Code Implementation

1) Initialization

We thought the code is too long to include it here, so we will explain only the crucial parts of the code.

The first lines of the code refer to the initialization of the variables (the word 'const' before int means that it won't be change during the experiment). We named it using the common keyword motorPin for the convenience where motorPin1 and motorPin2 refer to the motor A which as known as DC motor for the stirrer, and other two refer to the motor B which is a peristaltic pump. All four inputs (IN1 - IN4) of L298N module were connected to the corresponding pins in the Arduino.

```
//L298N //Motor A
const int motorPin1  = 9;
const int motorPin2  = 10;
//Motor B
const int motorPin3  = 6;
const int motorPin4  = 5;
```

2) Pin Setting

The pin setting is standard as we set every motorPin to perform an output. The new thing that is included into pinSet function is the float switch which is connected to the pin 12 in the Arduino and informs us about the liquid level.

```
///////////////////////////Pin setting
void pinSet (){
   pinMode(motorPin1, OUTPUT);
   pinMode(motorPin2, OUTPUT);
   pinMode(motorPin3, OUTPUT);
   pinMode(motorPin4, OUTPUT);
   pinMode(12, INPUT_PULLUP);
}
```

3) The liquid level parameter

We check the liquid level inside the bioreactor using the float switch as it is opened and returns the value of 0 when the liquid level does not exceed the maximum possible value; and it is closed and gives back 1 when the limit is exceeded. The liquid level function we have initialized as int value so it can return the value of 0 or 1.

```
////////////////////////liquid level
int liquidLevel (){
 return digitalRead(12);
}
///////////////////////printing liquid level
void prLevel (){
  Serial.print(liquidLevel());
  delay(DELAY);
}
```

The word DELAY was declared initially using the #define preprocessing command and is equal to 60000 (ms).

4) The peristaltic pump controlling

The peristaltic pump is connected to the motor B which corresponds to motorPin3 and motorPin4. The one of end of the tube is immersed into the bottle with our basis. Our basis consists of a baking soda mixed with order in order to increase pH.

```
void pumpingControl(float PH,int liquidLevel){
  if (PH<=PH_MIN_VALUE && liquidLevel!=1){
   analogWrite(motorPin3, 255);
   analogWrite(motorPin4, 0);
  }
  else{
   analogWrite(motorPin3, 0);
   analogWrite(motorPin4, 0);
  }
```

We chose to use the if-else function due to its convenience and readability; even after the code has been written it can be easily amended if the work of the peristaltic pump failed. The if-else statement combined two conditions: the first one refer to the pH value and it should be less the minimum pH value we declared at the beginning (it equals to 3,6); the second one states that the pump will work whenever the liquid level is not equal to 1.

The number 255 inside the analogWrite operation means the motor works at 100% of its total power. It was checked empirically when we set the low values and it didn't even work or worked slowly, so we concluded that 255 is the most optimal value.

5) The stirrer

The tuning of the stirrer in the code is easy, the only condition is that it should permanently work during the experiment. We decided to set the stirrer up working at nearly 40% of its total capabilities.

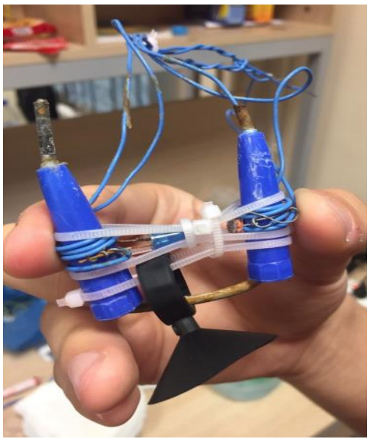
```
void stirrer (){
   analogWrite(motorPin1, 100);
   analogWrite(motorPin2, 0);
  }
```

6) The turbidity sensor

We were not provided with the pre-amplifier adaptor board for the turbidity sensor; therefore, we connected the turbidity sensor directly to the analog pin 5 on the Arduino. We are trying to get an output voltage by converting the analog reading from A5 to an exact value of the voltage. The Analog to Digital Converter (ADC) is a 10-bit ADC for the Arduino, so the maximum number of discrete analog levels is equal to 2 in a power of 10 which is 1024. So, the 1023(4) value corresponds to 5V.

```
/////////////////////////TURBIDITY
void turbidity ()
{
  Serial.println(analogRead(A5)*(5/1024.0));
delay(DELAY);
}
```

It looks like that:



7) The pH sensor

The type 2 pH sensor was connected in the same manner to the analog pin 2 on the Arduino, then to +5V and GND accordingly. It reads the analog value from the pin and first converts to a voltage value. Then, using the pH_voltage we derive the pH value itself via a formula that you can find in the code.

