Cost-Effective CyanoStat Array for Algal Cultures

Stephanie Conley
University of California, San Diego

Abstract

Biofuels are an emerging solution to provide global energy independence, offering a sustainable and biodegradable resource. Biofuels can be improved upon with the continuation of genetic research, which is not possible to accomplish with static fossil fuels. However, the production of biofuels has proven to be a costly endeavor. In order to advance research leading to more efficient biofuel production, a device known as the CyanoStat, which automates the process of growing algae, has been created. The design utilizes existing materials from open source projects, such as the Arduino microcontroller platform, to reduce cost and encourage further exploration in algae research. Overall, this device can culture six samples for under $500.

Keywords: CyanoStat, turbidostat, biofuel, biodiesel, algae, evolvinator, Arduino, embedded system.

I: Introduction

The production and usage of fossil fuels has raised many environmental and economic concerns amongst scientists, politicians and economists. The world's growing dependence on fossil fuels is causing an increase in demand that will eventually outstrip supply, because fossil fuels are ultimately nonrenewable. Additionally, there are convincing scientific studies indicating that the combustion of fossil fuels are increasing the levels of CO_2 and other greenhouse gases in the atmosphere causing climate change on a global scale. Although we are not certain about the effects that this climate change will have, most studies assert the delicacies of our global ecosystem to changes of this scale. Consequently, the issue of finding an alternative and safer energy source to replace fossil fuels has become more pressing.

One solution to the problem lies in using biofuels as an energy source. Biofuels boast several advantages over fossil fuels. These advantages include producing a non-toxic product that has biodegradable, sustainable, renewable, and portable capabilities [1]. There are different types of biofuel which exhibit varying levels of operating success. For this project, photosynthetic green algae are used as the model organism. In order to create algae biofuel, there needs to be a sufficient source of lipids. Algae produce 30 times more oil per acre than biofuel crops, like corn, and require less land and a fraction of the water to thrive. Another positive characteristic of algae is its rapid growth rate [2]. This is the defining characteristic that determines why biofuels are renewable and sustainable. Fossil fuels fail in this respect because there are no known ways to manufacture them. Since fast growing algae can be farmed and harvested in abundance, production can be handled on a large industrial scale.

The costs incurred in the production of biofuel have prevented this market from flourishing and becoming a more prominent contribution to our energy needs. Lowering the cost of producing biofuels is essential to their widespread market success. As the availability of fossil fuel declines, the urgency of a substitute grows stronger because industrialized societies are very dependent on fossil fuels for many aspects of daily life. For example, nearly half of our electricity is made from coal and vehicles depend on oil to run [3]. If the cost of biofuel production can be reduced, the manufacture of biofuel will increase allowing it to become a viable substitute for fossil fuels.

Biofuel research is restricted because of the energy intensive process involved in growing and harvesting the algae. This is due to algae needing to grow at optimal conditions. Additionally, the series of processes that involve separation and lipid extraction can be expensive and require a significant amount of time and work. In order to better grow algae and produce biofuel at the industrial scale, there are different aspects that need to be changed. One of these aspects focuses on the most fundamental part of the entire process – growing the algae. This process needs to be done as cost-effective as possible without comprising successful and optimal growth.

There have been several attempts by researchers to create cost-effective bioreactors with quite successful results. Arizona State University, for example, has conducted extensive research into designing bioreactors. In order to make a successful product that can be utilized on a larger scale, there are challenges that need to be met. First, there are thousands of algae species that need to be screened and evaluated for high growth rate and oil content. Arizona State University has been successful in completing this task, following up with genetic research to improve the way algae perform under diverse environmental conditions. This effort takes time and resources to accomplish.

The theories behind biofuel as a sustainable energy resource have gained increasing credibility but the cultivation process remains in infancy as challenges continue to arise. Funding and collaboration are equally important factors in the success of these projects also. Industry partners provide funding and the facilities to make the project feasible and marketing assistance helps to market the product. Without this assistance, developing a product that can be created on a large scale becomes difficult. Similar to many bioreactor researchers, I am in the beginning stages of designing and testing a viable product. The CyanoStat approach differs from other bioreactor endeavors in that existing materials from open source projects, such as the Arduino microcontroller platform, to reduce costs and increase the accessibility of algae research.

