YEAST BIOREACTOR

Building from scratch an automatize yeast bioreactor

Context and Inspiration

During the experimental evolution week, we had to create from scratch a yeast bioreactor that would be the most efficient as possible.

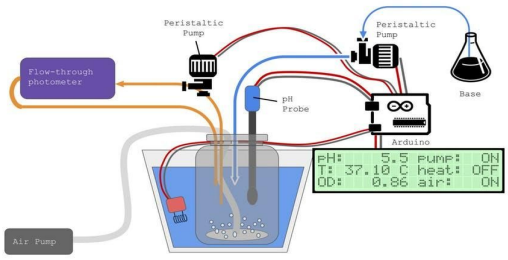
We decided that the efficiency of our bioreactor would be determined by its capacity to produce alcohol. Hence, the aim of our bioreactor is to produce as much alcohol as possible.

Yeast can produce alcohol through the fermentation process. This process corresponds to the transformation of sugar by yeast into ethanol and CO2. To realise this process in optimal conditions, yeast need to stay in a stable temperature; this induces the fact that our culture needs to be heated, but also to be agitated to get a homogenous media in which sampling can be performed. Due to a lack of liquid alcohol sensor, alcohol rate is not measurable directly. A roundabout way has been used to reach our goal: measuring the acidity. Indeed, the alcohol rate is correlated to the acidity increases, when one decreases, the other does also, leading to a decrease of the pH inside the bioreactor. Finally, the optical density was measured, in order to verify and control the growth rate of the culture.

Consequently, the following parameters are those chosen to our bioreactor : the temperature, turbidity, the optical density and the acidity.

Our inspiration comes from a microbial bioreactor, made by the team bioeconomy lab *on their website.* However, their project wasn't to make as much alcohol as possible, that's why modifications on parameters chosen for system have been performed. For example, the acid/base process : they regulated the pH to optimise the development of yeast, but in our case, we want to optimise the production of alcohol, so we use pH as a measurement.

But we kept the heater and flow through spectro-photometer system and optimized it to avoid the noise.



Moreover, our system is a closed system, that means that we didn't add anything, while their project is an open system. So, we don't have any material input or air input, it's an airtight system.

And because we decided to choose the alcohol, as a goal, we are also inspired by brewing system for the fermentation temperature.

Fermentation process and yeast culture

As previously said, we decided to build a yeast bioreactor and in order to do so, we need to cultivate yeast. However to determine the conditions for the yeast culture we had to take into account our goal. Indeed, we want to produce as much alcohol as possible. Yeasts undergo a process called "fermentation". Fermentation is nothing more than a chemical reaction. Glucose contained in the yeast medium reacts in a series of chemical reactions, releasing ethanol and carbon dioxide. The overall reaction scheme looks as :

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

One glucose molecule ($C_6H_{12}O_6$) is converted into 2 ethanol (C_2H_5OH) and 2 carbon dioxide (CO_2) molecules. Oxygen is not required for the reaction to occur.

Despite the advantages, fermentation is a challenging process since we're using a living organism to perform the chemical reactions. When dealing with a living microorganism, it's important that the circumstances are as ideal as possible for the yeast. Yeasts can die or stop fermenting if the conditions aren't ideal. Moreover, in our case, we want the fermentation to be as efficient as possible to produce alcohol. Through empirical observation, it has been proven that temperature and air exposure are key to the fermentation process. Indeed, yeasts will die at too high temperatures (most don't survive temperatures above 50°C). Also, their growth rate depends on the temperature. If it's too low, they will barely grow, the same goes up if it's too high. The ideal temperature for our yeast type is within a range of 22°C and 32°C. Moreover, as explained above the fermentation doesn't need any O_2 input in order to process. For this reason, we decide that our bioreactor system would be a closed system. This system will have no input such as sugar or yeast but also would be an airtight system in order to maximize the yield of alcohol in our bioreactor.

For our bioreactor, we bought dry yeasts from Naturalia (see picture below).

