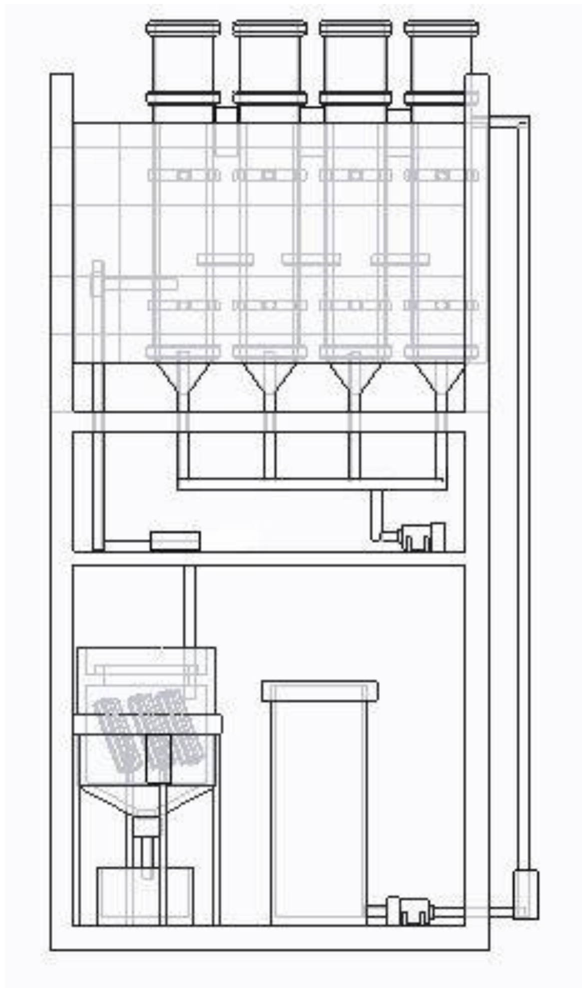
Operations Manual



Design and Development of an Automated Continuous Harvesting System for Microalgae Photobioreactors

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I. Functional Analysis

The automated microalgae harvesting system is a product that can cultivate, separate, and harvest microalgae to produce biomass constantly with little to no human interaction. This system has a set-up designed to be user friendly regardless of the scale in which the system is being used. Operational options include lab, pilot, and industry scale. Due to the fact that this system is automated, the main order of operations for the user involves initial cultivation of the microalgae to be put into the system and proper setup of all pumps, components, and chambers. However, even with an automated system, it is important to monitor system output to ensure that everything is running properly. Monitoring the system is also crucial in order to keep an eye on any potential problems as well as for general maintenance.

1. Relevant Terminology

Airlift/PBR – vertically tubed photobioreactor, a closed and controllable environment for the growth of algae with low contamination risk.

Cultivation – Growth of microalgae within the PBR set up of the system.

PEF – Pulsed electric field, pulsed electric field treatment involves applying a high voltage across the algae medium in order to neutralize the algae cell charge and has applications in cellular lysing.

Lysis – Breaking of a cellular membrane.

Harvesting – Extraction of algae biomass from the PBR system.

Lamella – thin plate (often flat) used to increase the speed of sedimentation.

Microcontroller – Circuit containing a processor, memory, and programmable input/output for the control of systems, electrical, and mechanical components.

2. User and Application Description

This product can be used in a variety of settings including but not limited to: university laboratories, private and public research institutions, as well as potential industry partners and companies.

This product is easily scalable due to a relatively simple and modular design in which the size of chamber and relative pumping power would be the most crucial parameters needing modification during scaling. Additionally, this automated photobioreactor will provide a useful starting point for continued optimization and development of more efficient biomass production facilities.

Biomass is a high value product which also has varied applications as a feedstock, medical supply, dye, and liquid carbon neutral biofuel source. The value of biomass makes this automated system an asset to not only algae production facilities but to agriculture, aquaculture, medical, transportation and energy industries.

3. System Components Overview

The product consists of three main stages: controlled cultivation of the algae within a airlift, algae cell lysis to facilitate biomass separation, and a sedimentation for extraction of products. The use of a photobioreactor decreases the needed area for cultivation and inhibits contamination. The algae cultivation time is primarily governed by its growth rate, which is monitored through optical sensors to determine optimal harvesting time. Once algae have reached harvesting potential, the cultivation medium is treated with a pulsed electric field (PEF). The treatment component consists of two aluminum electrodes separated by a specified distance which are fixed into place using custom designed chambers. The separation unit is a modified lamella separator with a specific geometry. Each system stage is listed below and includes a general inventory of components needed to accomplish the design function with the quantity given in parentheses.