Professional bioreactors can be purchased by suppliers for thousands. These types of bioreactors, however, handle only one sample at a time. The bioreactor can handle six samples for only $500. Not only have costs been cut dramatically but productivity has increased too. Therefore, the project goal of creating a cost-effective turbidostat has been achieved. The key focus of this approach has been to scale a model that is simple, effective and created with materials that are reasonably priced. Materials can become expensive if budgeting is not accounted for. Utilizing internet resources to buy parts has helped to budget the project. One of the main reasons why professional bioreactors are expensive is because of the costly parts that are used in construction.

Since this turbidostat involves small-scale algae production, the limitations are not quite the same as a large scale operation. That being said, both operations will experience the same issues, although not on the same level. A common issue with large scale operation is in obtaining enough CO2 to feed through the system because rapid algae growth will cause CO2 depletion. Although the turbidostat samples are small, CO2 levels need to be stable. The pH will shift if CO2 levels are not accurate. The basis of this project is to create an optimal environment for algae to flourish at low cost. Other limitations influencing this goal would involve solving the issues of how to harness the right amount of light energy, finding a decent temperature range, making sure enough nutrients are present, and ensuring that algae growth does not get excessively out of hand.

Biofuel production has a large DIY culture/community that could benefit from this device. Providing an easy to use, yet sophisticated, turbidostat device to the public would encourage further exploration into algae research. Computing and open source access is an inexpensive approach, which makes data acquisition and the dissemination of information easy to obtain and publish. Results would provide reference to those interested in creating their own biofuel.

Biofuels are important to the earth's well-being because they reduce greenhouse gases. Fossil fuels release gases, like carbon dioxide, when burned. Studies have shown that different types of biofuel can reduce emissions up to 65-87 percent. For example, biodiesel-powered cars benefit the environment by having fewer emissions and create less pollution. Furthermore, biofuel sustainability allows itself to regenerate. Fossil fuel is incapable of this and is used up once burned [4].

In terms of economic development, the biofuel industry helps to stimulate the economies of communities by providing a source of jobs. With this in mind dependence on foreign oil can be reduced. One of the most important qualities of biofuel production is how environmentally friendly it is when produced. Oil refineries release millions of pounds of carcinogens, like benezene, and other pollutants, which poison the environment and cause various health problems. Biofuel refineries, on the other hand, emit very few pollutants and are thus cleaner to run.

Put simply, this project is important because it is addressing an issue that affects the entire world. Research and development of biofuels means the resolution of fossil fuel dependency through the combined use of multidisciplinary fields such as technology, engineering, biology and chemistry. The turbidostat is an innovative device that is cost-effective, compact, and easy to use.

II: Related Work

Many researchers and companies have developed algae bioreactors using different designs. Companies like Algamerica, for example, develop large-scale photobioreactors that can handle 130 gallons for $1080.00. Algamerica's design features a cylindrical column that utilizes an air pump. This design could be considered successful because it can store an abundance of algae for larger scale production.

As previously stated in the introduction, Arizona State University has done extensive algae biofuel research through their Biodesign Institute. ASU has successfully used small-scale lab designs similar to the CyanoStat design. These designs typically use the same lab equipment as this project, such as flasks, test tubes, and some sort of container that will hold the algae culture. It would appear that neither Algamerica nor ASU appear to utilize an embedded system, like Arduino, to collect data in their small-scale and large-scale designs. Furthermore, they probably do not transfer data in the same way as the CyanoStat does by using a free service like Google Spreadsheet

via the Ethernet Shield. The CyanoStat's method of using reasonably priced parts that are powered by an embedded system, like Arduino, is the defining aspect of the project and its success.