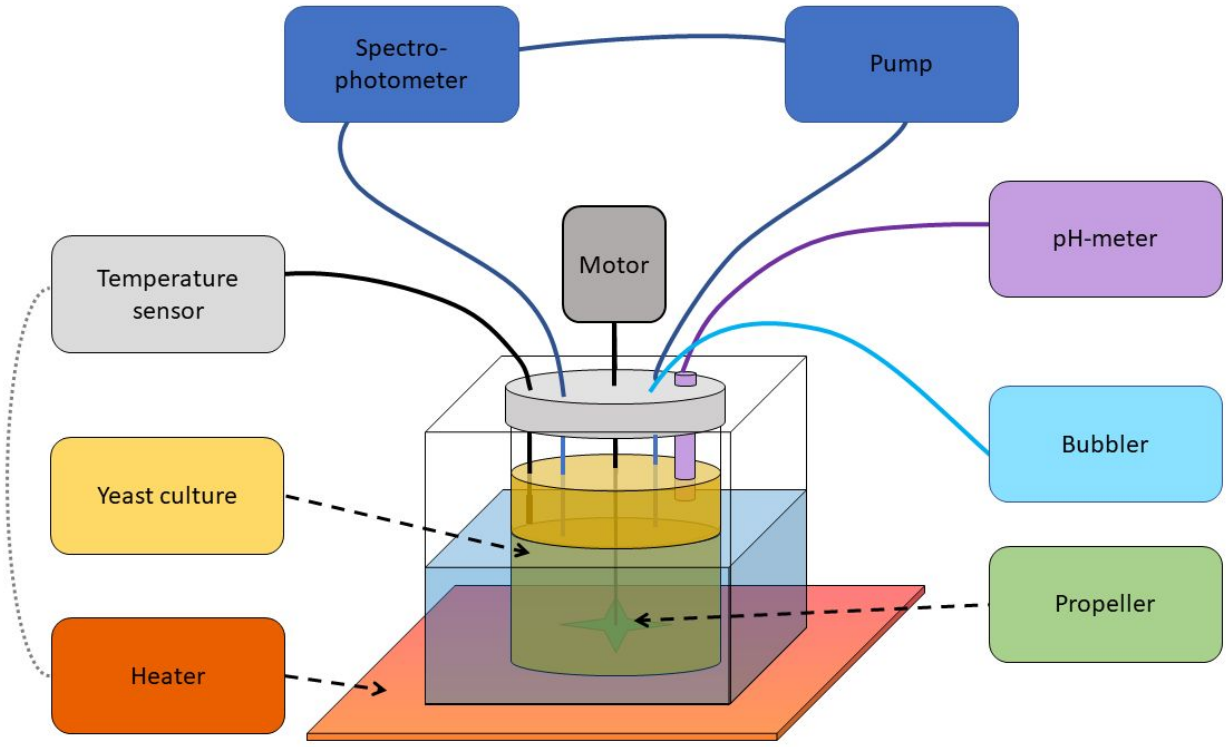
To activate them, we use 26g of yeast, add 260g of water and 74g of sugar[1]. Then, we maintained our yeasts in a bath at 27,7ºC, as this temperature is included within the optimal temperature range. In order to follow the growing rate, we measured the optical density of our culture at different times (dilution factor : 200).

We clearly see that the concentration is increasing, suggesting that our culture is multiplying itself. We could have verified this conclusion by counting our sample using Malassez cells.

Sources :

[1] F. Noé Arroyo-López et al., Effects of temperature, pH and sugar concentration on the growth parameters of Saccharomyces cerevisiae, S. kudriavzevii and their interspecific hybrid, International Journal of Food Microbiology 131 (2009) 120–127, doi:10.1016/j.ijfoodmicro.2009.01.035

