Contents

1 Appendix

1.1 Impedance

1.1.1 Summary

The impedance probe sub-task goal was to research the best settings to have the optimal readings of the solution's impedance, and consequently quantify cell growth. We begin by defining the position of the driving and sensing electrodes, followed by experimentally determining the frequency at which we can optimally monitor the cells' growth from minimum to maximum concentrations.

Within the frequency band of 1 to 20 kHz there is no concern for cell disruption, and ultimately we found the value of 4 kHz to give the highest output voltages. Through successive dilutions, we produced a calibration curve against which we could compare the output voltages during the 24h run. Unfortunately, due to a series of issues surrounding the precarious probe setup, the results collected during the 24h run could not be compared to our calibration curve in a meaningful way.

1.1.2 Introduction

The aim of the experiment is to establish a calibration curve against which we can track and compare the progress of our bioreactor during the 24h run. We do so by defining the appropriate frequency for best potential difference output on a phosphate buffer solution, as well as a 20 g/l yeast solution. Finally, we produce the calibration curve by analysing the potential difference for a series of yeast solution dilutions.

1.1.3 Background knowledge

Impedance spectroscopy is a widely used modality to establish cell composition, diagnosis of lymphatic edema and, most importantly for our purposes, quantify biological cell suspensions [5, 7]. Alternatively optical density can be used, which we in fact do use to verify our results.

Impedance (Z) is effectively resistance in an AC electrical circuit, which means that it follows Ohm's Law. Furthermore, we can represent a cell in extracellular liquid as capacitor in a electrical circuit, which allows us to use an important definition from AC theory; where V is potential difference and I is current, C is capacitance, f is the frequency, j is the imaginary unit:

$$V = ZI \qquad\qquad Z_c = \frac{-j}{2\pi f C} \tag{1}$$

In practice, we cannot determine C, but by combining both equations, we know the following relationship

$$V_{cell} \propto \frac{1}{f} \tag{2}$$

Which tells us that as we increase the function generator's frequency, the potential difference "lost" to the cells is reduced, and therefore, the potential that we measure in the extracellular liquid is higher.

$$V_{liquid} \propto f \tag{3}$$

1.1.4 Materials and Methods

1.1.5 Materials

- Combined amplifier, filter and driving circuit
- Function generator and oscilloscope. NI myDAQ used for both.
- Phosphate buffer solution (PBS)

- Activated yeast (*Saccharomyces cerevisiae*), a 20g/l and a 50 g/l solution.

1.1.6 Methods

1. Attach the probes to the cup, filled with 300 ml of each solution at a time.
2. For each solution measure the voltage output over the frequency range from 1 Hz to 20 kHz, using different gain ratios. Use this to establish the appropriate amplification.
3. Sweep the frequency range stated in step 2 by keeping function generator's peak-to-peak voltage input fixed at 10 V.
4. Finally, we generate the calibration curve using a 50g/l yeast solution, and repeating step 3 for a series of consecutive dilutions (expressed as % from the original): 100%, 20%, 10%, 4%, 2%, 1%.

Variables

- Control:
  - Output voltage on function generator: 10V.
  - Probes 180º from each other. Sense leads 30º from the probe and 180º from each other. See Figure 1.
  - Amplifier gain $\frac{15k}{150} = 100$.
  - Solution volume: 300 ml
- Independent: Frequency.
- Dependent: Potential difference

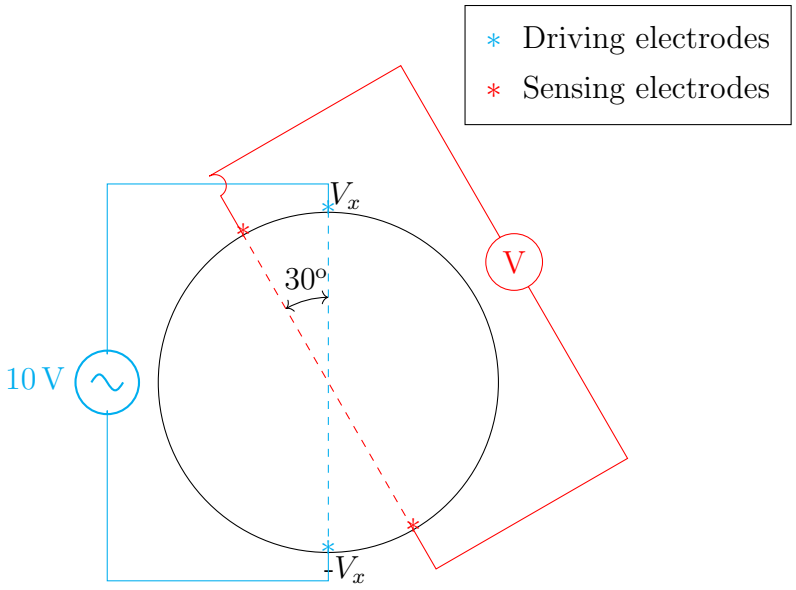


Figure 1: Orientation of electrodes

Considerations Due to a limited power input into the amplifier, a gain ratio of 1000 led to *gain saturation*, where we no longer observed the expected amplification. With gain of 10, the maxima appeared in different frequencies as to those of 100 and 1000, though we are unsure why this would happen.

Although we defined probe orientation, there are two factors we are disregarding: the cup is not perfectly cylindrical, and the leads are not straight – so the distance between electrodes is smaller than what the experimental setup implies.

1.1.7 Results and Discussion

Because there is no concern for cell disruption at the range of frequencies we are analysing (Yerworth, R., 2019 November 28), we can use Figure 2, to find the maximum amplitude. PBS is around 3 kHz, and the yeast solution, 5 kHz. We expect to see equally as reasonable results by picking a simple average of the two, 4 kHz.

