Temporary Immersion Bioreactor for Plant Propagation

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Executive Summary

This critical design review details a Temporary Immersion Bioreactor (TIB) intended to aid the propagation of seedlings during their initial stages of maturation. This project is being done in collaboration with Dr. Alireza Sarvestani and the Technological University of Matamoros (UTIM) in Mexico. As it currently stands, students in the agriculture department at UTIM do not have access to a temporary immersion bioreactor which is critical for plant research. With research heavily focused on propagating agave seedlings, UTIM students would greatly benefit from having a TIB in their lab to further their research.

The research team designing the Temporary Immersion Bioreactor comprises dedicated engineers with diverse expertise studying at Mercer University. Kendra Santillan Hernandez, a biomedical engineering student and team leader, is experienced in 3D printing and growing cells in bioreactors, ensuring cohesive project development. Carlie Ezell, an environmental engineering student, contributes experience working in hydrological research and hydraulics. Quyen Ong, a computer engineering student, brings an extensive background in robotics and web development.

The proposed solution involves the propagation of young plants, specifically cacti, in media under ideal conditions for the first stages of growth, thereby protecting propagated cacti in the critical stages of their maturation. This project should produce a small enclosed and automated container to feed and grow propagated plants while recording real-time measurements of the CO_2 and temperature of the bioreactor. One Temporary Immersion Bioreactor is estimated to cost under $300 and is designed to be 239.0 x 141.4 x 141.4 mm so that many can be placed in one lab room.

1. Introduction

The Mercer on Mission (MOM) trip to Mexico aims to address the lack of resources in the public education system as well as support degrees in agriculture at the Technological University of Matamoros Izúcar (UTIM). The agriculture department of UTIM does not currently have access to a Temporary Immersion Bioreactor (TIB), greatly limiting innovative research on native plants. Our client, Dr. Alireza Sarvestani, in association with UTIM, reached out to our team to develop a temporary immersion bioreactor for student research. The specifications provided for this project state that the bioreactor chamber should be 3D printed, the device should have a pump to fill the chamber with media, and an electronic circuit that will switch the pump on and off. It was also requested that the pump be remote-controlled and that the bioreactor be no larger than 239.0 x 141.4 x 141.4mm. The 3D printable chamber should be fitted with sensors to read several environmental factors including temperature, carbon dioxide levels, and light exposure. An ESP32 will be used to control the pump filling the chamber with growth media for the plants as well as gather the environmental readings from the sensors. Previous efforts to design a TIB for this use included a team of Mercer students designing a body for the TIB. Despite having several designs for the body of the device, they were never tested or printed. Ultimately, this is a low-cost, fully automated, and easily reproducible TIB with the hope that students at UTIM will be able to easily assemble and even expand on this design independently in the future. Different materials, pump systems, temperature sensors, and CO_2 sensors will be compared to create a suitable TIB for UTIM.

2. PDR Summary

This section includes a brief description of the initial design concept, potential quality tests and the criteria to be used in prototype evaluation.

2.1 Project Goal and Specifications

The goal of this project is to create a cost-efficient TIB for researchers at UTIM. The device will be 3D printed with a material that is autoclavable, translucent, and cost-effective. There will be a website where researchers can remotely monitor the temperature and CO_2 level of the TIB as well as turn a pump on and off to cycle media to the plant. The pump will be selected for optimal plant growth.

2.2 Roles and Responsibilities

Carlie Ezell is an environmental engineering student who is experienced in hydrological research and pipe hydraulics. She is responsible for ensuring the pump flow rate and drainage rate are functional and appropriate for cultivating a plant. Kendra Santillan Hernandez is a biomedical engineering student with experience in Fusion 360 and a good understanding of the use of bioreactors in a lab setting. She is responsible for printing the components in the resin printer and testing the material chosen. Quyen Ong is a computer engineering student with experience working with Arduino devices and components. She is responsible for the electrical components and web development.

2.3 PDR Design

The design will feature a temperature sensor (BMP180) and a CO2 sensor (MG811) inside the same compartment as the plant for a more accurate reading. There will also be a K Kaomer pump to pump media to the upper compartment. The Arduino controlling these electrical components will be located on the outside of the TIB so that it can be easily connected to a computer. The

Arduino will be connected to the sensors and the pump through holes in the TIB. The holes will also be for releasing air pressure. The final design will also have a body made out of Durable resin, which is 3D printable and autoclavable, as requested by the client. By selecting a 3D printable material that can also be autoclaved, we will be creating a design that is easily accessible and can easily be sterilized between uses. The dimensions of a fully assembled bioreactor are 239.0mm height x 141.4mm width x 141.4mm length. The design can be seen in Figure 1.

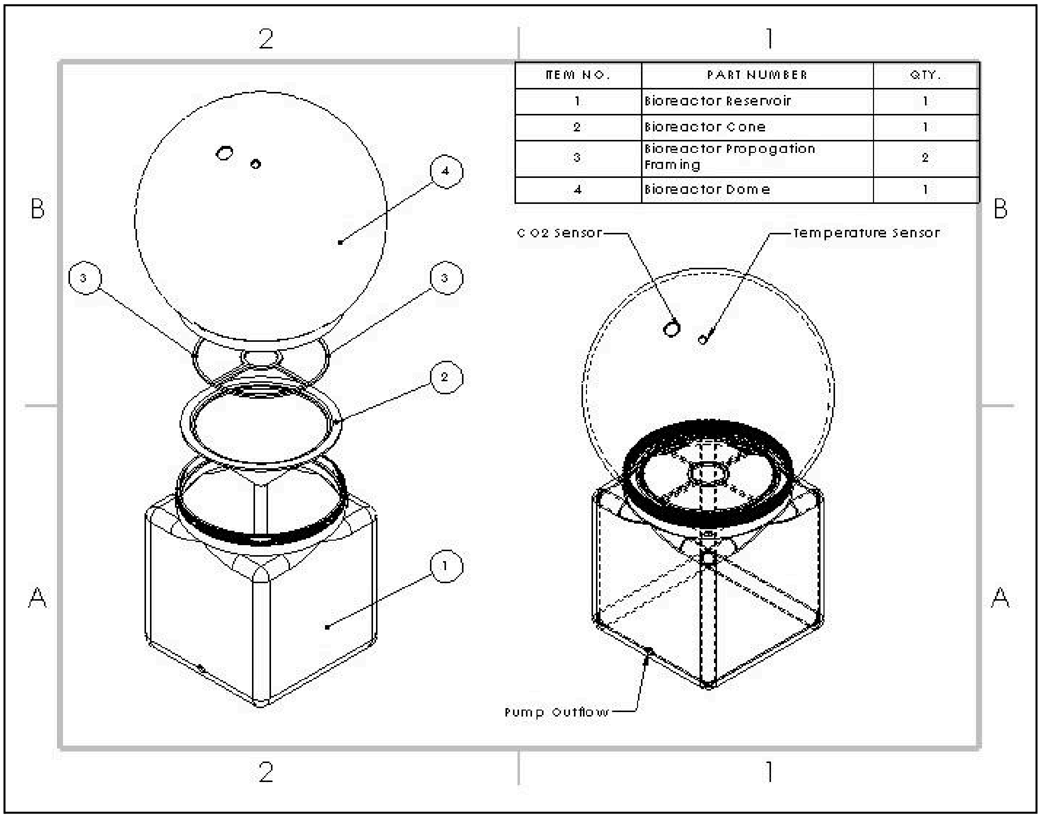

Figure 1: Bioreactor 3D Model Using SolidWorks2023

As for the website, all text will be left justified. There will be four sections separated by horizontal lines: the title, pump, temperature, and CO_2. The title will be “Temporary Immersion Bioreactor” in bold. The pump section will only consist of a green outlined button that says “Pump On”, which will turn the pump on. The temperature and CO_2 level will contain “Temperature:” and “CO_2”, respectively, with the measurements under the words. The website design can be seen in Figure 2.

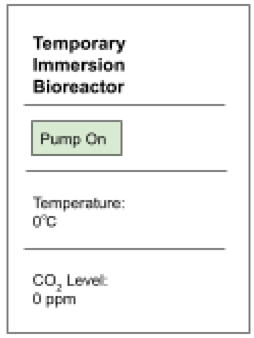

Figure 2: PDR Website Design

3. Work Accomplished

3.1 Design Revisions

The propagation framing lip within the drain cone was deemed to be unnecessary since the frame was adequately supported without the inner lip on the cone. The removal of the inner lip also lessened the amount of resin needed to print this component which was a priority for this project. Additionally, the 3D model was resized to 70% of its original design scale to facilitate printing on a Form 3B printer by Formlabs. Scaling the model down allowed for sufficient space to orient the individual components on the 3D printing platform in such a way that resin use and support placement would be minimized for each print. Scaling the overall size of the bioreactor down did change the interval for which the pump would remain on as well as the cone volume and drainage rates. Updated calculations for the inflow rates and drain hole radii can be found in Appendix C and are explained in Section 3.2.1.2.

The temperature sensor chosen in the PDR, the BMP180, was not used, rather DS18B20 was used. This was because the website we initially intended to use to purchase the BMP180 component required us to buy at least 49 components. Also, the DS18B20 is a water temperature sensor, so if the liquid in the bioreactor were to submerge the temperature sensor, there would be no issue.

Originally, an Arduino R4 Wifi was meant to be used as the microcontroller. However, the Arduino Wifi purchased only worked once. The code that was originally working was re-uploaded multiple times afterward over the span of 3 days, without success. Other people on forums have had the same issue, eventually switching to a different board (Dccontrarian, 2023). Due to this issue, it was decided that an ESP32 was used instead. Although an ESP32 is a bit more difficult to use, it is cheaper and more reliable than an Arduino R4 Wifi.

A relay was meant to be used to control the pump because it was initially intended to simply be turned on and off. However, the relay was replaced with a motor driver, specifically the L293D, to allow the speed to be controlled.

The only change made to the website is to the pump section. The new section has the current state of the pump stated before the button. The new button only has the desired state of the pump, without the word “Pump” before the desired state.

3.2 Testing and Engineering Analysis

The following analysis was done on the bioreactor. This includes three sets of tests: material evaluation, drainage cycling system evaluation, and sensor testing.

3.2.1 Testing and Results

The following tests were conducted to confirm the choices made previously based on feasibility and merit criteria for the final design.

3.2.1.1 Material Evaluation

An important feature of material selection is to ensure that the final product is sterilizable to avoid contamination between uses or unwanted mold growth. According to the Centers for Disease Control and Prevention (CDC) steam sterilization by autoclave is the "most widely used and most dependable"(CDC, 2016) method for sterilization, which is why the materials were autoclave tested using a 30-minute gravity cycle. Gravity cycles are typically at least 30 minutes long and reach a temperature of 121℃ (Steam Sterilization Cycles, 2018), meaning that the materials would have to withstand this high temperature for the entirety of the gravity cycle while also maintaining their integrity. While it was previously confirmed that Durable resin by Formlabs successfully endured a 30-minute gravity cycle, it was important to confirm that the same would be true with much bigger components that were now printed in specific shapes designed to fit together. The bioreactor body, made up of the dome and reservoir, were the largest pieces of the device and would need to maintain their shape perfectly in order to fit together well even after autoclaving. The dome and reservoir were autoclave tested by running a 30-30 gravity cycle and evaluating their shape once completed. If the materials melted or were visibly deformed, it was determined that they were not autoclavable. Table 1 below shows the results of autoclave testing.