To be more specific with regard to the CyanoStat design and process, not many other sources of similar method can be found. The best example is an "evolvinator", which is essentially another word for algae turbidostat. An evolvinator is different than a typical bioreactor used by ASU and Algamerica in that it uses an embedded system like Arduino. Gingko BioWorks, a company that sells engineered organisms, has provided a wiki article under OpenWetWare that outlines how to create an algae turbidostat/evolvinator. The CyanoStat approach almost mirrors their Arduino/Ethernet Shield data collection but the CyanoStat set-up is different because a simple algae culture display is used to cut costs. OpenWetWare has provided cost assessment of parts that is well above the $500 spent on the CyanoStat. In that regard, the CyanoStat is more cost-effective. In conclusion, the turbidostat created by Gingko BioWorks has more parts and is more developed. Their model and project tutorial could serve as a useful guide in the development of this project.

III: Technical Material

Obtaining data is straightforward in this project. The current display method consists of using photoresistors to take readings of the samples based on how much light is present. The code used to run the embedded system can be changed to take readings at any time interval indicated. An Ethernet Shield was utilized to allow the Arduino microcontroller platform to connect to the internet. The Ethernet Shield is an important part of the project because the user can send data through the Internet to a free service, like Google Spreadsheet, and further reduce the cost of running the system. Using the spreadsheet is an efficient method of handling data transfer because the code ensures that data is sent in real time. Essentially, the turbidostat will be functioning without the user needing to be there to supervise. In fact, the data is available to view anywhere just by accessing the Google Spreadsheet on a computer or smart device.

I. Structure Design

The CyanoStat has a modular structure, so that any number of small six experiments could be run in parallel. Test tubes were used as the containers for the cultures because they are inexpensive, save desk space, have many pre-constructed accessories, and have a low volume. The container is kept at a low volume in order to properly agitate the culture using air. Other containers have been used in this project, such as the erlenmeyer flask, but did not allow for adequate agitation. This caused algae to clump or stick to the walls of the glass.

The air pump is not only used for agitation, but also a source of aeration for the algae to grow. In order to drain the culture when it becomes too turbid (cloudy) a gravity-fed system, similar to the common IV fluid system used in hospitals, with media will drain into the test tube. A solenoid valve pinches the tube to stop the media from flowing in. In CyanoStat tests the solenoid valve worked perfectly, and no modifications were needed.

II. Circuit Design

The original prototype has extensive wiring that makes the system look cluttered and disorganized, as shown in **figure 1**. In order to fix this, the new printed circuit board (**figure 2**) used wires with different colors. This helps to differentiate which wires are used for solenoids and photoresistor sensors.

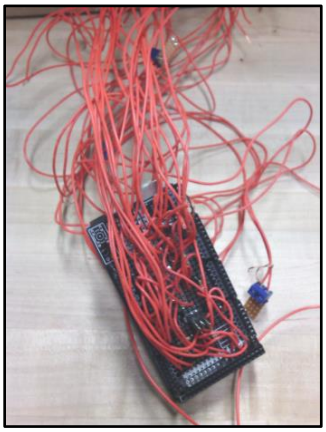


Figure 1: Original Arduino printed circuit board (PCB) with disorganized wiring.

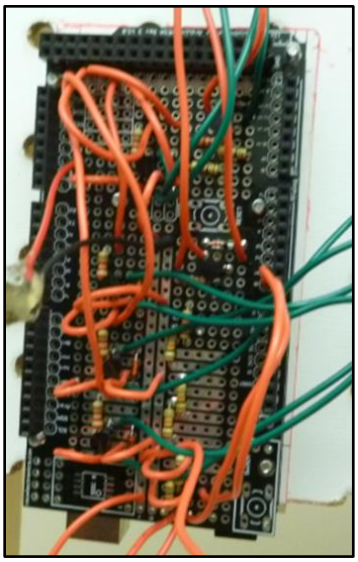


Figure 2: New Arduino printed circuit board (PCB) with reorganized.

