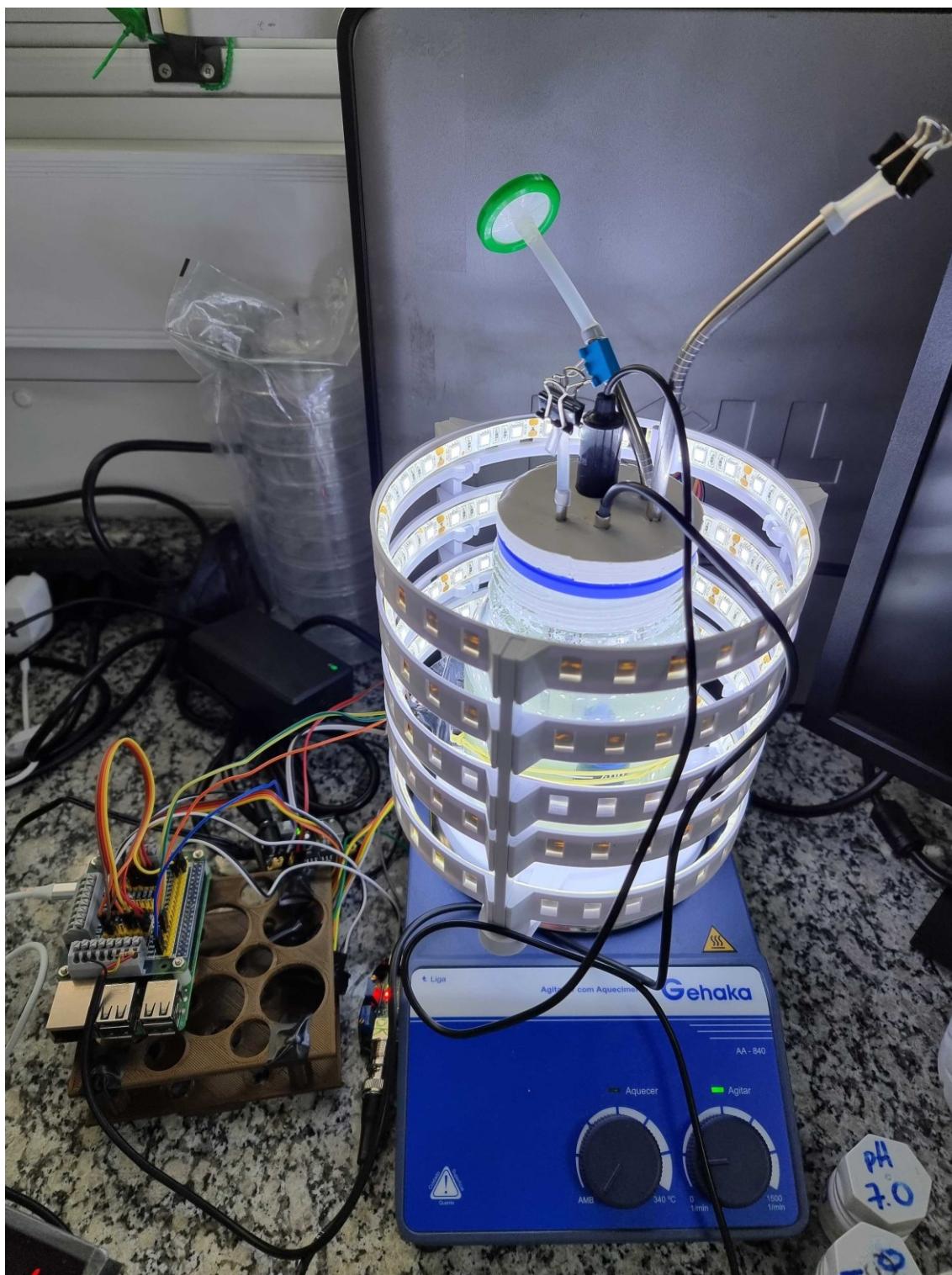


# TUTORIAL - How to build the bioreactor



## **REACTOR VESSEL:**

You will need a GLS-80 reagent flask, with 500mL or 1000mL size.

Alternatively, it is possible to use a mason jar with some limitations.