Table 1: Autoclave Results

Material	Reservoir	Dome
Autoclavable	Yes	Yes

After autoclaving the samples on a 30-minute gravity cycle, it is clear that Durable resin can not only withstand the autoclave gravity cycle, but the printed parts also maintain their shape and still fit together post-autoclaving. This also indicates that the post-processing method used during printing is appropriate for the demands of this project.

Previously the translucency of Durable resin was evaluated using a 2mm thick dogbone to determine if 10,000 Lux of light could indeed permeate through the material. This is a particularly important element to consider because according to the University of Melbourne, plants need between 807 - 1614 lux of light to allow for proper photosynthesis with a recommended minimum of 10,000 lux. The final bioreactor design includes a dome-shaped top component that was designed to better focus the light on the seedling it would be housing while maintaining the 2mm thickness that was previously tested. To evaluate the translucency of the dome, the Vernier Light Meter along with the Logger Pro program were used to determine the lux through the dome itself. The light testing was conducted in a lab under a grow light outside in order to simulate the setting in which this bioreactor would be used. The Vernier Light Meter was placed with the photocell pointed upwards so that the dome could be placed over it during test trials. Readings were taken with the light sensor fully exposed as well as covered by the dome. In order to test the calibration of the light meter a reading was also taken with the photocell fully covered to confirm that it would read 0 lux when exposed to no light. The results collected using the light meter are displayed in Table 2. The complete translucency testing process and additional results can be found in Table 18 in Appendix A.

Table 2: Dome Light Testing

Trial	Before (Lux)	After (Lux)	Change (Lux)
1	1562	1064	498
2	1513	1064	449
3	1580	1085	495

Taking the light readings in a lab setting under a grow light allowed for more consistent readings. The change in lux when the light sensor was fully covered by the material was on average 480.67 Lux indicating that the material does not heavily impede light. There was also light testing conducted outside to simulate a plant's natural growing environment. The results for light testing outside can be found in Appendix A. When using the grow light in the lab the recommended absolute minimum of 807 Lux was successfully met and exceeded in each trial. The light testing conducted outside indicated that the recommended minimum of 10,000 Lux of light was also met and exceeded during each trial of light testing. These results indicate that this material is appropriate for encouraging plant growth in both a lab setting and an outdoor setting. This also speaks to the versatility of our final material selection.

While translucency and autoclavability are two of the most important material properties for the bioreactor, it is also important to consider the material strength and reaction to liquids. The material strength is important because this design will need to be transported to Mexico and assembled upon arrival, a very brittle or weak material would likely suffer damage before arrival at the destination. The material's reaction to liquids is also very important since the lower chamber will be holding plant growth media and we need to ensure that the material does not significantly swell.

To evaluate the liquid exposure and the material's reaction to liquid, the salt water category was observed from the datasheet provided by Formlabs. The saltwater category was selected because the media that will be used in the TIB will be in liquid form and both "solid and liquid media [for plants] are generally composed of inorganic salts" (Ozier-Lafontaine & Jannoyer, 2014). Table 3 below shows the material properties of Durable resin according to the Formlab material datasheets.

Table 3: Other Material Properties

Material	Impact Strength (J/m)	24 hr weight gain % (for saltwater)
Durable	114	< 1

The Durable datasheet indicates that due to its low weight gain when exposed to saltwater, this is an appropriate material selection for the TIB.

3.2.1.2 Pump and Drainage Cycling System Evaluation

Four tests were conducted to either comprehensively test the bioreactor's ability to sustain life or to determine the adjusted settings at which the system must be operated given the design revisions. These are:

1. KHPP260 Pump Function Pretest
2. Function Check Calculations
3. Submersion Time and Physical Testing
4. Further System Calculations to Prevent Stalling
5. Plant Life Growth Test

The fifth test was not accomplished within the time frame for this project but is recommended for future analyses and is thus outlined here.

3.2.1.2.1 KHPP260 Pump Function Pretest

The goal of this pretest is to check that three of the same K Kamoer KHPP260 pumps can perform at a range of flow velocity settings and give similar values. Trials were repeated at different pump speed settings. Data for the volume of water displaced over time were used to calculate the flow rate at each setting interval. This data is available in Table 11 located in Appendix E.

Anytime a zero was recorded for flow rate data, this is an instance where the pump stalled out completely. It was noted during experimentation that jumping 20 or more mL/min between velocity settings causes the pump to stall. If the flow velocity is being adjusted, it is encouraged to move at increments of 10 mL/min or so, moving up or down from a starter middle velocity such as 180 mL/min. Also, the pump will always stall at flow velocities less than 120 mL/min when turned on from rest. This is assumed to be because the pump's peristaltic mechanics cannot use such low flows to overcome the static friction of the movement of the gears within the pump.

Though the pump's flow operation range is listed as functional up to 220 mL/min, the pump can function well even up to 400 mL/min. Very high velocities are not encouraged for long-term use, however, since this will likely cause early wear on the pump mechanics and seriously decrease the pump's life. Though this was necessary to mention, it is not a concern for this project since the pump will be operated at comfortable velocities.

If the drain system design does not allow for moderate pump flow settings, it must be redesigned. Therefore, the tests in this section will attempt to sort through 1) pump function, 2) theoretical appropriate values, 3) true operating values, and 4) design compensations to tune system features for streamlined function.

3.2.1.2.2 Function Check Calculations

This section entails the calculations to predict the inflow desired from the pump. First, we will take the Energy Equation, given in Equation 1, to derive the equations we will need. This equation is a more precise form of Bernoulli's Equation since it also accounts for head loss factors. It is provided in Appendix C.

The goal is to solve for the ideal inflow. The ideal velocity must be calculated first, and then multiplied by area to find the inflow using Equation 6. The formula for frictional losses is provided as Equation 3 in Appendix C.

Before solving for flow velocity using Equation 5, one must find the internal friction factor "f" of the tubing. The inner tubing diameter is 4 mm and has an internal roughness of 0.0015 based on the material (Houghtalen, 2017). With these one can find the relative roughness using Equation 7 in Appendix C and then find the friction factor using the Moody diagram in Figure 12 located in Appendix D. Equation 7 and Calculation 1 for solving for the friction factor are located in Appendix C, and the Moody diagram with relevant tracings is Figure 13 located in Appendix D. Assuming laminar flow, this gives an approximate friction factor of 0.0158.

Due to Equation 6, to find the flow rate one can use the area of the drain hole in the cone and the inflow velocity to calculate the ideal inflow rate. The inflow velocity can be used in this case because ideally, at the desired height used in the same calculations, the inflow and the outflow will equilibrate. The equation for the drain hole area is the same as the area of a circle, which is given in Equation 7. Equation 8 is the final equation for predicting the inflow flow rate at a certain desired height. These equations and calculations are located in Appendix C.

According to Calculation 2 in Appendix C, by using a drain hole with a radius of 1 mm the ideal inflow rate from the pump is 97.8 mL per minute.

3.2.1.2.3 Submersion Time and Physical Testing

The goal of this test is to determine the ideal flow setting for the pump, now that the target flow has been calculated. The volume of the cone is calculated in Calculation 3 using Equation 9. For design dimensions, refer to Appendix E.

Equation 8 can be rearranged as Equation 9 to calculate the waterline height at an inflow rate, so fill lines have been calculated for a range of flow rates. These are given in Table 12 located in Appendix E. An example calculation for these fill waterline heights is given in Calculation 4 located in Appendix C.

The most appropriate fill height and flow rate is very close to the value of 97.8 mL/min predicted in Calculation 2. The associated code setting of the ESP32 is "94".

The ideal submersion time for a plant is 5-10 minutes (Singh, 2021) so this time value is acceptable. Since this bioreactor is to be used for dry climate plants, a goal submersion time of 5-6 minutes was chosen. For inflows below 84 mL/min, the cone would reach an equilibrium at an insufficient fill point. For inflows above 108 mL/min, the cone would overflow and not provide sufficient submersion time.

At a pump setting of "94" the waterline approached overflow, so a setting of "92" was used. With the pump turned on for 5 minutes, the plant would be submerged for about 5.3 minutes which is within the ideal range. This submersion time is taken from the moment the waterline overtakes the propagation framing step until the moment the waterline drains below the step again.

Since the ideal TIB interval is 3-6 hours after each submersion (Singh, 2021), an interval of 6 hours was chosen due to the desert climate of the agave that this project is designed for. An interval of 6 hours also means that the pump will turn on at the same time each day. The total daily usage of the pump in this case will be approximately 32 minutes, spread out in 5.3-minute intervals throughout a day.

3.2.1.2.4 Further System Calculations to Prevent Stalling

Unfortunately, the KHPP260 pump cannot be turned on at any rate below a setting of 130 from rest without stalling completely. Though it will function if the setting is slowly decreased in increments to the desired rate, this is not a feasible option for the bioreactor's intended use. Thus, the pump must either be completely replaced with one that can accommodate lower flow rates without stalling, or the radius of the cone must be changed and the associated values recalculated so that the pump can be allowed to function at a higher rate.

The most reliable and comfortable place to operate a pump is somewhere in the middle of its operating range. With higher inflow, the drain radius must be larger. Rearranging Equation 8 as Equation 10 allows us to calculate the appropriate radius for a drain operating at a setting of "150", or a flow rate of 250 mL/min. Calculation 6 in Appendix C gives this calculation for a larger radius.

The new radius required to balance an inflow of 250 mL/min is 1.60 mm. With this value, the waterline fill heights were calculated again using Equation 10. Since no more resin was available to print a new cone with this drain radius, the values in Table 13 are theoretical. The new waterline heights at a range of theoretical flow rates is summarized in Table 13 located in Appendix E.

This predicts that the waterline height should still approach 30 mm with this new radius of 1.60 mm.

3.2.1.2.5 Plant Life Growth Test

The last test that was intended for the drain system of the bioreactor is a growth test. Unfortunately, due to the timeline for this project, we were unable to perform this test. The plan for this test was to use a similar plant to propagate and measure leaf area per day as the bioreactor ran as it was intended to. Leaf area would be measured once every two days for two to three weeks using the "Leafscan" application. This data could be compiled into a trend graph along with images of the growing plant. Figure 3 is the logo for the intended app for testing.

Figure 3: Leafscan App Logo

<u>3.2.1.3 Temperature Sensor Testing</u>

The three temperature sensors bought were tested in room temperature, hot, and cold water. The measurements were compared to a Taylor folding food thermometer with an accuracy of ±2℉

(*Taylor*, n.d.). Using the equation $℃ = \frac{℉-32}{1.8}$, ±2℉ is ±16.67℃. As for the DS18B20 sensor, it has an accuracy of ±0.5℃ (*DS18B20*, n.d.).

