1 Appendix

1.1 Impedance

1.1.1 Summary

State the aims and objectives of the experiment.

Give a brief outline of the experiments you performed.

Summarise the most important results and major findings/trends, expressed quantitatively.

State the major conclusions which can be drawn from the results of the experiment

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. 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 at a range of yeast concentrations.

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 [2, 3]. 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.

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

1.1.3 Materials and Methods

1.2 Materials

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

1.3 Methods

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 $\frac{15k}{150} = 100$.
  - Liquid volume 250 ml, 60mm height measured from inside the cup.
- Independent: Frequency.
- Dependent: Potential difference

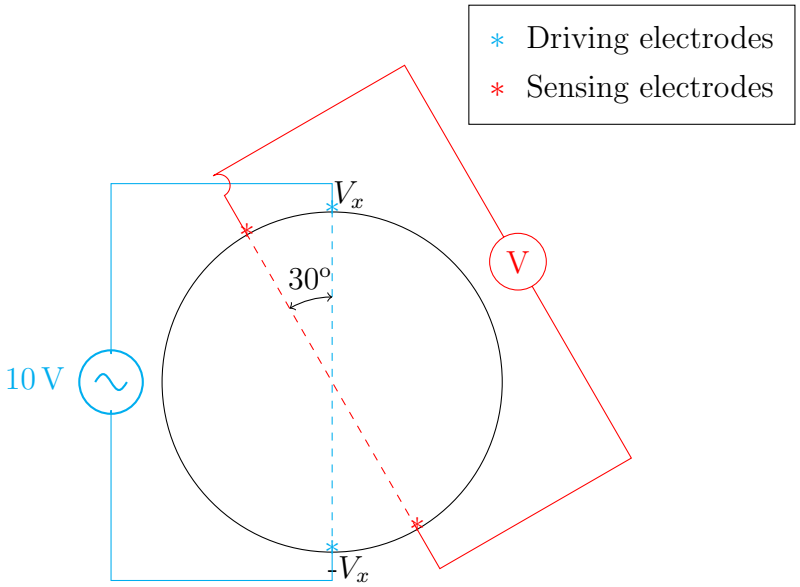


Figure 1: Orientation of electrodes

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. Instrumental uncertainty becomes largely irrelevant, as we expect there to be some movement in the probes as we

Maximum amplitude for PBS around 3000hz, maximum amplitude for yeast at 5000hz. We pick 4000 Hz. There is no concern for cell disruption at the range of frequencies we are analysing (Yerworth, R., 2019 November 28)