General use

(N/A) Algae and algae growth medium (traditionally water supplemented with nutrients)
(N/A) Variable lengths of 0.25” flexible rubber tubing
(N/A) JB ClearWeld Waterproof Quick Setting Epoxy Cement
(#) Brass tube adapters
(1) Arduino Mega 2560 Microcontroller

Stage 1 – Cultivation: Growth and transfer of algae

(4) Airlift PBR tubes
(1) Air pump for CO_2 distribution
(4) Cellular density sensors (Black Box)
(1) Shelving/ cabinet unit
(3) Solenoid Valves
(3) Peristaltic Pumps
(N/A) Ample UV lights, 2 lamp panels recommended

Stage 2 – PEF Treatment and Separation: Charge neutralization and potential lysis of algae

(8) Delrin based PEF treatment chamber halves
(8) Bruce Profile Electrodes
(1-4) PEF circuits or high voltage power supplies

Stage 3 – Extraction: Removal of produce biomass from system

(1) Modified lamella separator (chamber, lamellas)
(1) Peristaltic pump
(1) Modified black box
(1) Biomass and water collection tanks

II. Product Specifications

The performance of the automated PBR system is governed by the need for the automatic and continuous production of biomass. The system must cultivate and harvest the algae with minimal human interference and maintenance. The collection of the produced biomass must be somewhat continuous and in a manner that will avoid a short circuit to the system i.e overflow, backflow, and algal contamination of recycled medium..

1. Dimensions of Crucial Systems

Our system is approximately 71 inches tall, 40.5 inches wide, and 18.5 inches deep. There are four clear, acrylic airlift chambers at the top of the system that each hold 2 L of water, creating a total cultivation volume of 8 L. The airlift tubes are spaced 4 inches apart, have a length of 30 inches and a diameter of 3 inches. A Hydrofarm Active Aqua air pump is used to infuse the airlift chambers with carbon dioxide through air bubble circulation which passes through diffuser to ensure uniformity. Quarter inch clear flexible hosing is cut between 3 inches and 48 inches to connect the air pump and the bottom of the airlift. These lengths of tubing are somewhat arbitrary, would vary from system to system, and depend on how components are spaced during system installation. Four 22.5 inch lights in two light panels powered by a wall socket are used to provide UV light to the algae. The modified lamella separator has a large volume capacity which will semi-continuously have mixed medium-biomass input and separated biomass and medium output.

2. Technical Specifications

The main components utilized in this design are the microcontroller, pumps, and solenoid valves. Below the components are is listed with their important operational parameters.

Table 1. Selected specifications for Arduino Mega[1].

Arduino Mega 2560 (ATmega2560)	
Operating Voltage	5V
Input Voltage	7-12V
Digital I/O Pins	54 (15 provide PWM)
Flash Memory	256 KB
Clock Speed	16 MHz
Length	101.52 mm
Width	53.3 mm
Weight	37 g

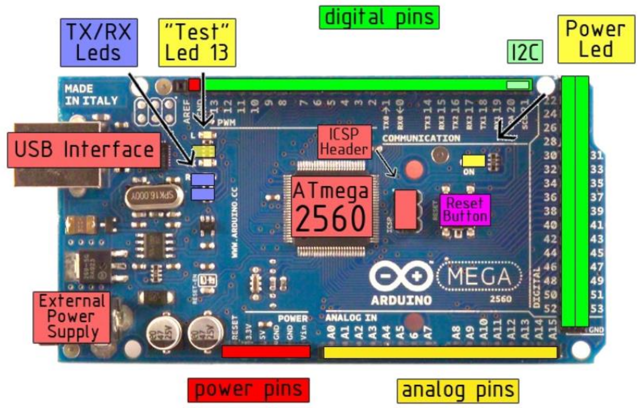

Fig 1. Image of Arduino Mega 2560[2].

Table 2. Selected specifications for air pump[3].

Active Aqua Air Pump	
Air Outlets	4
Power	6 W
Operating Voltage	120 V
Volumetric Flow	240 GPH
Max Amperage	0.08 A
Max Decibels	45 db

Fig 2. Image of Hydrofarm Active Aqua air pump[3].