General setup



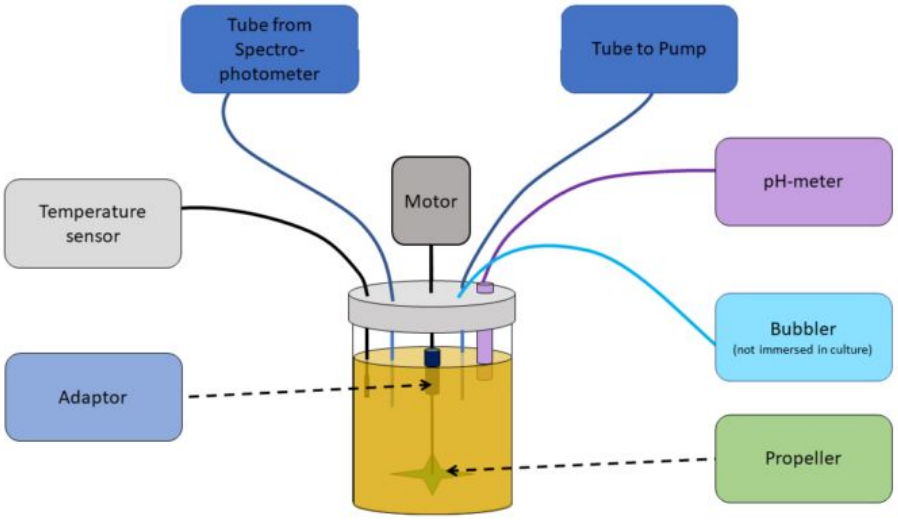
A closed jar has been used as bioreactor to keep the yeast culture in a closed environment. Since the optimal temperature of yeast development is between 22°C and 32°C, we decided to keep the setup at a temperature of 27,7°C. For that purpose, the bioreactor has been put in a water bath heated by a heater plate. The temperature inside the bioreactor is checked continuously and once upper to 27,7°C, the heater plate stop working.

For the aim of keeping the culture mixed, the setup is composed of a propeller, activated by a motor, to mix the culture.

A flow through spectro-photometer takes sample continuously from the yeast culture solution thanks to a pump and releases it inside the bioreactor to create a closed circuit. The in and out tubes were placed at the opposite sides of the bioreactor in order to avoid the circuit to take the same sample uninterruptedly.
A pH-meter has been installed to verify the evolution of the acidity of the culture.

Finally, a bubbler is connected to the jar in order to release CO2 from the bioreactor and to avoid air, from the outside, coming in.

Main Body



The main components in the system are a propeller connected to a motor to mix the culture, two tubes connected to the pump for water in and water out (immersed in the culture), and a tube connected outside to a beaker for out-gasing (not immersed in culture).

The mixer system consists of a motor powered and controlled by the Thermoregulator System, and a propeller connected to the motor by an adapter.

The adaptor is 1cm hollow tube on each end, with 2cm filled tube in the middle to ensure the propeller is deep enough to reach almost the bottom of the culture.

Caution!

The holes are not precise enough for 3d printed material, further drilling of the hole may be required.

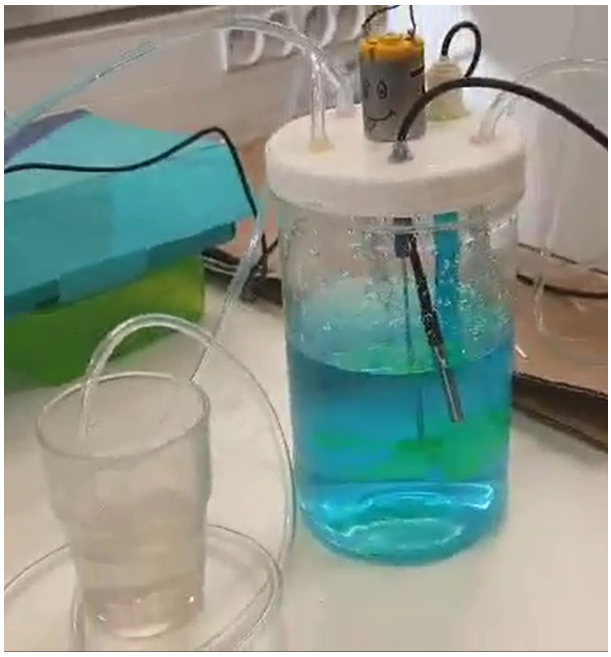
The speed of the motor should be kept low in order not to kill the yeast.

For the code, please refer to the blog post Thermoregulator System.