Figure 1 and **figure 2** were organized and soldered with circuit schematics as a guide. The first circuit as shown in **figure 3** is for the photosensors. The light detection sensor is a photo resistor because they are simple to use and low cost. The photoresistor changes the amount of current flowing through the circuit based on the amount of light it is receiving. The Arduino reads the analog signal, which is generated by the current flowing through the photo resistor. The second circuit shown in **figure 4** is the solenoid valve circuit. The solenoid valve allows media to drain into the culture tube, and flushes out some of the algae in order to make the culture less turbid. The solenoid valve needs 12 volts in order to operate so we needed a second power source connected to the circuit. A transistor was used to open and close the solenoid valve to protect the Arduino from high current. A digital pin on the Arduino was used to trigger the transistor. Finally, a flyback diode was implemented to protect the circuit from voltage spikes.

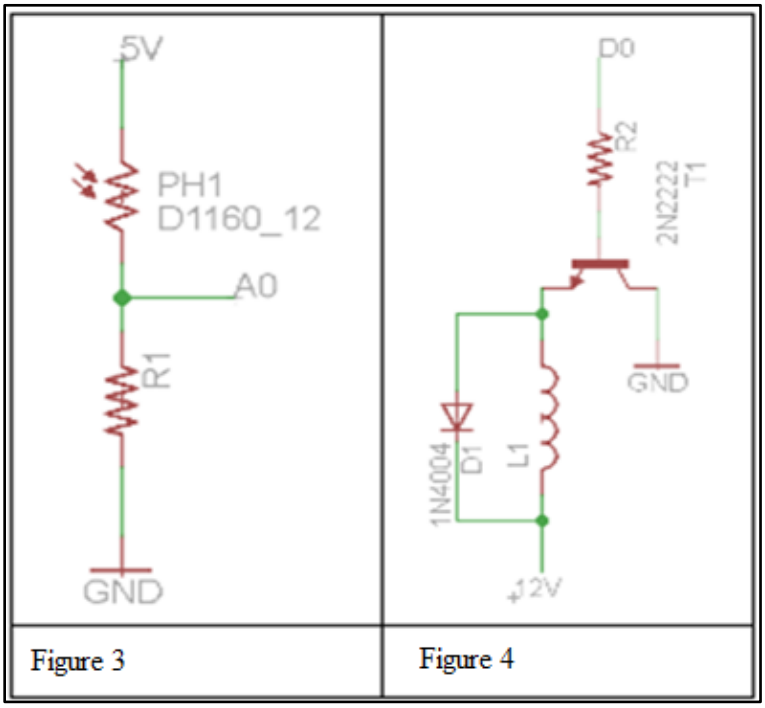


Figure 3 is for a circuit schematic for photoresistor sensors. **Figure 4** is for the solenoid valves.

Initially, there was difficulty triggering the solenoid due to not supplying the transistor with enough current at the base. The resistance was lowered between the Arduino digital pin and the transistor, and the solenoid began to switch properly.

III. Device Programming

A small and simple program was utilized in order to test the two circuits independently. The photoresistor sensors were tested to model their behavior on a larger array, and found that they were still extremely sensitive. Any movement within the room would affect the readings. The photoresistor sensors were covered with small black rubber tubing (previously used in the first prototype) as a means of directing the light into the sensor. Doing this limits the fluctuations because ambient light is minimized. To ensure that all the solenoids function properly a program tutorial for a toy DC motor was utilized for testing. The motor and the solenoid had similar properties such as high current draw. At first some of the solenoid valves would not open. After increasing the voltage applied to the transistor's base the solenoids began switching properly.

The logic portion of the program is straightforward and reads like plain English. For example, if the user changes the code for the light sensor reading to a value that is less than '500', then the code will trigger media to enter the tube and displace any over-abundance of algae. This is important because anyone with a limited knowledge of programming to be able to modify the program to suit their own needs. The code language for this system is C for the Arduino microcontroller processor.