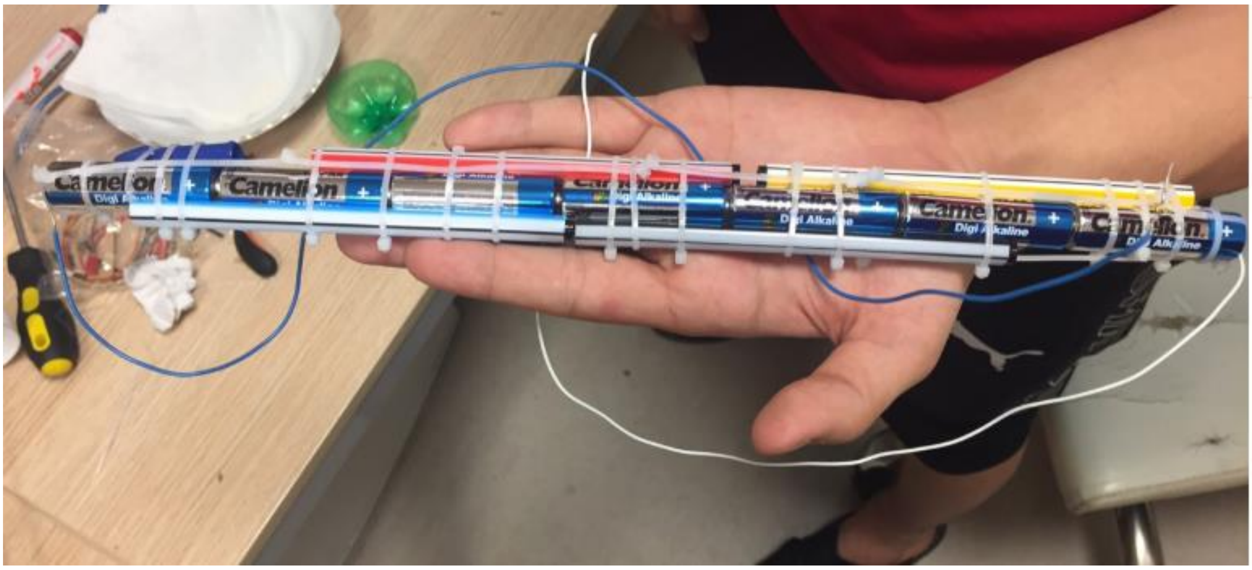
```
float measurePH2()
{
  int pH_sensorValue = analogRead(A2);
  float pH_voltage = pH_sensorValue * 5.0 / 1023.0;
  float pH_value = PH_CALIBRATION*(28.2+PH_OFSET  - 6.5 * pH_voltage) ;
  return pH_value ;
}
void PHsensor(){
     Serial.print(",");
  Serial.print("pH:");
  Serial.print(measurePH2());

  delay(DELAY);
}
```

The parameters 28.2 and 6.5 were provided in one of the labs and then was checked by us empirically. It turned out that these parameters are also suitable for our type of pH sensor in order to start measuring. Also there are PH_CALIBRATION and PH_OFSET macro parameters that are appeared into formula and which equal to 2.2 and -1 respectively.

8) The power supply

We didn’t have an everyday access to the laboratories especially on the weekends, but we wanted test the bioreactor and conduct experiments even on Saturdays and Sundays; therefore, we made the handmade power supply from bunch of batteries and pens.



Benchmark cases

Benchmark case №1

The first benchmark case is based on checking the stirrer’s work. We added some food colouring into the jar, turned off the stirrer via the code and recorded the values of the turbidity. The speed of the impeller was equal to 155 RPM.


video].mp4


video3.mp4

There you see the Turbidity diagram vs Time

Benchmark case №2

The second benchmark case was taken in order to see how the peristaltic pump works. As you see from the video it pumps the liquid from the buffer solution into the jar. Our main buffer solution was baking soda ($NaHCO_3$) with water (H_2O).


video4.mp4

Here you can see the diagram of the Liquid Level vs Time:

Benchmark case №3

In the 3rd benchmark case we added some vinegar and recorded the change of pH medium. Theoretically, the vinegar should decrease the value of pH, so which is happening in the video.