Originally it was expected that the impeller would not affect our results, but during the 24h run, we saw that the magnetism that drives the impeller had a very large effect on the numbers seen. It is reasonable to take measurements while the impeller is in full use – it is

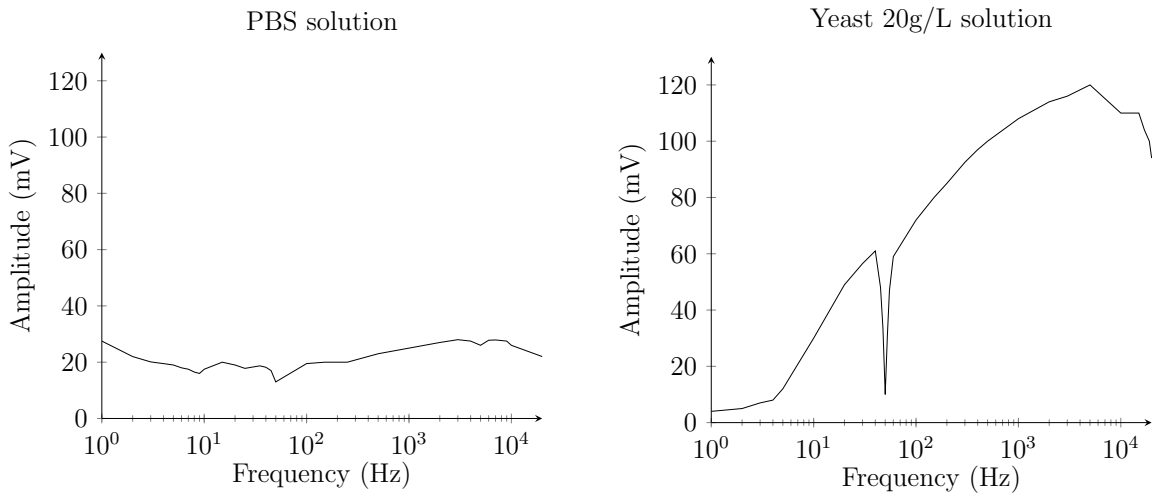


Figure 2: Amplitude based on frequency. Note the dip at 50 Hz due to the filter.

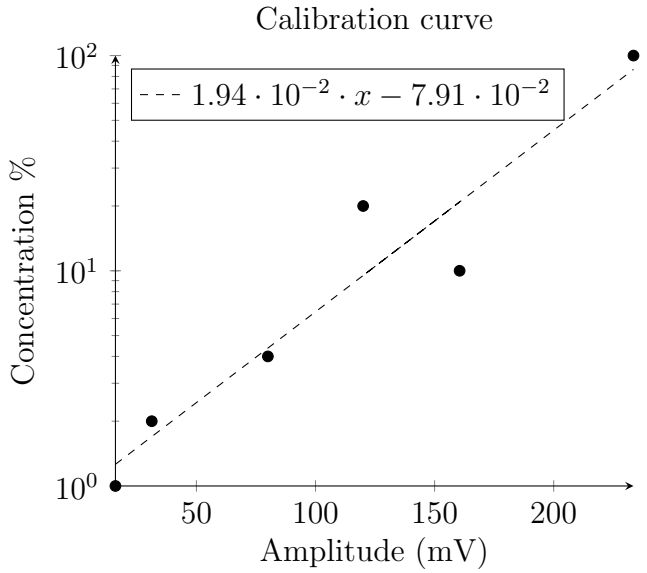


Figure 3:

simply something to be account for in scaling up this project. Furthermore, as can be seen in Graph 4, the very first reading of the 24h run was 400 mV, far greater than any result we observed during calibration. We suspect a few issues in addition to those described in section 1.1.6: Some deterioration of the tape that held the electrodes in place, as we produced the serial dilutions may have contributed to them floating in the liquid – adding random error to our data. This was exacerbated by having to stir the solutions by agitating the cup, further displacing the electrodes.

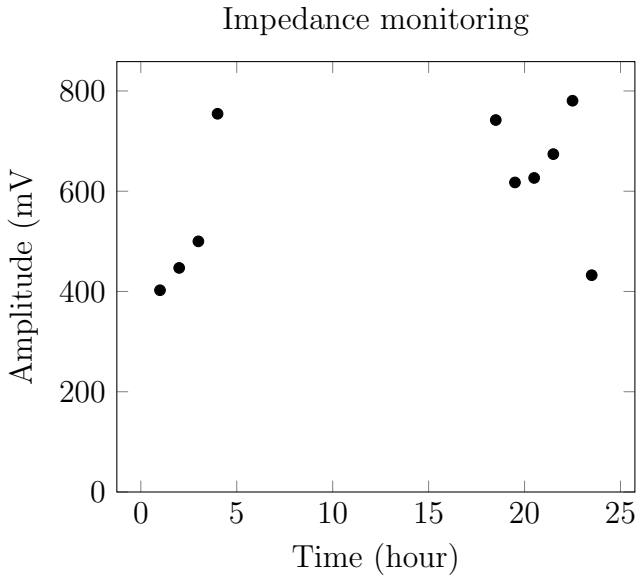


Figure 4: Impedance monitoring during 24h run. The large jumps in numbers are due to accidental movement of the probes, invalidating the results.

The 24 h setup was made ex novo, with the stirring and heating built in, and upon arrival there was a probe completely dislodged – possibly it was bumped against while it was set up. No corrections were allowed, so adjusting the values to be in line with our calibration curve was not a possibility; and neither could we see through the dark liquid to adjust orientation. During the run, our magnetic stirrer would loosen out of its socket every few minutes, and in attempting to fix it, amplitude values ranged anywhere from 200 mV to 800 mV, completely hindering our ability to somehow normalise the data and compare to our calibration curve.

important comments on results, relate to theory and knowledge

1.1.8 Conclusion

In conclusion, in order to determine optimal frequency, we graphed the amplitude vs frequency response for the equivalent of saline, or in our case a phosphate buffer solution (PBS), and 100% concentration with 20 g/L yeast cell solution. By analysing the frequency responses for both, we arrived at the best averaged between the two, 4 kHz.

