DHF 1: Background and Mark 1

UBC-V iGEM 2024

Project: NuCloud

Arduino Microcontroller Bioreactor

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1.0 Introduction

1.1 Background

The International Genetically Engineered Machine (iGEM) competition has been running since 2003 with a central focus on synthetic biology. Teams utilize synthetic biology to produce unprecedented solutions and innovations that help to shape and advance science and research [1]. An essential part of synthetic biology is designing, culturing, and producing cells and bacteria. This is an expensive, time-consuming, and complex process as culturing cells can take up to several weeks to yield results [2][6]. The cost of this for small labs and student teams can quickly deplete budgets as reagents or unexpected expenses can quickly accumulate upwards of several hundreds of dollars to a few thousand varying with the quantity and type [3][4][5]. The goal of this bioreactor project is to produce and design a small (> 1 L) affordable bioreactor for the purpose of culturing cells, specifically Escherichia coli or E. coli.

1.2 Needs

A suitable bioreactor contains several components and has a multitude of functions, all to enable the precise control of the environment inside. Since organic matter is sensitive to the environment, a certain degree of precision in control and monitoring is required to successfully culture bacteria and cells. Some factors that could require monitoring and control can include the following:

- Temperature
- pH/acidity
- Oxygen levels/Aeration
- Sustenance amounts
- Pressure
- Agitation/Mixing
- Biomass
- Volume

In light of the limited time given to complete this bioreactor, media control will be handled manually and controlled and monitored by technicians. In addition, light levels and pressure are not a primary growth factor in E. coli growth, and thus are outside of this project's scope.

Table 1: List of needs' description and plans

Need #	Description	Metrics and Methods:
1	Temperature: keeping the temperature stable is important to maintain cell function and health	Monitored by Arduino thermometer, kept in 30 deg C room Temperature will be updated at a minimum frequency of 1 minute
2	Acidity: Maintaining pH levels is another factor in cell health	Media will contain a buffer and pH probe will be added if needed pH is not expected to change significantly
3	Aeration: Oxygen levels evenly distributed	E. coli can grow in both aerobic and

	around the bioreactor helps to promote cell growth	anaerobic conditions, but to keep growth consistent, O2 will be added via fans/pumps Bubble rate will be consistent and adjusted to the growth curve optimization
4	Biomass: Measuring the amount of biomass is important to determine whether growth is occurring and what conditions can optimize the curve	Optical density sensor will be added to the clear walls of the bioreactor Sensor should be able to measure biomass with a maximum error of ±25%
5	Agitation: Mixing/agitating is a key function of the bioreactor that helps keep the components thoroughly mixed and evenly distributed	Serological pipette pumps will mix via displacement This can be tested qualitatively with particles and the media should assume a homogeneous mixture within a minute.
6	Volume: The bioreactor should be able to hold a certain amount of media, cell culture and space for all components to function appropriately.	The volume will be decided by wet lab team members and is estimated to be 100-200 mL. The container will be a flask that is commonly available in labs and online. Mk.1's vessel is mentioned in Section 3.0.
7	User Interface: The bioreactor should be able to have various parameters adjusted in real time via a physical device that can regulate oxygen, agitation, and temperature levels.	The interface must have a display capable of showing the various parameters and their values. Interfaces should have signals that let the user know what features/functions are active/inactive.

2.0 Objectives

2.1 Goals

The goal is to culture E. Coli and produce consistent control and monitoring mechanisms for the bioreactor they will be produced in. The Mark 1 Bioreactor has passive oxygen inflow and outflow (filtered). A fan will also be attached externally to pump air into the vessel.