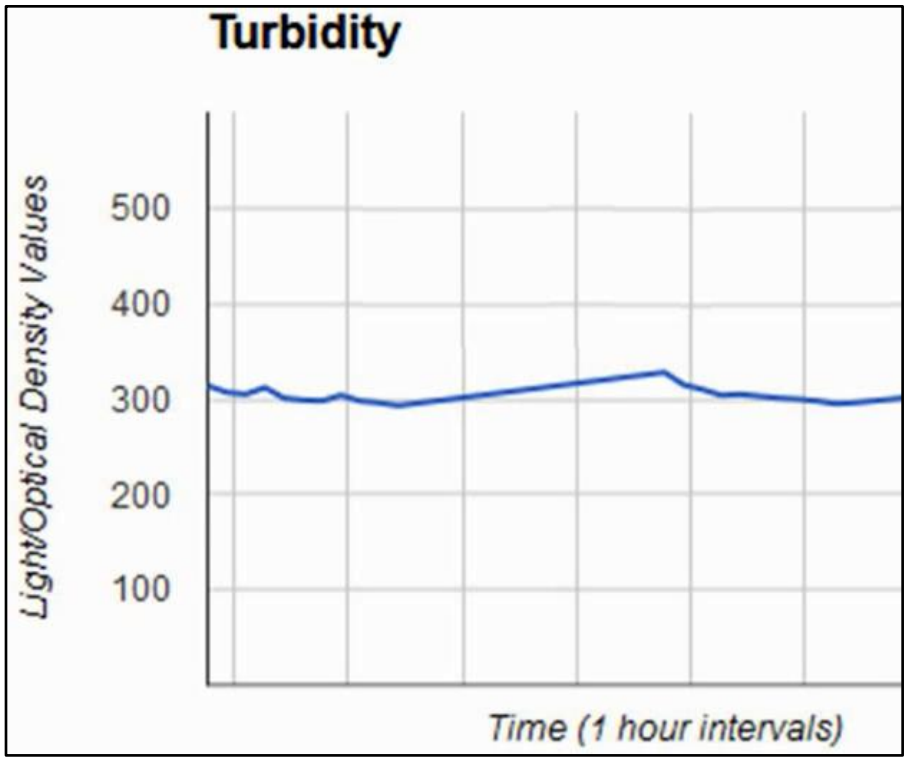
IV: Lighting Options

The initial system used a heat lamp with a fluorescent bulb as the provided light source. This light is supposed to stay on for 12 hours of a day cycle. The user can indicate in the code how they would like to divide day and night cycles. It does not have to be 12 hours for day and 12 hours for night. When the night cycle is triggered the system will keep bubbling because the system is still running. The only thing that is deactivated is the data collection. This can be observed more clearly in **graph 2**. If the data collection does not turn off, then the photoresistor sensor will assume the lack of light is the result of an over-abundance of algae. The reading will be so extreme that the photoresistor will send the Google Doc a data point of zero. This is bad because the user can also indicate in their code which optical density value signals nutrient injection (dilutes the algae sample). If the user has decided that they would like a nutrient injection when optical density reaches a value below 185, then every time the photoresistor sensor picks up a value of zero (for whichever time interval the user has picked. i.e. every hour, half day, half week, etc.) the system will flush every time. The system doesn't know that it is in a night cycle and will keep diluting until it reaches above 185. This will never happen in the night cycle. The result of this is an over-diluted sample and possible overflow of the output container.

Other lighting options were looked at (see Milestones section). The decision was to stay with the fluorescent heat lamp lighting was the end result.

IV: Results Section

Results from Google Docs show algae growing at an exponential rate. Since the device measures relative brightness (instead of growth) it would be expected to see an exponential decay in the graph. Referencing **graph 1**, the near linear blue line at the 300 range represents a test tube filled with diluted algae. This sample provided very steady readings. **Figure 5** provides the numerical data and dates for the **graph 1** representation.