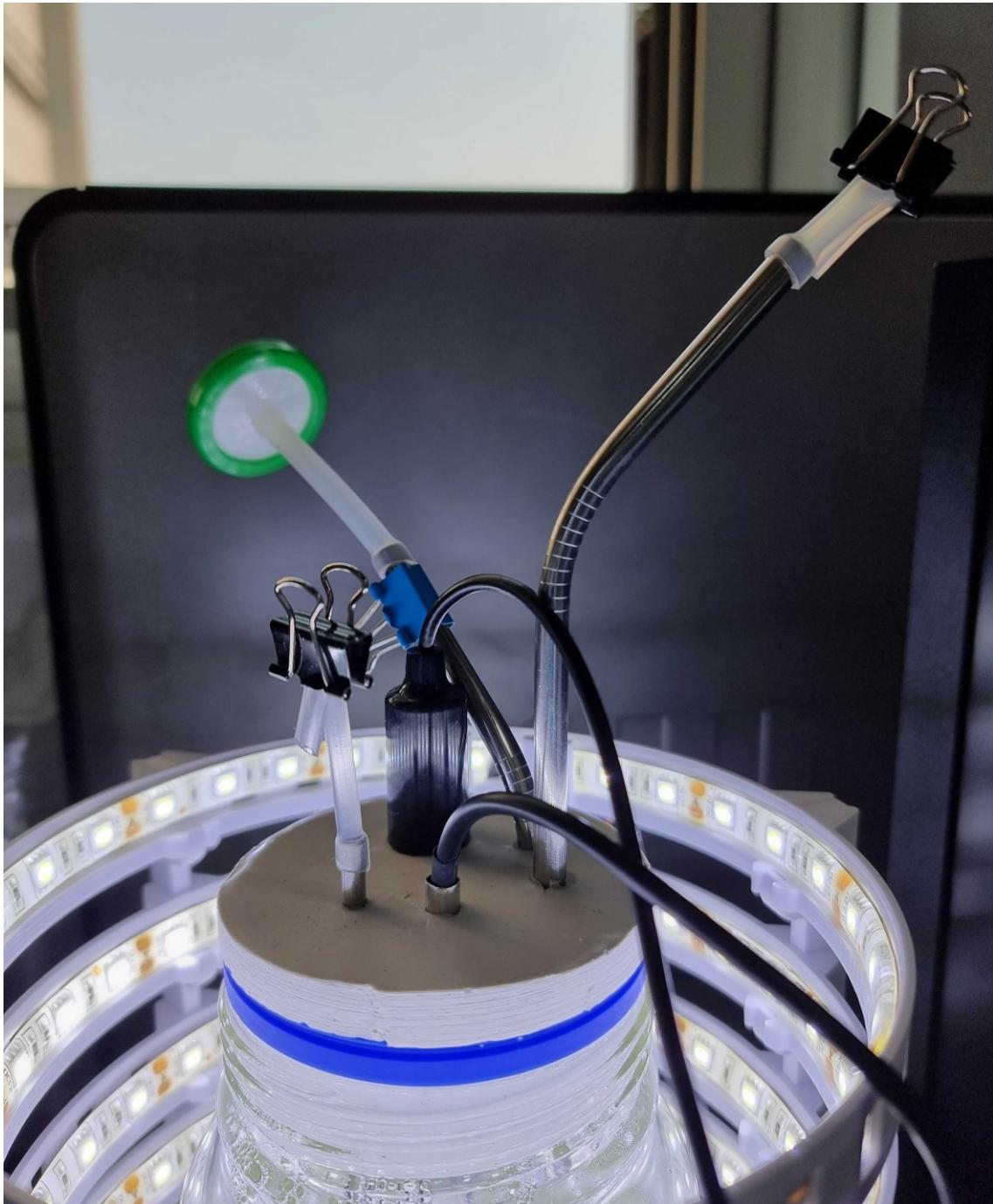


## REACTOR LID

There are two options:

- 1- build a 3D printed part to use with GLS80 lab flask
- 2- build a less tight LID to use with mason jar.

### LID VERSION 1:



## YOU WILL NEED:

- Shore 15 A to Shore 25 A silicone rubber. We do recommend using platinum silicone
- 3D printer to print the mold
- Modeling clay to seal the mold
- Silicone spray or Vaseline (petroleum jelly)

## STEPS:

1. 3D printing the mold.