Table 3. Selected specifications for fluid pump[4].

Adafruit Peristaltic Pump	
Weight	200 grams
Working Temp	0℃ - 40 ℃
Motor voltage	12 V
Motor current:	200-300 mA
Flow rate	< 100 mL/min

Fig 3. Image of Adafruit peristaltic pump[4].

Table 4. Selected specifications solenoid valve.

Adafruit Liquid Solenoid Valve	
Nominal NPS	0.5 in
Working Temp	-5℃ - 80 ℃
Working Pressure	0 Mpa - 1.0 Mpa
Actuating voltage	6 - 12 V
Actuating life	≥ 50 million cycles

Fig 4. Image of Adafruit solenoid valve[4].

If more information is needed for design, installation, or operation full data sheets, diagrams, and specified operating conditions from the manufacturer are available in Appendix A.

III. Product Assembly

Below is a color CAD model of the automated microalgae photobioreactor system. The system is separated into three stages: Cultivation, PEF Treatment, and Harvesting. A general overview of components is given in section 1.3.

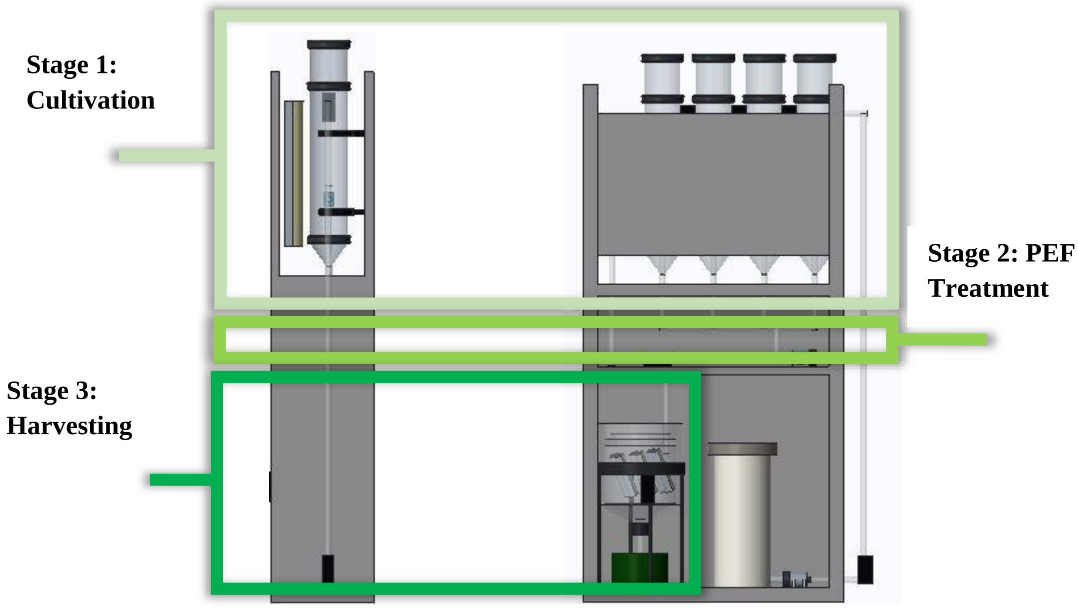

Fig 5. 3D color CAD of airlift system.