1.2 Mixing

1.2.1 Summary

This lab includes four comparative experiments to investigate the effects of rotational speed, impeller type, baffles, and number of impellers on liquid mixing time. Based on the results, a bioreactor prototype involving one Rushton Turbine and one Pitched-blade operating at 280rpm under baffled condition was proposed. This design is a scale down model for the optimization of mixing in large scale vaccine production in the Uganda vaccination project.

1.2.2 Introduction

This lab attempts to design a prototype of an optimal bioreactor at the bench scale producing yeast cells to mimic vaccine production in the Uganda tuberculosis vaccine project. An optimal bioreactor should be able to achieve liquid homogeneity under minimum liquid mixing time, $\theta_{99\%}$ which is defined in the following equation:

$$\theta_{99\%} = \frac{6.34}{N\left(\frac{D}{T}\right)^{2.3}\left(\frac{Z}{T}\right)^{-0.5}} \tag{4}$$

$\theta_{99\%}$ = blend time (corresponding to 99% uniformity)

N = rotational speed

D = impeller diameter

T = tank diameter

Z = liquid level

According to equation (4), we can analyze the effects of rotational speed and impeller diameter on liquid mixing time, given that the tank diameter and liquid level remain unchanged throughout the lab.

1. Rotational speed is inversely proportional to mixing time, as shown below

$$\theta_{99\%} \propto \frac{1}{N} \tag{5}$$

   Which means that a higher rotational speed will result in a shorter mixing time. However, this relationship is only valid until a certain point due to the formation of vortex which would increase the time to achieve homogeneity, thus would result in a longer mixing time. Therefore, we are conducting this experiment to find out the optimal speed which gives the least liquid mixing time.

2. Impeller diameter is inversely proportional to mixing time, as shown below

$$\theta_{99\%} \propto \frac{1}{N} \tag{6}$$

   Pitched-blade has the largest diameter (4.0 cm) compared to Rushton impeller (2.5cm) and paddle impeller (2.3cm). Therefore, pitched-blade would achieve the least mixing time among all three types of impellers. Verifying this hypothesis would be vital for our prototype design as it will help us to determine other variables such as the liquid level which is affected by impeller diameter.

In order to optimize the rate of mixing, the distance between the bottom of the reactor and the impeller should equal the diameter of the impeller [2]. Considering multiple impellers might be used, the liquid level should be above the top impeller without jeopardizing the aeration space. Since we do not have the equipment to sparge air into the liquid media, we will use the top impeller to create a disturbance on the surface, allowing oxygen to cross the barrier. Aeration system is important as healthy and stable cells can be produced hence maximizing the production [5, p.245].

On the other hand, eliminating vortex is crucial. Vortex is the swirling of fluid that disrupts creating a homogenous mix. With the help of a four-petal baffles (obstructing

panels) that are mounted vertically against the wall of the reactor, gross vortexing and swirling of the liquid can be reduced hence allowing us to achieve homogeneity of liquid within a shorter period of time.

1.2.3 Materials and Methods

The following lab materials are provided:

1. 2 plastic cups
2. 6 baffles: 2 Rushton, 2 Pitched-blade, 2 Paddle
3. Magnetic Stirrer and plate
4. Shaft and lid
5. Blue dye

Four comparative experiments were designed to investigate the optimal bioreactor prototype by considering the following aspects:

1. Which one of the three types of impellers is the most efficient? In this experiment, individual impellers of each type were assessed on its mixing time over various rotational speed, starting from 60rpm. For each impeller, mixing time is plotted against rotational speed. The rotational speed giving the least time is the optimal rotational speed. The impeller that achieves mixing under the least time and least rotational speed is the optimal choice.
2. Do baffles assist mixing? This element was tested using Rushton under baffled versus unbaffled conditions. To test whether baffles are helpful, both conditions are tested under the optimal rotational speed, 280rpm. Whichever condition with the less mixing time is the preferred.

3. Do multiple impellers work better than a one? 2 Rushton turbines were tested for rotational speeds 260rpm, 280rpm, and 300rpm. The mixing time under each speed is compared to that for 1 Rushton turbine.

4. If using two impellers, which combination is the most efficient? From experiment one, the least efficient impeller can be eliminated. Thus, in this experiment, different combinations of the most efficient impeller types will be assessed for various rational speed.

Procedures of the experiments are described in detail as follows:

1. Assemble the impeller of choice with the shaft, stirrer, and mixing vessel.

2. Add 300mL of water to the vessel.

3. Put the vessel on the magnetic plate.

4. Adjust the rotational speed to the one of choice and wait until the number blinks on the display panel suggesting that the desired speed is achieved.

5. Add 5 drops of blue dye at 2cm from the top of the cup to the water. Must keep the height at 2cm each time to ensure consistency throughout the experiment.

6. Start the timer when the first drop is added; stop the timer when the color is evenly distributed in water.

7. Repeat 1-6 for different settings including different types of impellers, various rotational speeds, baffled versus unbaffled conditions, and different numbers of impellers. Specific set up is determined by the four comparative experiments mentioned above.

1.2.4 Results and Discussion

Data from the four experiments were recorded and plotted in figures. The results were analyzed to propose mixing settings.