1.3.1 Results and Discussion

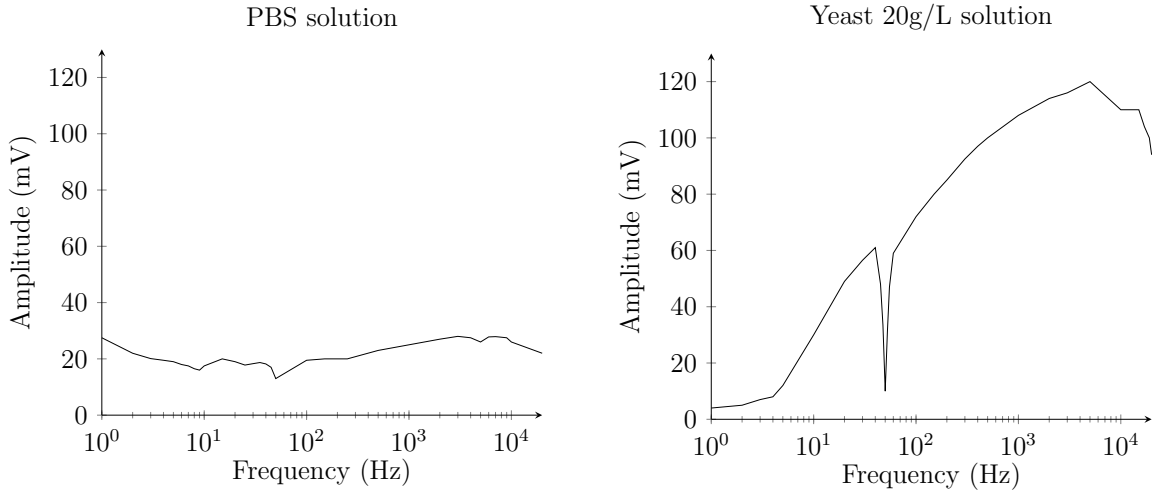
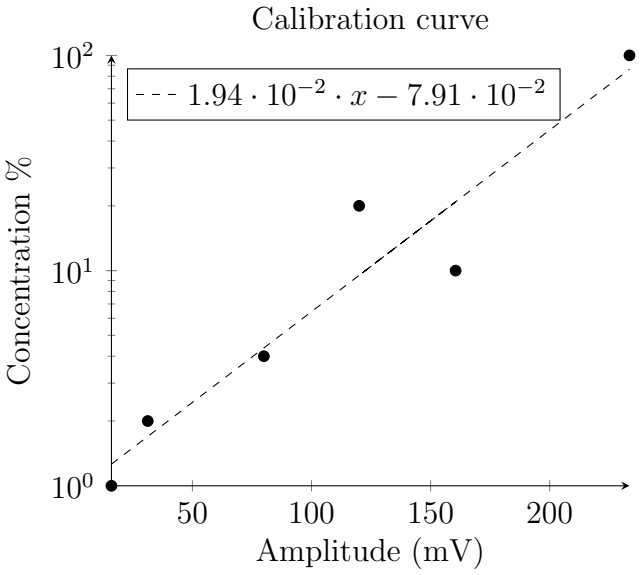


Figure 2: Amplitude based on frequency. Note the dip at 50 Hz due to the filter.

Limitations and improvements 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. This was not taken into consideration in our design, but it definitely should have been.

Assess performance of reactor prototype; cell density with final design

important comments on results, relate to theory and knowledge



1.3.2 Conclusion

Conclusion

Future

1.4 Mixing

1.4.1 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.

1.4.2 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 [1]. 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 [2, 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.

1.4.3 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.

1.4.4 Results and Discussion

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

According to Figure 4, 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.

1.4.5 Conclusion

References

[1] P. M. Doran. *Bioprocess engineering principles: Second edition*, volume 9780080917. 2012.

[2] V. F. Lvovich. *Impedance spectroscopy : applications to electrochemical and dielectric phenomena / Vadim F. Lvovich.* 2012.

[3] A. K. Polat, U. Karabacak, V. Mutlu, L. Tomak, and A. Bilgici. Early diagnosis of lymphedema after breast cancer treatment: Bio-impedance spectroscopy. *The journal of breast health*, 13(2):83, 2017.

