in Appendix C Table **C-2** and plotted Figure **C-1**. The pH is then put into a chart which is in the front panel that can be called on later for other loops.

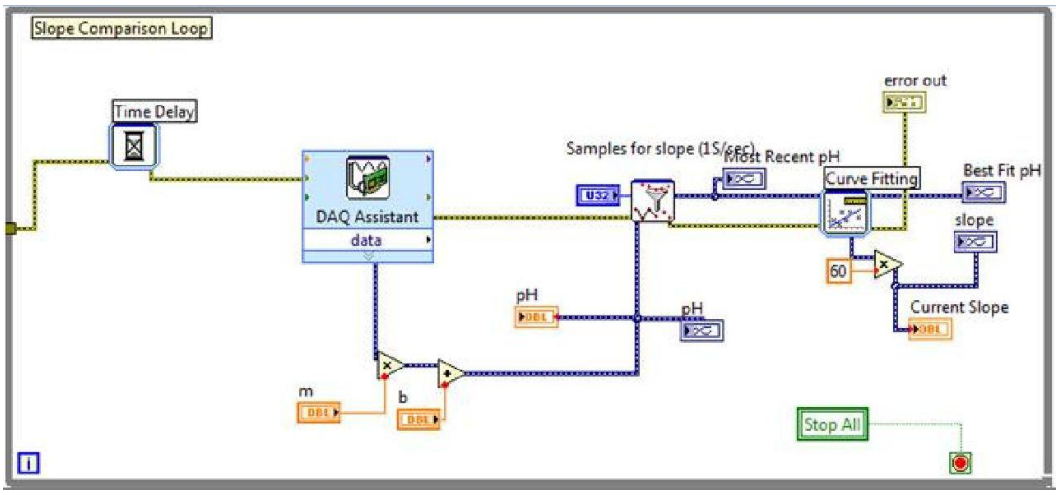

Figure 2.1.3-2 The data acquisition and slope calculation loop with the modified collector.vi.

The pH data is then used to find the slope via two different vi's. The first vi is a "collector.vi" that is slightly modified and this new modified sub-vi needs to be contained in the same folder as the main vi. The new "collector.vi" now has the ability to change the number of data points that are collected in the vi without having to click on the vi and change its properties. This allows a controller to be connected to the "collector.vi" and the program therefore does not have to be stopped in order to change the number of data points that are collected. After a certain number of points have been collected, the slope can then be calculated by the "curve fitting.vi" and then sent to two charts on the front panel, one for the pH best fit line and one for the slope of the pH. This loop has a time delay of one second so there is only one pH and slope sample taken per second.

The pH and slope are not recorded in the data acquisition and slope calculation loop but a separate RECORD loop as shown in Figure **2.1.3-3**. The reason for this is so that the number of recorded data points can be adjusted to the desired amount by the user, in the case of this experiment one sample every ten seconds. This loop also is only dependent on the "Stop All" local variable. The pH and slope are put into an array and record into a .lvm file which can open

as a spread sheet. The first column is relative time, second column in the pH, and the last column is the slope of the pH.

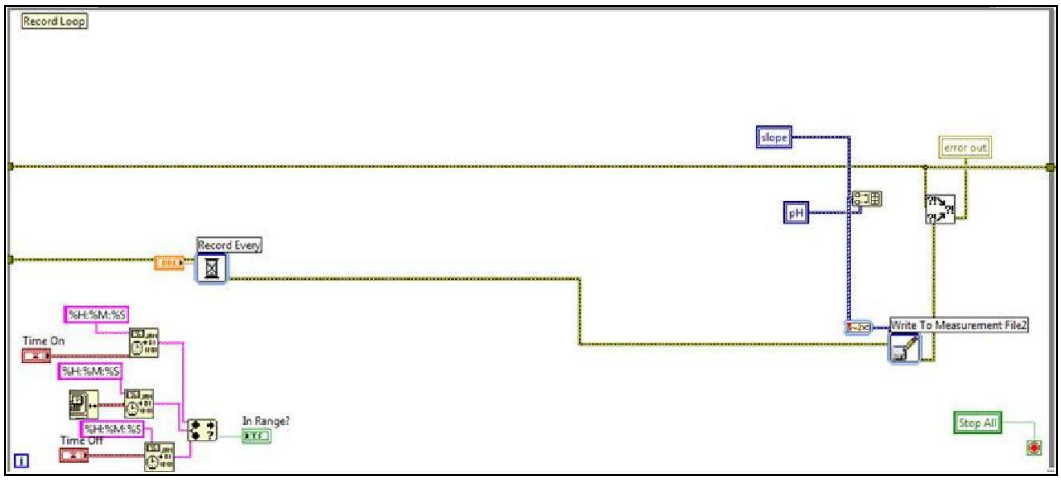

Figure 2.1.3-3 The RECORD loop that contains the logical check for when the CONTROL loop should be occurring; during the hours the light is on in the Conviron or daylight hours.

The other part of the RECORD loop is the logical check for when the CONTROL loop is turned on and off during the day based on daylight. For this experiment the Conviron turns on at 9:00 a.m. and off at 1:00 a.m. This check did not necessarily have to be put in this loop but it could have also been added to the data acquisition loop. For this experiment it does not matter since the RECORD loop repeats every 10 seconds and the data acquisition loop repeats every second. In either case it will not affect the functionality of the program turning on and off the CONTROL loop at these times.

The slope check or SLOPE COMPARISON loop shown in Figure **2.1.3-4**, is used continuously determine if the pH is leveling off, and if HCl should be added or not within the non-leveling additions. The pH is considered "leveling out" when the slope is in the specified range for the specified time which is inputted on the front panel. The main loop, what is contained in the entire figure, is only stopped by the "Stop All" local variable. To determine whether or not the pH is leveling off, the loop compares the current slope value that has been

determined from a select amount of past data points to a high and low slope value in a sub-loop which is contained in the red box in Figure **2.1.3-4**. The two threshold values will be better determined at the end of the experiment. Also for the program to determine if the slope is leveling out, the current slope has to stay within the threshold values for a period of time comparing to the "elapsed time.vi" also shown within the red box. If the slope falls out of range, the elapsed time is reset and more time must pass before the program determines if the pH is leveling out. Once the slope is in range and the time has elapsed, the sub-loop in red will stop and be shut off temporarily by the case structure highlighted by the blue box in Figure **2.1.3-4** until the "Slope Check Pause" is reset in the CONTROL loop by an addition to the reactor.

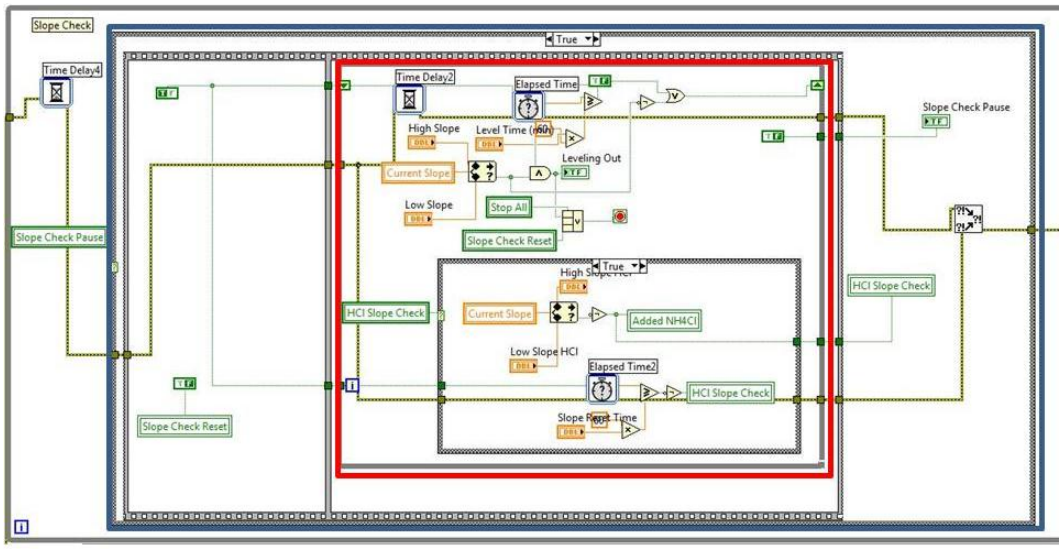

Figure 2.1.3-4 The SLOPE COMPARISON loop that uses the current slope of the pH to determine it the pH is leveling off and if NH_4Cl can be added to the reactor and not HCl again.

There are two other things that will stop the sub-loop in red, the "Stop All" and "Slope Check Reset" local variables. The "Slope Check Reset" is turned "True" only after an addition has been made to the reactor, otherwise it is "False" so the program can continue to compare the slopes and determine if the pH is leveling off.

The other important part of the SLOPE COMPARISON loop is the case structure within in the sub-loop highlight in red. This case structure is activated after NH_4Cl is added to the reactor. If the current slope is between the two values inputted on the front panel, it will reset and NH_4Cl can be added again, if not HCl will be added on the next iteration that NH_4Cl was to be added. This check will only occur for a short period of time, which is what the "Elapsed Time.vi" is there for because if not the slope will become positive after the addition. Once the time has passed, the HCl slope check will cease until HCl or NH_4Cl are added to the reactor again.