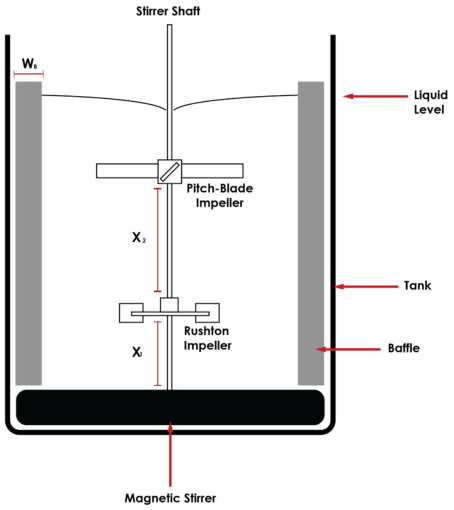


Figure 5:

According to Figure 6, the minimum of each curve indicates the least mixing time for each impeller. Overall, Paddle's mixing time is longer than that of Pitched-blade or Rushton for the various rotational speed tested. Thus, paddle has been eliminated from our choices of impellers. Comparing the curves of Pitched-blade to Rushton turbine, Pitch-blade achieves mixing under less time for all rotational speeds. This observation is consistent with our hypothesis that Pitched-blade would give the smallest mixing time due to its large impeller diameter. Both Pitched-blade and Rushton reach minimum mixing time at 280rpm, indicating that 280 is the optimal rotational speed that will be used in the final set up.

According to Figure 7, 2 Rushton turbines take longer time to achieve homogeneity than 1 Rushton turbine. This result differs from our expectation that 2 impellers would be more effective. One explanation could be that when more impellers are used, larger vortex is generated, and since this experiment is conducted under unbaffled condition, vortex would hinder the mixing, hence slow down mixing time.

Figure 8 shows that when 2 impellers are used, the combination of 1 Rushton and 1 Pitched-blade is the most ideal since is achieves a homogeneous solution in the shortest amount of time. It is distinctive that the liquid mixing time of this combination is less than that of any other settings in the previous experiments. This figure also reassures that

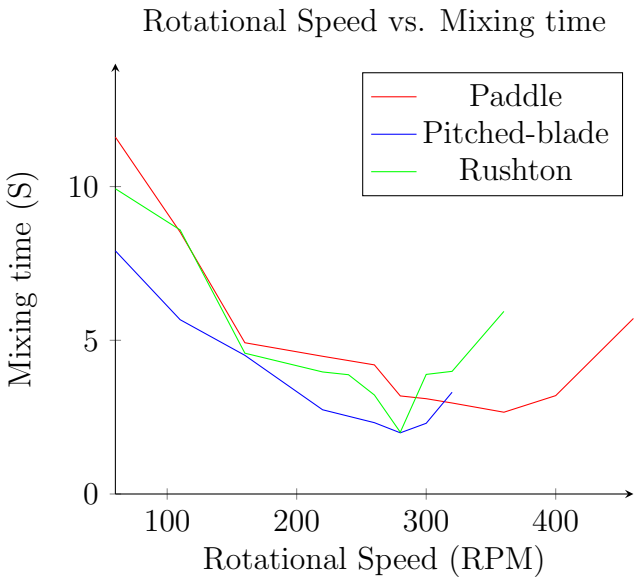


Figure 6: Rotational speed against liquid mixing time for paddle, pitched-blade, and Rushton turbine.

280rpm is the optimal rotational speed because both curves reach minimum mixing time at 280rpm.

Figure 9 demonstrates that baffles help reducing mixing time as the baffled setting achieves homogeneity in 1.42 seconds whereas it is 1.48 for the unbaffled.

The design and dimensions of our bioreactor prototype as well as impeller layout are discussed below.

1. Mixing vessel (bench-scale reactor)
    - Upper diameter = 10cm
    - Bottom diameter = 6cm
    - Height = 12cm; however, we used a maximum **filling height** of 10cm to allow for the increased volume during stirring.
2. Baffle dimensions: Optimal ratio of baffle width to cup diameter is 1/10 - 1/12 [2, p.258], therefore, the width of baffle is $\frac{1}{10} \cdot 10\,\text{cm}$. We chose to account for the diameter at the top as the vortex is the greatest there. Based on real life examples, the thickness

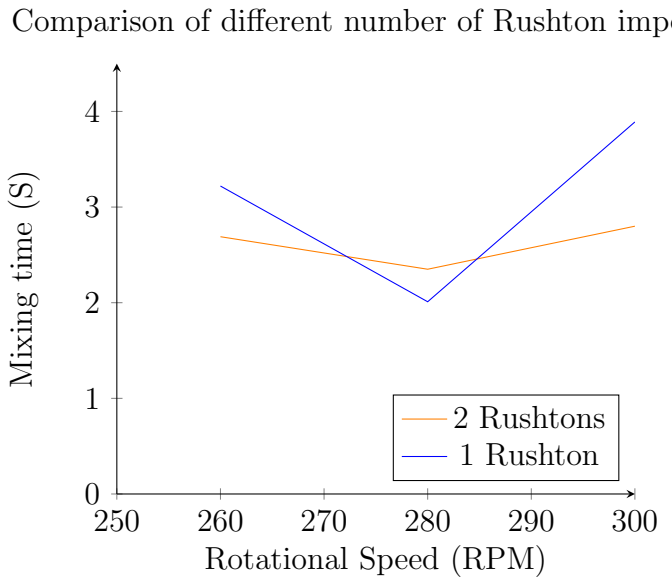


Figure 7: Rotational speed against liquid mixing time for paddle, pitched-blade, and Rushton turbine.

of a baffle is typically 1/100 the length of the baffle, which is $\frac{1}{100} \cdot 10\,\text{cm} = 1\,\text{mm}(0.1\,\text{cm})$. This is unrealistically thin for a scale-down of this magnitude hence a thickness of 0.3cm was used.