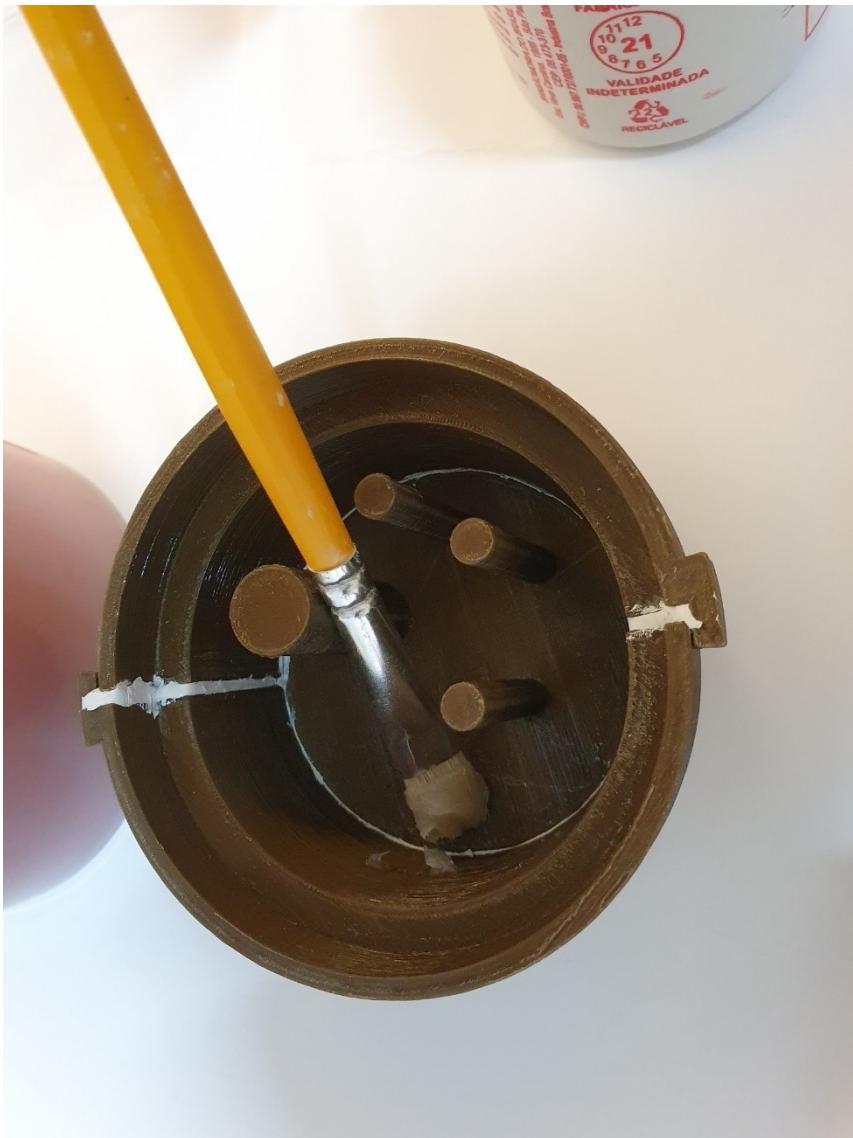


2. Applying a clay/plasticine/putty on the connections to get a good seal. Put a rubber around it to keep all parts close..