The last main loop in the LabVIEW programing is the CONTROL loop. The main part of the loop is shown in Figure **2.1.3-5**. The CONTROL loop is used to activate the pumps used to add the different solutions to the reactor. As mentioned before, the CONTROL only operates during the daylight hours, between 9:00 a.m. to 1:00 a.m. as controlled by the RECORD loop which controls a case structure around all of the addition loops which is not shown in Figure **2.1.3-5**. The next case structure, which is also not shown in Figure **2.1.3-5**, is either "True" if the pH is leveling out or "False" if it is not as determined by the SLOPE COMPARISON loop. If "True" then either NH_4NO_3 or KNO_3 will add or if "False" then either NH_4Cl, HCl, or KOH will add. These are based on the pH logic in Section **2.1.2**. If at any time the operator wants to stop the pumps, pressing the "STOP PUMPS" button on the front panel will stop the add loop and record the amount that was added to the reactor and stop the CONTROL loop from adding anything further until the button is pressed again which effectively resets the CONTROL loop.

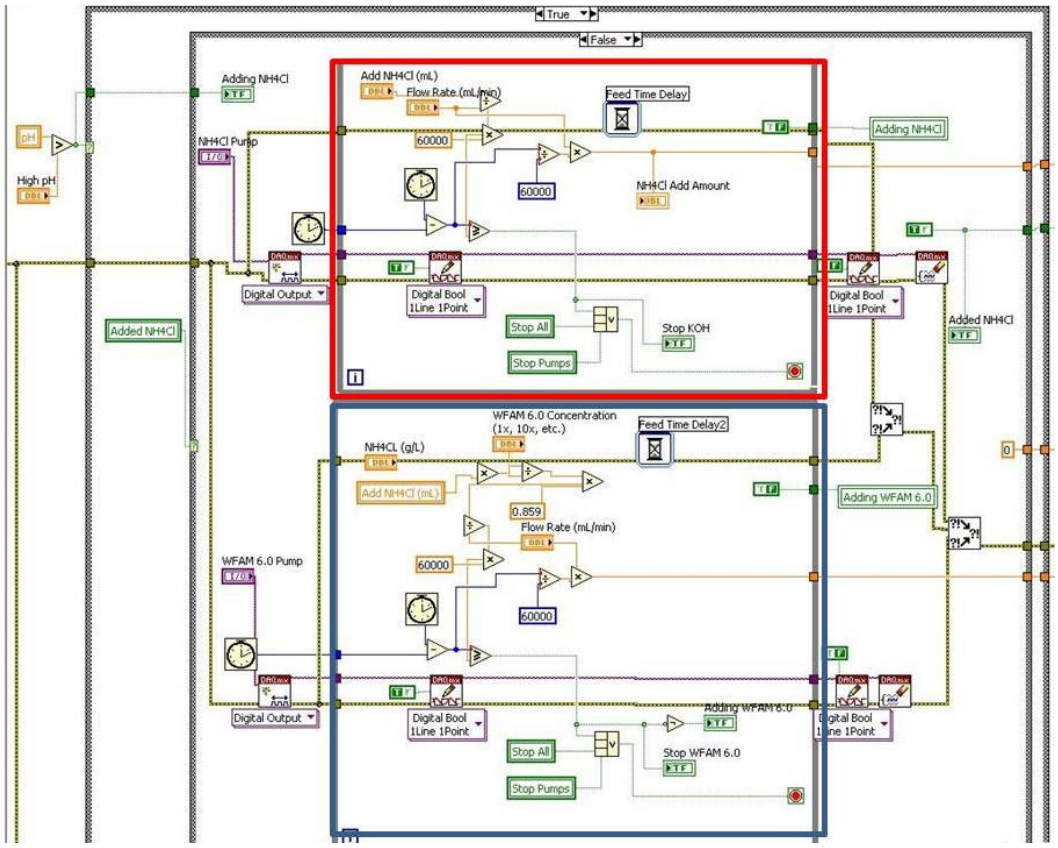

Figure 2.1.3-5 The "False" case when either NH_4Cl or KOH can be added to the reactor. The NH_4Cl add loops are only shown here which include the WFAM 6.0 addition loop.

Starting with the "False" case where the system is not leveling out and the NH_4Cl addition as shown in Figure **2.1.3-5**, the pump will add the amount inputted in the front panel in the "Add NH_4Cl" field as shown in Figure **2.1.3-1** when the pH is greater than the "High pH". The program divides the add amount by the pump flow rate which is also inputted in the front panel under "Flow Rate (mL/min)" to get the time the pump needs to be on as shown in the loop highlighted by red in Figure **2.1.3-5**. The pump will run until milli-second timer equals the amount of time needed for the pump to run and add the amount needed then end the NH_4Cl add loop. The case will end once the pump for the WFAM 6.0 stops.

The WFAM 6.0 volume is calculated from a nitrogen balance from WFAMC which contains KNO_3, these calculations are found in Appendix B. Depending on the solution, a

constant is multiplied by the solution and the amount added and divided by the WFAM 6.0 concentration. The WFAM 6.0 loop works the same way that the NH_4Cl loop does to run the pump and stops the same way as highlighted by the blue box in Figure **2.1.3-5**. For this experiment there was no WFAM 6.0 pump and was added manually with a pipette.

Once the WFAM 6.0 loop ends, the case structure ends and the amount of all six solutions added is recorded. There is at most two solutions added at a time, NH_4Cl, NH_4NO_3, or KNO_3 with WFAM 6.0, but for HCl and KOH no media is added to the reactor. On the front panel, there is an indicator so the total reservoir amount for each solution can be inputted by the controller under each indicator. When there is volume added to the reactor it is subtracted from the total amount and reflected on the front panel via the visual indicator and the controller. This programing can be seen in Figure **2.1.3-6** for NH_4NO3 and KNO_3.

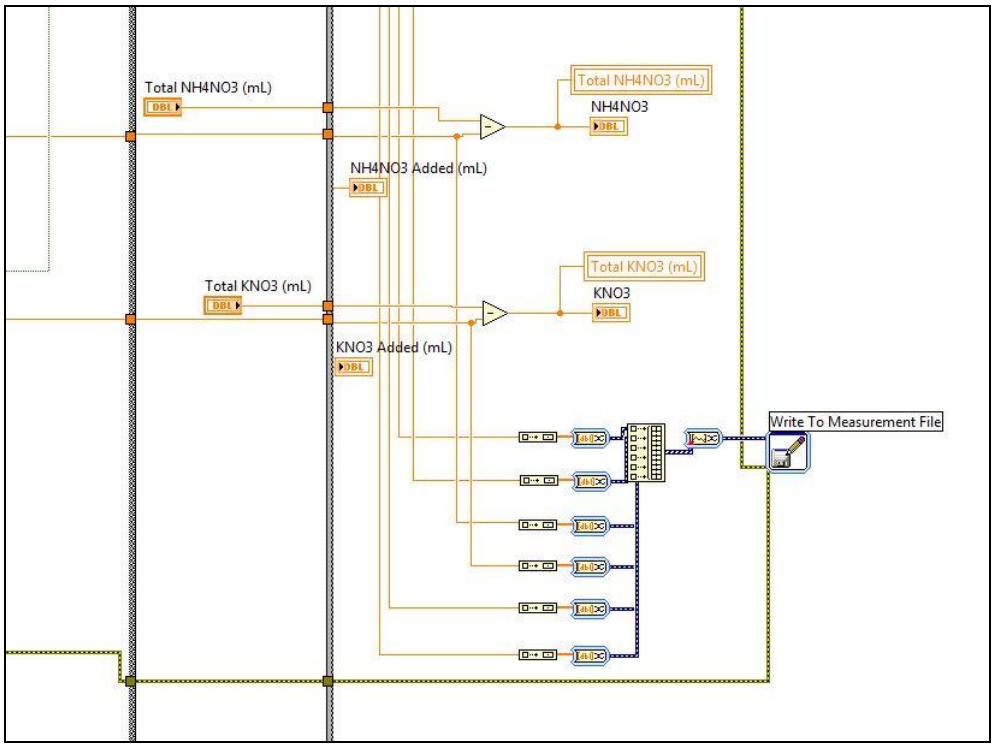

Figure 2.1.3-6 The subtraction from the total solutions is shown for NH_4NO_3 and KNO_3. The array structure that combines the amount of each solution added and then is recorded to a .lvm file is also shown.

When NH_4Cl finishes adding, the variable "Added NH_4Cl" becomes "True" and activates the HCl slope check in the slope comparison loop and changes the case structure around the NH_4Cl loop to "True" and becomes the loop shown in Figure **2.1.3-6**. This loop operates the same way as the NH_4Cl loop but does not added any media. Once a solution is done adding to the reactor, the variable "Slope Check Rest" becomes true.

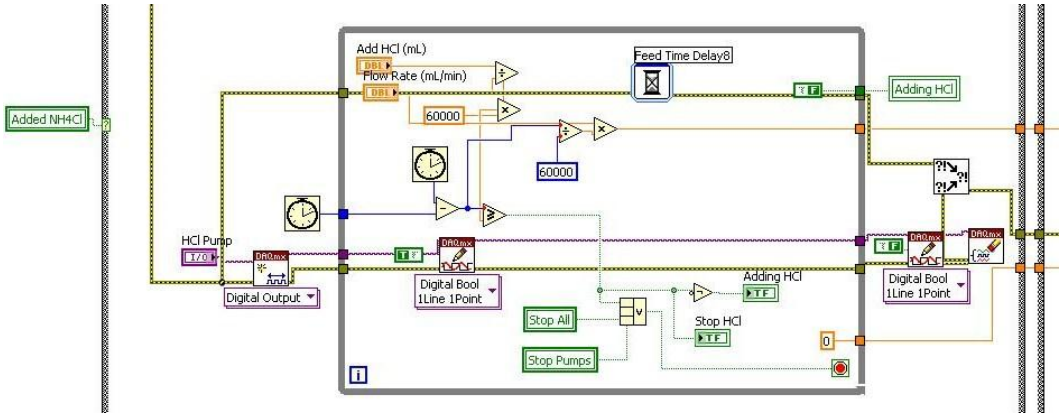

Figure 2.1.3-7 When the local variable "Added NH_4Cl" becomes true after NH_4Cl has been added, this loop becomes activated to add HCl on the next iteration until it is reset by the slope comparison loop.