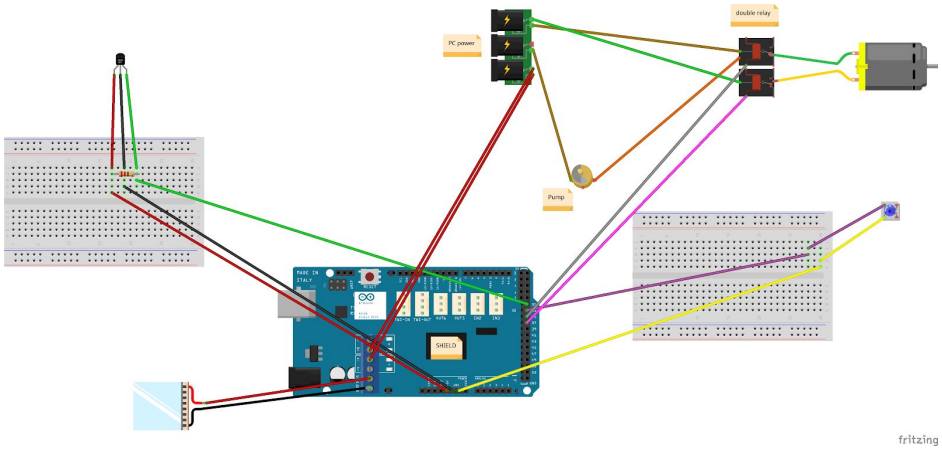
Two tubes are immersed in the culture so as to keep the culture flowing through the photo-pH meter. Please refer to the specific blog posts for the details of the pump or photo-pH meter

As one of the products from fermentation of yeast is CO_2, we need to release the gas in order not to build up an air pressure inside the bioreactor, and prevent the culture to be acidic (When the pressure is built up, more CO_2 would be dissolved in the culture and turns to carbonic acid which is harmful for yeast growth). The gas-out side of the tube is immersed in water in a beaker to prevent inflow of oxygen. We do not want oxygen as respiration would be carried out by the yeasts, instead of fermentation.

Design



Thermoregulator System

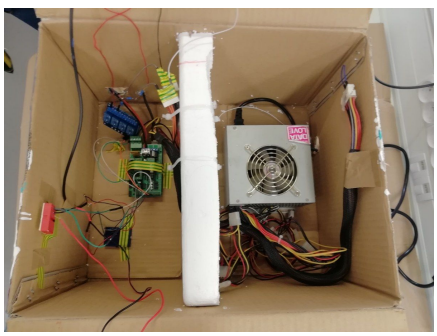


Because yeast are living into a specific temperature range, we decided to create a thermosensor system, linked to a heater. To this, we can't use a leonardo arduino but a Mega Arduino, coupled to a Ramps 1.4 (Shield). The shield is more efficient by integrating a powerful relay of 20 A inside it, instead of 5A for a single relay.

So, we began with the sensor system who is only connected to the Mega Arduino and coupled to a "If" loop to detect the temperature, that we can see into the monitor series. Then, we linked the heater to the shield relay because the heater needs a powerful alimentation, superior than a simple generator, that's why we used a computer generator to aliment the Mega Arduino. Inside the code, we coupled the heater to the temperature sensor so that the heater warms when the temperature is inferior to 27,7°C.

To be warn when the temperature is decreasing, we implement a LED system connected to the Mega Arduino. Thanks to the code, the blue LED is switched on when the temperature isn't good, when is it, the LED switches off.

Thanks to another double relay of 10A, we connected the pump and the motor system to the generator and to the arduino because they're turning continuously and are needing less power than the heater. These electrical connections are made to compact the global system and reduce the number of Arduino around the bioreactor.



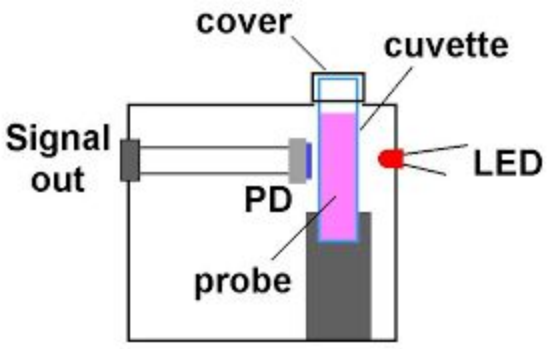
And to compact all this thermoregulator system and to protect it from water and risks, we put everything into a hand-made cardboard box. But we can also make the box with the LaserCut, but we didn't have the time to make it correctly.