Actually, we made this benchmark twice as one of them did not show the change explicitly or we did not wait for this visible alteration enough time

video5.mp4

video2.mp4

There is the diagram showing the change of pH level during time:

The experiment

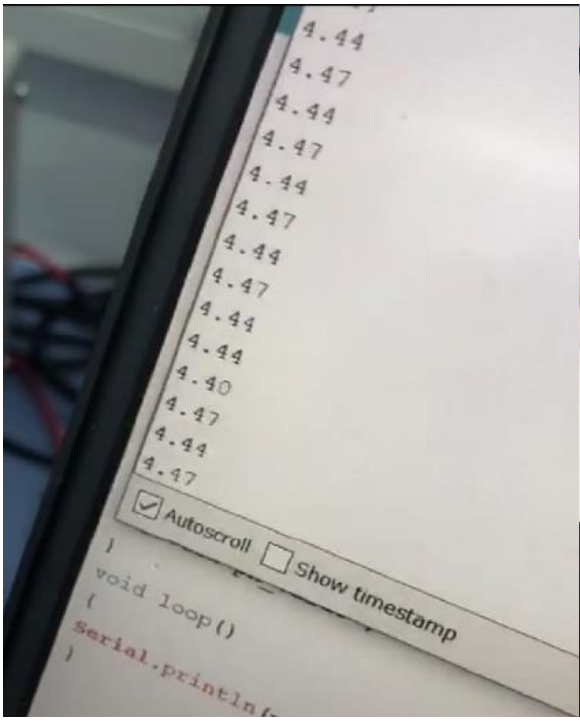
Before demonstrating the results let us show the process of yeast production itself. This is how it looks like when the process has been started:

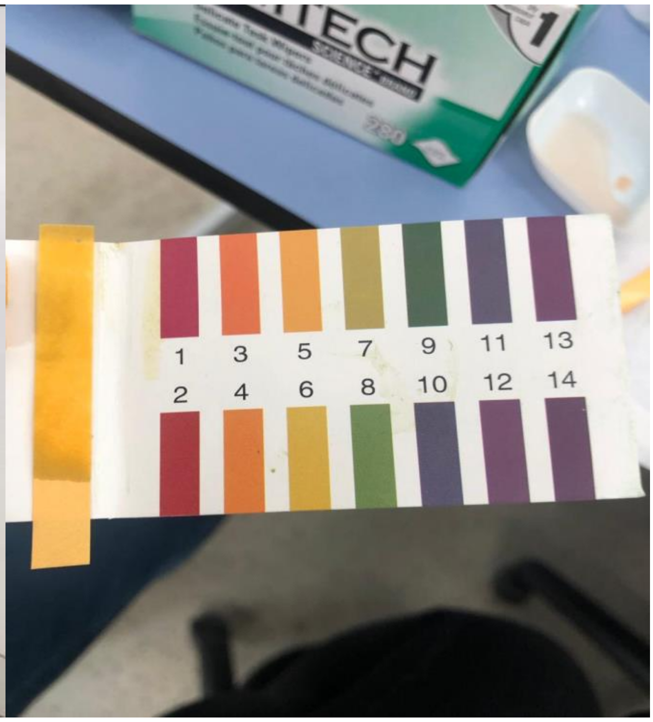
process.mp4

Results

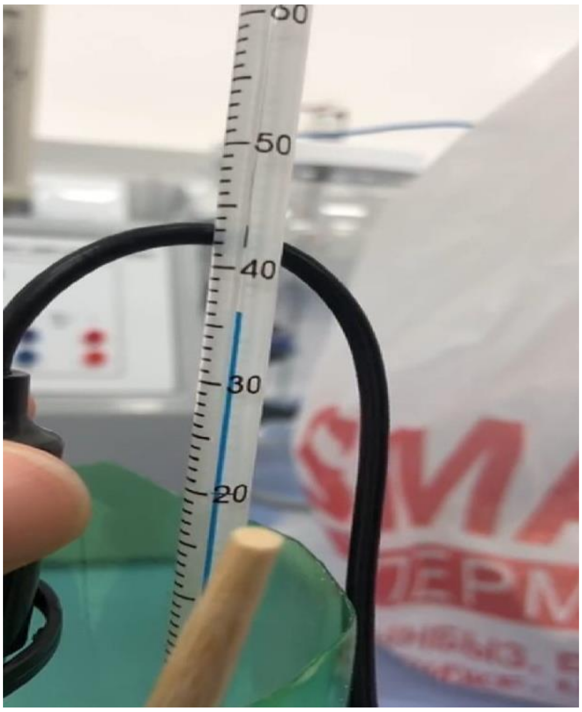
You’re all waiting for the results of the experiment but let us first show the conditions in which the yeast was able to grow peacefully.

The auspicious pH value that was sustained during the process yeast production was equal to nearly 4.5 according to the pH sensor and around 5 according to the litmus paper.