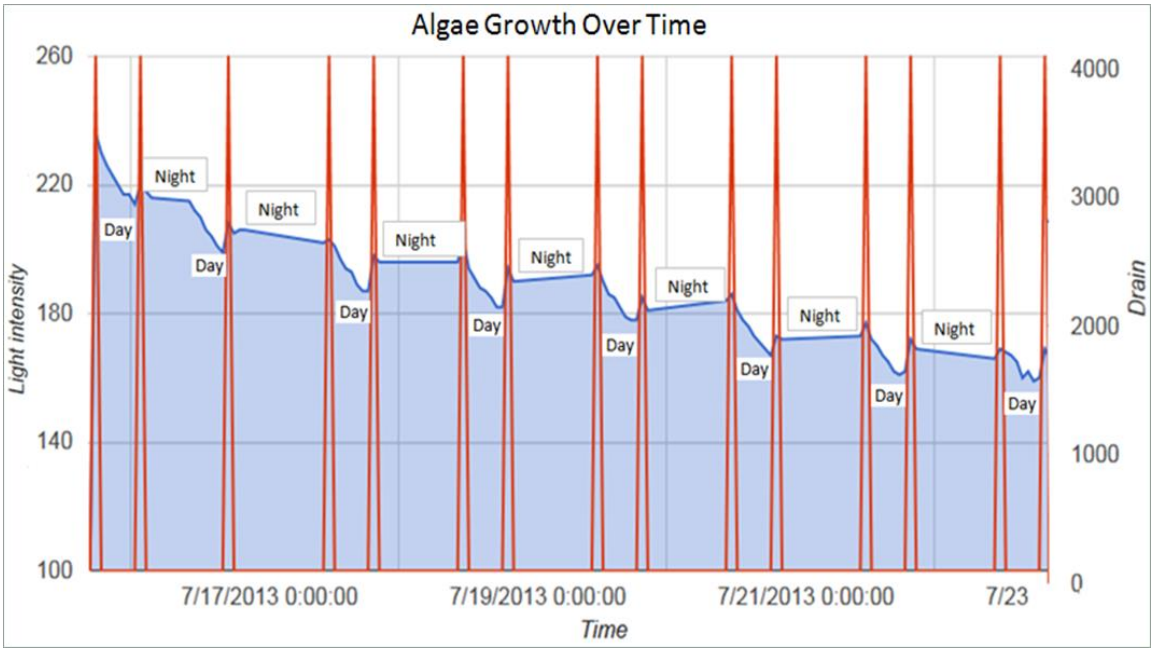


Graph 1: The blue line represents a growing algae culture. The line slopes downward as more algae grow.

	A	B
1	**Timestamp**	**PS1**
2	5/15/2014 17:36:17	307
3	5/16/2014 18:36:21	305
4	5/17/2014 19:36:28	312
5	5/18/2014 20:36:32	301
6	5/19/2014 21:36:36	299
7	5/20/2014 22:36:39	298

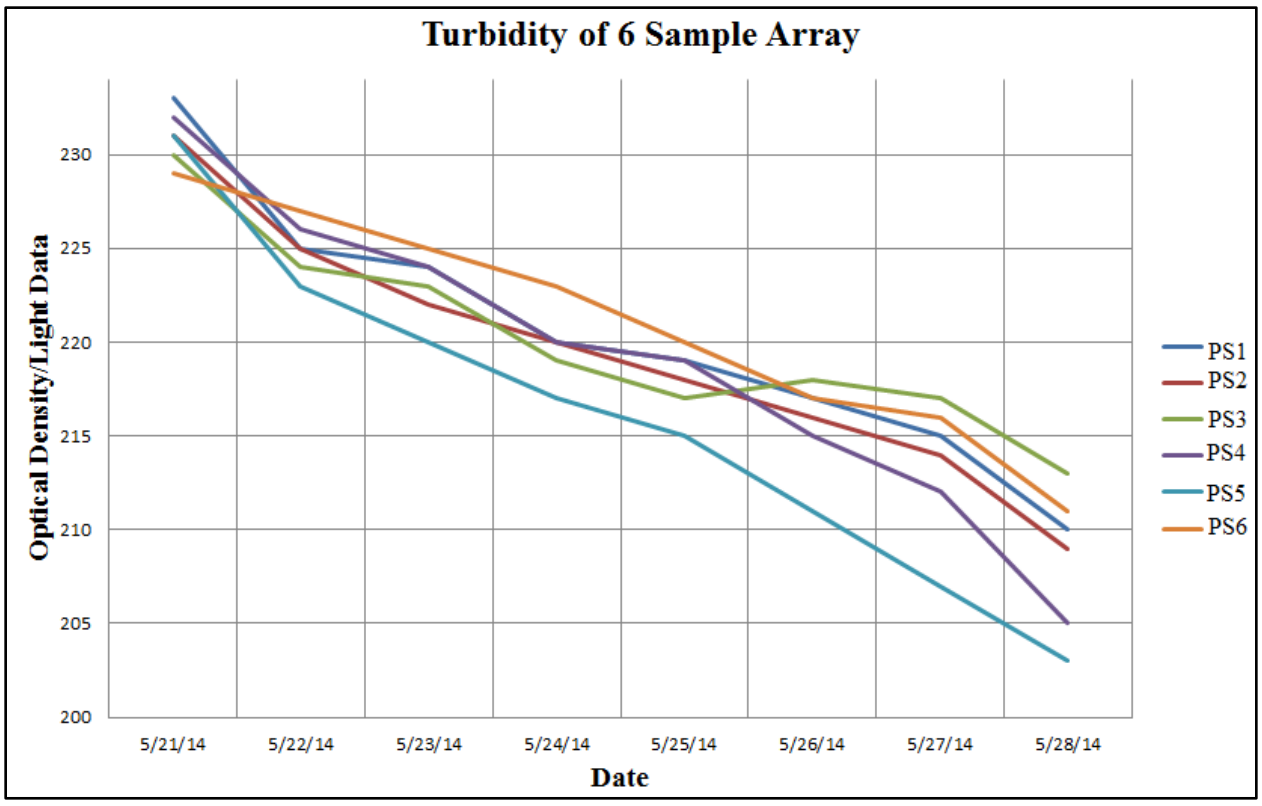
Figure 5: The numerical data and timestamps for **graph 1**.