For the first sensor, the measurements for room temperature, hot, and cold water were 28.37℃, 73.44℃, and 2.44℃, respectively. The thermometer measurements were 27.8℃, 73.1℃, and 2.0℃, respectively. So, the difference between the sensor and thermometer measurements in ℃ are 0.57℃, 0.34℃, and 0.44℃, respectively.

For the second sensor, the measurements for room temperature, hot, and cold water were 23.06℃, 84.31℃, and 1.87℃, respectively. The thermometer measurements were 23.2℃, 84.0℃, and 0.8℃, respectively. So, the difference in ℃ are -0.14℃, 0.31℃, and 1.07℃, respectively.

For the third sensor, the measurements for room temperature, hot, and cold water were 24.19℃, 70.00℃, and 1.75℃, respectively. The thermometer measurements were 24.8℃, 70.6℃, and 1.4℃, respectively. So, the difference in ℃ are -0.61℃, -0.60℃, and 0.35℃, respectively.

The results of the tests can be seen in Table 4.

Table 4: Temperature Sensor Test Results

Water Temperature	Room Temperature	Hot	Cold
Sensor 1 (℃)	28.37	73.44	2.44
Thermometer (℃)	27.8	73.1	2.0
Difference (℃)	0.57	0.34	0.44
Sensor 2 (℃)	23.06	84.31	1.87
Thermometer (℃)	23.2	84.0	0.8
Difference (℃)	-0.14	0.31	1.07
Sensor 3 (℃)	24.19	70.00	1.75
Thermometer (℃)	24.8	70.6	1.4
Difference (℃)	-0.61	-0.60	0.35

The largest |℃| difference is 1.07℃ from sensor 2 in cold water. A 1.07℃ difference is within the accuracy of the Taylor thermometer of ±16.67℃, meaning the temperature sensor is working properly.

3.2.1.4 CO_2 Sensor Testing

For the CO_2 sensor testing, three holes were drilled into a bottle. Two holes were made to fit the CO_2 sensor and the tube connecting to a CO_2 tank. One small hole was made to easily be covered with a finger to seal or release the air inside the bottle. The setup can be seen in Figure 16 in Appendix I. To use the CO_2 sensor, it must first be left on for at least 48 hours and calibrated according to DFRobot (n.d.). The code used to test the sensor, from DFRobot (n.d.), can be seen in Appendix J, Figures 17-19. The test code was uploaded onto the ESP32 to calibrate the code, and the voltage received at 400 ppm and 1000 ppm were taken. The voltage measurement at 400 ppm divided by 8.5 was placed inside the parentheses in line 13 of the code. The difference in the voltage measurements at 400 ppm and 1000 ppm divided by 8.5 was placed inside the parentheses in line 14. After calibrating, the code was reuploaded to the ESP32, and the measurements were taken. The first set of measurements were taken on a separate day from the second and third set of measurements. The calibration process was completed before each of the three measurements. The values from calibrating can be seen in Table 5.

Table 5: CO_2 Sensor Calibration

ppm	Measurement 1 Volts	Volts/8.5	Measurement 2 Volts	Volts/8.5	Measurement 3 Volts	Volts/8.5
400	2.31	0.272	2.62	0.308	2.66	0.313
1000	2.15	0.253	2.45	0.288	2.49	0.293
400-1000		0.019		0.020		0.020

Five measurements were taken in each set of measurements. The measurements were taken when the Pasco sensor read 400 ppm, 600 ppm, 750 ppm, 900 ppm, and 1000 ppm. The first set of measurements are <400 ppm, 413 ppm, 657 ppm, 835 ppm, and 812 ppm, respectively. The second set of measurements are <400 ppm, 553 ppm, 720 ppm, 1299 ppm, and 1537 ppm, respectively. The third set of measurements are 440 ppm, 576 ppm, 996 ppm, 1309 ppm, and 1591 ppm, respectively. The measurements can be seen in Table 6.

Table 6: CO_2 Sensor Test Results

Pasco Sensor (ppm)	Measurement 1 (ppm)	Measurement 2 (ppm)	Measurement 3 (ppm)
400	<400	<400	440
600	413	553	576
750	657	720	996
900	835	1299	1309
1000	812	1537	1591

From the results, it can be seen that the measurements are not consistent, especially in measurement sets 2 and 3. In measurements 2 and 3, there is a large increase from the 750 ppm to 900 ppm measurement. This could be explained by how the code is written because the code uses the voltages from calibration to infer what the CO_2 measurement should be based on the voltage received. Since the calibration voltages for measurements 2 and 3, this explains why they behave similarly compared to measurement 1. However, even taking into account the accuracy of the Pasco sensor, ±5% of reading + 100 ppm, and the accuracy of the MG-811, ±100 ppm at 400 ppm, the sensor is not performing as intended (*Wireless CO_2 Sensor, n.d.*; *Gravity: Analog for Arduino*, n.d.). For the MG-811, it only specifies the accuracy at 400 ppm, however that accuracy will be used for all measurements. So, the maximum variance for 1000 ppm should be ±250 ppm, meaning the measurement can range between 750 ppm to 1250 ppm. However, in measurements 2 and 3, the measurements are 1537 and 1591, well above the 1250 value. While testing, it was also found that slightly moving the wires will require recalibration. As a result of the testing, it can be seen that the performance of the CO_2 sensor is not reliable.

3.2.1.5 Website Testing

Five different devices were used to access the website to ensure the website functions properly. The devices used were an iPhone, an iPad, an Android phone, and two different laptops. Each device would wirelessly access the website to check the temperature and CO_2 measurements as well as turn on and off the pump. The website worked on all devices.

3.2.5 Failure Mode and Effects Analysis (FMEA)

When analyzing the possible weak points of this design, the two possible issues that stood out the most were the possibility of the bioreactor not being airtight and the possibility of the circuitry being exposed to water or media. If the bioreactor is open to the atmosphere it will render the autoclaving useless and expose the plant and media to mold growth. If the circuitry is exposed to water or media it could short circuit and cause full system failure or it could send current through a liquid leak. To keep the bioreactor airtight and watertight the 3D model threading securing the globe to the reservoir must be thoroughly reviewed by the team and appropriate faculty. The circuitry must also be securely waterproofed with redundancy. The full FMEA can be found in Appendix F.

3.3 Socio-cultural and Ecological Considerations

The SLA resin used to print the bioreactor components is not recyclable, however, measures have been taken to ensure that this product is not single-use. This product was designed to be reusable to limit the use of non-recyclable resin by selecting a material that can be sterilized. Providing a reliable sterilization option ensures that this product can be reused until failure therefore mitigating the potential drawbacks of using a non-recyclable material.

For the electrical components, the components are properly grounded to prevent shock hazards. The only components that will be exposed to the humidity inside the TIB will be the temperature sensor and the CO_2 sensor. The temperature sensor, DS18B20, is waterproof. As for the CO_2 sensor, MG811, the operation has a humidity of 0-95% RH, meaning the sensor can work in dry to almost saturated air (*Gravity: Analog for Arduino*, n.d.).

The KHPP260 pump is heat resistant and the tubing is autoclavable. Both products can be used continually until failure. The pump may have a shortened lifespan if exposed to very high humidity levels for extended periods of time, but even in this case it should be useable for several months before failure and is affordable to replace. This particular pump is designed to be easy to take apart and clean, which is necessary since it cannot be autoclaved along with the tubing.

3.4 Final Design and Construction

In this section, the construction process and final design are presented in detail.

3.4.1 3D Printing

A feasibility criterion for the bioreactor design was that it should be 3D printable. This was accomplished with the final design by using a Formlabs 3B printer along with engineering-grade Durable resin also by Formlabs. The individual components were oriented and supported using the PreForm 3D printing software, and one of the top priorities while printing was to maximize the resin cartridge which contained 1 liter of resin. Initially, the larger components of the bioreactor such as the reservoir and the dome were set to print flat on their base as shown on the left side of Figure 4, however, it was quickly discovered that this was not the most efficient way to orient the print on the printing platform.

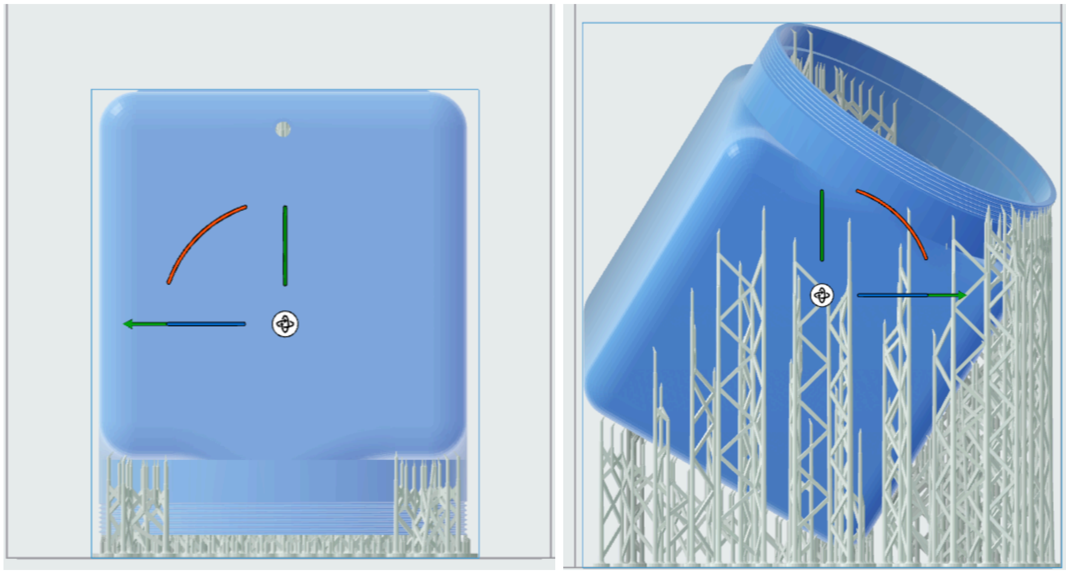

Figure 4: Reservoir component before and after tilting on print platform using Preform

By tilting the reservoir as displayed on the right side of Figure 4, less resin was used in the printing process leaving more resin that could be used for another complete bioreactor print. Due to its shape, the dome was also easier to print at a tilt because this avoided any major areas lacking support that could potentially lead to a failed print. During the printing process, there were some issues encountered with the printer itself where the prints would fail by having a hole in the middle as seen in Figure 5 below.

Figure 5: Failed Dome Print

It was discovered that the printing bed was damaged and the printer itself needed cleaning. The issue was resolved by replacing the printing bed used and removing built-up resin in the printer that was preventing proper printing.

After successfully printing all components, they were post-processed according to Formlabs instructions on washing and curing times and temperatures. Table 7 below displays the wash and cure time and temperatures that were used for post-processing.