2.2 Timelines

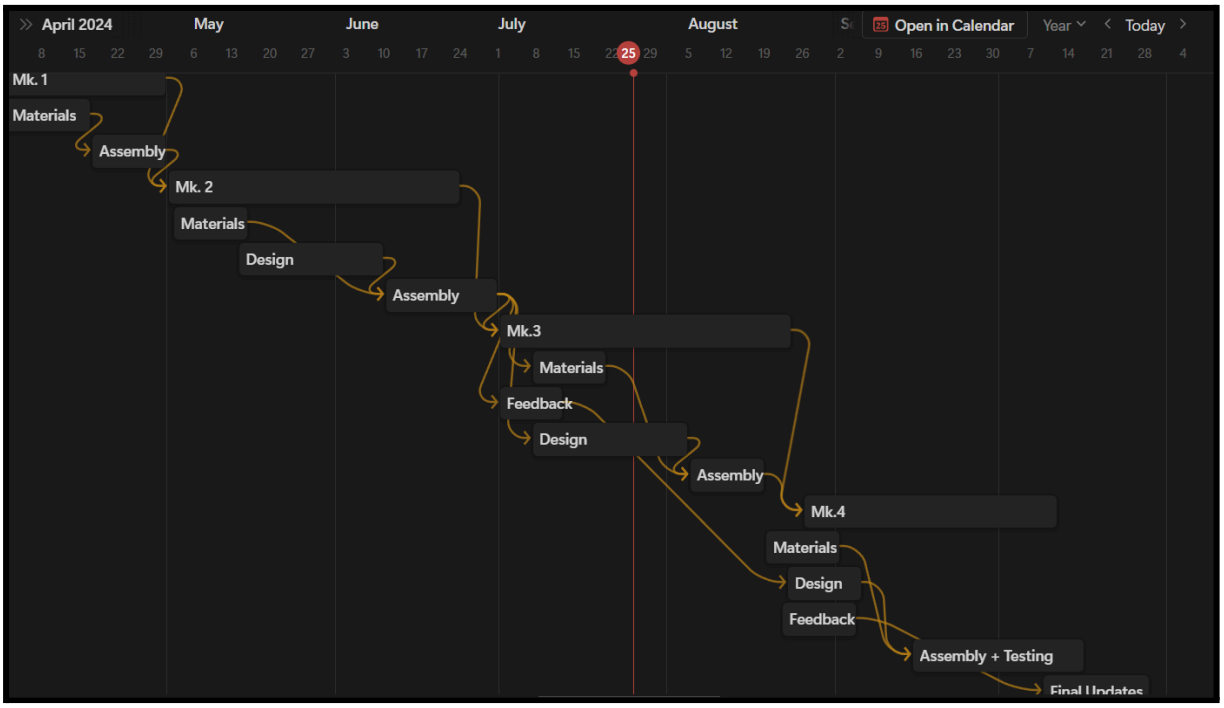

Figure 1: Gantt chart timeline as of July 25th

3.0 Design

After purchasing a 2 L, clear, plastic, auto-clavable vessel, we designed custom accessories for airflow and attached air filters. The material we chose for these parts was polycarbonate, which was used to 3D print these parts. Polycarbonate was chosen for its durability, relatively high thermal stability during autoclaves, and transparency.