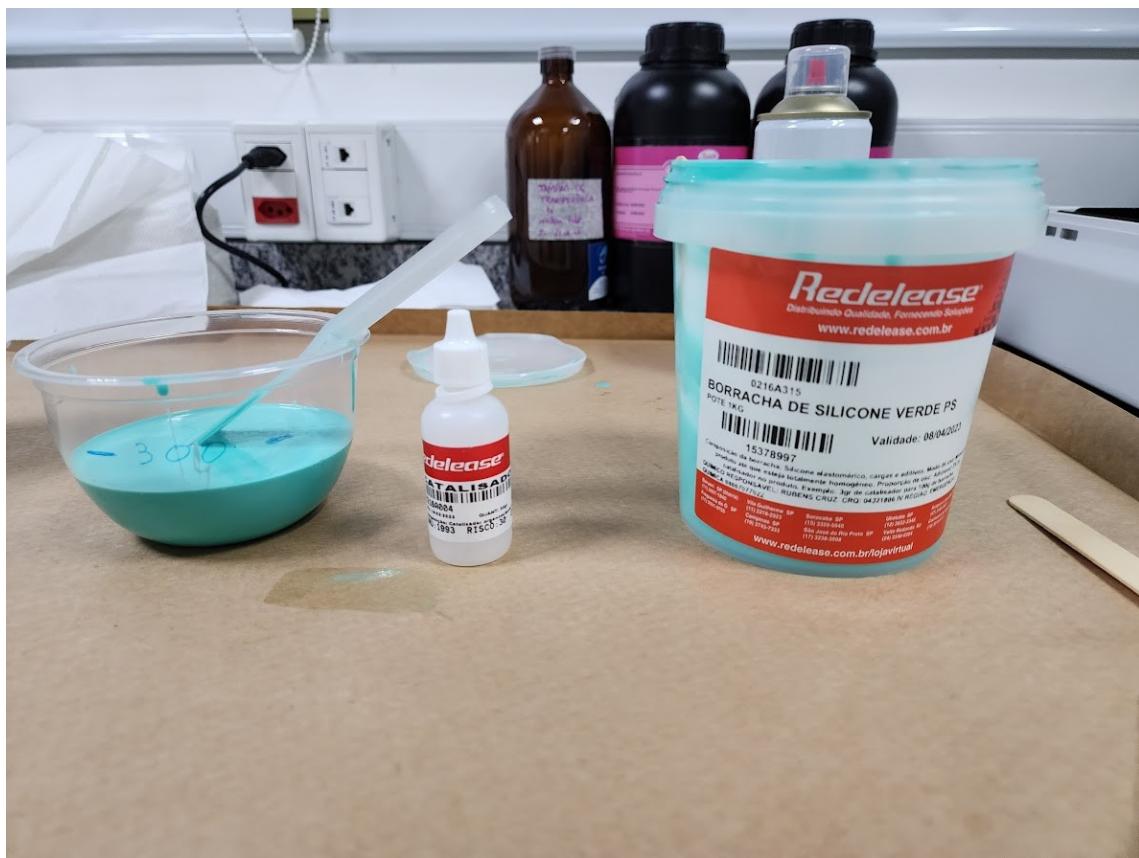




3. Apply Vaseline (petroleum jelly) on all internal parts, to avoid the rubber sticking to it.

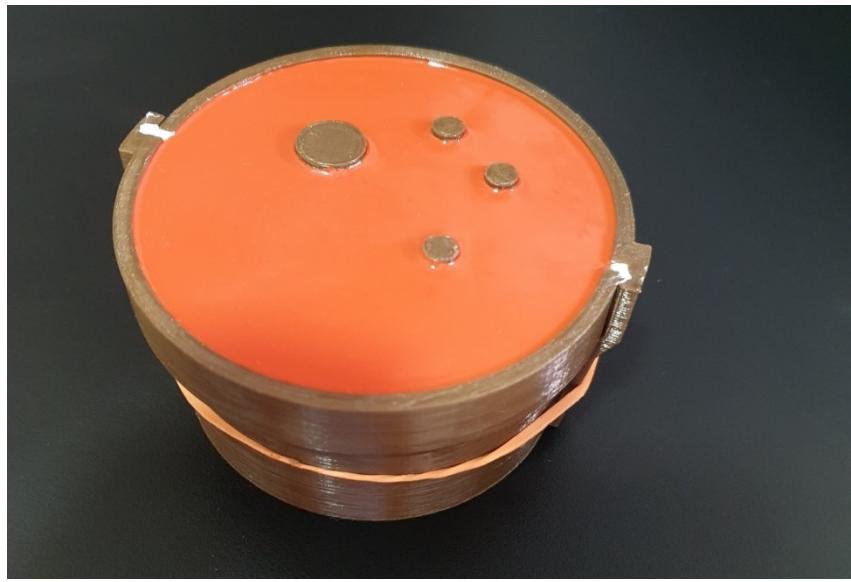


4. Catalyze the rubber in a disposable cup, according to manufacturer instructions.

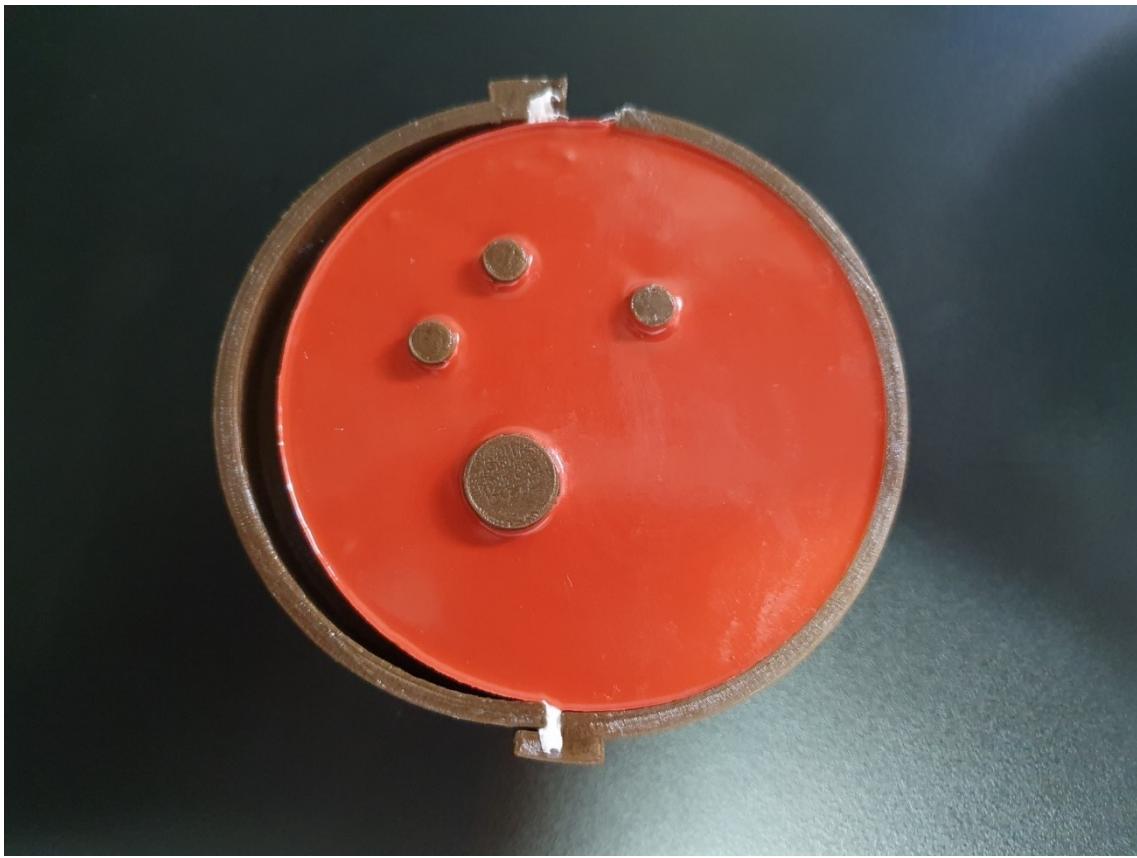


5. Pour the rubber slowly into the mold to avoid bubbles.

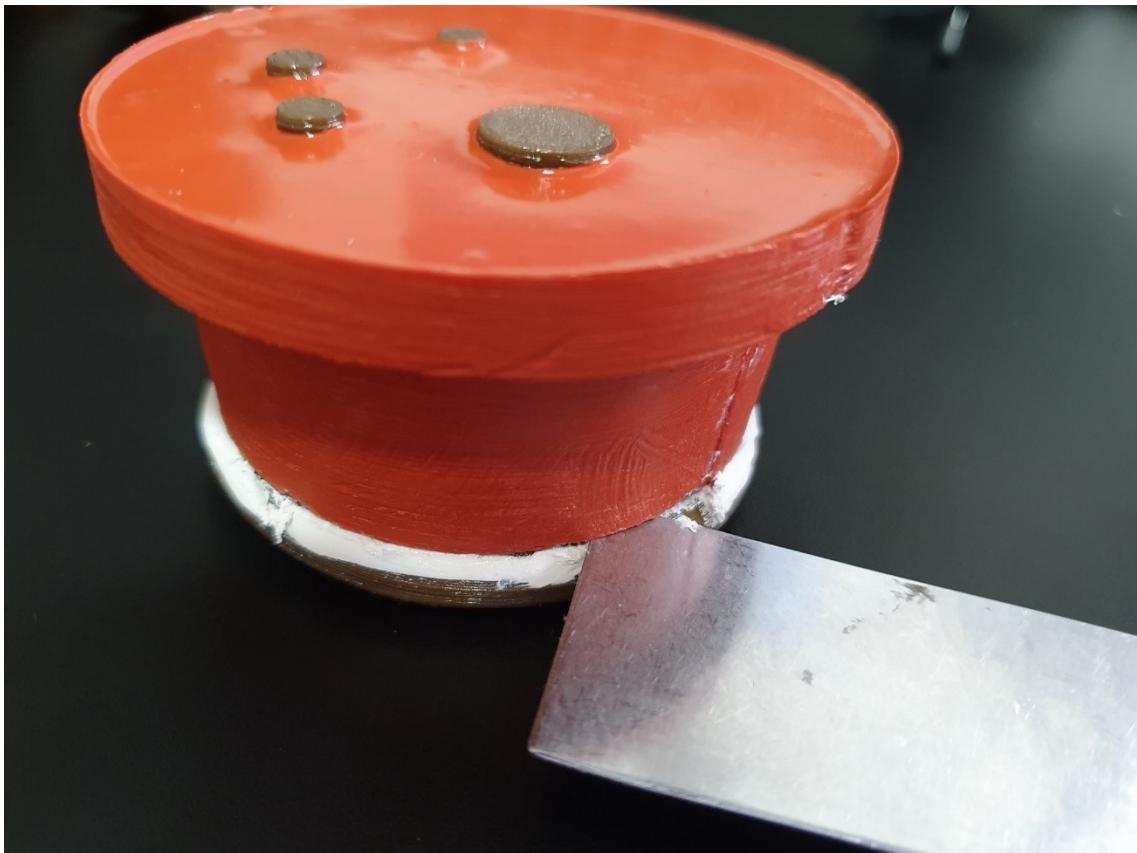
6. Wait for it to cure.



7. Carefully remove one side, then the other side.



8. Put slowly a thin object between the rubber and the bottom mold, to free it up. Attention to don't break the thin plastic tubes.



### **LID VERSION 2:**

This is the lid for the mason jar

### **YOU WILL NEED:**

- Mason Jar
- Shore 15 A to Shore 25 A silicone rubber. We do recommend using platinum silicone