Spectrophotometer

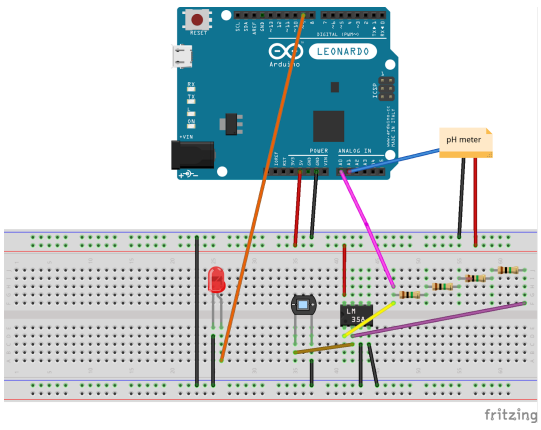
As there are two sensors used for detecting parameters of yeast in this system, we decided to combine the two sensors control in one Arduino so data monitoring and further calculation could be done at once.

We decided to measure the optical density and the pH of the yeast solution. In order to do so, we used an Arduino. The spectrophotometer will give information regarding the growth rate of yeast, using the optical density.

For the spectrophotometer, we need a LED and a photodiode. The light of the LED will pass through the cuvette and then, reach the photodiode. The evolution of absorbance will give us insight of evolution of yeast population within our system. The optical absorption of yeast cells was investigated in the wavelength range 250 - 500 nm. In order to recreate this wavelength, we used a white LED, and a blue filter.



A Semiconductor diode is a component in which incident light radiation causes a variation in intensity. However, because this variation is pretty weak, it needs to be amplified. In order to increase the gain of the amplifier, we put resistance, we put four 1 Mohm resistances in series because we didn't have a more powerful resistance available. Moreover, we decide to develop a flow-through photometer to determine the culture density continuously.



For the pHmeter, we used the pH control system that consists of a traditional pH probe and we plugged it into an analog input of the arduino and to power.

Additional Material: Silicone

A 2mm hole is drilled at the bottom of the cuvette for this bioreactor. The cuvette should be held vertical in order to fill up all of the gaps inside the cuvette.

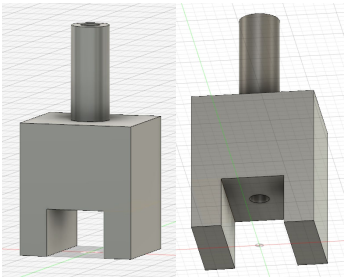
The water-in adaptor is to adapt the water-in hole from the cuvette to the tube connected to the pump. The one used in this project is 3D printed (refer to Designs.zip).
The dimensions worth mentioning is :

The side connected to cuvette should cover the cuvette.

The side connected to tubes to pump should be slightly bigger than the inner diameter of the tube.

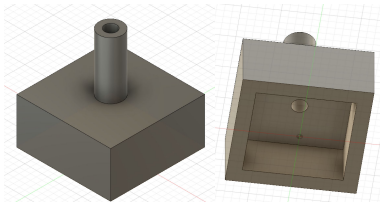
Caution!

Please be reminded that dimension of 3d printed materials are not precise enough as it shrinks as it cools down. Additional drilling may be required.
It is not necessary to build the hollow space as in this design



Link to design (Water-in Adapter):

The water-out adapter is pretty much the same with water-in adapter mentioned above, only the hollow space inside is removed.