The "Slope Check Reset" variable does two important things; it resets the SLOPE COMPARISON loop and it also the delays the CONTROL loop so that nothing else adds for a selected amount of time to account of any pH lag. The "Slope Check Reset" variable will be reset to "False" when nothing is added by the control loop. This is shown in Figure **2.1.3-8** along with the last piece of code in the program. There is a "HCl Reset" button on the front panel so if the operator does not want to add anymore HCl or the HCl does not reset when necessary, this button will set the variable "Added NH_4Cl" back to "False".

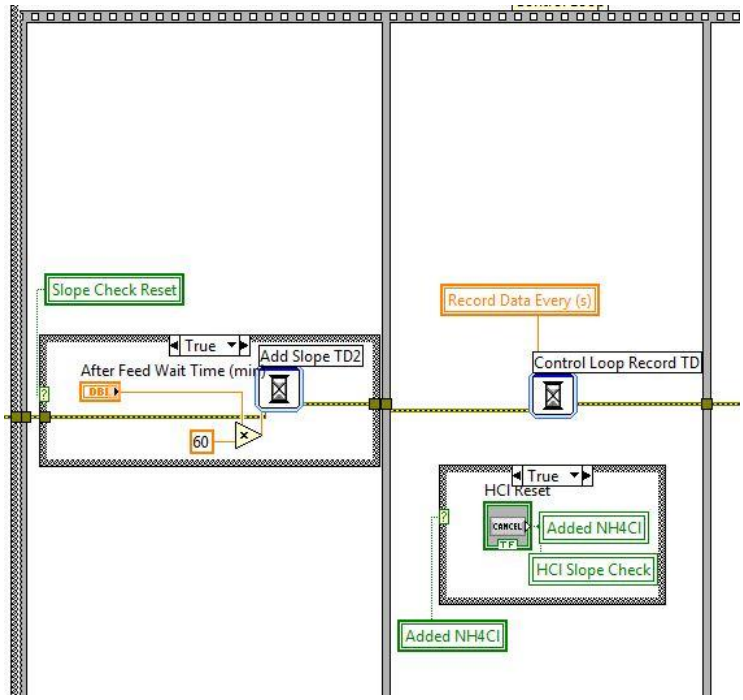

Figure 2.1.3-8 The case structure that will delay the CONTROL loop for a certain amount of time after an addition as inputted in the front panel. The coding for HCl reset button is also shown here.

The KNO_3 coding is same as the NH_4Cl coding, just a different constant for the WFAM 6.0 loop. If the system is leveling out, the pH is within range for the specified time; either NH_4NO_3 or KOH is added to the reactor. The coding for these two loops is also the same.

2.2: Bag Reactor Setup

The plastic bag reactor's original design along with the silicone tubing for low level CO_2 delivery and buffering was designed by Robert Hendrix for 1.5 L and later up scaled to the 15 L size used in this experiment. The reactor was designed to have a short light path to increase growth densities. Each of the two reactors used in this experiment were constructed out of two sheets of plastic that were sealed together and wedged between two 17" by 48" Metro storage shelves. The Metro storage shelves were held together by four zip ties on each side of the pair of

shelves. Each side of the pair of shelves was also laced with nylon string to keep the shelves from spreading apart when filled with media. The silicone tubing was inserted into each bag before the Metro storage shelves were tied together and then the media was added. The 15 L reactors are shown in Figure **2.2-1** before being inoculated with algae.

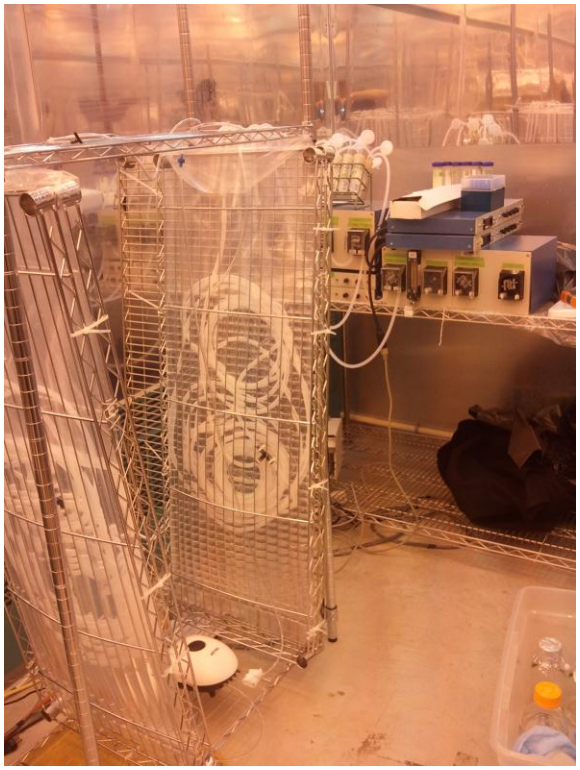

Figure 2.2-1 The experiment setup with media in each bag before inoculation with the automatic bag delivery system in the background.

Each bag was inoculated with 15 L of 1/8thx concentrated WFAMC with the formulation in Appendix B and with algae from a previous bag that was grown to an OD_{550} = 0.648. The silicone CO_2 tubing was purged periodically during the experiment to rid the lines of nitrogen and oxygen that diffused into the line from the reactor system and restore the driving force for CO_2 diffusion through the silicone tubing into the reactor system. The silicone line was kept at approximately 9 psig and the regulation was adjusted accordingly during the experiment to keep at this pressure.

2.3: National Instruments USB 6008 DAQ Setup

The digital output channels are assigned to the pumps based on Table **C-4** in Appendix C. Three pieces of 25 feet of phone wire were run from the NI USB 6008 DAQ to the relay that powered the five pumps. Two Valley Instruments 1517MC-L-0-0-0-S foam controllers were hotwired directly to the relays to bypass the controller itself and just use the relays. This was done so only one relay box had to be built from scratch as shown in Figure **2.3-1**. A Crydom D1202 SSR, 2.5 amps with a 3-32 VDC input and 24-140 VAC output, was used to build the box. The switch on the left can be used to turn on the pump manually and the switch on the right is an overall power switch. A phone jack is used to connect to the input on the SSR.

Figure 2.3-1 The relay box that was built to trigger one of the support module pumps.

Since 25 feet of phone wire was run to the relays, there was too great of a voltage drop from the DAQ to actually trigger the relays. A circuit board needed to be built with an op-amp for each channel to increase the voltage going to the relays, from approximately 3 VDC to 10 VDC to ensure the relays would trigger. A 18 VDC and 1.7 amp HP AC power adapter was used to power the circuit board which was constructed from a Radio Shack module breadboard. Two Radio Shack LM324 Quad Op Amps were used to amplify the five relays that were used in this

experiment. Each op amp circuit used a 2200 Ω and a 4700 Ω resistor as shown in Figure **2.3-2** for a gain of 3.1 found using Eqn. 2.3-1 (Horowitz, P. and Hill W.). The op amp could run on

$$V_{out}/V_{in} = 1 + R_3/R_4 \quad (2.3\text{-}1)$$

either 16 VDC or 32 VDC, in this case it was run on 16 VDC with a 18 VDC power adapter using a 560 Ω and a 56 Ω resistor.

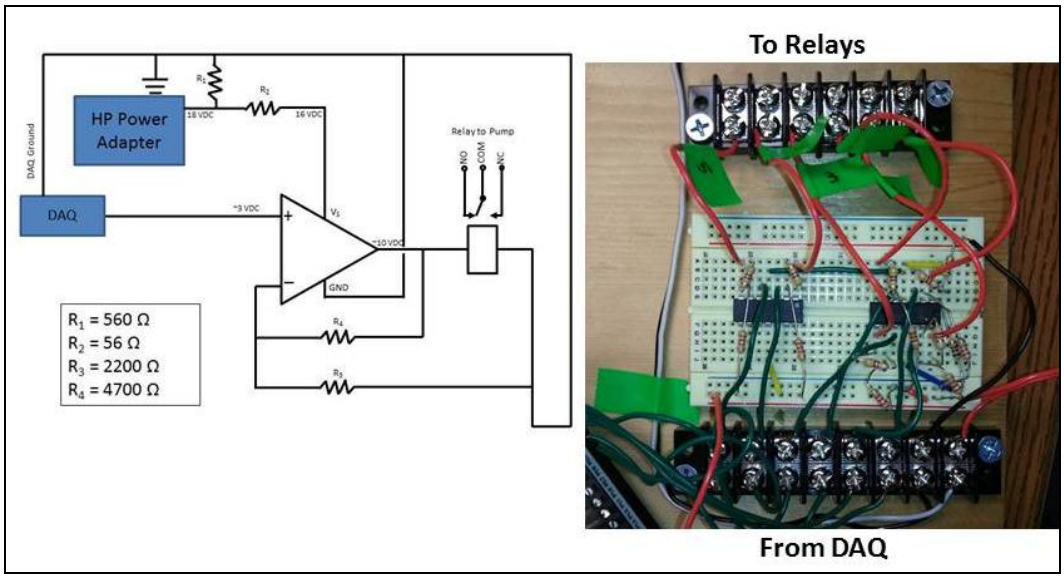

Figure 2.3-2 The circuit diagram for each op amp leading to the relay and a picture of the circuit board that was used in this experiment to increase the voltage from approximately 3 VDC to 10 VDC.