- Agar-agar for 1L (or enough to fill the jar)
- Aluminum foil
- Masking tape
- Silicone spray

1. Mix agar-agar with water and heat it to the indicated temperature to jellify it.
2. Fill the jar a little bit above the jar neck. Now let it get back to room temperature and solidify.

ATTENTION, if the agar-agar contract, add more agar till it reach the vertical part of the jar.



If this happens, fill until you reach the green line

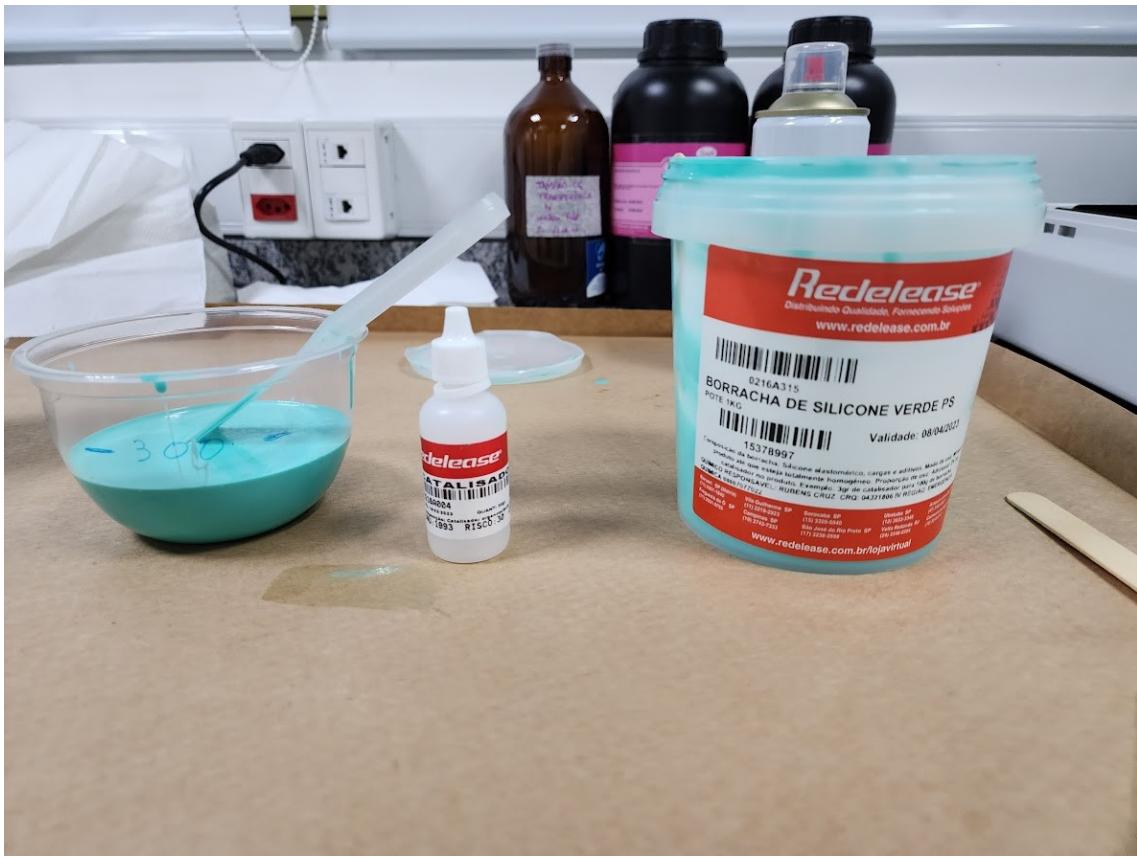
3. wrap the upper part of the jar with aluminum paper with the help of the masking tape. Make it tight and vertical. Put masing on every interface between the aluminum foil and the jar, and where the foil ends. The aluminum foil must exceed the jar mouth at minimum 3cm in height.