3.1 CAD files for initial bioreactor vessel (2 L) [7]

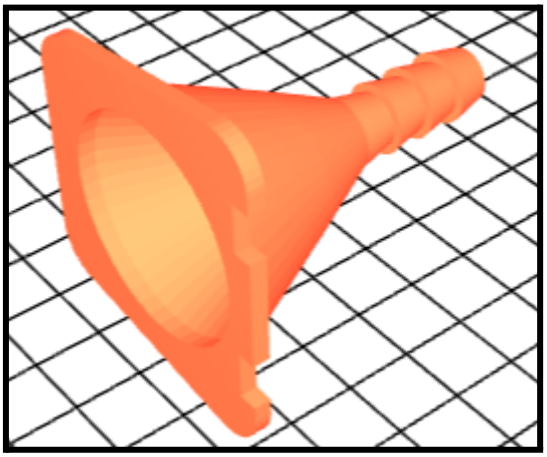

Figure 2: Fan air outflow nozzle

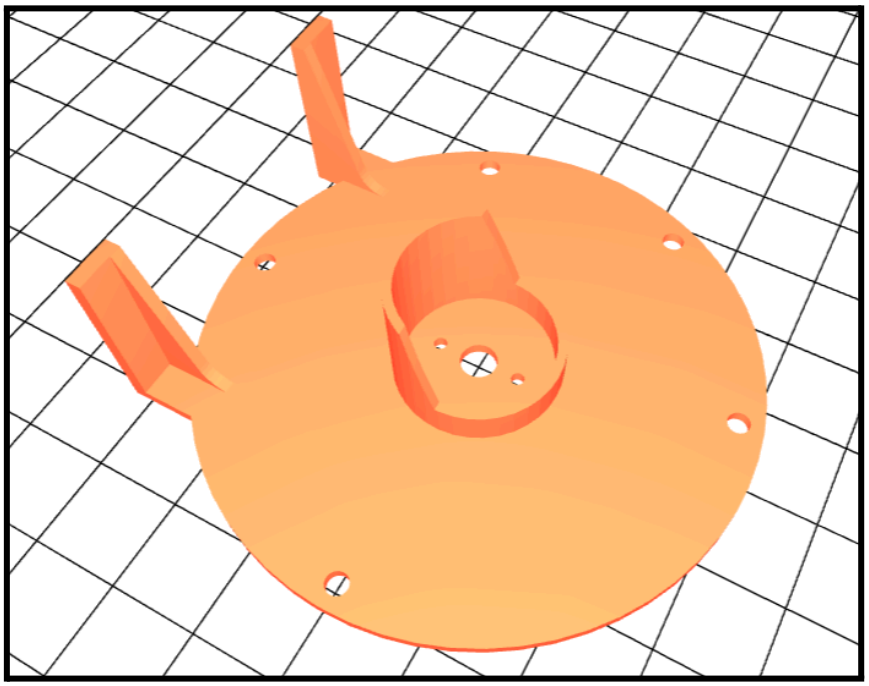

Figure 3: Back of Fan casing with slot for DC Motor

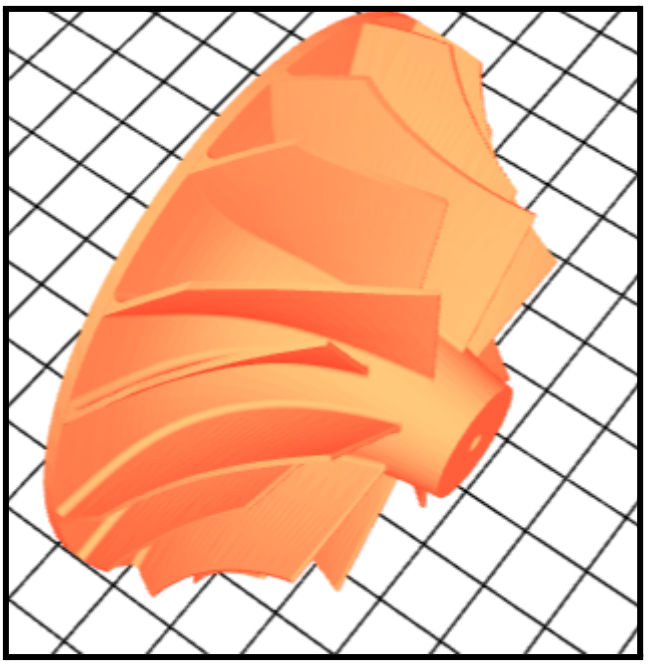

Figure 4: Fan blades and rotor

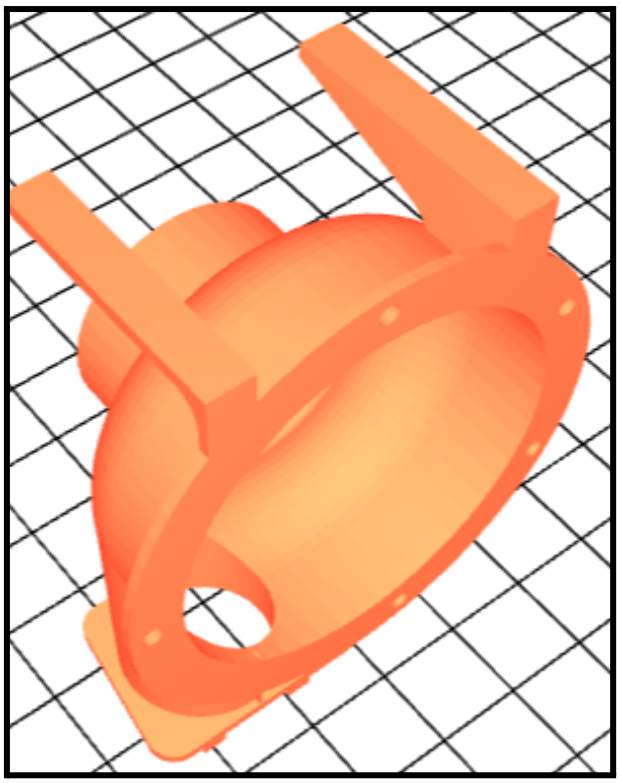

Figure 5: Front casing for fan

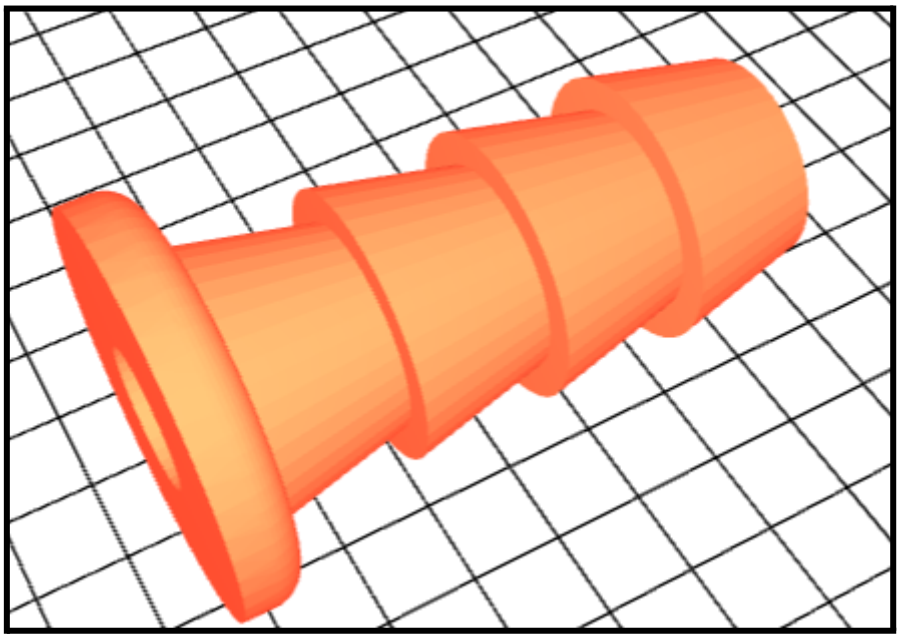

Figure 6: Nozzle (portable) for tubing or airflow

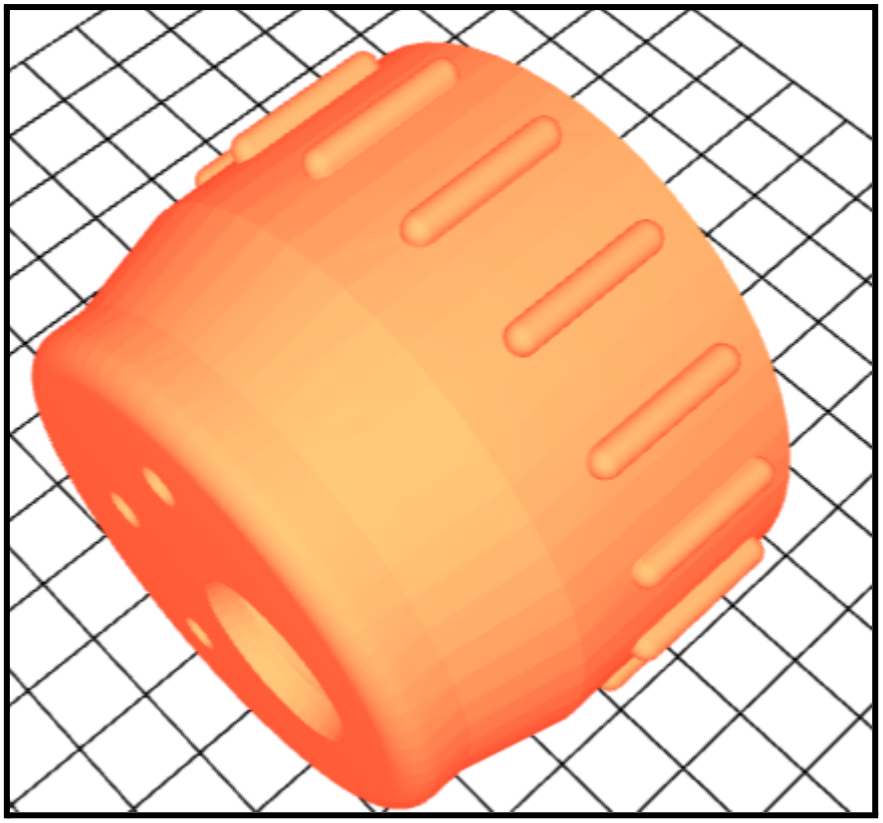

Figure 7: Lid for 2 L vessel with 3 slots for nozzles and 1 for excess

3.2 Circuit Design

To build the circuit, we first tested the motor on its rated 3-12 V with a current of 1 A and determined what voltage would be sufficient to produce steady bubbling. Since the motor would be battery-powered, 9 V and 1 A was tested first and was found to produce the desired bubbling rate of 2 per second at a depth of 200 mL.