Chapter 3: Results and Discussions

3.1: LabVIEW Programing Changes Made During the Experiment

The original RECORD loop for the pH was not recording the pH every second but approximately 1000 sample/s, causing too much data to be recorded in the spreadsheet which is unnecessary for the slow pH changes observed in this experiment. The error was fixed on September 29th by changing the number of samples in the "DAQ Assistant.vi" to on demand and moving the vi to the SLOPE COMPARISON loop as shown in Figures **3-1** and **3-2**.

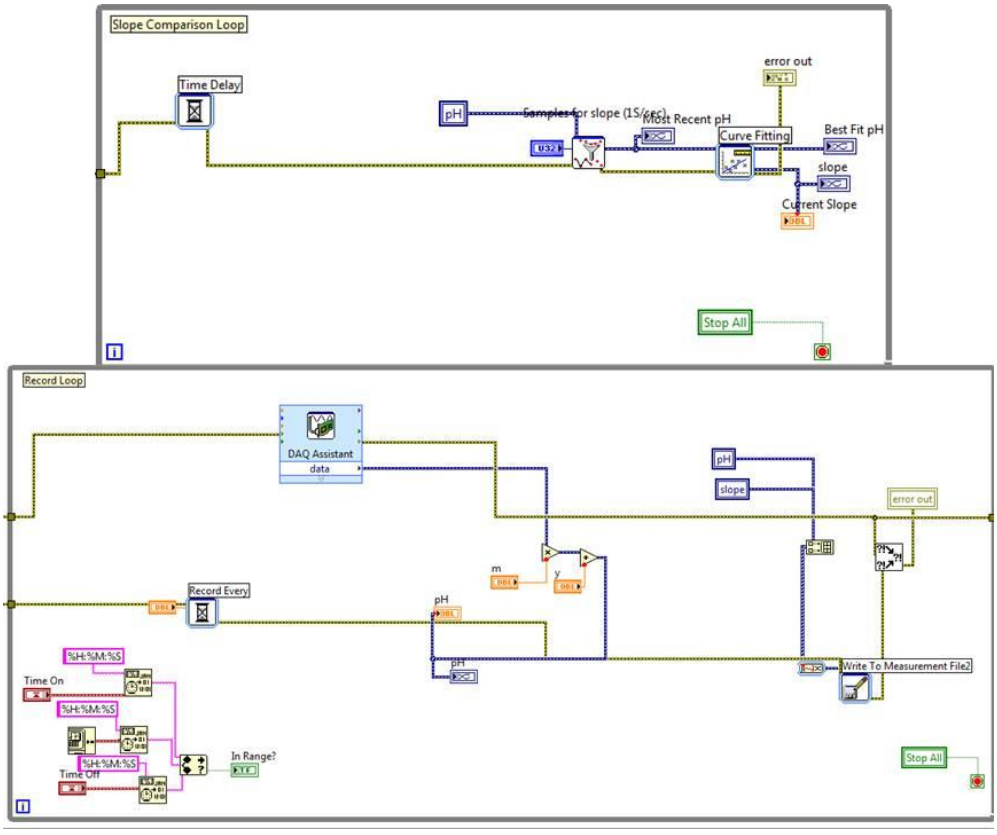

Figure 3.1-1 The SLOPE COMPARISON loop and RECORD loop before the changes were made.

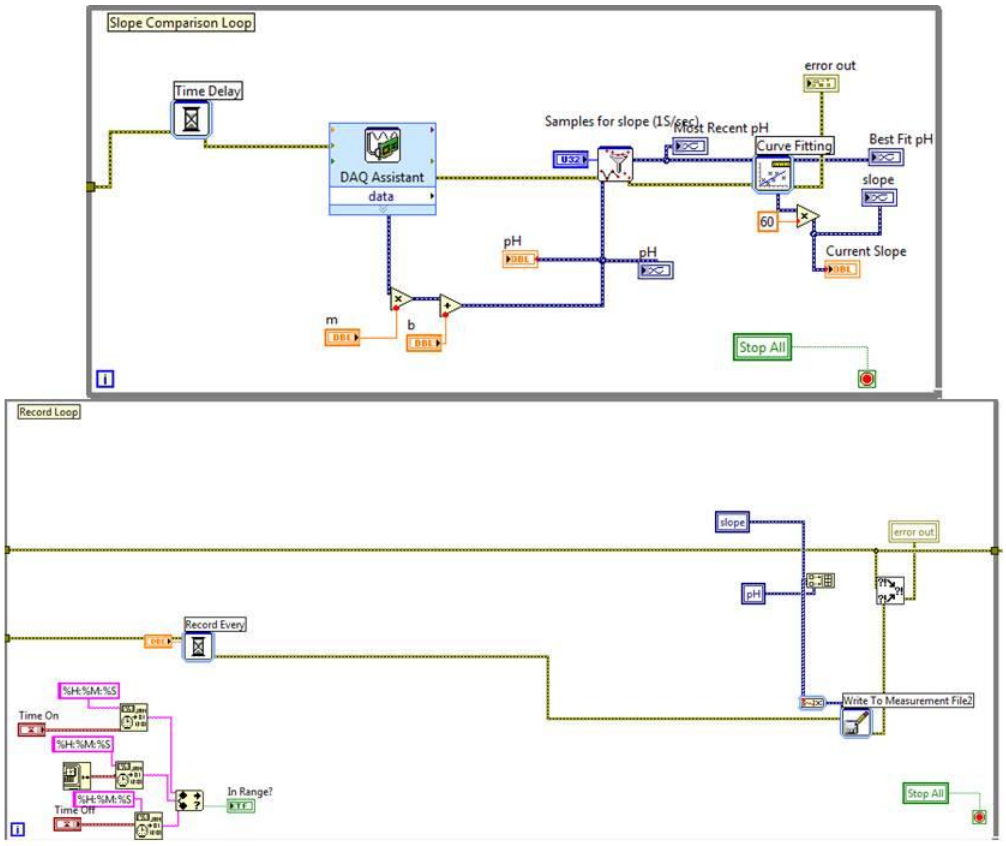

Figure 3.1-2 The SLOPE COMPARISON loop and RECORD loop after the changes were made.

Moving the "DAQ Assistant.vi" to the SLOPE COMPARISON loop allowed more flexibility in the RECORD loop. The way the original program was written, if "Record Data Every (s)" was greater than 1 second, the pH would be successfully recorded but the calculated slope would no longer be instantaneous. For example, if 10 samples were taken to calculate the slope, and the pH was recorded every 10 seconds, it would take 100 seconds before a slope would become available for any kind of comparison.

Another change that was made to the program on September 29th was made because the program would always trigger additions if it thought the pH was leveling off and this was undesirable when the culture was first inoculated. pH fluctuations remained small during the culture's lag phase and the pH was not leveling off, therefore nutrient additions were

unnecessary. A “Flat Sequence” was added around the CONTROL loop so that the program would not add anything until the pH was greater than 7.2 as advised by Waqas Khatri. This logic was placed in a loop before the control sequence. This alleviated some problems, but because the CO_2 supplementation was turned off when the lights were turned off at 1:00 am, the loss of the carbonic acid buffering allowed the pH to rise above 7.2 which could trigger the pumps to turn on in the morning if not carefully watched and intervened by pressing the “STOP PUMPS” button. What needs to be fixed in the program so this does not occur is to have the 7.2 pH check turned off during the night and back on when the CO_2 acid buffer takes effect in the morning.

On October 1st at around 12:45 pm, the computer was running slow. The program was stopped. For the SLOPE COMPARISON loop, there was no time delay for the loop causing it to take 100% control of the CPU which caused the computer to slow. The LabVIEW program was still operating but since the CPU was dedicated to a single loop in LabVIEW, no other program on the computer could be operated. A time delay of 1ms was added to the loop so it did not have 100% control of the CPU. This was a minor error that was over looked and changed during the experiment.

The last change that was made to the program during the experiment was also another minor programing error that occurred for the WFAM 6.0 nitrogen balance and was noticed on October 4th. The constant from the equations in Appendix B and Figure **2.1.3-5** in the red highlighted loop; 0.859 for NH_4Cl as shown in Figure **3-3** was actually switched with the constant 1.1482 that was for NH_4NO_3 and vice versa. This was done because the WFAM 6.0 pump would run for the incorrect amount of time and therefore the media added to the reactor system would not be properly balanced.

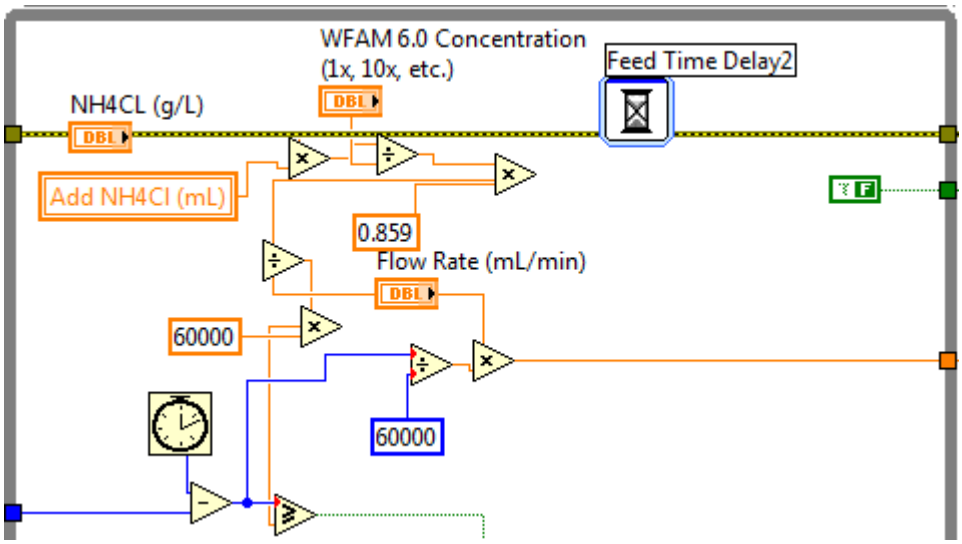

Figure 3.1-3 The constant that was found for the media balance depicted correctly as 0.859. This constant was found from the original balanced media in Methods and Materials.