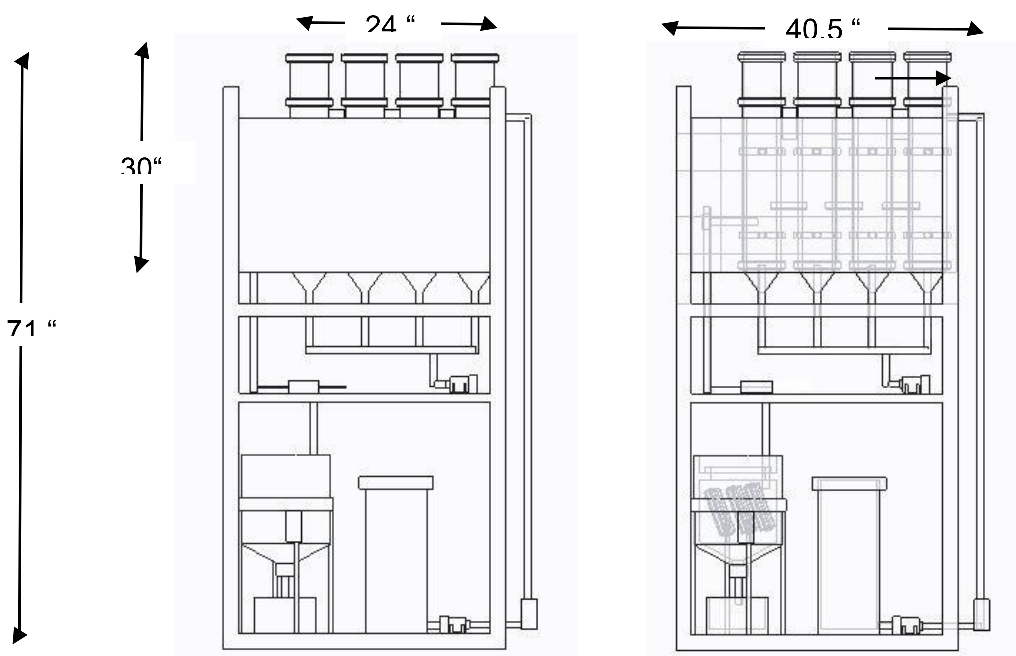

Fig 6. (Left) Wireframe view of airlift assembly, (right) hidden line view of airlift assembly. Both views are presented for installation and size reporting purposes.

1. Stage 1: Cultivation Airlift Assembly

Before using the airlift, make sure all components are in place and secure. The four clear airlift chambers must be secured into their black clips with the open end of the tube facing up. Ensure that the bottom valve is turned to the off position so the mixture inside will not leak out. Connect one end of the clear rubber hosing to the connector at the bottom of the airlift and the other end through the top shelf and onto one of the connections on the air pump. Ensure that the black box density sensor is secured tightly to the back of the airlift panel directly behind the airlift and approximately 3 inches from the top. Secure the pump and solenoid valve to the panel as well making sure all rubber tube connections are secure and all valves between the pumps are open. Repeat this process for each of the airlift chambers. Hang the 2 light panels vertically, facing the airlift chambers, approximately 10.75 inches from the front of the airlift chamber. Make sure all airlift tubes receive an appropriate amount of UV light. The CAD for the cultivation stage of this produce can be seen on the next page.