Table 7: Post Processing Details

Task	Time (minutes)	Temperature (℃)
Wash	20	N/A
Dry	60	N/A
Cure	60	60

An additional step was added between washing and curing the resin which was to let components dry for an hour before placing them in the cure station. The drying stage was especially important for the large prints, the reservoir, and dome because they had very dense internal supports that could hold onto a lot of liquid. Another important feature in setting up the prints for the bioreactor was the support settings. As previously stated, it was crucial to set up prints in such a way that the 1 liter of resin would be maximized. In order to accomplish this all components were printed using the mini raft option in PreForm. Additionally, the touch point sizes were minimized in order to facilitate the removal of supports after printing while also leaving the surface of the components as smooth as possible. Easy support removal was especially important in areas where there were threads that would need to fit together. Overall, a full bioreactor print including all individual components used 469.41 ml of resin meaning two complete bioreactors can be printed using a 1 liter cartridge of resin. The complete breakdown showing how much resin was used for each component can be found in Appendix B.

3.4.2 Electrical and Website Components

The circuit schematic can be seen in Appendix J, Figure 20, while the code for the final design can be seen in Figures 21-28 in Appendix J. Essentially the code will check for clients, then get the temperature, and set the pump speed. Next, if there is a client, it will continue into a loop that will loop until the client disconnects, otherwise, it will start from the beginning. While the client is connected, the website will be sent to the client, specifically the heading, the pump section, the temperature section, and the CO_2 section, in that order. The code flowchart can be seen in Figure 6.

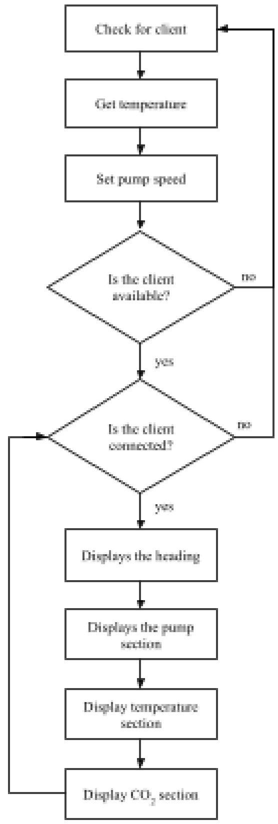

Figure 6: Code Flowchart

As for the website, all text is left justified. There are four sections separated by horizontal lines: the title, pump, temperature, and CO_2. The title will be “Temporary Immersion Bioreactor” in bold. The pump section has the current state of the pump stated before the button. The button has the desired state of the pump. The temperature and CO_2 level will contain “Temperature:” and “CO_2”, respectively, with the measurements under the words. The website only works if the ESP32 and the client are connected to the same network. The website design can be seen in Figure 7.

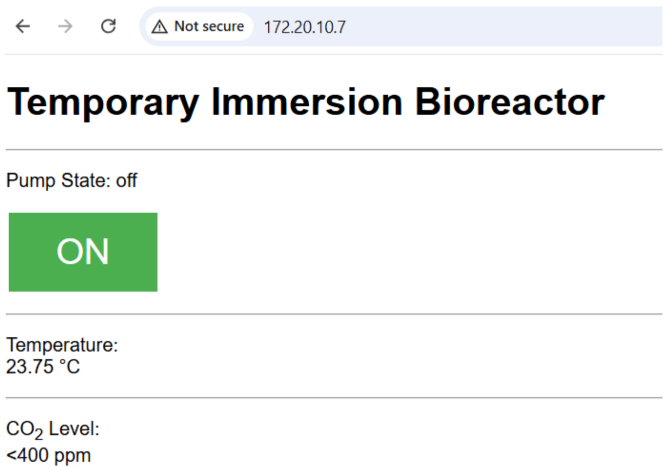

Figure 7: Final Design Website

3.4.2 Final Assembly

The final design was constructed by combining the 3D printed components, the pump selected, and the electrical components into one functioning device. Figure 8 below shows the final model created.

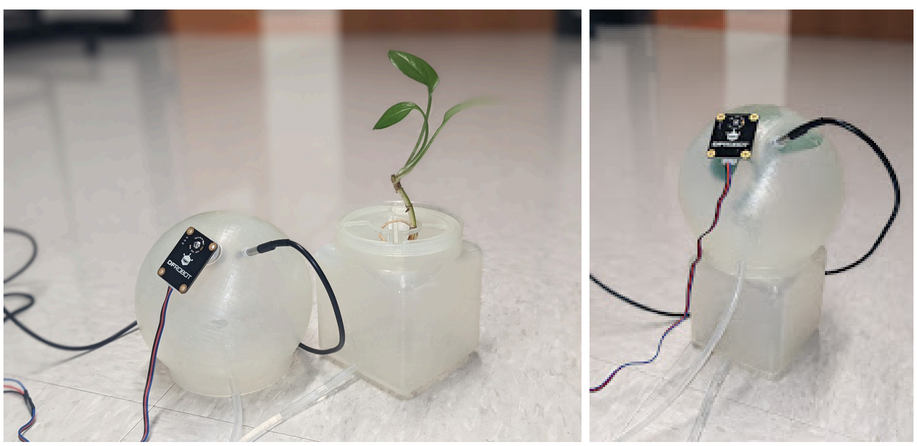

Figure 8: Bioreactor final assembly with plant

The propagation frame proved to be an effective method of maintaining a plant in place inside the bioreactor. The temperature sensor and the CO_2 sensor were both secured in place using a rubber washer in order to ensure no leakage.

The completed circuit built, using the schematic in Figure 20 in Appendix J, can be seen in Figure 9.

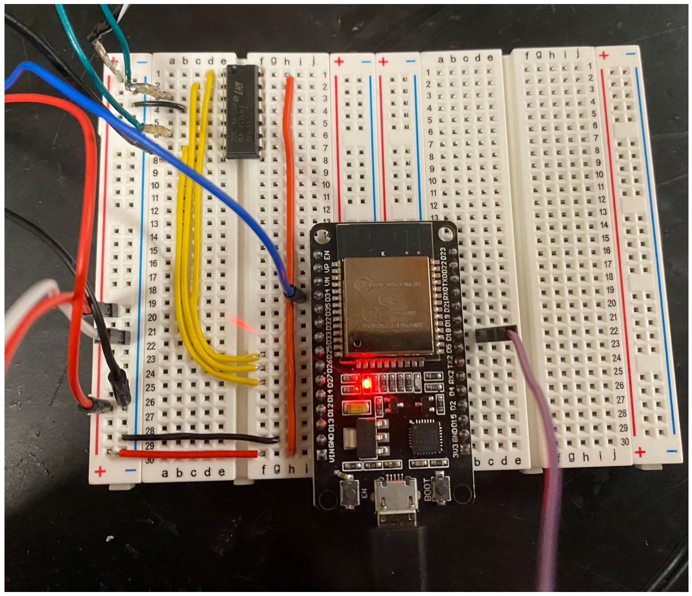

Figure 9: Final Circuit Assembly

The pump and drain system was initially designed using a 1.00 mm drain opening and is for use with a pump code setting of “92.” This gives an inflow of close to 93 mL/min and a total plant submersion time of close to 5.3 minutes every 6 hours. With a 6-hour interval, the pump can be turned on at the same time each day. However, since the pump stalls at “92” unless worked down by increments to that setting from “130”, the system was reevaluated for use at a high flow rate that does not stall. This gave a final drain hole radius of 1.60 mm at a pump setting of “150”, which is an inflow of 249 mL/min. The submersion time of the plant should remain generally the same.

The final product very closely resembles the initial proposed design and is fully functional.

4. Other Factors

An important factor to consider about this final design is the software that was used to not only design it but also prepare it for 3D printing. During the construction stages of the final design a combination of SolidWorks 2023, Fusion360, and PreForm was used. It is recommended that in the future the design be rendered in Fusion360 and prepped for printing in PreForm. This would expedite the printing process, especially if part sizes need to be resized during construction. As previously mentioned, there were some issues during the printing stages which led to delays due to the long printing time of larger components such as the dome. In order to avoid similar issues, the 3D printer was cleaned and the printing bed was inspected thoroughly after large prints.

5. Costs

The overall budget for this project was provided by Mercer University and it was in the amount of $300.00. Table 8 below shows the budget breakdown based on individual components.

Table 8: Budget Breakdown

Item	Cost
Wires	6.98
Temperature Sensor	13.48
CO2 Sensor	48.9
Hi-Temp Washers	15.99
Durable Resin	199
K Kamoer Pump	17.85
Silicone Tubing	8.99
L293D Pump Motor Chip	8.99
ESP32	13.99
TOTAL	334.17

The majority of the budget was allocated toward the material for the body of the TIB. This is because the resin selected meets all of the client's requests for the bioreactor such as being 3D printable, autoclavable, and letting sufficient light through for plant growth. Despite the material accounting for the majority of the budget, after printing it was discovered that two complete bioreactors can be printed using one cartridge of resin. This would mean that if cost were broken down per ml of resin, each bioreactor uses approximately $100 worth of resin. However, the smallest resin cartridge available for purchase is $199.00. The budget was further distributed among the electrical components which all need to be ordered individually. The last component in the budget is the pump that will be used to move the media from the lower chamber of the device to the upper compartment with the plant. Although the original budget was that of $300.00 our client has approved the new budget of $334.17.

6. Conclusion

The production of this simple Temporary Immersion Bioreactor will give agriculture students at the Technological University of Matamoros Izúcar direct experience measuring and recording plant growth and growth conditions. The team of Mercer engineering students Santillian, Ezell, and Ong will be responsible for planning and producing the first fully designed simple prototype for this purpose. The students and faculty of the University in Mexico will be able to make further modifications according to their desires or plant specialty, as well as turn the bioreactor project into a large-scale propagation method. The cost to produce one bioreactor is $334.17, which was approved by our client. Referencing the results from the feasibility and merit analysis previously conducted, the final design was successfully assembled and proved to be fully functional. For future recommendations, more sensors could be added and a different CO_2 sensor should be used. The website could be improved to work when connected to any network rather than just the network the ESP32 is connected to. If no further changes are to be made, a PCB board can be produced for a more permanent and neat circuit. Replication of this project should apply a pump setting of “150” and a drain hole radius of 1.60 mm, or select a pump with similar features and advantages that can reliably function from rest at around 90 mL/min without stalling. Growth testing is also recommended for comprehensive testing of the TIB and can be done easily by using the Leafscan application and tracking growth trends. Lastly, it is recommended that the walls of all individual components be designed to be 1mm thick rather than the produced 2mm thick. This allows for the conservation of resin used and potentially more parts being printed from a 1-liter cartridge of Durable Resin.

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Appendix A: Translucency Testing

The Vernier Light sensor shown in Figure 10 below was used for material translucency testing. The component with orange ends contains the photosensor which was first exposed to light without any obstructions and then each material was used to cover the photosensor.

Figure 10: Vernier Light Sensor and Reading from Logger Pro program

The light readings were collected outside of the engineering building. Figure 11 below shows the testing set up with the material blocking the photosensor.