3. Impeller layout: Two impellers were used. The first, Rushton, is 2.5cm from the top of the stirrer in the cup because to achieve optimal mixing, the distance between the bottom of the tank and the impeller should equal to the diameter of the impeller which in this case is 2.5cm [2, p.286] The second impeller, Pitched-blade was placed 4.0cm away from the center of the Rushton since the distance between the two impellers should equal to the diameter of the pitched-blade, 4.0cm [2, p.286].

4. Volume of water used: The maximum height of liquid should be 1.25 times the bottom diameter of vessel which is $1.25 \cdot 6 = 7.5$cm [2, p.257]. Since the height of the magnetic stirrer is 1cm, the maximum height is $7.5 + 1 = 8.5$cm from the bottom of the vessel. This is equivalent to a liquid volume of 312mL. A liquid volume of 300ml was used because this value was a good compromise between the maximum solution volume of 315ml and the requirement of a value to easily work with calibration curves.

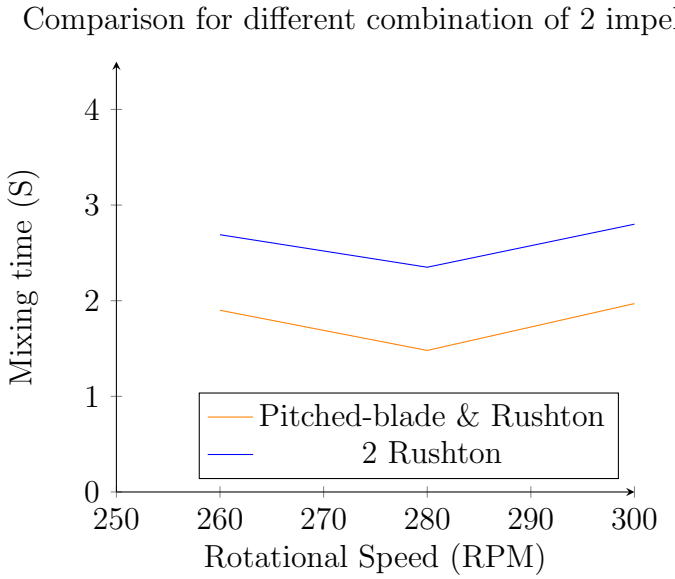


Figure 8: Comparison of 1 Rushton & 1 Pitched-blade and 2 Rushtons under unbaffled conditions

1.2.5 Conclusion

In this lab, four comparative experiments were conducted to determine the optimal bioreactor set up. The impacts of rotational speed, impeller type, baffles, and impeller numbers on liquid mixing time were explored by operating the mixing system under various conditions. Data were recorded throughout all experiments and plotted in figures. The results were consistent with our hypotheses that Pitched-blade would be the best choice due to its size, baffles would assist mixing by reducing vortex, and multiple impellers would work more efficiently than individual ones. Therefore, the optimal setting is decided to be operating the mixing equipment with 1 Rushton turbine and 1 pitched-blade at 280rpm under baffled condition.

For future work, baffles of the precise dimension could be made via 3D printing to optimize their vortex elimination function. A cylindrical vessel could be used in real culturing condition. A monitoring system should be developed to replace the manual timing and supervising of mixing. In this lab, blue dye was added above the liquid level; however, when culturing cells, the position of feed addition point should be under liquid level and should ensure optimal distribution.

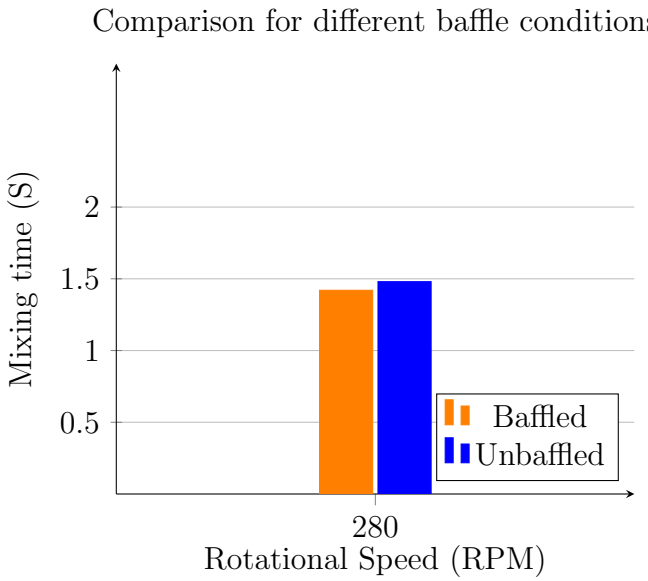


Figure 9: Liquid mixing time of Rushton turbine under baffled and unbaffled conditions at 280rpm.

1.3 Heating

1.3.1 Summary

The heating lab conducted 4 experiments to study the impacts of insulation material, mixing and hot plate temperature on maintaining a heating temperature within the optimal range for yeast cell growth. The final prototype includes using a 2cm thick cotton insulation layer and 35°C as the heating temperature. Based on the 24-hour run results, temperature was maintained between 28.5-29.5°C, suggesting the prototype to be effective.

1.3.2 Introduction

Fourier's Law of Heat conduction is given by the following equation:

$$Q^{'} = -kA\frac{dT}{dx} \tag{7}$$

where $Q^{'}$ = Heat flux (rate of heat transfer)

k = The thermal conductivity of the material to which heat is being transferred

A = Cross-sectional area of surface that heat is being transferred to

$\frac{dT}{dx}$ = Temperature gradient

Fourier's Law assumes that the system is in steady state – i.e. the temperature of the source of heat stays constant [1]. Heat flux (Q') is the rate at which heat energy is transferred between two points in the system. We can analyse that the heat flux is proportional to temperature gradient.