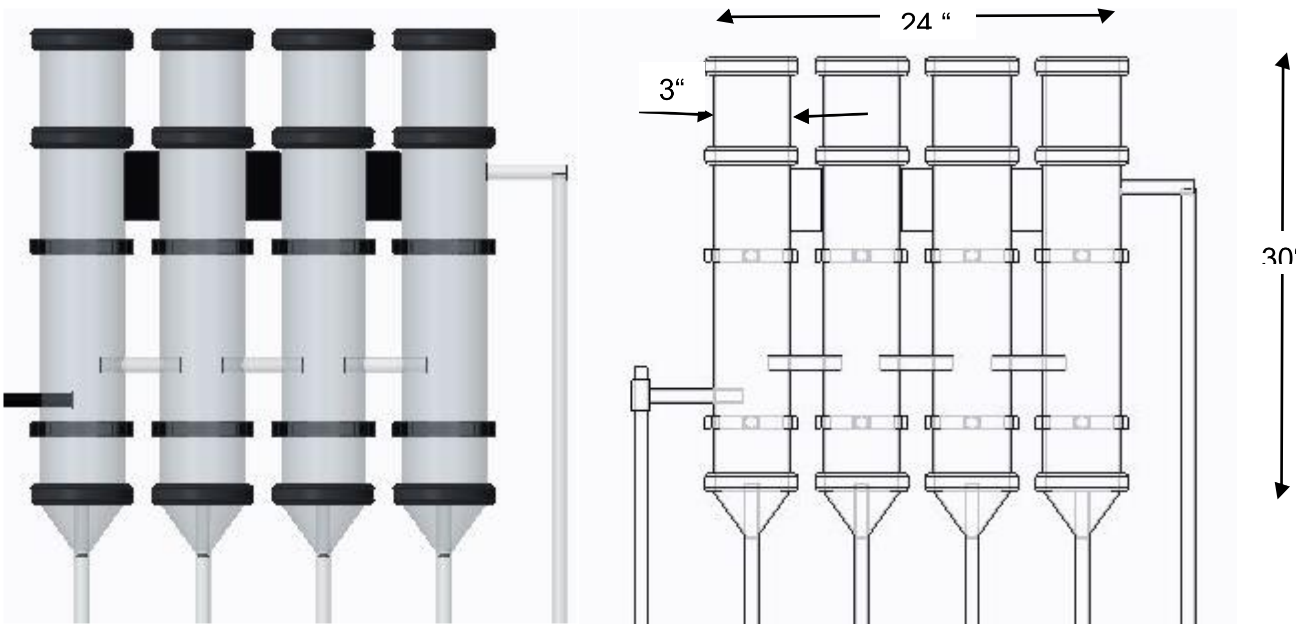

Fig 7. (Left) Color view of airlift tubes assembly, (right) hidden line view of airlift tubes assembly.

2. Stage 2: PEF Lysis Treatment Assembly

In order to install the PEF treatment chambers, the user will need two half chambers, two Bruce electrodes, two segments of 0.25 inch flexible tubing, two screws, and a flexible sealant. Gently screw the electrodes into the threaded chamber, proceed with caution so that the electrodes do not get scratched or dented as this can affect the uniformity of the electric field generated within the chamber. These chamber halves can then be sealed using any waterproof sealant of choice. A watertight but nonpermanent sealant is recommended. Insert each segment of flexible tubing into a chamber hole, ensure the tubing is fully connected and sealed with a waterproof epoxy. The CAD for this component can be seen below.

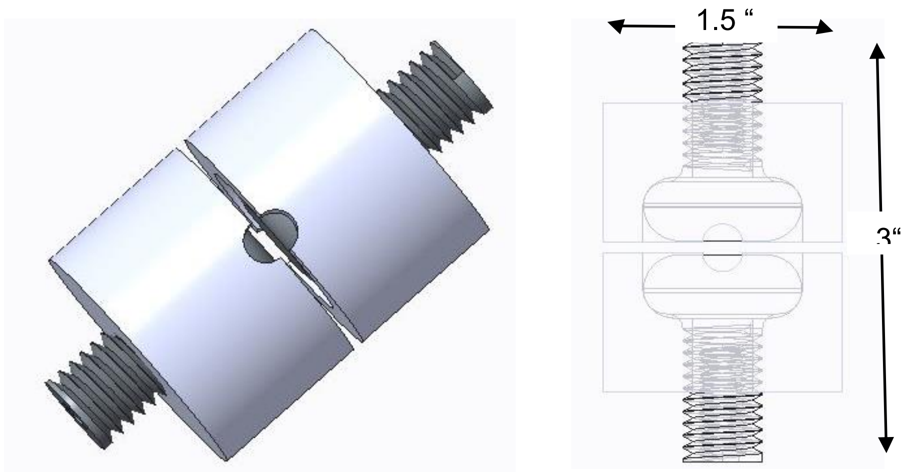

Fig 8. (Left) Color view of PEF assembly, (right) hidden line view of PEF assembly.

3. Stage 3: Lamella Separator Assembly

The separator stage, used to harvest the produced biomass, is constructed of a large separator tank, three 45° corrugated and angled lamella plates. These lamellas are fixed to the walls of the separator chamber using water proof epoxy. Flexible tubing is used to route the PEF treated biomass and medium mixture into the lamella separator. A peristaltic pump is installed with a cell density box here as well to extract the biomass when it has sedimented sufficiently and is ready for extraction. Flexible tubing can also be connected to the top port, pictured above the clarifier lip, to extract clarified water for potential recycling. The CAD for this component system is feature below.