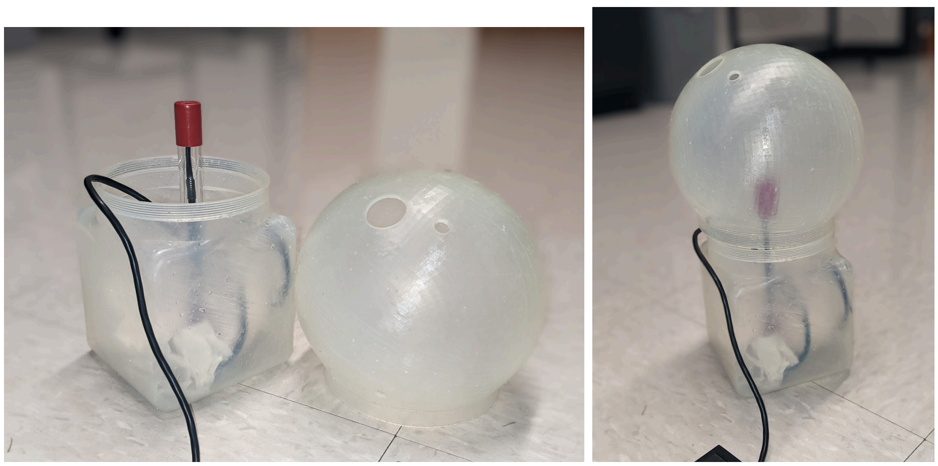

Figure 11: Light Sensor covered by Dome

This testing site was selected because plants are successfully grown here, and this area allows for testing light that has already been through a glass. We can assume based on our clients description that the lighting in this area is similar to where the TIB will be placed. Table 9 below displays the light readings from outdoor trials.

Table 9: Outside Light Testing

Trial	Before (Lux)	After (Lux)
Trial 1	39499	30145
Trail 2	45843	41293
Trial 3	35152	24947

Appendix B: Total ml of resin used per component of bioreactor

Table 10 below shows the resin volume used for each component of the bioreactor.

Table 10: Volume of Resin Used

Component	Volume of Resin (ml)
Dome	212.73
Reservoir	206.48
Cone	35.17
Frame	15.03
Total	469.41ml

Appendix C: Equations, Calculations, and Derivation Explanations

Equation 1. "The Energy Equation" (Houghtalen, 2017).

$$h_1 + \frac{P_1}{\gamma} + \frac{\nu_1^2}{2g} = h_2 + \frac{P_2}{\gamma} + \frac{\nu_2^2}{2g} + H_L$$

This equation divides the flow problem into an "initial" and "final" state, on either side of the equals sign and renders all factors into units of length. The "$h_\#$" accounts for the vertical height displacement of the liquid. The "$\frac{P_\#}{\gamma}$" factor accounts for changes in pressure between states. The "$\frac{\nu_\#^2}{2g}$" factor accounts for the kinetic energy difference between the states, such as changes in flow velocity. And finally, the "H_L" factor accounts for the overall changes in the system due to friction and other losses.

Before looking at values, several of these factors can be ignored. The pressure factors cancel out because the system starts and ends at atmospheric pressure. The second height factor can also be ignored because there is no height of the waterline when the cone is empty. The first kinetic energy factor cancels out as well because the inflow is being caught and held in place before being allowed to drain. After removing these terms, the equation can be simplified as follows in Equation 2.

Equation 2. Simplified Energy Equation for this scope.

$$h_1 = \frac{\nu_2^2}{2g} + H_L$$

Equation 3. The Darcy-Weisbach Equation for frictional losses (Houghtalen, 2017).

$$H_L = f\left(\frac{L}{D}\right)\left(\frac{\nu^2}{2g}\right)$$

The Energy Equation for this project, seen in Equation 2, can be rewritten as Equation 4. Algebraically rearranging terms for flow velocity achieves Equation 5.

Equation 4. Expanded Energy Equation for this scope.

$$h_1 = \frac{\nu_2^2}{2g} + f\left(\frac{L}{D}\right)\left(\frac{\nu_2^2}{2g}\right)$$

Equation 5. Equation 4 rearranged to solve for input velocity.

$$\nu_2 = \sqrt{\frac{2gh_1}{[1+f(\frac{L}{D})]}}$$

Equation 6. Finding the friction factor (Houghtalen, 2017).

$$Relative\ roughness = \frac{\varepsilon}{D}$$

The factor "$\frac{\varepsilon}{D}$", which is inner roughness divided by inner diameter, will give a value that can be traced across the Moody Chart to find the friction factor. Calculation 1 shows the calculation for this roughness factor, and Figure X in Appendix V shows the traced line to find "f".

Calculation 1. Calculating the friction factor within the peristaltic tubing.

$$Relative\ roughness = \frac{0.0015\ mm}{4.0\ mm} = 0.000375$$

Equation 6. Fundamental flow equation.

$$Q = Av$$

Equation 7. Fundamental area of a circle.

$A = \pi r^2$

Combining these two equations gives us Equation 8.

Equation 8. The resulting equation for ideal flow rate.

$$Q = \pi r^2 \sqrt{\frac{2gh_1}{[1+f(\frac{L}{D})]}}$$

One can now solve Equation 8 for the ideal inflow. The height of the waterline in the cone should stop rising and equalize at about 30 mm high which is ¾ full, so "h_1" is 30 mm. The length of tubing used, "L", is very close to one foot, or 0.30 m. The inner diameter of the tubing, "D", is 4 mm. This calculation is provided as Calculation 2.

Calculation 2. Finding the desired input flow rate.

$$Q = [\pi(1\ mm)^2] \sqrt{\frac{2(9810\ mm/s^2)(\frac{3600\ s^2}{1\ min^2})(30\ mm)}{[1+(0.0158)(\frac{300\ mm}{4\ mm})]}}^{1} = 97833.0\ mm^3/min = 97.8\ mL/min$$

Equation 9. Equation 8 rearranged for waterline height.

$$h = (\frac{Q}{\pi r^2})^2 (\frac{1+f(\frac{L}{D})}{2g})$$

Calculation 4. Example calculation for waterline height.

$$h = (\frac{(80\ mL/min)(\frac{1\ min}{60\ s})(\frac{10^3 mm^3}{1\ mL})}{\pi(1\ mm)^2})^2 (\frac{1+(0.0158)(\frac{300\ mm}{4\ mm})}{2(9810\ mm/s^2)}) = 20.06\ mm$$

Equation 10. Equation 8 rearranged for hole radius.

$$r = \sqrt{\frac{Q}{\pi\sqrt{\frac{2gh}{1+f(\frac{L}{D})}}}}$$

Calculation 6. Calculating the radius required to balance the desired inflow.

$$r = \sqrt{\frac{(250\ mL/min)(\frac{1\ min}{60\ s})(\frac{10^3 mm^3}{1\ mL})}{\pi\sqrt{\frac{2(9810\ mm/s^2)(30\ mm)}{1+(0.0158)(\frac{300\ mm}{4\ mm})}}}} = 1.600\ mm$$

Appendix D. Moody Diagrams

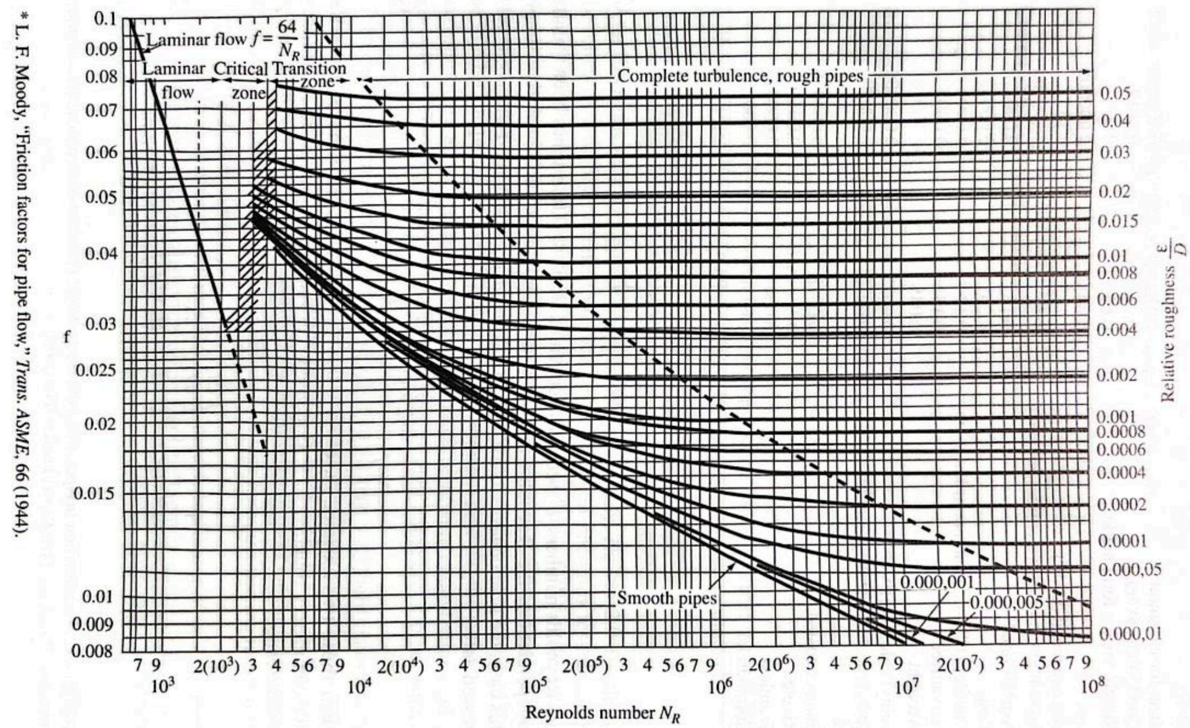

Figure 3.8 Friction factors for flow in pipes: the Moody diagram. *Source:* From L. F. Moody, "Friction factors for pipe flow," *Trans. ASME*, vol. 66, 1944.