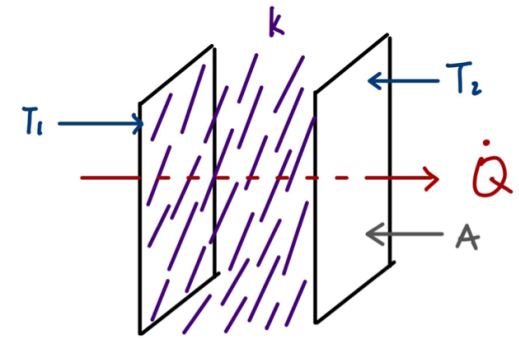


Figure 10: Fourier's equation

According to the equation, we designed 6 experiments to find out the variation of heat flux by finding the thermal gradient of each experiments. At high temperature, yeast will denature and become chemically inactive, and at low temperatures, fermentation will be inefficient. Neither will give a good yield production. Thus, the temperature of heating plate needs to be considered to maintain the temperature within the range. We aimed to design an insulation system that minimises heat loss to the surroundings while heating temperature range being kept within 28-33°C [4].

1.3.3 Materials and Methods

1.3.4 Equipment

- Hot plate
- Plastic cup
- Digital thermocouple
- Magnetic stirrer

- Cotton wool
- Aluminium foil
- Scissors
- Tape

1.3.5 Method

To find out which insulator is better out of aluminium foil and cotton, an experiment was conducted as follow:

1. Wrapped the cup with cotton jacket. Set the hot plate at 100°C with 300 cm^3 of water in the cup and measure the time taken for it to reach 33 °C.
2. Repeat step one with aluminium wrapped around the cup. To find out the effect of heat conduction, experiments were conducted as follow:
3. Mark dots at regular intervals using ruler along the bottom and side of the cup.
4. The plastic cup was then placed on the hot plate, which is set at 35°C and the temperature was measured and recorded at different positions using dots marked. No insulation and mixing method were used. Horizontal measurements taken at 0 cm, 1.5 cm and 3 cm from the side of the cup towards the centre. Vertical measurements taken at 1.5 cm, 4.5 cm and 7.5 cm from bottom to the top of the cup. All horizontal measurements were taken at a vertical distance of 7.5 cm as the impeller system was in the cup therefore could not be taken at the bottom of the cup.
5. Repeat step 4 under mixing condition using a magnetic stirrer.
6. Repeat step 5 with insulation layer around the cup using 1cm thick cotton.
7. Repeat step 5 with insulation layer around the cup using 2cm thick cotton.

Time (min)	Temperature (°C) - Cotton	Temperature (°C) - Aluminium
0	22.6	22.6
1	23.8	23.3
2	24.5	24.6
3	25.8	25.5
4	26.7	26.3
5	27.5	27.1
6	28.5	27.9
7	29.4	28.9
8	30.1	29.6
9	30.9	30.6
10	31.9	30.9
11	33	31.9
12	–	32.5
13	–	33

Table 1: Comparison between insulation methods

8. Repeat step 7 with an aluminium foil layer on top of the cup.

9. Results of the temperature at each point were recorded on tables in section 4.

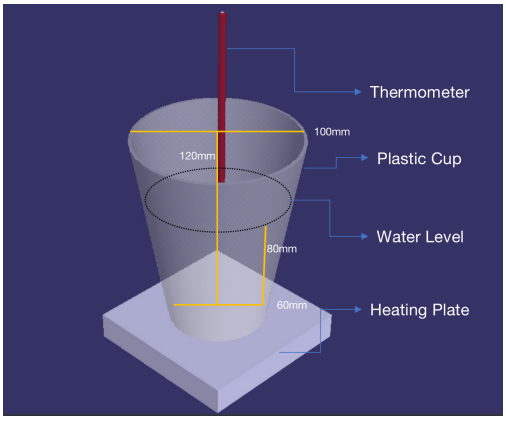


Figure 11: Apparatus setup

1.3.6 Results and Discussion

Comparing figures **??** and **??**, although not much difference in temperature change along X-direction, the temperature change along Y-direction is significant, with a range between 26.7-29.4 °C and a gradient of -0.45 under non mixing condition. In comparison to that