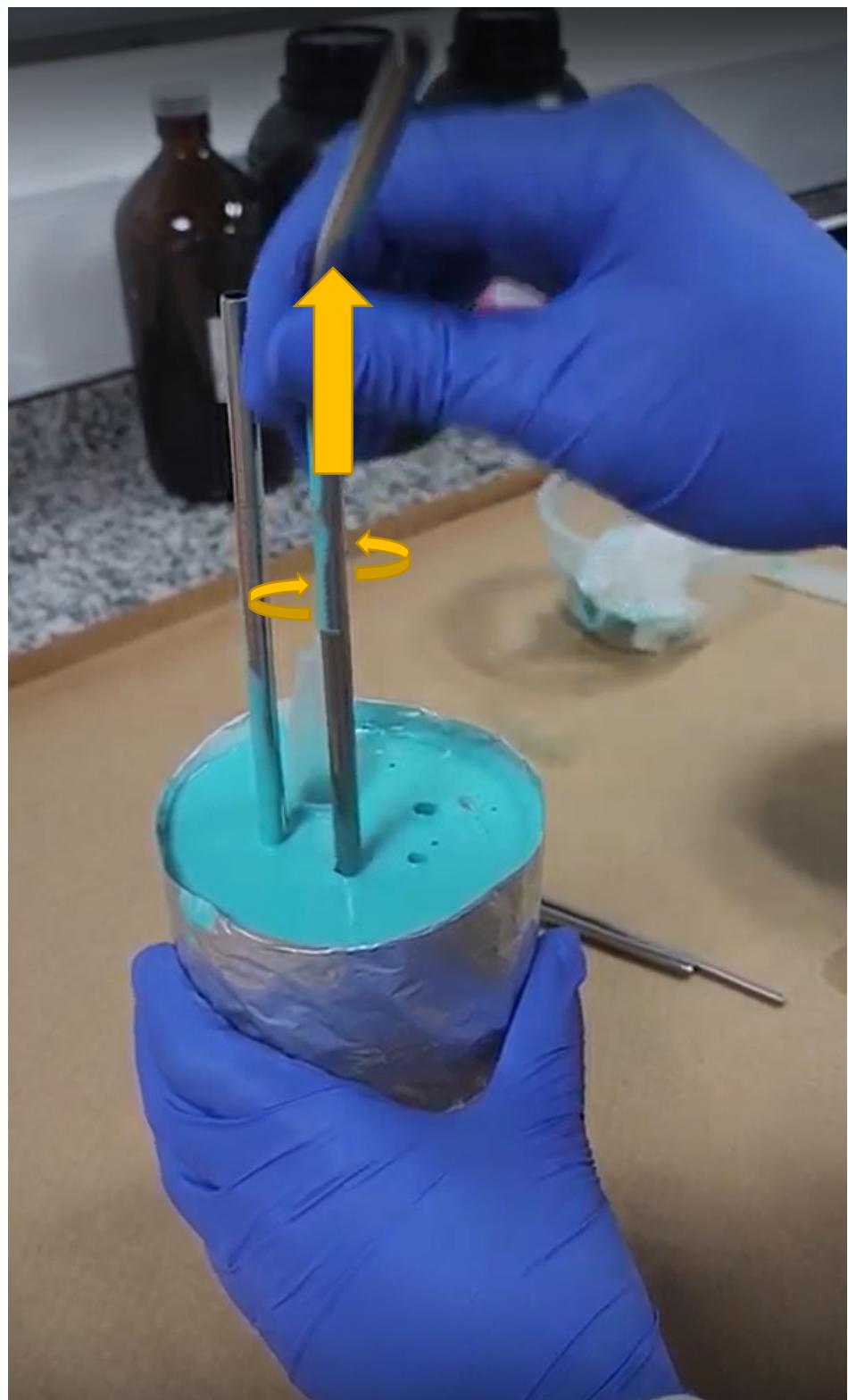
4. Insert all the tubing into the agar-agar



5. Catalyze the rubber in a disposable cup, according to manufacturer instructions.



6. Pour the rubber slowly into the mold to avoid bubbles.
7. Wait for it to cure.
8. Remove everything you inserted before. A slow twist movement helps to reduce friction



9. Remove the aluminum foil

10. Remove the lid with extra care to avoid silicone rupture. First unglue the bottom part near the neck, and then twist and pull up from the jar.

## Gas Inlet & Outlet

As a simple and easy solution to autoclavable stainless-steel tubes are stainless straw tubes. They are implemented as inlet and outlet gas tubes.