In order to show the significance of the data in **graph 1**, **graph 2** has been provided. **Graph 2** shows a very successful test run of the original system with one test tube. Like in **graph 2**, the line trend in **graph 1** suggests that the same line trend will eventually occur.



Graph 2: Original single test tube test. The downward slope indicates less light is hitting the photoresistor sensor. Therefore, the light/optical density readings will decrease. The decline is steady and not sporadic.

Graph 3 shows the set-up for a six sample array. As seen in **graph 2**, all six samples have a successful linear decline. This means that all samples have data collection that indicates the algae cultures are growing.



Graph 3: Six algae samples are declining linearly. These are successful curves because they indicate algae is growing.

Over the span of eight days the CyanoStat was tested to run the six test tube array. The results were expected to mirror that of the single test tube experiment in **graph 2**. What does that mean? Essentially, the code is supposed to run the system successfully enough as to create a sustainable environment for the algae to thrive. **Graph 3** shows six samples in a parallel decline. Therefore, not only did each sample grow but all six samples grew together.

V: Milestones

Over the duration of this research, not all the milestones that were established in the beginning were achieved. This, however, is not necessarily an unfortunate thing. These milestones consisted of changing the existing C for Arduino code to Python, changing the existing Arduino microcontroller processor with BeagleBone Black, cleaning up the proto board wiring, purchasing glassware and other related parts from the stockroom in Urey Hall, completion of the casing/stand that will hold the system and testing of IR sensors with LED lights.

The goal of the project was to create and test a six turbidostat array. This proved to be a task that would not be completed on BeagleBone Black due to time constraints. I found that six turbidostat schematics would work the best because of space availability and I would not have to spend a lot of time soldering in the component pieces. I still plan on trying to implement BeagleBone Black in another design over the summer. Another issue with changing microcontroller processors is that the code language had to change too. I was super ambitious with learning Python to replace the Arduino C code. This proved to be more ambitious than planned. I set out to learn Python via CodeAcademy.com. Although this website has a nice interface, time did not allow for learning the language well enough to recreate the Arduino code and make it more efficient. By efficient, I mean that I wanted to shorten the code quite a bit and make it more simplified. Furthermore, the code would have been easier to read and thus distinguish functions right away. This doesn't quite happen with the other code. Having said that, the part that the user would change is easy to follow because the user is changing numerical values.