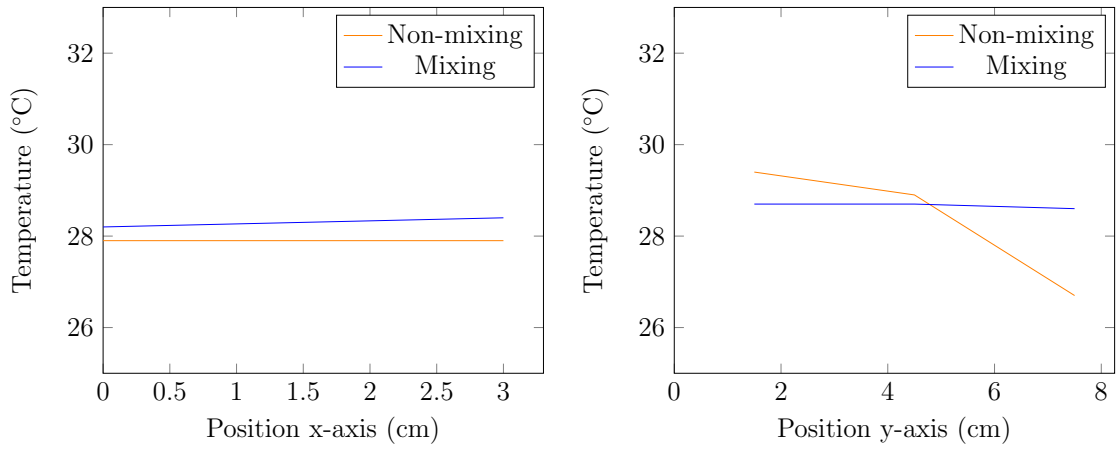


Figure 12: Non-insulated, Mixing compared to Non-mixing

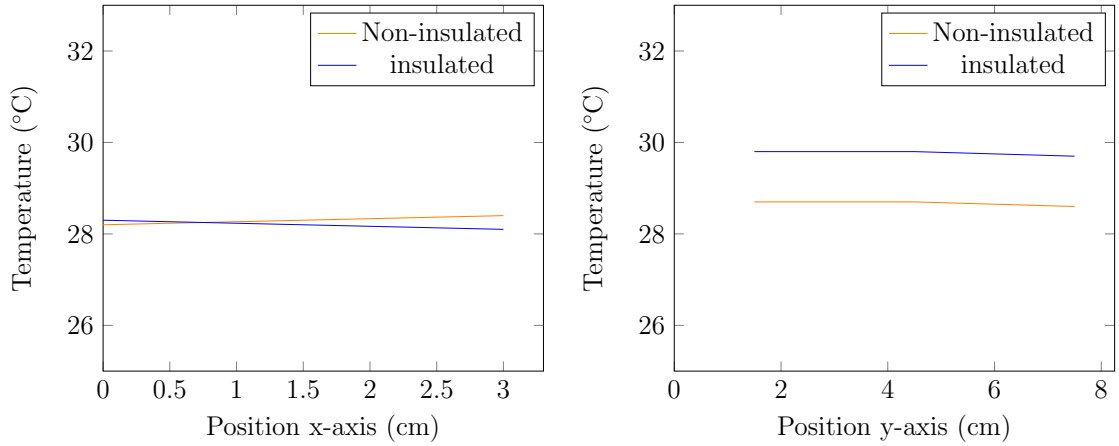


Figure 13: Mixing, Insulated compared to non-insulated

figure **??** has a much stable gradient with a value of -0.017 under mixing condition, showing that heat is being distributed evenly along both horizontal and vertical directions within the cup. Therefore, we concluded mixing condition is required and should be included in our design in order to meet our objectives of the experiment.

Comparing figure **??** and **??**, both graphs seems to have a steady gradient along both directions, as a result of uniform distribution of heat. The slight difference come from that graph **??** is shifted upwards generally in contrast to **??**. This suggest that a higher y-intercept and greater temperature of the system, due to less heat loss to the surroundings. Our desired temperature is around 30°C, as even if heat is lost or accumulated the system's temperature is still within the range of 28-33°C by allowing some small fluctuations. The graph **??** shows

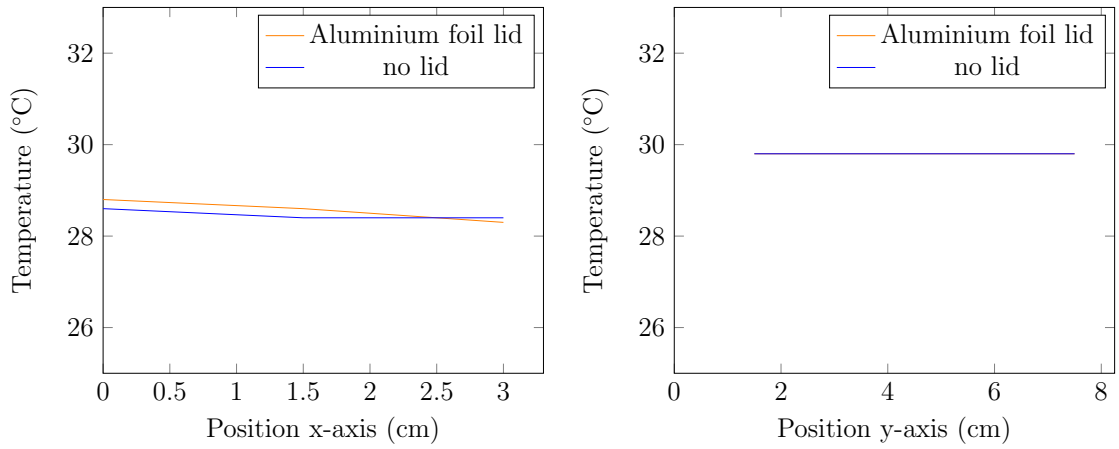


Figure 14: 2 cm thick insulation, Comparison for use of lid

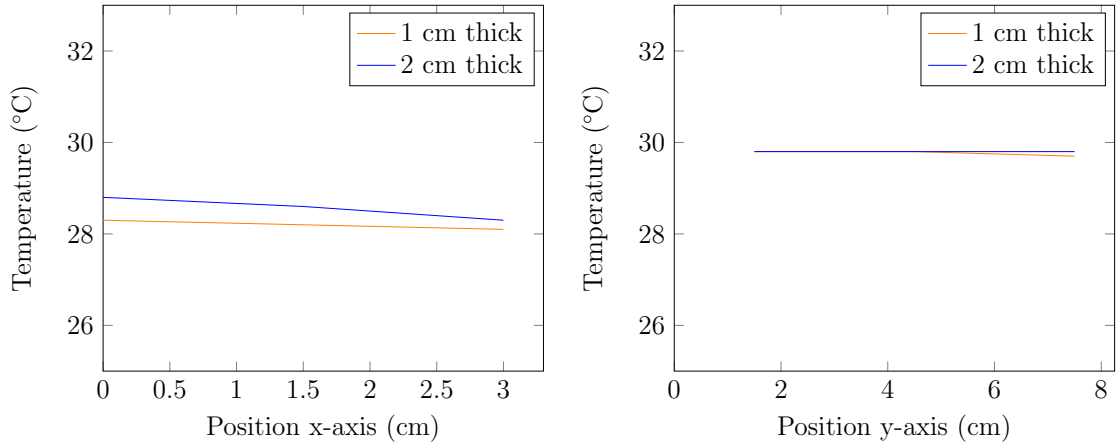


Figure 15: Mixing, Comparison for thickness of insulation

under mixing condition with insulation allows closer approach to our desired temperature, therefore we concluded that insulation is also required. Follow by that we compared **??** to **??**, where first one used 1 cm thick cotton jacket and second used 2 cm thick, the temperatures don't have much difference vertically, however horizontally there is an increase by 0.5°C, which we were then able to decide 2 cm thick cotton jacket should be used. An additional experiment was conducted with an aluminium foil layer placed on the top of the bioreactor for testing, but the effect it made on minimising heat loss was insignificant.