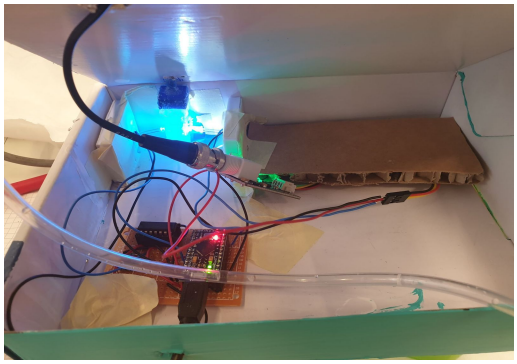


Link to design (Water-out Adapter):

The tubes are connected to the adapter by covering the tube extended from the adapter. The gaps between the adapters, cuvette and tubes are covered with silicone to prevent leakage.

Figure: Silicone surrounded the connection between adaptor and cuvette

We put all the system within a box because the photodiode shouldn't be excited by ambient light. Also, make the circuit more compact, we have welded the cables on a breadboard and used an Arduino pro micro.

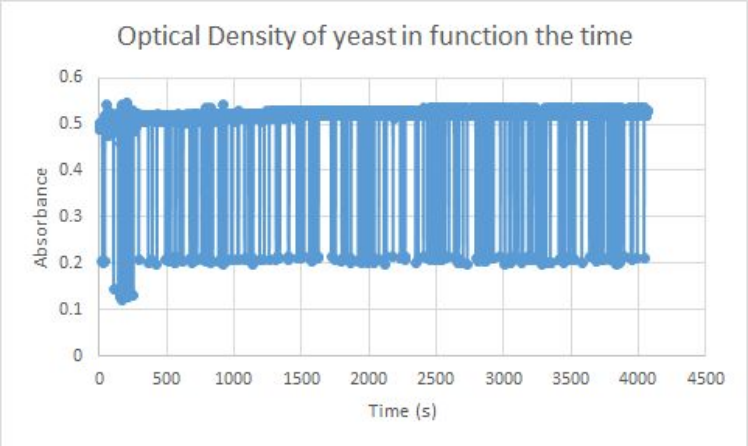


Data Presentation

Spectrometer

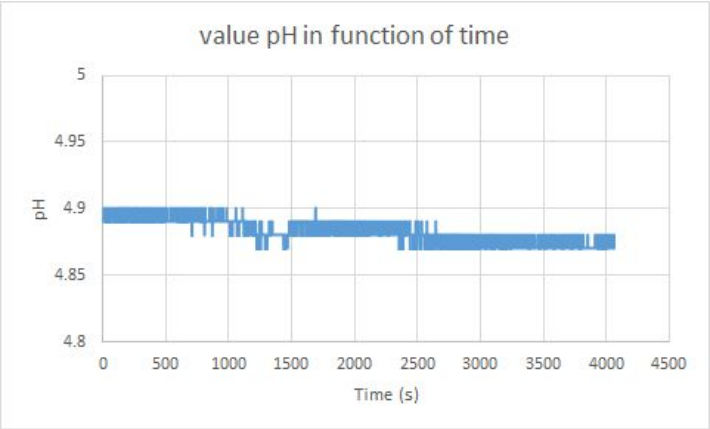
We collected data from the pHmeter and also, from the spectrometer during one hour.

Here below is the graph for the optical density. Indeed, we see a large variation within the data. Many different parameters could explain this variability, for example, the precision of the photodiode, the fact that the turbidity of the yeast solution might be too high to be read by the spectrophotometer. We can't really analyse those data because there is too much variability.



pHmeter

Here below is the graph for the pH. We observe the acidity is decreasing (being closer from 1). This decrease could be a proof of the fermentation occurring in the yeast culture. Indeed, during fermentation organic acids are produced which would lead to a decrease in pH. However, the variation is pretty low so it's hard to know if it was caused by the noise or a real difference.



Conclusion

To conclude, we built a yeast bioreactor that allow the survival and proliferation of yeast . We succeeded in automatizing the heating process using a thermo sensor and a heater. Moreover, we automatized the pHmeter and the flow-through photometer in our system. However, it's hard to determine if our bioreactor is efficient or not regarding the alcohol production, indeed we can't conclude with our current data.