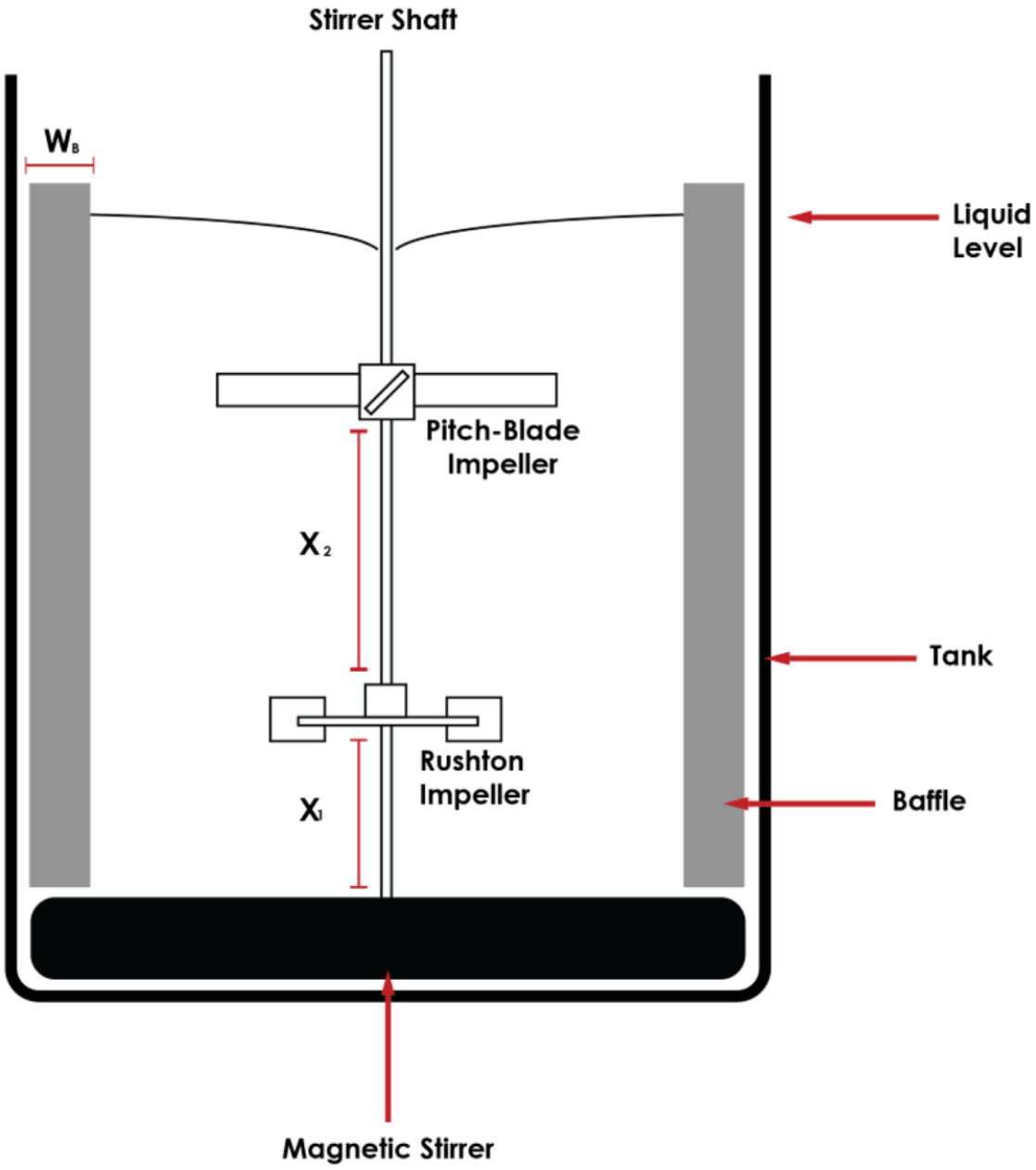

Stirrer Shaft
W_B
Liquid Level
Pitch-Blade Impeller
X_2
Tank
Rushton Impeller
X_1
Baffle
Magnetic Stirrer

Figure 3:

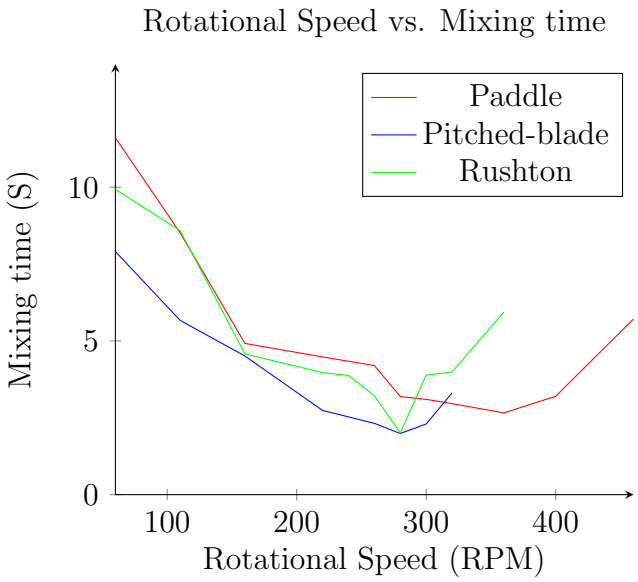


Figure 4: Rotational speed against liquid mixing time for paddle, pitched-blade, and Rushton turbine.