	Heat flux (x-direction) $\mathrm{W} \cdot 10^{-4}$	Heat flux (y-direction) $(\mathrm{W}) \cdot 10^{-4}$
1 cm thick cotton	8.48	2.12
Without insulation	-8.48	2.12

Table 2: Heat flux under different conditions

1.3.7 Calculations

Temperature recorded ranged from 28 to 30 °C, therefore we take an average between the values for 27 °C and 32 °C [8]. Furthermore, the cup is a truncated cone, and therefore its area can be calculated as:

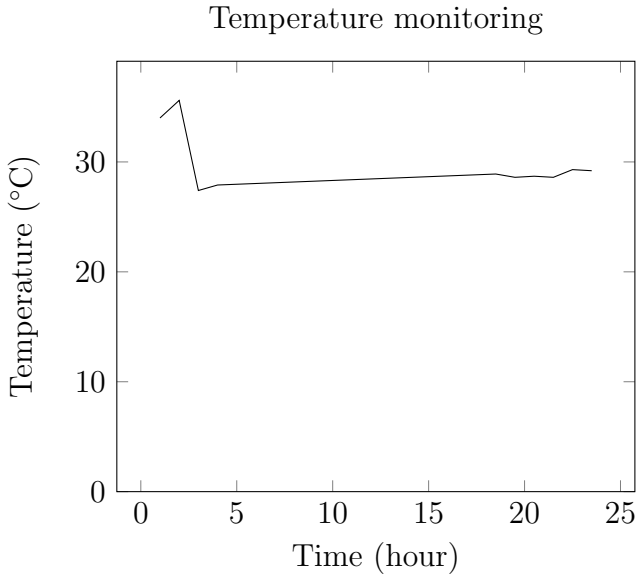
$$\begin{aligned} A &= (5+3)\pi\sqrt{(5-3)^2} + 8^2 = 0.0207\,\mathrm{m} \\ k_w &= 0.614\,\mathrm{W\,m^{-1}\,{}^{\circ}C^{-1}} \end{aligned} \tag{8}$$

Utilising Fourier's law of heat, equation (7), we calculate heat flux in each direction and summarise in Table 2.

From Table 2, it can be observed that heat induction with insulation and without insulation don't seem to have much difference here, with a magnitude of 10^{-8}. This difference may be really small, but as this prototype is a lab scale of the bioreactor, the difference in an industry size reactor can be huge therefore cannot be ignored. We can conclude that insulation is better at keeping the temperature constant, and it will make a significant difference on large scale. One consideration for scaling this prototype is that it is neither efficient nor possible to reactor with 1m thick cotton – a different design is needed for better insulation.

1.3.8 24 h run prototype results

The prototype was set up as described in section 4.3, and temperature of the bioreactor was taken at regular interval. A graph of the recorded temperature was plotted. As it can be observed from the graph, the temperature raised quickly at the beginning, reaching 35.6°C two hours after starting. This temperature was unexpected, and we decided to turn



the heating plate down to 30 °C instead. Straight after that temperature dropped down to 27.4°C, and after five hours started it became steady, maintain in the range of 28.5-29.5 °C, which is out desired temperature range.

1.3.9 Fouling prevention techniques

Fouling is the accumulation of unwanted materials such as scale, biomass and insoluble salts, on the internal or external surfaces of reactors. Fouling significantly impacts the efficiency of the reactors and increases the thermal resistance reducing the flow of heat. One technique to prevent fouling from occurring would be to use anti-fouling coatings such as Notak™ and Dursan® which can be applied to the metal, ceramic and glass surface of the reactor. The anti-fouling coating is initially introduced as a gas then penetrates on the surface of the reactor to create a micro thin coating which bonds to the surface. This then reduces the risk of fouling from occurring as the coating can act as a barrier. Another method to prevent fouling from occurring is through the material selection when designing the equipment. Using materials that do not easily corrode or using materials with a low-fouling surface such as surfaces with ions, very smooth surfaces or surfaces of low surface energy will reduce the chance of fouling from occurring [3]. If fouling has already occurred steam cleaning would

assist in reducing the unwanted materials on the surfaces of the equipment, however this is not always effective and impacts the process productivity [6].

1.3.10 Conclusion

The aim of this experiment is to maintain the temperature of the system within the optimal range for maximum yeast cell production, by designing a prototype that considers insulation, mixing condition and heat conduction, and test it on the lab scale reactor under different conditions allowed us to find the best way to create an uniform heat distribution within the reactor as well as steady temperature. From results we obtained from various experiments suggested us mixing condition is required for an even distribution of heat, insulation should also be included to minimise heat conduction. After testing out various insulation methods we concluded 2 cm think cotton is the best insulator, and the optimal mixing conditions is determined by mixing group. The heat plate is set at 35 °C, to allow some heat loss to surroundings while keeping the temperature within the range of 28-33 °C.

For future design jacketed vessels [9] can be used to minimise heat loss to the surroundings. Temperature sensor and control system can be placed to monitor and changes the temperature when required by adding or removing heat energy. (High-throughput reactor system with individual temperature control for the investigation of monolith catalysts)

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