Figure 12: The Moody diagram (Houghtalen, 2017)

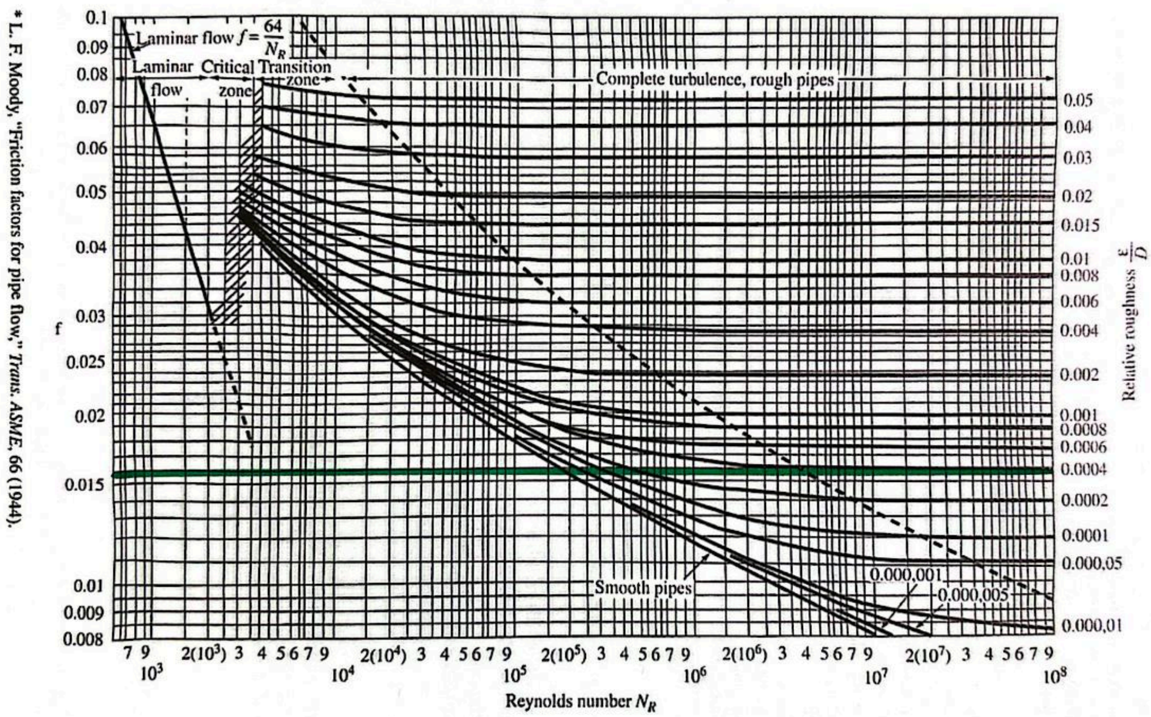

Figure 3.8 Friction factors for flow in pipes: the Moody diagram. *Source:* From L. F. Moody, "Friction factors for pipe flow," *Trans. ASME*, vol. 66, 1944.

Figure 13: The Moody diagram with a green trace line to find "f" (Houghtalen, 2017)

Appendix E. Pump and Flow Data Tables

Table 11: Performance data of all three of the K Kamoer pumps

Code Setting	Q Pump 1 (mL/s)	Q Pump 2 (mL/s)	Q Pump 3 (mL/s)
255 (true max)	4.30	4.18	3.91
220 (listed max)	3.76	3.31	3.54
100	1.83	1.66	1.70
90	1.35	1.81	1.45
80	1.20	0	0.95
70	0	0	0

Table 12: Flow rates measured at different code settings with a hole radius of 1 mm

Code Setting	Flow Rate Measured (mL/min)	Calculated Waterline Height (mm)
86	80	20.06
87	82	21.08
88	84	22.02
89	86	23.08
90	88	24.27
91	92	26.53
93	94	27.70
94	97	29.49
95	100	31.34
96	102	32.61
98	104	33.90
100	108	36.56
102	115	*Overflow*
105	124	*Overflow*
107	130	*Overflow*

Table 13: Flow rates calculated at different code settings with a hole radius of 1.6 mm

Code Setting	Flow Rate Measured (mL/min)	Calculated Waterline Height (mm)
130	181	*Inadequate Fill*
135	199	*Inadequate Fill*
142	222	23.57
150	249	29.89
155	268	34.35
170	319	*Overflow*

Appendix F. Mechanical Measurements and Data for Pump and Drain.

Table 14: Relevant pump-specific data from seller and listing

	Name/Brand	Pipe Inner Fit	Pipe Outer Fit	Advertised Max Flow	Power
Pump 1	**Adafruit**	3.5 mm	5 mm	100 mL/min	6V/0.50A or 12V/0.25A
Pump 2	**K Kamoer**	4 mm	6 mm (5/32)	220 mL/min	6V/0.50A
Pump 3	**Intllab**	3 mm	5 mm	100 mL/min	6V/0.50A
Pump 4	**Oswoo**	3 mm	5 mm	100 mL/min	12V/0.35A

Table 15: Relevant Bioreactor Design Specifications

Part	Original Size	Resized (70%)
$r_{cone\ top}$	57.255 mm	40.079 mm
$r_{cone\ hole}$	0.500 mm	1.00 mm or 1.60 mm [calculated features]
h_{cone}	60.000 mm	42.000 mm
$h_{assembly}$	341.0 mm	239.0 mm
$w_{assembly}$	202.0 mm	141.4 mm
$l_{assembly}$	202.0 mm	141.4 mm

Appendix G: Failure Mode and Effects Analysis

Failure Modes Effects Analysis

Process or Product Name:	EGR 487; Senior Design	Prepared by:	Carlie Ezell	Page:	1 of 1
Process Owner:	Carlie Ezell	FMEA Date (Orig):	26-Mar-23	Rev.	

Design Function	Potential Failure Mode	Potential Failure Effects	SEV	Potential Causes	OCC	Current Controls	DET	RPN	Actions Recommended	Resp.	Actions Taken	SEV	OCC	DET	RPN
What is the desired design function or goal?	In what ways can the Design fail?	What is the impact on the Key Output Variables once it fails (customer/design requirements)?	How **Severe** is the effect to the customer?	What causes the Key Input to go wrong?	How **often** does cause or FM **occur**?	What are the existing **controls** and procedures that prevent either the Cause or the Failure Mode?	How well can you **detect** the Cause or the Failure Mode?		What are the actions for reducing the occurrence of the cause, or improving detection?	Who is Responsible for the recommended action?	Note the actions taken. Include dates of completion.				
growth of agave	mold grows inside	plant diseased	7	autoclave misapplication	1		9	63							0
			4	open to atmosphere (seals)	4	secure seals and tight threading	8	128	faculty review of 3D model mating parts	Carlie		4	2	6	48
	pump breaks	plant dehydrated, malnourished, dies	4	misalignment of rotating shaft	2		7	56							0
			9	overpressurized system failure	1	slow flow and low system pressure	8	72							0
	not translucent enough	hinders growth	8	thick material	2	material testing	2	32							0
			8	cloudy material	2	material testing	2	32							0
	automation fails	total system failure	10	website down	2		1	20							0
			10	electronics destroyed	3	waterproofing	4	120	waterproofing of electronics	Quyen		10	1	3	30
			10	no connection	1	does not require internet	2	20							0

Figure 14: Failure Modes Effects Analysis for Bioreactor Assembly

Appendix H: Temperature Testing

```
// Make sure to include Onewire and Dallas Temperature libraries
#include <OneWire.h>
#include <DallasTemperature.h>

// Connect signal pin to Pin18
#define ONE_WIRE_BUS 18

// Setup a oneWire instance to communicate with any OneWire devices
OneWire oneWire(ONE_WIRE_BUS);

// Pass our oneWire reference to Dallas Temperature sensor
DallasTemperature sensors(&oneWire);

void setup(void)
{
  // initialize the Serial Monitor at a baud rate of 9600.
  Serial.begin(9600);

  sensors.begin();
}

void loop(void){
  // Call sensors.requestTemperatures() to issue a global temperature and Requests to all devices on the bus
  sensors.requestTemperatures();

  Serial.print("Celsius temperature: ");
  Serial.print(sensors.getTempCByIndex(0));
  Serial.print(" - Fahrenheit temperature: ");
  Serial.println(sensors.getTempFByIndex(0));
  delay(1000);
}
```

Figure 15: Temperature Sensor Testing Code

Appendix I: CO_2 Testing

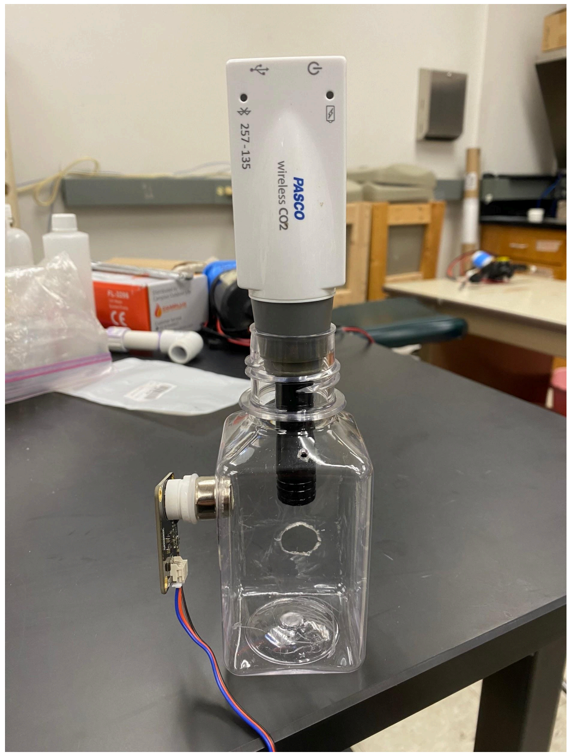

Figure 16: CO_2 Testing Setup

```
/************************Hardware Related Macros************************************/
#define         MG_PIN                          (34)     //define which analog input channel you are going to use
#define         BOOL_PIN                        (2)
#define         DC_GAIN                         (8.5)   //define the DC gain of amplifier

/***********************Software Related Macros************************************/
#define         READ_SAMPLE_INTERVAL            (50)    //define how many samples you are going to take in normal operation
#define         READ_SAMPLE_TIMES               (5)     //define the time interval(in milisecond) between each samples in
                                                        //normal operation

/**********************Application Related Macros**********************************/
//These two values differ from sensor to sensor. user should derermine this value.
#define         ZERO_POINT_VOLTAGE              (0.282) //define the output of the sensor in volts when the concentration of CO2 is 400PPM
#define         REACTION_VOLTGAE                (0.030) //define the voltage drop of the sensor when move the sensor from air into 1000ppm CO2

/*****************************Globals***********************************************/
float           CO2Curve[3]  =  {2.602,ZERO_POINT_VOLTAGE,(REACTION_VOLTGAE/(2.602-3))};
                                                        //two points are taken from the curve.
                                                        //with these two points, a line is formed which is
                                                        //"approximately equivalent" to the original curve.
                                                        //data format:{ x, y, slope}; point1: (lg400, 0.324), point2: (lg4000, 0.280)
                                                        //slope = ( reaction voltage ) / (log400 -log1000)

void setup()
{
    Serial.begin(9600);                                 //UART setup, baudrate = 9600bps
    pinMode(BOOL_PIN, INPUT);                           //set pin to input
    digitalWrite(BOOL_PIN, HIGH);                       //turn on pullup resistors

   Serial.print("MG-811 Demostration\n");
}
```

Figure 17: CO_2 Testing Code Part 1

```
void loop()
{
    int percentage;
    float volts;

    volts = MGRead(MG_PIN);
    Serial.print( "SEN0159:" );
    Serial.print(volts);
    Serial.print( "V           " );

    percentage = MGGetPercentage(volts,CO2Curve);
    Serial.print("CO2:");
    if (percentage == -1) {
        Serial.print( "<400" );
    } else {
        Serial.print(percentage);
    }

    Serial.print( "ppm" );
    Serial.print("\n");

    if (digitalRead(BOOL_PIN) ){
        Serial.print( "=====BOOL is HIGH======" );
    } else {
        Serial.print( "=====BOOL is LOW======" );
    }

    Serial.print("\n");

    delay(500);
}
```