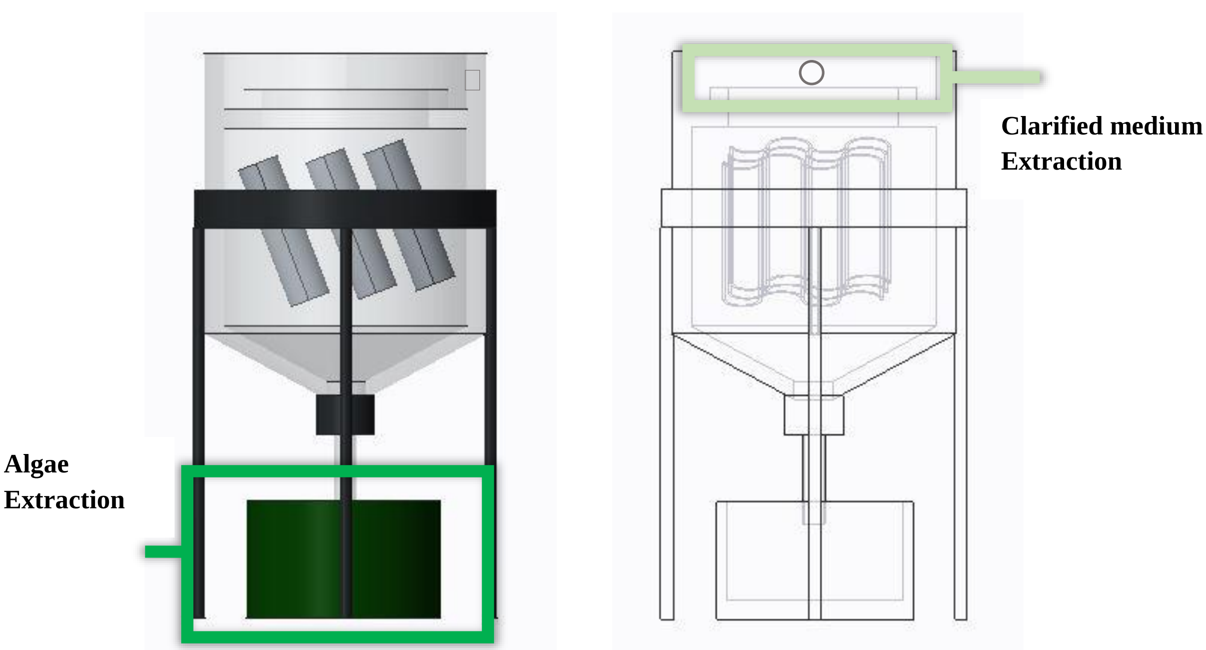

Fig 9. (Left) Color view of harvesting assembly, (right) hidden line view of harvesting assembly.

IV. Operation Instructions

1. Preparation of Algae and Airlift Operation

Sterilization of Instruments and Growing Chambers

Microalgae is sensitive to foreign bacteria and other substances so it is vital that the user handles the algae with sterile instruments in order to minimize contamination. All metal instruments used to transfer the algae and all glass containers that will hold algae at any stage of cultivation must be sterilized before use. To do this, the glass containers are filled with at least 75% distilled water brought to a rolling boil before the heat is turned off. At this point, if possible, submerge any instruments that will be handling algae into the water while it is still boiling and leave in for at least ten seconds. Remove the sterile instrument from the water using protective gloves. When the instrument is removed make sure it is placed on a sterile surface as it cools to avoid needing re-sterilization. Use protective eyewear and gloves when handling the hot containers.

Preparation of Nutrient Medium

It is highly recommended that instead of growing the algae in pure distilled water, a diluted medium containing the algae's essential minerals and trace elements required for algae growth is prepared.

Begin by measuring out 990 mL of distilled water into a sterilized container. Add 10 mL of concentrated medium (modified CHU for example) to the sterilized water and add 1 mL of baking soda to the batch. Stir with a sterilized instrument until all ingredients have dissolved and mixed thoroughly. This diluted mixture needs to be kept at a refrigerator temperature. This mixture can be scaled up or down to fit any volumetric need as long as the ratios of ingredients are kept the same.

Transferring between Containers

The first stage of growth will occur in the shipping test tube. Break up the agar in the test tube with a sharp and sterile instrument. Add the diluted medium mixture until test tube is 80% full. Once the microalgae in the test tube is ready to transfer, gently pour the entire algae and nutrient mixture into a sterile, glass, 250 mL Erlenmeyer flask. Add the diluted nutrient mixture until the entire fluid volume reaches 200 mL. Make sure the diluted nutrient mixture is at room temperature before adding it to the microalgae. Add the air supply to the stopper in the top of the flask and secure stopper on top of the flask creating a tight seal. Place a 1/4" glass tube (cut to 8" for the 250 mL flask and 18" for the 2000 mL flask) through a hole drilled through the stopper. Make sure the glass tube is submerged in the algae mixture and is approximately ½" from the bottom of the glass container. Connect a ¼" rubber tube (cut to necessary length) to the open end of the glass tube. Connect the other end of the rubber hose to the air pump. Turn the air pump on until there is a steady and continuous flow of bubbles rising through the mixture. This process continues to 2000 mL when it is then appropriate to transfer to the airlift cultivation stage. Ensure the bottom valves on the airlift are closed and that the entire chamber has been sterilized. Gently pour the entire algae mixture into the first airlift chamber and make sure air pump is turned on and running through the bottom of the airlift chamber.