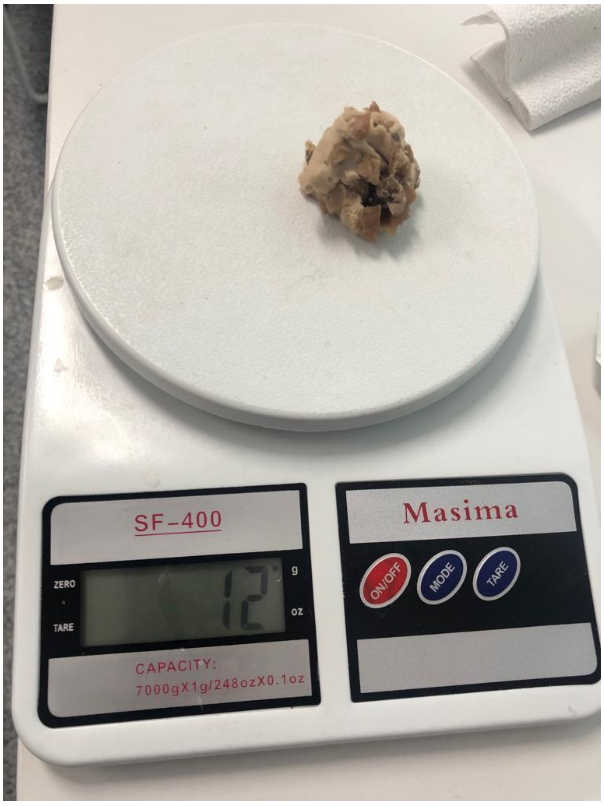
We measured temperature using thermometer and were trying to achieve optimal temperature for the yeast growth, which is 34.5°C. The heating process has been maintained with the help of the heater provided.



These is the amount of the “dirty” yeast we got which will be filtered afterwards:

The final step

This amount of yeast was exposed to the process of filtration. We filtered it several times through the coffee filter and obtained the mass of 12 g, while the initial mass of the yeast put was 7g. This is how it looks after the filtration.



We have also made some calculations for finding the coefficient of the growth rate:

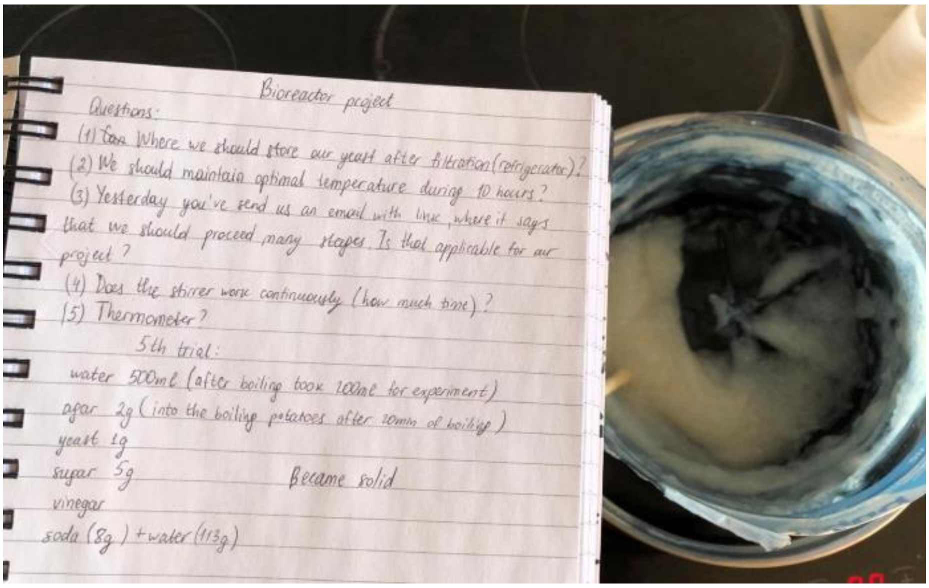
$$\mu = \frac{logN_f - logN_i}{t_f - t_i} = \frac{\log(2.63 \times 10^{22}) - \log(1.55 \times 10^{22})}{7.5hours} = 0.031\ hrs^{-1}$$

Difficulties

1)Many trials

In order to get close to the favorable conditions of yeast growth we made several attempts. There you can see the notes that we're taking during each of the trials.

trials.mp4



2) Agar usage

Initially we were using agar, but it brought us several problems as the medium became solid. The turbidity in that case too high, so that the turbidity sensor could not show the output voltage properly.

3) Foam and bubbles

As we find the way how to proceed the experiment, at some point there was a sudden high formation of bubbles and foam. We thought that there was too much air supply and the stirrer was revolving at a higher speed, therefore we limited the amount of air going into the medium for some amount of time.

4) Halting of the pH sensor

Another difficulty was connected to the work of the pH sensor. For some amount of time during the experiment it halted and did not return any values. We suppose it happened due to the high resistance of the medium where the voltage supply is constant. However, after some time being halted it again started working normally, so there was not any disaster.