Given this circumstance, the C code was worked around with for a few weeks to optimize for six test tubes. This goal was actually achieved. This shows that the task is possible and transfer to BeagleBone Black will be easier. Since my fellowship was awarded 6/13/14, I did not want to start buying materials for casing. My reasoning behind this is attributed to not wanting to spend my own money on a design I did not fully agree with. This device is supposed to be cost-efficient for the user and for me. Also, I had old glassware supplies in my office used for testing. I do plan on finishing the entire design and project by the end of the summer.

Since six samples were finally achieved, Google Docs was worked with in order to transfer the data for six photoresistor sensors and six solenoids. This took three days of testing to do. A more challenging portion was recording the data being received. Data transfer to an SD card was researched but time did not permit any changes to the CyanoStat. In Arduino version 1.0 the Ethernet class was changed to accept DHCP leases. One of the biggest challenges was to maintain the Ethernet connection. After a few hours of the CyanoStat running, the DHCP lease would end. This would cause the device to stop recording. After many attempts to fix the problem, a simple DHCP lease called Ethernet maintain () was added to the code to maintain DHCP lease. After this fix the CyanoStat would record data for three and a half days, and the connection was again lost. Two more attempts were made and neither test cycle lost a connection. This may have been an isolated incident.

On the top of IR sensors, the discussion of lighting was a big issue. I went as far as buying a blue-red LED grow light and using the original photoresistor sensors. Although the light was nice to stare at, it did hurt my eyes. So, this goes back to design. For a light such as this, I want it to be placed in a closed space. I changed the lighting back to the fluorescent bulb because the experiments were working well in the previously set-up. Since the main focus was scaling to six samples and actually being able to acquire data, the previously lighting was not changed. The idea was to mount three LED lights and three IR sensors on each tube. This was another ambitious part of the project that will be revisited at another time.

Previously, I mentioned that I wanted to gain more client labs. Although I have yet to communicate with a finished product (as opposed to some promise), I did manage to get a portion of the Chancellor's Interdisciplinary Fellowship from the California Center for Algae Biotechnology for $2500 (I assume Dr. Stephen Mayfield is the PI). Victory!

VI: BitBucket Organization

The files that are featured in the BitBucket account consist of previous PowerPoint presentations, milestone reports (a good reference for some mistakes or issues along the way), this final technical paper, a parts list with pricing estimates, a drawing of the set-up, and the Arduino code used to run the system. The parts list and set-up

documentation is featured in a construction manual-type pdf. The documentation of pricing is based on what can be purchased online because I know that I am privy to services on campus (i.e. the Urey Hall Stockroom and Prototyping Lab) that were free to use or relatively cheap. That being said, I did much of my purchasing online, so the price differences shouldn't be that much. Another file featured in the BitBucket account is a README file for the code. This is standard practices and ensures that the user has some idea of what the code can do.

VII: Conclusion

The goal of this project was to design and construct a six sample array algae turbidostat, or bioreactor, using cost-effective tactics. The goal was achieved by using an embedded system featuring an Arduino microcontroller platform to run the algae turbidostat array. The vital component pieces of the display are photoresistor sensors to collect the data and solenoids to control air, water, and nutrient input and output. Typically, a single sample algae bioreactor sold by a supplier costs thousands. The CyanoStat bioreactor handles six samples and costs only $500.

Production of biofuel is expensive and we have been successful in cutting costs. Producing biofuel in some cases has resulted in costs that amount to more than the value of product generated. In order for the biofuel industry to advance its methods of obtaining biofuel at a cheaper cost, processes need to be reevaluated. The algae turbidostat serves to carry out this purpose.

Future goals for this project consist of further research into data collection and design refinement. Other future goals for this project include manufacturing PCB, switching to BeagleBone Black with Python code, creating a web app control system, and creating an intuitive graphical display that integrates with the control system.

There has been discussion about moving the algae array to the algae growing facility next to Warren College and continuing data collection there. Furthermore, Dr. Vineet Bafna and Nathan Schoepp (CAL-CAB) would like to publish this research in a scientific journal. Another goal in talks for this project would be to start a small-scale company that would sell these turbidostats to labs.

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