All further cultivation transfers will be made by the system. It is important to transfer the algae between containers when it has reached its maximum growth level. This is determined by viewing a small sample of the algae mixture under a microscope and making sure the cell count is approximately what it is on the growth curve.

Growing Conditions

For each stage of the cultivation, the light, temperature, and air supply conditions are mostly the same. The microalgae requires 12 hours of UV light on and then 12 hours of no light. If using an artificial light source make sure the light is between 12 and 48 inches from the container to help prevent photo-inhibition. The surroundings should be room temperature and must not fall below 15.5°C. Make sure to refill all containers with room temperature, diluted nutrient mixture to the appropriate amount if the mixture begins to evaporate. The microcontroller will function with density light boxes to automatically transfer mass between tubes.

Additional Notes on Airlift Operation

Because the system is designed to be automated, there is not much human interaction after the first batch of algae is cultivated and placed into the first airlift tube. The lights hanging in front of the airlift chambers need to be cycled on and off every 12 hours, or set on an electronic timer. Keep the air-pump running through the bottom of the chambers on constantly. Ensure that all sensors are running properly and that once the algae has reached it's maximum growth count, it is being distributed into the next airlift chamber.

2. PEF Treatment

The PEF treatment uses high voltage and low current to neutralize the cell potential and cause irreversible cellular poration which allows for algae to not repel and therefore expedites sedimentation without chemicals. Screws are inserted into threaded area on inside of electrode stem and alligator clips can be used to connect the PEF apparatus to a high voltage source or circuit. Be sure to use proper personal protective equipment (i.e. high voltage rated gloves) and, prior to power connection, insulate the remaining exposed stem of the electrode.

3. Lamella Separation Stage

The lamella separator works with a peristaltic pump and black box sensor that uses a LED-phototransistor pair to return a digital voltage. The microcontroller is programmed to collect data and monitor this density to determine when to draw (when digital voltage corresponding to appropriate density is reached) and when to stop drawing (biomass does not register high enough digital voltage (density) for acceptable extraction). Flexible tubing can be attached to upper water collection port and can allow for gravity collection, i.e. any water above or at port level will draw, or user can install additional black box to ensure only pure water is collected from the separator.

V. Troubleshooting

Although this is an automated system there are a handle of problems which may arise during operation including algae death, fatigue leaking, process disruptions, and poor lysing results.

The initial sample of microalgae arrives in a test tube and is very sensitive to many things. It is essential to follow cultivation procedures carefully and to handle the algae with care when it is still in the initial stages of growth. However, even with proper care there is still the chance that the algae will begin to die. It is important to keep a close eye on if the algae cell count is following the growth curve to see if the algae ever begins to become stressed and begin dying. If it is found that the algae is dying, it is important to locate the source of the problem and try to salvage all remaining algae cells. After the mixture has reached a certain point all algae cells will be dead and it will be impossible to restart the growth process.

Because of the water pressure constantly sitting in the airlift chamber, this constant strain may cause potential leaks and seal breaks in the airlift. Ensure that all threads on connections have been taped to ensure a water tight seal. If leaking begins to occur, stop the system, empty the contents of the chamber into a sterile container, and seal the open leak with a strong epoxy. Allow the epoxy to harden for at least one hour before refilling the chamber with the mixture.

There are many sensors involved in this system that have the potential to have a problem. If this should occur at any time while the system is running, you must first turn off the system so that it does not keep running with a faulty sensor. Carefully disconnect any tubing running to the sensor or valve and secure the open connection with a plug. Visually inspect the sensor to see if you can identify any problems. If no problems are found after inspection, troubleshoot the sensor using a computer and the Arduino board to see if the problem can be identified and fixed. If troubleshooting is unable to solve the problem, it may be required to purchase a new sensor.

In the event that the lysing stage of the system is producing insufficient results, it is important to inspect all components for faults. Poor lysing outputs may be the result of either faulty electrodes or a problem in the voltage timing chip. Inspect these parts before resuming the lysing process.