3.2: pH Data, Solution Additions, and Growth Data

For the automated bag, the WFAM 6.0 media balance was under by 3.7% and for the manual bag over by 2.4% which resulted from human error by either forgetting to add media to the reactor or adding too much media to the reactor. The slight error in the media balance did not seem to affect the growth of the cultures or the outcome of the experiment. Looking at Figure **3.2-1**, the graph of automated bag pH, compared to Figure **3.1-2**, the graph of the manual bag pH; there are a few basic trends to describe. The pH drop from 12:00 a.m. to 1:00 a.m. is due to the dimming of the lights and the algae not metabolizing as much NH_4^+ during this time due to reduced photosynthesis rates. The rise in pH from about 2:00 a.m. to 8:00 a.m. is because the CO_2 line has been turned off and the reactors lose the carbonic acid buffer, but the pH then drops again around 9:00 a.m. when the CO_2 is turned back on.

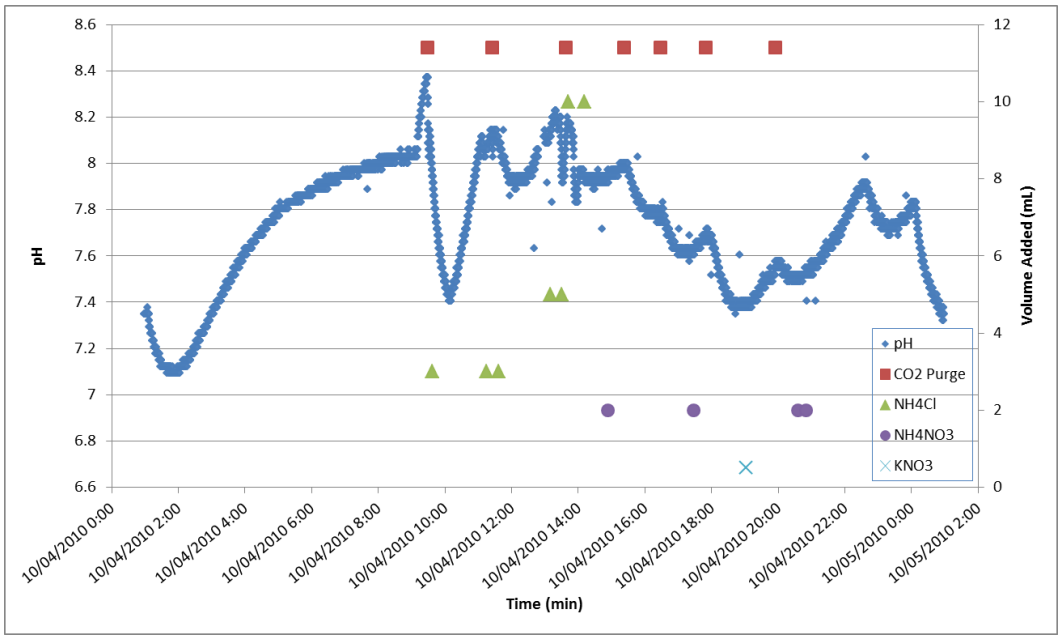

Figure 3.2-1 The graph of the pH data, the addition data, and CO_2 purge data for the automated bag on October 4, 2010.

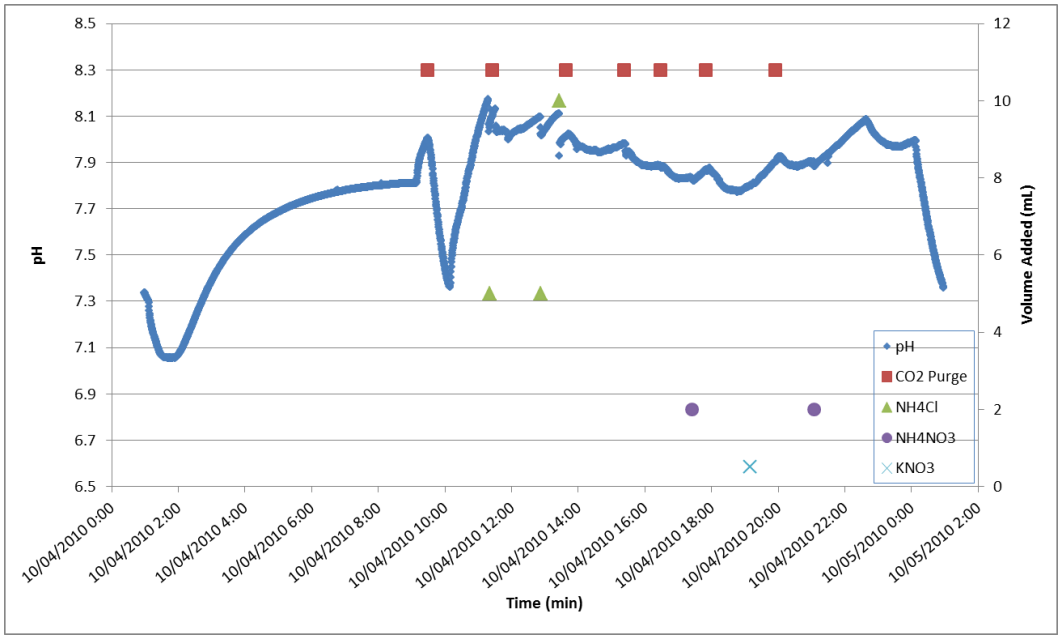

Figure 3.2-2 The graph of the pH data, the addition data, and the CO_2 purge data for the manual bag on October 4, 2010.

The automated bag tended to add more NH_4Cl and NH_4NO_3 than what is needed as compared to the manual bag. The manual bag has a more stable pH, between 7.7 and 8.0, whereas the automated bag tends to be between 7.3 and 8.2. Both of these ranges are acceptable for growing the algae though. This is the trend throughout the experiment, where the automated bag is slightly overfed and the pH is less stable than the manual bag. Looking at both Figures **3.2-1** and **3.2-2**, the purging of the dead ended silicone CO_2 line has a much more significant effect with the carbonic acid buffering in both systems then previously known which can really be seen by the automated bag. The automated bag had a large buildup of NH_4^+ that was added early in the day and it caused the pH of the reactor to continuously drop every time the CO_2 line was purged.

Comparing Figure **3.2-3**, the graph of the automated OD_{550} plotted with the manual OD_{550}; both growth curves are nearly identical. The automated bag grew to an OD_{550} = 3.56 and the manual bag to an OD_{550} = 3.19; 11.6% more than the manual bag. Looking at the linear growth phase that was most likely due to light limited growth, the automated bag grew 12.7% faster than the manual bag. There is no way to correlate the growth differences to anything since both bags had enough media to grow to over an OD_{550} = 4.0 at the end of the experiment and neither bag was under fed during the experiment.

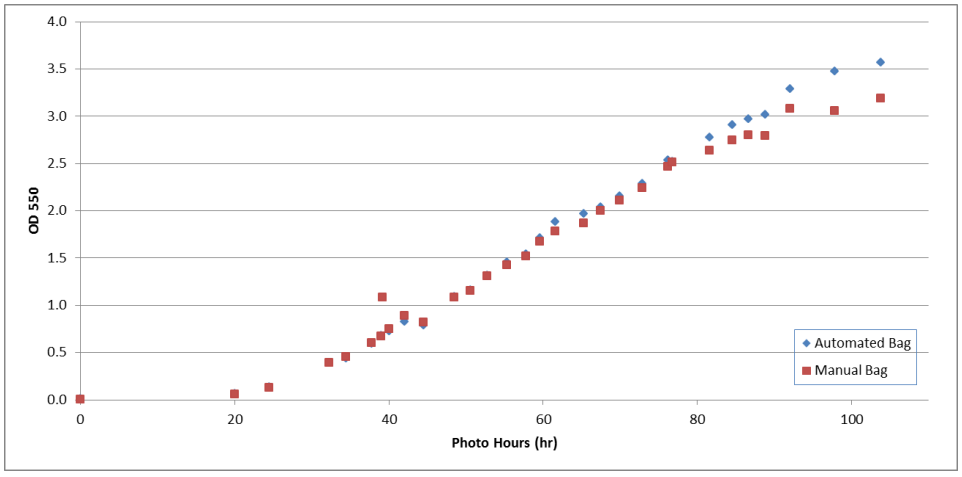

Figure 3.2-3 The graph of OD_{550} verses the number of photo hours for the automated bag that grew to an OD_{550} = 3.56 and the manual bag that grew to an OD_{550} = 3.19.

3.3: LabVIEW Front Panel Parameters

All of the values used during the experiment for the front panel are found in Appendix C, Table **C-5**. Most of the values in the table are self-explanatory and could be easily found, but some of the values were not predetermined before the experiment started. These values include the high and low slope values and the time that the slope had to stay between these values, how many samples to take to calculate the slope, how much time to wait after an addition before another addition, and the HCl reset parameters.