Figure 18: CO_2 Testing Code Part 2

```
/*****************************  MGRead *********************************************
Input:   mg_pin - analog channel
Output:  output of SEN-000007
Remarks: This function reads the output of SEN-000007
************************************************************************************/
float MGRead(int mg_pin)
{
    int i;
    float v=0;

    for (i=0;i<READ_SAMPLE_TIMES;i++) {
        v += analogRead(mg_pin);
        delay(READ_SAMPLE_INTERVAL);
    }
    v = (v/READ_SAMPLE_TIMES) *5/1024 ;
    return v;
}

/*****************************  MQGetPercentage **********************************
Input:   volts   - SEN-000007 output measured in volts
         pcurve  - pointer to the curve of the target gas
Output:  ppm of the target gas
Remarks: By using the slope and a point of the line. The x(logarithmic value of ppm)
         of the line could be derived if y(MG-811 output) is provided. As it is a
         logarithmic coordinate, power of 10 is used to convert the result to non-logarithmic
         value.
************************************************************************************/
int  MGGetPercentage(float volts, float *pcurve)
{
   if ((volts/DC_GAIN )>=ZERO_POINT_VOLTAGE) {
      return -1;
   } else {
      return pow(10, ((volts/DC_GAIN)-pcurve[1])/pcurve[2]+pcurve[0]);
   }
}
```

Figure 19: CO_2 Testing Code Part 3

Appendix J: Final Design Circuit and Cod

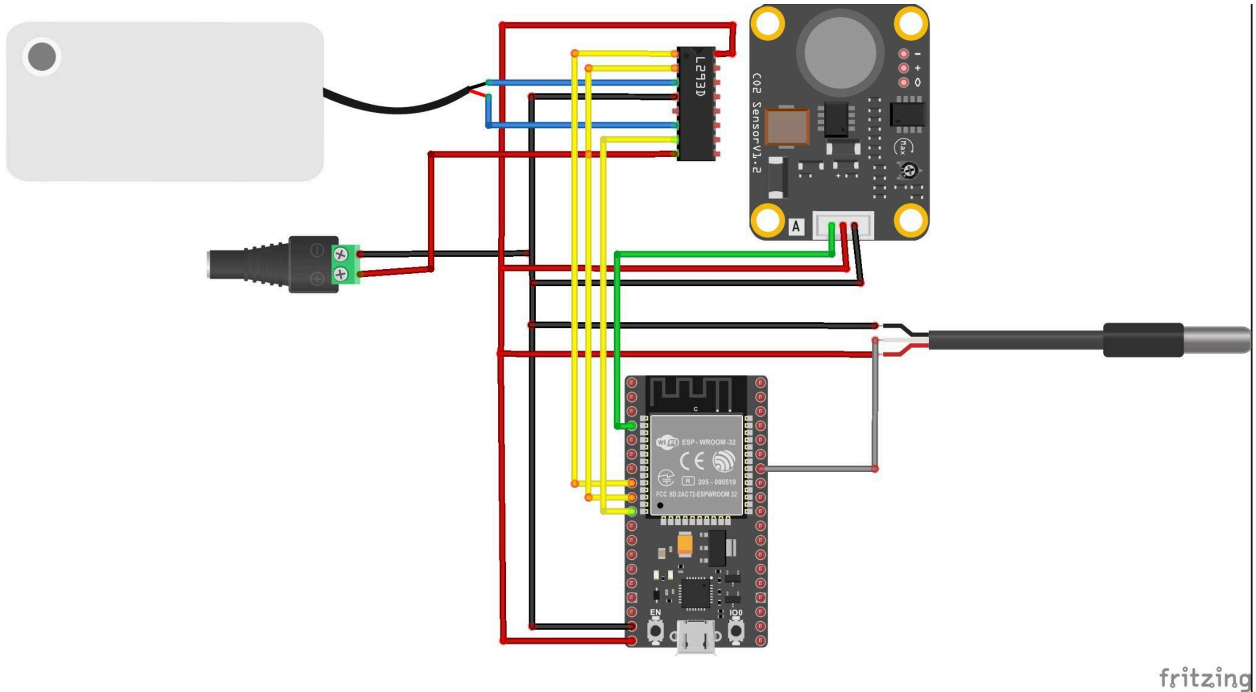

Figure 20: Final Design Schematic

```
#include <WiFi.h>
#include <ESPAsyncWebServer.h>
#include <OneWire.h>
#include <DallasTemperature.h>

/*Temperature*******************************************************************/
// Connect signal pin of the temperature sensor to to D18
#define ONE_WIRE_BUS 18

// Setup a oneWire instance to communicate with any OneWire devices
OneWire oneWire(ONE_WIRE_BUS);

// Pass our oneWire reference to Dallas Temperature sensor
DallasTemperature sensors(&oneWire);

/*Website***********************************************************************/
// Replace with your network credentials
const char* ssid = ""; //Enter your WIFI SSID
const char* password = "";   //Enter your WIFI password

// Set web server port number to 80
WiFiServer server(80);

// Variable to store the HTTP request
String header;

/*Pump**************************************************************************/
// Auxiliar variables to store the current output state
String output26State = "off";

// Assign output variables to GPIO pins
int enA = 25;
int in1 = 26;
int in2 = 27;

```

Figure 21: Final Design Code Part 1

```
// Current time
unsigned long currentTime = millis();
// Previous time
unsigned long previousTime = 0;
// Define timeout time in milliseconds (example: 2000ms = 2s)
const long timeoutTime = 10000;

/*CO2****************************************************************************/
#define         MG_PIN                       (34)     //define which analog input channel you are going to use
#define         BOOL_PIN                     (2)
#define         DC_GAIN                      (8.5)   //define the DC gain of amplifier
#define         READ_SAMPLE_INTERVAL         (50)    //define how many samples you are going to take in normal operation
#define         READ_SAMPLE_TIMES            (5)     //define the time interval(in milisecond) between each samples in
                                                     //normal operation

//These two values differ from sensor to sensor. user should derermine this value.
#define         ZERO_POINT_VOLTAGE           (0.761) //define the output of the sensor in volts when the concentration of CO2 is 400PPM
#define         REACTION_VOLTGAE             (0.02) //define the voltage drop of the sensor when move the sensor from air into 1000ppm CO2

/*****************************Globals***********************************************/
float           CO2Curve[3]  =  {2.602,ZERO_POINT_VOLTAGE,(REACTION_VOLTGAE/(2.602-3))};
                                                     //two points are taken from the curve.
                                                     //with these two points, a line is formed which is
                                                     //"approximately equivalent" to the original curve.
                                                     //data format:{ x, y, slope}; point1: (lg400, 0.324), point2: (lg4000, 0.280)
                                                     //slope = ( reaction voltage ) / (log400 -log1000)

/////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////////

void setup() {
  Serial.begin(115200);

  // Set all the motor control pins to outputs
  pinMode(enA, OUTPUT);
  pinMode(in1, OUTPUT);
```

Figure 22: Final Design Code Part 2

```
  pinMode(in1, OUTPUT);

  // Turn off motors - Initial state
  digitalWrite(in1, LOW);
  digitalWrite(in2, LOW);

  // Connect to Wi-Fi network with SSID and password
  Serial.print("Connecting to ");
  Serial.println(ssid);
  WiFi.begin(ssid, password);
  while (WiFi.status() != WL_CONNECTED) {
    delay(500);
    Serial.print(".");
  }
  // Print local IP address and start web server
  Serial.println("");
  Serial.println("WiFi connected.");
  Serial.println("IP address: ");
  Serial.println(WiFi.localIP());
  server.begin();

  //Temperature
  sensors.begin();

  //CO2
  pinMode(BOOL_PIN, INPUT);                          //set pin to input
  digitalWrite(BOOL_PIN, HIGH);                      //turn on pullup resistors
}

void loop(){
  WiFiClient client = server.available();   // Listen for incoming clients

  sensors.requestTemperatures(); // Send the command to get temperatures

```

Figure 23: Final Design Code Part 3

```
//sets pump speed (can be 0-255)
analogWrite(enA, 150);

//CO2
int percentage;
float volts;

if (client) {                             // If a new client connects,
  currentTime = millis();
  previousTime = currentTime;
  Serial.println("New Client.");          // print a message out in the serial port
  String currentLine = "";                // make a String to hold incoming data from the client
  while (client.connected() && currentTime - previousTime <= timeoutTime) {  // loop while the client's connected
    currentTime = millis();
    if (client.available()) {             // if there's bytes to read from the client,
      char c = client.read();             // read a byte, then
      Serial.write(c);                    // print it out the serial monitor
      header += c;
      if (c == '\n') {                    // if the byte is a newline character
        // if the current line is blank, you got two newline characters in a row.
        // that's the end of the client HTTP request, so send a response:
        if (currentLine.length() == 0) {
          // HTTP headers always start with a response code (e.g. HTTP/1.1 200 OK)
          // and a content-type so the client knows what's coming, then a blank line:
          client.println("HTTP/1.1 200 OK");
          client.println("Content-type:text/html");
          client.println("Connection: close");
          client.println();

          // turns the pump on and off
          if (header.indexOf("GET /26/on") >= 0) {
            Serial.println("Pump On");
            output26State = "on";
            digitalWrite(in1, HIGH);
            digitalWrite(in2, LOW);
```

Figure 24: Final Design Code Part 4

```
} else if (header.indexOf("GET /26/off") >= 0) {
  Serial.println("Pump Off");
  output26State = "off";
  digitalWrite(in1, LOW);
  digitalWrite(in2, LOW);
}

// Display the HTML web page
client.println("<!DOCTYPE html><html>");
client.println("<head><meta name=\"viewport\" content=\"width=device-width, initial-scale=1\">");
client.println("<link rel=\"icon\" href=\"data:,\">");
// CSS to style the on/off buttons
// Feel free to change the background-color and font-size attributes to fit your preferences
client.println("<style>html { font-family: Helvetica; display: inline-block; margin: 0px auto; text-align: left;}");
client.println(".button { background-color: #4CAF50; border: none; color: white; padding: 16px 40px;");
client.println("text-decoration: none; font-size: 30px; margin: 2px; cursor: pointer;}");
client.println(".button2 {background-color: #555555;}</style></head>");

// Web Page Heading
client.println("<body><h1>Temporary Immersion Bioreactor</h1>");
client.println("<hr>");

////////////////////////////////////////////////////////////////////////////////
//Pump
// Display current state, and ON/OFF buttons for the pump
client.println("<p>Pump State: " + output26State + "</p>");
// If the output26State is off, it displays the ON button
if (output26State=="off") {
  client.println("<p><a href=\"/26/on\"><button class=\"button\">ON</button></a></p>");
} else {
  client.println("<p><a href=\"/26/off\"><button class=\"button button2\">OFF</button></a></p>");
}

////////////////////////////////////////////////////////////////////////////////
```