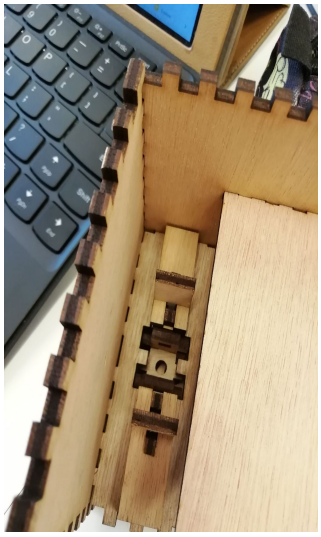
We succeed in almost all objectives we determined:

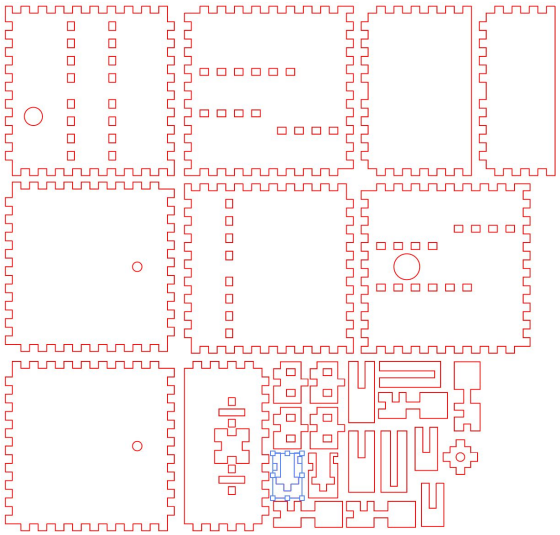
ONGOING	Collecting coherent data
COMPLETE	Propeller to mix the yeast culture in the bioreactor
COMPLETE	Bubbler
COMPLETE	Thermo sensor connected to a heater
COMPLETE	pH meter
COMPLETE	Flow through photometer
COMPLETE	Building bioreactor setup

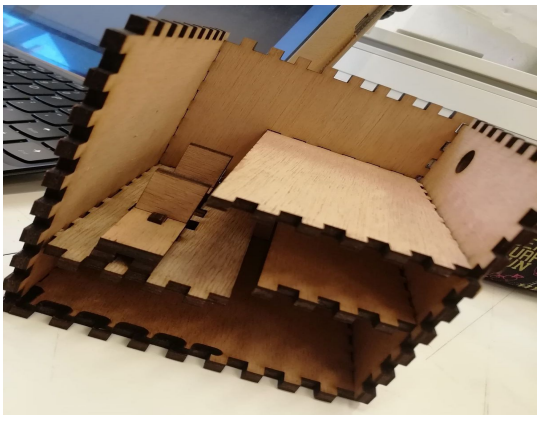
Further improvements

The Lovely Box

To improve the accuracy of the photometer and have a more compact structure, a wooden cover box could be made from a laser cutter. Arduino board is placed on the top level on the left, adapter board of pH meter on the middle level, and the cuvette placed on the left. Holes are drilled on the shell of the box for wires and tubes to pass through. 4mm plywood is used in the design of the box.







Caution! Glue might be used to fix the wood together.

A continuous harvesting model

Most yeasts can only handle a certain maximum alcohol content. If the content goes over this value they will simply stop growing or die. This is the reason drinks with a high alcohol content are generally not fully made through fermentation. What we propose is that a harvesting model could be introduced by a scheduled automatic addition of glucose solution to the system and a scheduled automatic collection of the yeast culture which we could extract alcohol from.

Dilution of culture

This could only be done with the presence of continuous input of glucose (food of yeast), which we suggest the continuous harvesting model (refer to above).
A possible improvement for the system could be adding a dilution system to the culture before passing through the photometer. This is because when the yeast are densely populated, the photo-diode is not sensitive enough for slight concentration change.

Our suggestion is that, instead of using a flowing-through design, we could adopt a scheduled automatic data collection design. In this design, the cuvette is also connected to a pump that pumps yeast free culture. A few milliliters of culture in the bioreactor is extracted each hour and added to the cuvette, and then filled up by the yeast free culture. After the reading is recorded, the sample in the cuvette is discarded and washed by yeast culture and wait for another hour's measurement.