VI. Regular Maintenance

To keep the system running smoothly and properly, it is important that the components are kept clean and components and sensors are monitored and replace when necessary. To ensure the algae does not become contaminated with mold or debris during the cultivation process, it is important to inspect for mold on a weekly basis. The individual airlift chambers and connecting hosing must be cleaned monthly or bi monthly depending on how large the system is scaled to. Be sure to check the air pump on a monthly basis to see if it has collected any hair and debris or if any water has back-flowed into the system. The electrodes in the lysing stage must be replace every 6 months as they will degrade from cavitation and become less efficient over time. With each of these maintenance repairs, make sure the system is turned off and that either all disconnected pipes are sealed, or that the algae is transferred into a sterile holding container while repairs are being done.

VII. References

[1] "Arduino - ArduinoBoardMega2560." Arduino - ArduinoBoardMega2560. Web. 31 Mar. 2016.

[2] "Arduino - ArduinoBoardMega2560." *Arduino - ArduinoBoardMega2560*. Web. 31 Mar. 2016.

[3] "Featured Brand." *Hydrofarm — Active Aqua Air Pump, 4 Outlets, 6W, 15 L/min*. Web. 01 Apr. 2016.

[4] *Adafruit Industries Blog RSS*. Web. 01 Apr. 2016.

VIII. Acknowledgements

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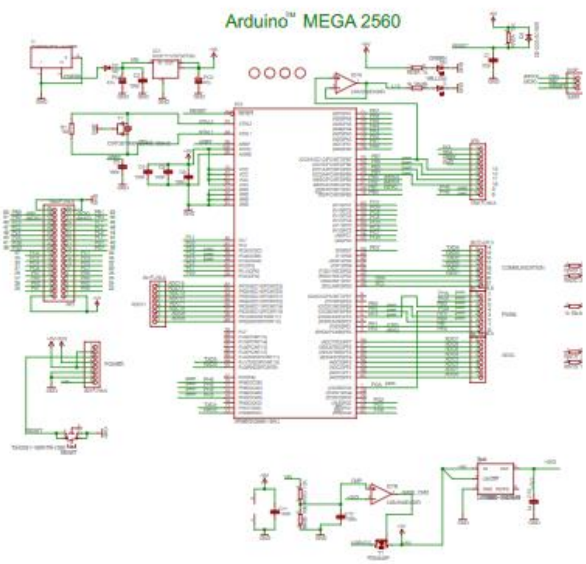
XI. Appendices

1. Appendix A



Microcontroller ATmega2560	
Operating Voltage	5V
Input Voltage	7-12V
Input Voltage (limit)	6-20V
Digital I/O Pins	54 (15 PWM output)
Analog Input Pins	16
DC Current per I/O Pin	20 mA
DC Current for 3.3V Pin	50 mA
Flash Memory	256 KB
SRAM	8 KB
EEPROM	4 KB
Clock Speed	16 MHz
Length	101.52 mm
Width	53.3 mm
Weight	37 g

Hydrofarm Active Aqua Air Pump	
Package Dimensions	6.0L x 4.1W x 9.4H
Output Ports	4
Warranty	1 year
Rated LPM	15LPM, 240 GPH
Voltage	120 V
Rated Amperage	0.08 A
Max. Decibels	45 db

Adafruit Peristaltic Pump	
Dimensions	27mm diameter, 72mm length
Working Temp	0℃ - 40 ℃
Flow Rate	<100 mL/min
Voltage	12 V
Rated Amperage	200 – 300 mA
Mounting Holes	2.7mm diameter, 50mm distance

Adafruit Fluid Solenoid Valve	
Dimensions	105mm tall x 55mm wide x 65mm long
Working Temperature	-5℃ - 80 ℃
Working Pressure	0 Mpa - 1.0 Mpa (140 PSI)
Actuating voltage	6 - 12 V
Actuating life	≥ 50 million cycles
Weight	1.7lbs

Adafruit Fluid Solenoid Valve	
Voltage	**Current**
6V	1.6 A
7V	1.86 A
8V	2.0 A
9V	2.3 A
10V	2.5 A
11V	2.7 A
12V	3 A

2. Appendix B – Spare Parts

List of spare
parts:
Electrodes
1/4" OD tubing
Brass connectors
RGB LED's
Resistors
Solenoid Valve
Epoxy
BJT's
Diodes
Spare wiring