Figure 25: Final Design Code Part 5

```
//Temperature
float temp = sensors.getTempCByIndex(0);
String temperatureStr = String(temp, 2);

client.println("<hr>");
client.println("<p>Temperature: <br>");
client.println(temp);
client.println("&deg;C</span></p>");

//////////////////////////////////////////////////////////////////////////////////
//CO2 sensor

//prints volts received from the CO2 sensor in the serial monitor
volts = MGRead(MG_PIN);
Serial.print( "SEN0159:" );
Serial.print(volts);
Serial.print( "V           " );

//prints ppm in the serial monitor
percentage = MGGetPercentage(volts,CO2Curve);
Serial.print("CO2:");
if (percentage == -1) {
    Serial.print( "<400" );
} else {
    Serial.print(percentage);
}
Serial.print( "ppm" );
Serial.print("\n");

//prints ppm in the website
client.println("<hr>");
client.println("<p>CO<sub>2</sub> Level: <br>");
if (percentage == -1) {
    client.println("<400");
} else {
```

Figure 26: Final Design Code Part 6

```
              client.println(percentage);
            }
            client.println("ppm</p>");


            client.println("</body></html>");

            // The HTTP response ends with another blank line
            client.println();
            // Break out of the while loop
            break;
          } else { // if you got a newline, then clear currentLine
            currentLine = "";
          }
        } else if (c != '\r') {  // if you got anything else but a carriage return character,
          currentLine += c;      // add it to the end of the currentLine
        }
      }
    }
    // Clear the header variable
    header = "";
    // Close the connection
    client.stop();
    Serial.println("Client disconnected.");
    Serial.println("");
  }
}
```

Figure 27: Final Design Code Part 7

```
/*****************************  MGRead *********************************************
Input:   mg_pin - analog channel
Output:  output of SEN-000007
Remarks: This function reads the output of SEN-000007
************************************************************************************/
float MGRead(int mg_pin)
{
    int i;
    float v=0;

    for (i=0;i<READ_SAMPLE_TIMES;i++) {
        v += analogRead(mg_pin);
        delay(READ_SAMPLE_INTERVAL);
    }
    v = (v/READ_SAMPLE_TIMES) *5/1024 ;
    return v;
}

/*****************************  MQGetPercentage *********************************
Input:   volts   - SEN-000007 output measured in volts
         pcurve  - pointer to the curve of the target gas
Output:  ppm of the target gas
Remarks: By using the slope and a point of the line. The x(logarithmic value of ppm)
         of the line could be derived if y(MG-811 output) is provided. As it is a
         logarithmic coordinate, power of 10 is used to convert the result to non-logarithmic
         value.
************************************************************************************/
int  MGGetPercentage(float volts, float *pcurve)
{
   if ((volts/DC_GAIN )>=ZERO_POINT_VOLTAGE) {
      return -1;
   } else {
      return pow(10, ((volts/DC_GAIN)-pcurve[1])/pcurve[2]+pcurve[0]);
   }
}
```

Figure 28: Final Design Code Part 8

Appendix K: Translucidity Testing

Objective: The Objective of this test is to ensure that the selected material allows at least 10,000 lux of light through before and after autoclaving.
Equipment: Vernier Light Sensor, Go!Link, Logger Pro Program, Material Sample Printed at 2mm thick.
Location(s): EGC first floor near entrance where plants are located. Potentially in an environmental engineering lab under a grow light to simulate different settings.
Time Required to Complete: Approximately 1-2 days
Personnel: Kendra Santillan
Success Criteria: Above 10,000 lux of light are detected by the light sensor when the material is introduced as a barrier.
Data: The data collected will include a reading from the light sensor when it is fully exposed to the selected light source without a material sample as a barrier. A second reading will be recorded from the light sensor that is exposed to the selected light source with the material sample as a barrier.
Procedure: The test will begin by securing a reading with the light sensor fully exposed to the environment/selected light source. After the initial reading the material sample will be introduced in front of the light sensor completely blocking the photosensor and a second reading will be recorded. After the initial readings are collected as previously stated, the material samples will be autoclaved and the process will be repeated. This will be done with a sample size of n=5.

Appendix L: Autoclave Testing

Objective: The Objective of this test is to determine if the material can successfully be autoclaved.
Equipment: Autoclave, Material samples that are 2mm thick
Location(s): Biology Department Autoclave
Time Required to Complete: 1 week (depending on autoclave availability)
Personnel: Kendra Santillan
Success Criteria: Material shows no signs of melting after a 15 minute gravity cycle in the autoclave
Data: This test will be more of a check meaning no quantitative data will be collected. The information collected will be qualitative based on material appearance.
Procedure: A total of 5 material samples will be printed to ensure that they are 2mm thick. These samples will then be placed in an autoclave bag and autoclaved on a 15 minute gravity cycle. They will be inspected for signs of melting after the 15 minute gravity cycle.

Appendix M: Temperature Sensor Accuracy Testing

Objective: The objective of this test is to test the accuracy of the temperature sensor we are using.

Equipment: a laptop, a circuit connecting the sensor to the Arduino, a thermometer, a refrigerator, a heat lamp

Location(s): Quyen's room

Time Required to Complete: 1 hour

Personnel: Quyen Ong

Success Criteria: The sensor will succeed if the sensor measurements are within ±1℃ of the thermometer measurements.

Data: The data will consist of the measurements from the sensor and the thermometer for comparison.

Procedure: The temperature will be taken using the thermometer and the temperature sensor at normal room temperature, in a refrigerator, and under a heat lamp. The sensor and thermometer will be left in each location for 10 minutes before measurements are recorded.

Appendix N: CO_2 Sensor Accuracy Testing
Objective: The objective of this test is to test the accuracy of the CO_2 sensor we are using.
Equipment: a laptop, a circuit connecting the sensor to the Arduino, a CO_2 monitor, a box, dry ice
Location(s): Cruz Plaza, Fabrication lab on the 3rd floor of Willet
Time Required to Complete: 2 hours
Personnel: Quyen Ong
Success Criteria: The sensor will succeed if the sensor measurements are within ±100 ppm of the monitor's measurements.
Data: The data will consist of the measurements from the sensor and the monitor for comparison.
Procedure: To get a box of CO_2, dry ice will be left in the box for five minutes before the sensor and monitors are placed in the box to take measurements. The measurements will be taken on Cruz Plaza, in the Fabrication lab, and in a box full of CO_2. The sensor and monitor will be left at each location for 10 minutes before measurements are recorded.

Appendix O: Temperature Sensor Range Testing

Objective: The objective of this test is to test the range of the temperature sensor we are using.

Equipment: a laptop, a circuit connecting the sensor to the Arduino, a thermometer, a refrigerator, a heat lamp

Location(s): Quyen's room

Time Required to Complete: 1 hour

Personnel: Quyen Ong

Success Criteria: The sensor will succeed if the sensor measurements are able to measure the different temperatures.

Data: The data will consist of the measurements from the sensor and the thermometer for comparison.

Procedure: The temperature will be taken using the thermometer and the temperature sensor at normal room temperature, in a refrigerator, and under a heat lamp. The sensor and thermometer will be left in each location for 10 minutes before measurements are recorded.

Appendix P: CO_2 Sensor Range Testing
Objective: The objective of this test is to test the range of the CO_2 sensor we are using.
Equipment: a laptop, a circuit connecting the sensor to the Arduino, a CO_2 monitor, a box, dry ice
Location(s): Cruz Plaza, Fabrication lab on the 3rd floor of Willet
Time Required to Complete: 2 hours
Personnel: Quyen Ong
Success Criteria: The sensor will succeed if the sensor measurements are able to measure the different CO_2 levels.
Data: The data will consist of the measurements from the sensor and the monitor for comparison.
Procedure: To get a box of CO_2, dry ice will be left in the box for five minutes before the sensor and monitors are placed in the box to take measurements. The measurements will be taken on Cruz Plaza, in the Fabrication lab, and in a box full of CO_2. The sensor and monitor will be left at each location for 10 minutes before measurements are recorded.

Appendix Q: Website Testing
Objective: The objective of this test is to ensure the website is operating properly.
Equipment: Three different devices, the bioreactor
Location(s): Fabrication lab on the 3rd floor of Willet
Time Required to Complete: Approximately an hour
Personnel: Quyen Ong
Success Criteria: The website is displaying the temperature and CO_2 levels. The pump turns on when the button on the website is pressed.
Data: The information collected will be either a yes or a no for whether the website displays the sensor measurements and turns on the pump.
Procedure: The website will be accessed on each of the three devices. The website will be observed to see if the sensor measurements are displayed correctly and the pump button will be pressed to see if the pump turns on.

Appendix R: Material Properties Testing

Objective: The Objective of this test is to evaluate the selected materials properties before and after autoclaving.

Equipment: Material Samples (2mm thick), tensile tester

Location(s): Science and Engineering Building

Time Required to Complete: approximately 1 week including print time and test time

Personnel: Kendra Santillan

Success Criteria: This test will evaluate the several material properties such as Ultimate tensile strength before and after autoclaving selected material. The goal is to note if there is a significant difference in selected material properties before and after autoclaving.

Data: The data collected will be based on ultimate tensile strength.

Procedure: First a total of 10 dog ones that are 2mm thick of the selected material will be printed. 5 of the dogbones will be autoclaved while the other 5 are not. All of the samples printed will then be tensile tested to compare effects of the 15 minute gravity cycle.

Appendix S: Flow Adjustment
Objective: Adjust the flow rate from the pump to produce ideal submersion.
Equipment: An accurate timer, a ready-printed bioreactor (full assembly not required), media supply
Location(s): Engineering Building (EGC)
Time Required to Complete: 4 Weeks
Personnel: Carlie Ezell
Success Criteria: Total submersion time must be within 5-10 minutes
Data: Detention time, drainage time, submersion time, media flow or pump rate, pump voltage
Procedure: Adjust pump voltage and pump flow rate so that the total residence time of the media within the drain system is within 5-10 minutes. The pump flow rate based on the voltage will be measured. At each test setting the timer will be run three times. All timers will be started when the pump is turned on. The first timer will be stopped when the drain fills up. This is the pump time. The second timer will be stopped when the drain empties completely. This is the detention time. The third timer will be stopped when the drain empties halfway. This is the submersion time. The first two timers are for data collection. The third timer is for the success criteria. If the time is not ideal, the pump voltage or pump rate will be adjusted and the test will be re-run.

Appendix T: Verify Plant Growth

Objective: Use the bioreactor to grow a plant and record comparative growth data. Answer whether the bioreactor affects growth.

Equipment: A ready-printed bioreactor assembly, media supply

Location(s): Engineering Building (EGC)

Time Required to Complete: 4 weeks

Personnel: Carlie Ezell

Success Criteria: The growth levels of bioreactor plants are equal to or better than those grown in soil under the same environmental conditions.

Data: Propagate height, propagate “leaf” count

Procedure: Insert one propagate into the bioreactor and one propagate into soil. When the pump interval is turned on, water the soiled plant. Each day, record the height of the propagated plant above the full media level (in millimeters). Each day, record the number of leaflets. Use the number of leaves as a health indicator and the height as a nutrition indicator. Compare data from the two plants. Create growth curves.