The wait time between additions was adequate during this experimental run for maintaining algae growth, but looking at Figure **3.2-1**, there should have been more time between additions to improve pH stability so that a pH crash does not occur if pH slope changes are not as steep as expected. High and low slope values worked for this experiment but other values should be determined with later experiments since the goal of this experiment was to determine if a computer program could control the pH of an algae reactor. To determine the slope, a 9 minute period of historical pH data was used at the end of the experiment. This allowed for less harsh changes in the slope over time by utilizing more data points.

For the HCl reset parameters, this system did not work at all. Too much NH_4Cl was added, which can be seen in Figure **3.2-1** and HCl needed to be added to the reactor instead. This programing needs to be studied in greater detail and not added to the programing right before the reactor run without the proper testing.

The last thing that was changed frequently for the automated bag and the manual bag was the amount of each solution that was added at a time. As the culture density increased, the amount of any metabolite added to the system had to be increased to see any significant change in the pH. This should have really been changed according to the OD of the culture. For this experiment, the amount that was added to the bags was increased to a point, but then the frequency of additions

was also increased. What could also be studied is how much of each solution should be added to target a greater or lesser degree of precision in the pH range by either adding more or less metabolite volume per each addition.

Chapter 4: Conclusions and Future Work

Overall this experiment was a success because the pH of a large batch algae reactor was maintained by LabVIEW within the photosynthetically active pH range as shown in comparison to the manual control bag. However, the degree of control demonstrated by the automated system is not yet sophisticated enough to exceed the control imposed by a manual, human imposed system, which leaves room for future development of the LabVIEW program. Furthermore, this experiment allowed the working parameters for the program to be determined for later experimentation. The main purpose of this program will be to control a continuous algae reactor.

The purging of the CO_2 silicone tubing was found to be more important than was initially expected. More nitrogen and oxygen diffused into the line then what was originally thought decreasing the CO_2 concentration in the line and therefore diminishing the driving force for CO_2 diffusion into the system. This resulted in a decreased carbonic acid buffering capacity until the CO_2 line was purged again. For future experiments with automatic pH control, this aspect will be better controlled by an automatic purge controlled by a solenoid so a person does not need to be continuously monitoring and purging the CO_2 line. This has already been successfully implemented in the Curtis lab by controlling the solenoid by a timer.

There are three additional aspects that could be monitored by the LabVIEW program; the OD, total nitrogen balance, and ion specific measurement for potassium. What was not implemented in this experiment but will be in future runs is a continuous inline OD measurement. This will be done using a LED and a photo diode in an inline cell with a very short path length to enable measurements even at high densities. A continuous stream of algae culture will be circulated from the algae bag, through the cell and returned to the bag. This would allow for continuous monitoring of the culture growth and better growth curves. Since there is a limited amount of nitrogen that the algae can consume in a day, the total amount of nitrogen added to the

reactor should be kept track of and once the daily limit is reached no additional nitrogen should be used to control the pH. Further pH control would need to be implemented by KOH and HCl additions only. This leads into the LabVIEW program to be utilized for a continuous reactor system. For this experiment, other nutrients such as potassium were not kept track of. For future studies, a continuous conductivity should be taken because if the algae are lacking certain nutrients such as potassium, it will not grow to its full potential due to osmotic pressure issues in the cell.

An important study that should be done that has not been done before is to see which delivery method of these solutions, fast additions or slow additions, is better and what effect each has on the pH of the system. For the manual bag, the additions were fast, but for the automated bag, the additions were a lot slower. Another thing is to determine if WFAM 6.0 can be added automatically. For this experiment it was not added automatically because it was too concentrated and precipitated out of solution. Even if a stir plate was used, there would still be a buildup of sediment in the pump tubing. A more dilute solution could be used with a higher flow pump, but there is a risk of possibly flooding the reactor since the volumes were already high for the 20x WFAM 6.0 solution.

Since this experiment was run in a climate-controlled chamber, there is a lot of important variables that affect the growth and pH of the reactor that were meticulously controlled in this experiment, but that would fluctuate much more significantly if this reactor were run outdoors. The program would have to respond differently depending on the amount of light due to clouds and the temperature. Further experimentation would have to be done to determine the effects each of these parameters has on the culture.

References

Getting Started With LabVIEW. National Instruments Corporation, 2010.

Grady, L. *A Bioprocessing Comparison of High Density Botryococcus Braunii and Chlorella vulgaris Verifying Light Limited Growth*. The Pennsylvania State University, 2010.

Horowitz, P. and Hill W. *The Art of Electronics*. Cambridge [England], New York: Cambridge University Press, 1989. 178-179.

Appendix A: OD's Taken for Both Bags during the Experiment

The OD's were recorded by Waqas Khatri and myself during the experimental run that were used to construct Figures **3.2-3** and **3.2-4**.

Table A-1 The OD data that was collected for the automated bag during the experiment. The first three ODs are the diluted OD and the last three are the actual ODs found by multiplying by the dilution.

Time	Photo Hours	Dilution	550nm	600nm	680nm	550nm	600nm	680nm
09/29/2010 09:00:00.0	0.000	0x	0.002	0.002	0.002	0.002	0.002	0.002
09/30/2010 13:00:00.0	20.000	0x	0.060	0.059	0.079	0.060	0.059	0.079
09/30/2010 17:30:00.0	24.500	0x	0.129	0.130	0.196	0.129	0.130	0.196
10/01/2010 09:15:00.0	32.250	0x	0.387	0.394	0.613	0.387	0.394	0.613
10/01/2010 11:25:00.0	34.417	3/2x	0.285	0.288	0.436	0.428	0.432	0.654
10/01/2010 14:45:00.0	37.750	2x	0.292	0.298	0.450	0.584	0.596	0.900
10/01/2010 16:00:00.0	39.000	2x	0.335	0.343	0.521	0.670	0.686	1.042
10/01/2010 17:02:00.0	40.033	2x	0.360	0.370	0.578	0.720	0.740	1.156
10/01/2010 19:05:00.0	42.083	3x	0.272	0.279	0.422	0.816	0.837	1.266
10/01/2010 21:30:00.0	44.500	3x	0.260	0.269	0.423	0.780	0.807	1.269
10/02/2010 00:13:00.0	39.217	3x	0.358	0.368	0.538	1.074	1.104	1.614
10/02/2010 09:30:00.0	48.500	3x	0.362	0.367	0.568	1.086	1.101	1.704
10/02/2010 11:34:00.0	50.567	3x	0.384	0.391	0.609	1.152	1.173	1.827
10/02/2010 13:46:00.0	52.767	4x	0.327	0.331	0.501	1.308	1.324	2.004
10/02/2010 16:20:00.0	55.333	4x	0.363	0.371	0.563	1.452	1.484	2.252
10/02/2010 18:45:00.0	57.750	4x	0.383	0.392	0.596	1.532	1.568	2.384
10/02/2010 20:36:00.0	59.600	6x	0.284	0.289	0.426	1.704	1.734	2.556
10/02/2010 22:33:00.0	61.550	6x	0.313	0.319	0.468	1.878	1.914	2.808
10/03/2010 10:15:00.0	65.250	6x	0.327	0.334	0.505	1.962	2.004	3.030
10/03/2010 12:30:00.0	67.500	6x	0.338	0.343	0.523	2.028	2.058	3.138
10/03/2010 14:55:00.0	69.917	6x	0.358	0.366	0.558	2.148	2.196	3.348
10/03/2010 17:50:00.0	72.833	6x	0.380	0.390	0.595	2.280	2.340	3.570
10/03/2010 21:11:00.0	76.183	10x	0.253	0.255	0.367	2.530	2.550	3.670
10/03/2010 21:47:00.0	76.783	10x	0.251	0.253	0.369	2.510	2.530	3.690
10/04/2010 10:35:00.0	81.583	10x	0.277	0.280	0.412	2.770	2.800	4.120
10/04/2010 13:31:00.0	84.517	10x	0.290	0.294	0.430	2.900	2.940	4.300
10/04/2010 15:39:00.0	86.650	10x	0.296	0.301	0.436	2.960	3.010	4.360
10/04/2010 17:50:00.0	88.833	10x	0.301	0.307	0.444	3.010	3.070	4.440
10/04/2010 21:00:00.0	92.000	10x	0.328	0.335	0.483	3.280	3.350	4.830
10/05/2010 10:51:00.0	97.850	10x	0.347	0.354	0.517	3.470	3.540	5.170
10/05/2010 16:50:00.0	103.833	10x	0.356	0.364	0.523	3.560	3.640	5.230

Table A-2 The OD data that was collected for the manual bag during the experiment. The first three ODs are the diluted OD and the last three are the actual ODs found by multiplying by the dilution.