The following figure is a design of a simple circuit used to power the fan on Mk. 1. It consists of a 9V battery with a toggle switch to turn on and off the fan. The battery connection was constructed with a commercial battery clip and crimped into a Dupont connector. It is placed in series with the switch and the motor.

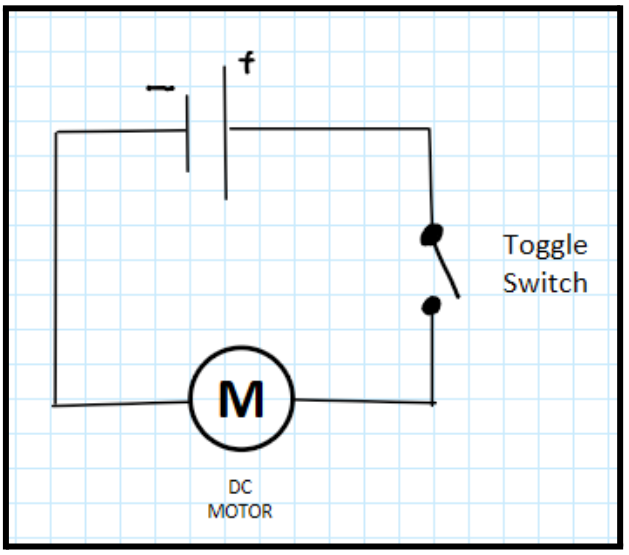

Figure 8: Simple circuit design for Mk. 1 using a 9 V battery

4.0 Validation

To ensure that this entire project was not a complete waste of time, the bioreactor needs to be tested against traditional methods of culturing E. coli and produce them more efficiently. To do that, experiments were run with the mk.1 vessel, using the fan and circuit shown previously. E. coli was cultured in the same media in both the bioreactor and a flask in the same 37 degree Celsius room. The only differences were the agitation method and oxygen delivery which was a stir bar and fan for the bioreactor and lab shaker and no active oxygen delivery for the flask.

4.1 Mk.1 Trials [8]

For trial 1: Optical density (OD) was the dependent variable and was measured as a quantity over time at a wavelength of 600 nm. Samples were taken manually via pipetting from both sources and passed through an OD sensor. The results of the experiment are presented in the following table:

Table 2: OD sensor readings

Time	Bioreactor	Control
21:05:00	0.033000	0.033000
21:25:00	0.037333	0.041333
21:45:00	0.073667	0.067333
22:05:00	0.158000	0.144000
22:00:00	0.399333	0.343000
22:20:00	0.718667	0.596333
22:40:00	1.196333	0.893333