Time	Photo Hours	Dilution	550nm	600nm	680nm	550nm	600nm	680nm
09/29/2010 09:00:00.0	0.000	0x	0.001	0.001	0.001	0.001	0.001	0.001
09/30/2010 13:00:00.0	20.000	0x	0.058	0.057	0.076	0.058	0.057	0.076
09/30/2010 17:30:00.0	24.500	0x	0.127	0.128	0.194	0.127	0.128	0.194
10/01/2010 09:15:00.0	32.250	0x	0.394	0.401	0.631	0.394	0.401	0.631
10/01/2010 11:25:00.0	34.417	3/2x	0.301	0.304	0.461	0.452	0.456	0.692
10/01/2010 14:45:00.0	37.750	2x	0.303	0.308	0.496	0.606	0.616	0.992
10/01/2010 16:00:00.0	39.000	2x	0.336	0.344	0.528	0.672	0.688	1.056
10/01/2010 17:02:00.0	40.033	2x	0.373	0.344	0.528	0.746	0.688	1.056
10/01/2010 19:05:00.0	42.083	3x	0.296	0.304	0.461	0.888	0.912	1.383
10/01/2010 21:30:00.0	44.500	3x	0.273	0.283	0.448	0.819	0.849	1.344
10/02/2010 00:13:00.0	39.217	3x	0.360	0.372	0.552	1.080	1.116	1.656
10/02/2010 09:30:00.0	48.500	3x	0.361	0.367	0.573	1.083	1.101	1.719
10/02/2010 11:34:00.0	50.567	3x	0.384	0.340	0.616	1.152	1.020	1.848
10/02/2010 13:46:00.0	52.767	4x	0.328	0.334	0.508	1.312	1.336	2.032
10/02/2010 16:20:00.0	55.333	4x	0.356	0.365	0.556	1.424	1.460	2.224
10/02/2010 18:45:00.0	57.750	4x	0.380	0.391	0.592	1.520	1.564	2.368
10/02/2010 20:36:00.0	59.600	6x	0.279	0.285	0.416	1.674	1.710	2.496
10/02/2010 22:33:00.0	61.550	6x	0.297	0.304	0.444	1.782	1.824	2.664
10/03/2010 10:15:00.0	65.250	6x	0.311	0.315	0.482	1.866	1.890	2.892
10/03/2010 12:30:00.0	67.500	6x	0.333	0.337	0.512	1.998	2.022	3.072
10/03/2010 14:55:00.0	69.917	6x	0.351	0.357	0.540	2.106	2.142	3.240
10/03/2010 17:50:00.0	72.833	6x	0.373	0.380	0.571	2.238	2.280	3.426
10/03/2010 21:11:00.0	76.183	10x	0.247	0.248	0.357	2.470	2.480	3.570
10/03/2010 21:47:00.0	76.783	10x	0.251	0.253	0.364	2.510	2.530	3.640
10/04/2010 10:35:00.0	81.583	10x	0.264	0.267	0.386	2.640	2.670	3.860
10/04/2010 13:31:00.0	84.517	10x	0.275	0.278	0.400	2.750	2.780	4.000
10/04/2010 15:39:00.0	86.650	10x	0.280	0.283	0.403	2.800	2.830	4.030
10/04/2010 17:50:00.0	88.833	10x	0.279	0.283	0.405	2.790	2.830	4.050
10/04/2010 21:00:00.0	92.000	10x	0.308	0.312	0.444	3.080	3.120	4.440
10/05/2010 10:51:00.0	97.850	10x	0.306	0.310	0.450	3.060	3.100	4.500
10/05/2010 16:50:00.0	103.833	10x	0.319	0.324	0.463	3.190	3.240	4.630

Appendix B: Media Formulation

Included in this appendix are the media formulations and concentrations used in this experiment. The formulations for WFAMC and WFAM 6.0 are from Curtis lab. Following is the mass balance used to calculate how much WFAM 6.0 needs to be added for each solution that contains nitrogen.

Table B-1 The media formulation for WFAMC from Curtis Lab used for inoculation and to feed the algae early in the experiment**.**

	MW	[final]	[stock]	**prep / L**	250 mL
KNO_3	101.11	--	**na**	**2.2 mL**	0.55 mL
MR26 Phosphates (50x, 1M) (pH 6.8) 1M				**1.3 mL**	0.325 mL
K_2HPO_4 (dibasic)	174.18	0.150 g/L	115 g/L		
KH_2HPO_4 (monobasic)	136.09	0.059 g/L	44.9 g/L		
pH to 6.8 with KOH or H_3HPO_4					
WFAM MICROnutrients (1000x)			g/L stock	**1 mL**	0.25 mL
H_3BO_3 (boric acid)	61.83	1/1000th or mg/L	1.86		
$MnCl_2•4H_2O$	197.41		0.54		
$ZnSO_4•7H_2O$	287.56		0.066		
$ZnSO_4•H_2O$	179		0.0411		
$ZnSO_4$ (anhydrous)	161.47		0.0371		
Na_2MoO_4-$2H_2O$	241.95		0.031		
$(NH_4)_6Mo_7O_{24}•4H_2O$	1235.86		0.0229		
$CoCl_2•6H_2O$	237.93		0.03		
$CuSO_4•5H_2O$	249.7		0.0075		
Fe-EDTA•$2H_2O^{(F)}$	403.1	0.024 g/L	4.0 g/L	**6 mL**	1.5 mL
After autoclaving add Mg and Ca solutions aseptically					
Magnesium Solution (1M, filter sterilized)			g / 50mL stock	**1 mL**	0.25 mL
$Mg(NO_3)_2•6H_2O$	256.41	0.132 g/L	6.6		
$MgSO_4•7H_2O$	246.5	0.121 g/L	6.03		
$MgSO_4$ (anhydrous)	120	0.0588	2.94		
Calcium Solution (1M, filter sterilized)			g / 50mL stock	**0.088 mL**	0.022 mL
$CaCl_2•2H_2O$	147	0.0132 g/L	7.5		
$CaCl_2$ (anhydrous)	111	0.01	5.66		

Table B-2 The media formulation for WFAM 6.0 from Curtis lab added to the reactor to balance the nitrogen addition made during the experiment**.**

	MW	[final]	[stock]	**prep / L**	250 mL
KNO_3	101.11	--	**na**	**2.2 mL**	0.55 mL
Diabasic Only MR26 Phosphates (50x, 1M) (pH 6.8) 1M				**1.3 mL**	0.325 mL
K_2HPO_4 (dibasic)	174.18	0.224 g/L	172.47 g/L		
pH to 6.8 with KOH or H_3HPO_4					
WFAM MICROnutrients (1000x)			g/L stock	**1 mL**	0.25 mL
H_3BO_3 (boric acid)	61.83	1/1000th or mg/L	1.86		
$MnCl_2•4H_2O$	197.41		0.54		
$ZnSO_4•7H_2O$	287.56		0.066		
$ZnSO_4•H_2O$	179		0.0411		
$ZnSO_4$ (anhydrous)	161.47		0.0371		
Na_2MoO_4-$2H_2O$	241.95		0.031		
$(NH_4)_6Mo_7O_{24}•4H_2O$	1235.86		0.0229		
$CoCl_2•6H_2O$	237.93		0.03		
$CuSO_4•5H_2O$	249.7		0.0075		
Fe-EDTA•$2H_2O^{(F)}$	403.1	0.024 g/L	4.0 g/L	**6 mL**	1.5 mL
After autoclaving add Mg and Ca solutions aseptically					
Magnesium Solution (1M, filter sterilized)			g / 50mL stock	**1 mL**	0.25 mL
$Mg(NO_3)_2•6H_2O$	256.41	0.132 g/L	6.6		
$MgSO_4•7H_2O$	246.5	0.121 g/L	6.03		
$MgSO_4$ (anhydrous)	120	0.0588	2.94		
Calcium Solution (1M, filter sterilized)			g / 50mL stock	**0.088 mL**	0.022 mL
$CaCl_2•2H_2O$	147	0.0132 g/L	7.5		
$CaCl_2$ (anhydrous)	111	0.01	5.66		

The concentrations for the solutions used in this experiment:

NH_4Cl = 53.89 g/L
KOH = 0.4M
NH_4NO_3 = 48.88 g/L
KNO_3 = 88 g/L
HCl = 1M
WFAM 6.0 = 20x

The following is the calculations used to make the formula shown in Figure **2.1.3-5** for the WFAM 6.0 addition loop.

The following was used to calculate the amount of nitrogen there was per mL of WFAMC to determine how much was needed per mL of WFAM 6.0.

2.2gKNO_3/L (for 1xWFAM 6.0)

14.01g N/(101.1g KNO_3) = 0.1386

2.2 g*0.1386 = 0.3049gN/(L (1xWFAM 6.0))

So as a function of the concentration of solutions added, the volume added, and the concentration of WFAM 6.0 (1x, 10x, 20x), the amount of WFAM added per addition will be calculated in LabVIEW. The equation in bold is the equation that was used in the LabVIEW program.

NH_4NO_3: 0.3501 gN/g NH_4NO_3

(0.3501gN/g NH_4NO_3)/(0.3049gN/L WFAM 6.0) = 1.1482 L WFAM 6.0/g NH_4NO_3

mL WFAM 6.0 to Add = 1.1482*[NH_4NO_3]*(mL NH_4NO_3 added)/[WFAM 6.0]

KNO_3: 0.1386 gN/g KNO_3

(0.1386gN/g KNO_3)/(0.3049gN/L WFAM 6.0) = 0.4546 L WFAM 6.0/g KNO_3

mL WFAM 6.0 to Add = 0.4546*[KNO_3]*(mL KNO_3 added)/[WFAM 6.0]

NH_4Cl: 0.2691 gN/g NH_4Cl

(0.2691gN/g NH_4Cl)/(0.3049gN/L WFAM 6.0) = 0.8590 L WFAM 6.0/g NH_4Cl

mL WFAM 6.0 to Add = 0.8590*[NH_4Cl]*(mL NH_4Cl added)/[WFAM 6.0]

*Note: All concentrations are in g/L except for WFAM 6.0 which is 1x, 10x, 20x, etc.

Appendix C: Calibration Data and LabVIEW Parameters for the Front Panel

Table C-1 pH data of when NH_4NO_3 was added to the reactor from Lisa Grady from the semi-steady state trickle film reactor run. The graph of the data is shown in Figure **2.1.2-2** (Grady, L.)**.**

Time	pH
4/20/2010 19:13	7.2473
4/20/2010 19:14	7.2645
4/20/2010 19:15	7.2725
4/20/2010 19:17	7.2716
4/20/2010 19:18	7.2728
4/20/2010 19:19	7.2766
4/20/2010 19:20	7.2782
4/20/2010 19:21	7.2837
4/20/2010 19:22	7.2822
4/20/2010 19:23	7.2817
4/20/2010 19:24	7.2885
4/20/2010 19:25	7.2855
4/20/2010 19:26	7.2866
4/20/2010 19:27	7.2902
4/20/2010 19:28	7.2919
4/20/2010 19:29	7.2971
4/20/2010 19:30	7.2944
4/20/2010 19:31	7.2981
4/20/2010 19:32	7.2944

Table C-2 pH calibration data for the Valley Instruments pH controller and Cole-Parmer pH electrode used for the slope and intercept values in LabVIEW.

Voltage (V)	pH
1.331	3.73
2.495	7
3.587	10

Slope	Intercept
2.779584795	0.041645972
Model	
1.331	3.741273334
2.495	6.976710035
3.587	10.01201663

m	b
2.78	0.04

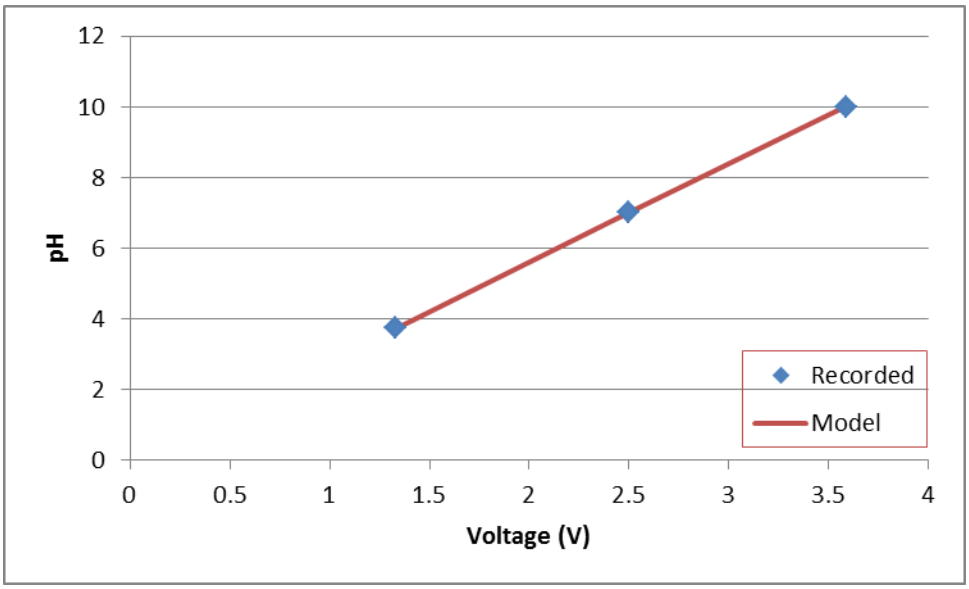

Figure C-1 The graph of the pH probe calibration data from Table **C-2** with an $R^2 = 1.0$. Since no multiple regressions were taken, this is a good linear model to use to find the pH in LabVIEW.

Table C-3 The raw data collected for the Valley Instruments Support Module pumps used to find the average flow rates including the variance and percent error in the calculated flow rate.

	Time (min)	Volume 1 (mL)	Volume 2 (mL)	Volume 3 (mL)	Variance	Average Volume (mL)	±	Flow Rate (mL)
PS00104279 Acid	2	2.6	2.4	2.4	0.01	2.47	0.54%	1.23
PS00104279 Base	2	2.5	2.2	2.4	0.02	2.37	0.99%	1.18
PS00104279 Anti-Foam	2	2.6	2.4	2.5	0.01	2.5	0.40%	1.25
PS00104279 Aux. Pump	2	2.2	2.2	2.2	0	2.2	0.00%	1.1
PS00104280 Acid	2	2.3	2.5	2.4	0.01	2.4	0.42%	1.2
PS00104280 Base	2	-	-	-	-	-	-	-
PS00104280 Anti-Foam	2	2.5	2.5	2.5	0	2.5	0.00%	1.25
PS00104280 Aux. Pump	2	-	-	-	-	-	-	-

Table C-4 The average flow rates for each Valley Instruments Support Module pump and the digital line assigned to each pump for the NI UBB 6008 DAQ.

Pump	port/line	Solution	Flow Rate mL/min
PS00104279 Acid	0/0	NH4Cl	1.23
PS00104279 Base	0/1	KOH	1.18
PS00104279 Anti-Foam	0/2	NH4NO3	1.25
PS00104279 Aux. Pump	0/3	KNO3	1.1
PS00104280 Acid	0/4	HCl	1.2
PS00104280 Base	-	-	No Head
PS00104280 Anti-Foam	0/5	WFAM 6.0	1.25
PS00104280 Aux. Pump	-	-	No Cover

Table C-5 The LabVIEW parameters that were inputted into the front panel along with the final parameters that were used and inputted into the front panel.

	pH Calibration					Leveling Out			For NH4Cl and KOH Limits								
Time	m	b	Level Time (min)	After Feed Wait Time (min)	Samples for slope (1S/sec)	High Slope (per min)	Low Slope (per min)	Record Data Every (s)	High pH	Low pH	NH4Cl (g/L)	Add NH4Cl (mL)	Flow Rate NH4Cl (mL/min)	Total NH4Cl (mL)	Add KOH 0.4 M (mL)	Flow Rate KOH (mL/min)	Total KOH (mL)
9/29/2010 9:00	2.78	0.04	5	5	60	0.0020074	-0.01	10	8.3	6.3	53.89	0.5	1.23	-	1	1.18	-
9/30/2010 15:32	2.78	0.04	5	5	120	0.0020074	0	10	8.3	6.3	53.89	0.5	1.23	-	1	1.18	-
10/4/2010 9:25	2.78	0.04	5	10	240	0.0020074	0	10	8.3	6.3	53.89	0.5	1.23	-	1	1.18	-
10/4/2010 1:00	2.78	0.04	10	10	540	0.0020074	0	10	8.1	6.3	53.89	5	1.23	-	1	1.18	-
10/4/2010 15:20	2.78	0.04	10	10	540	0.0002007	0	10	8.1	6.3	53.89	5	1.23	-	1	1.18	-
10/4/2010 16:47	2.78	0.04	10	10	540	0.0020074	-0.002009	10	8.1	6.3	53.89	5	1.23	40.99	1	1.18	96.98
ON	9:30:00																
OFF	1:00:00																

For NH4NO3 and KNO3 Limits										For HCl			For WFAM 6.0			HCl Addition Reset		
High pH	Low pH	NH4NO3 (g/L)	Add NH4NO3 (mL)	Flow Rate NH4NO3 (mL/min)	Total NH4NO3 (mL)	KNO3 (g/L)	Add KNO3 (mL)	Flow Rate KNO3 (mL/min)	Total KNO3 (mL)	Add HCl (mL)	Flow Rate (mL/min)	Total HCl (mL)	Concentration (1x, 10x, etc.)	Flow Rate (mL/min)	Total WFAM 6.0 (mL)	High Slope	Low Slope	Reset Time Hack (min)
7.5	7.5	48.88	0.5	1.25	-	88	0.5	1.1	-	0.6	1.2	-	20	1.25	-	1	-0.1514	5
7.5	7.5	48.88	0.5	1.25	-	88	0.5	1.1	-	0.6	1.2	-	20	1.25	-	1	-0.1514	5
7.5	7.5	48.88	0.5	1.25	-	88	0.5	1.1	-	0.6	1.2	-	20	1.25	-	1	-0.1514	5
7.5	7.5	48.88	2	1.25	-	88	0.5	1.1	-	0.6	1.2	-	20	1.25	-	1	-0.1514	5
7.5	7.5	48.88	2	1.25	-	88	0.5	1.1	-	0.6	1.2	-	20	1.25	-	1	-0.1514	5
7.5	7.5	48.88	2	1.25	97.88	88	0.5	1.1	99.5	0.6	1.2	100	20	1.25	-65.85	1	-0.1514	5

Academic Vita

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The Pennsylvania State University – University Park, PA
Bachelors of Science in Chemical Engineering with Honors in Chemical Engineering
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Leadership and Activities:

Penn State Army ROTC – Received a four and a half year scholarship and participated for four and a half years Fall 2006 –Fall 2010

LDAC Graduate	Summer 2009
Air Assault Graduate	Summer 2009
S.A.M.E. Michael Baker, Jr. Scholarship Award	2008 – 2009
National Sojourners Award	2008 – 2009
The Military Order of the Loyal Legion of the United States	2007 – 2008
Department of the Army Superior Cadet Decoration Award	2006 – 2007
Pittsburgh Post: Society of Military Engineers Award	2006 – 2007

Pennsylvania National Guard – A member of Co A(-) 2-112th IN (SBCT) 2006 – 2010

Penn State Ski Club

An active member	2006 – 2010
Treasurer in charge of $50,000 plus in trip and dues money	2009 – 2010

Work Experience:

Project and Process Engineering – An intern at Creative Engineers, Inc. a consulting company. Performed mass balance and batch sizing for non-ideal systems, level control design for emissions scrubber, sample cylinder scenario sizing, and creation and revision of P&IDs utilizing AutoCAD. Summer 2009

Special Skills:

Computer Skills – Proficient with Microsoft Windows, MAC OSX, and Linux; Microsoft Office, Mathematica, Aspen HYSYS, MATLAB, AutoCAD, and LabVIEW.

Student Pilot Certificate – 90.6 total Flight hours

NAUI – Basic and Advanced SCUBA certification to 80ft of depth

Security Clearance – Eligibility of Secret on 2007 01 04