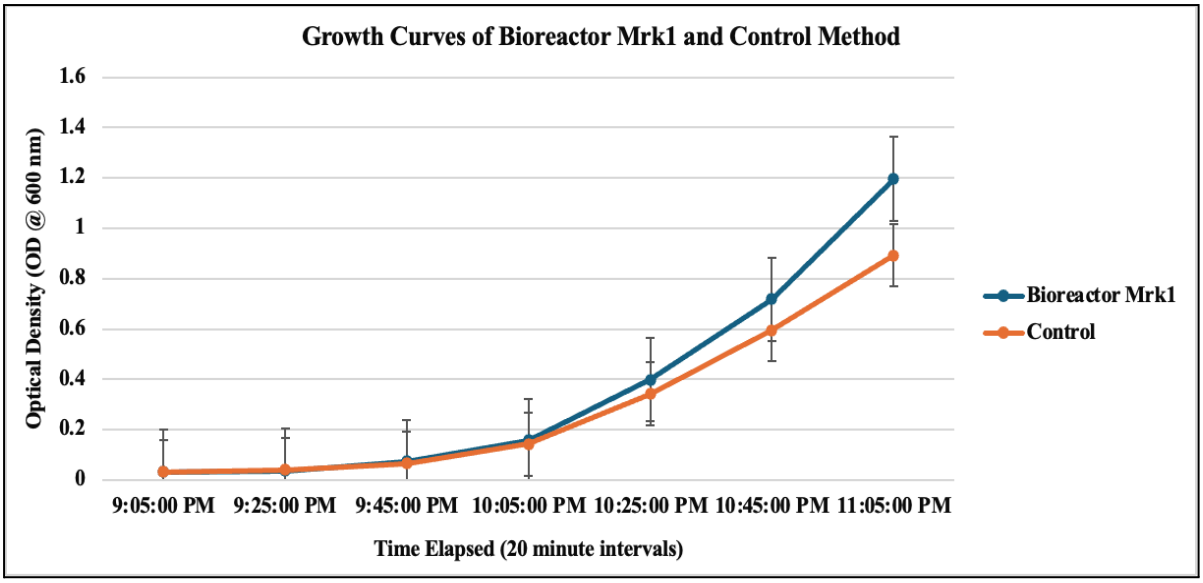

Figure 9: Trial 1 growth curve comparison between Mk.1 and traditional flask

Appendix

[1]

'iGEM'. Accessed: Jul. 18, 2024. [Online]. Available: https://igem.org

[2]

'Bioreactor - an overview | ScienceDirect Topics'. Accessed: Jul. 18, 2024. [Online]. Available: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/bioreactor

[3]

B. Sargent, 'The True Cost of Cell Culture', Cell Culture Dish. Accessed: Jul. 18, 2024. [Online]. Available: https://cellculturedish.com/the-true-cost-of-cell-culture/

[4]

'Pricing'. Accessed: Jul. 18, 2024. [Online]. Available: https://www.coriell.org/1/NIA/How-to-Order/Pricing

[5]

'Cell Media Facility Cost of Services | School of Chemical Sciences | Illinois'. Accessed: Jul. 18, 2024. [Online]. Available: https://scs.illinois.edu/resources/cores-scs-research-and-service-facilities/cell-media-facility/cell-media-facility-cost

[6]

'Isolating Cells and Growing Them in Culture - Molecular Biology of the Cell - NCBI Bookshelf'. Accessed: Jul. 18, 2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK26851/

[7]

'Pattarin Blanchard / Bioreactor · GitLab', GitLab. Accessed: Jul. 25, 2024. [Online]. Available: https://gitlab.igem.org/pblan/bioreactor

[8]

UBC Vancouver iGEM, "2024.09.10 - Mk. 1 Bioreactor Growth Rate Measurements (Trial 1)" iGEM, 2024. [Online]. Available: https://2023.igem.wiki/ubc-vancouver/.

Inventory:

Name	Use/Description	Quantity of items	Additional Info
2L Polycarbonate Vessel	Primary vessel for cell culture	1	Transparent, 2L volume
ID tubing	air Filtering/pumping	1	4 meters, 1/4" in, "likely not silicone"
Arduino	CPU for readout + measurement	1	provided by SBME?
Stir rod + hotplate	Mk.1 mixing and temp control	1	provided by Hallam lab?
Peristaltic Pump	O2/fluid cycling	4	Source
Air fan/blower	gas cycling	1	.sldprt file available for reprint
53B Closure (lid?)	isolating bioreactor system	1	standard part - specs available - 2 ports
Custom 53B closure		1	.sldprt file available for reprint
O-rings pack		1	
carbonation stone	aeration	2	Source
280 DC motors	blower motor	5	Source
inline air filters	O2 filtering	4	Source
permatex RTV silicone		1	
1.75mm polymaker PC filament		750 g	Source