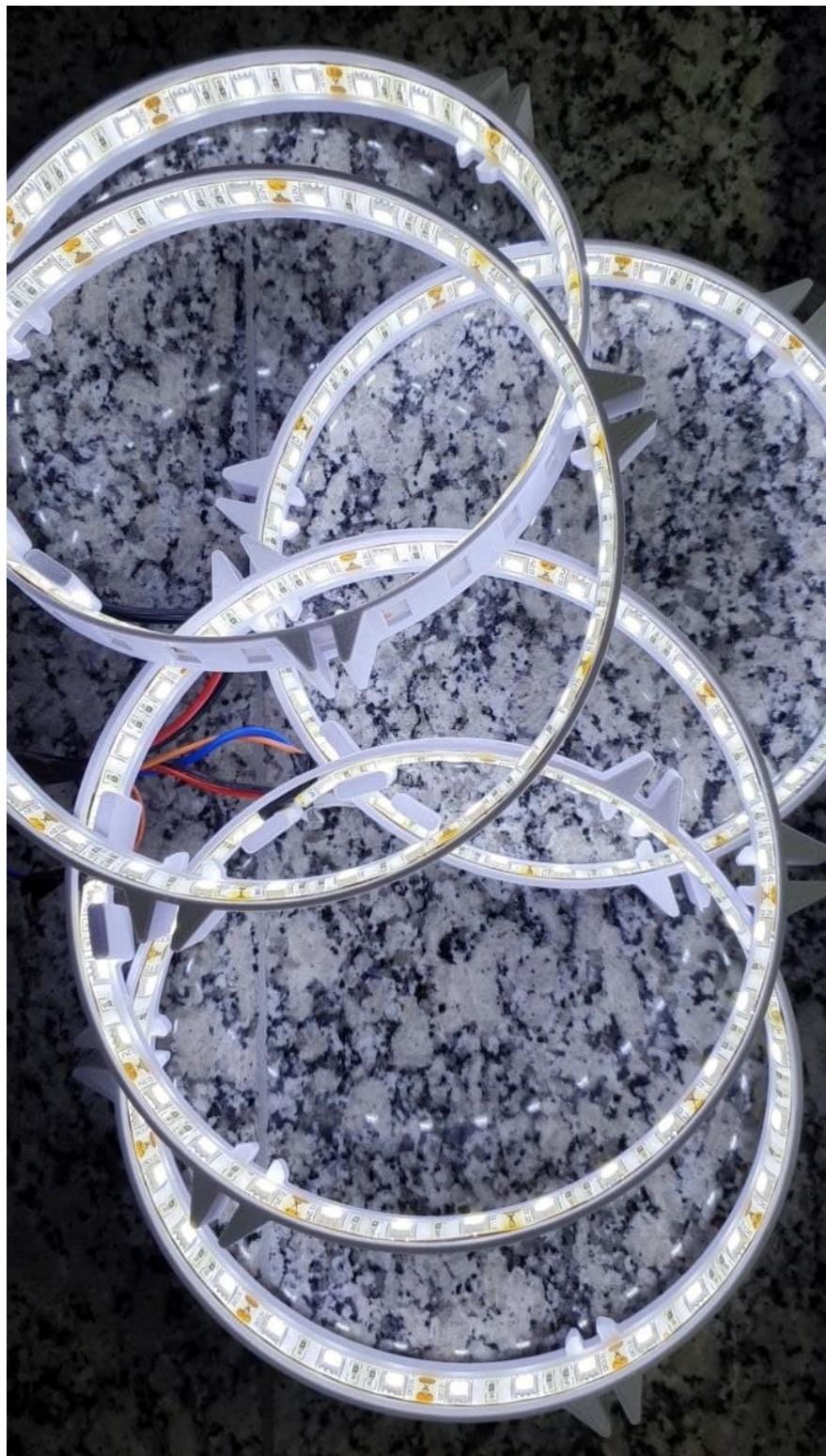


To keep everything sterile, we add sterile syringe filters.



## LIGHT SOURCE AND CONTROL

As a light source, it was developed a printable structure to hold commercial 12V Led light strips. This support file is available with the 3D printable parts



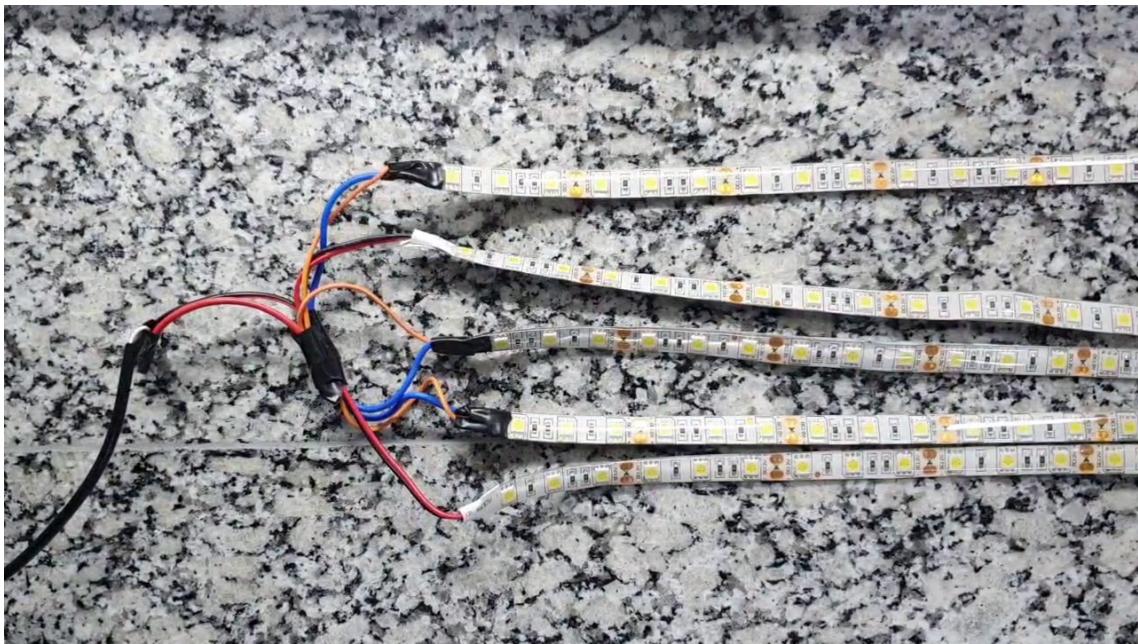
To build it, you will need:

- 3D printer and filament/resin
- LED light strips (50cm total)
- Some wiring
- 12V power source for LEDs

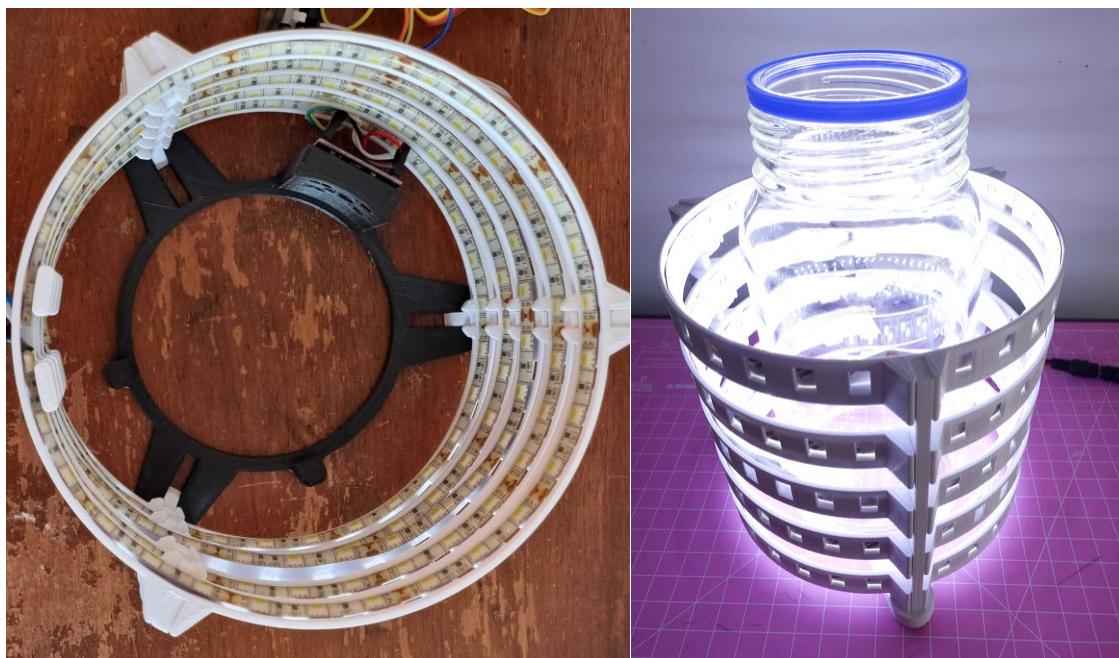
1- The first step is to print the rings, supports and the base.



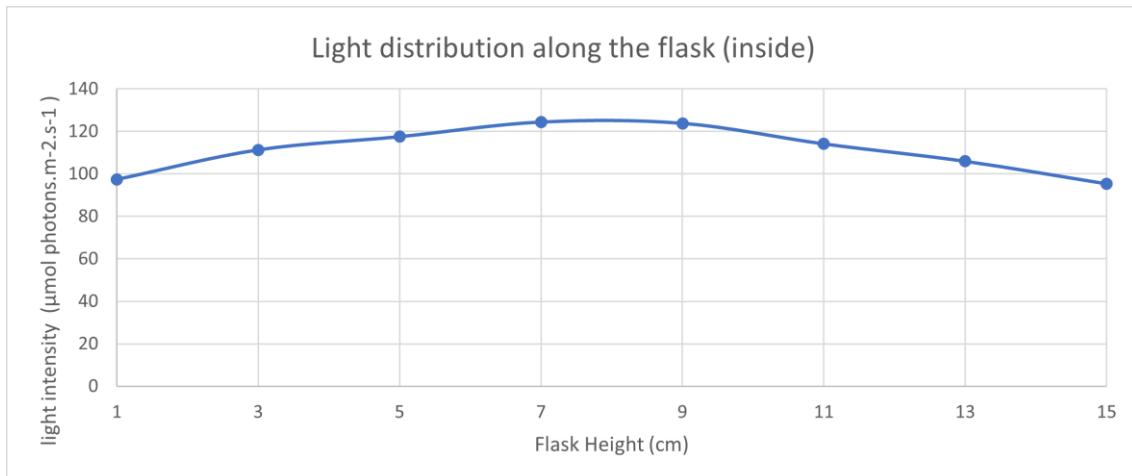
- 2- Cut the LED strips in 10cm sections.
- 3- Add 15 to 25cm of wire to each independent section (attention to the voltage indication using +/- signs).
- 4- Solder the other end together or use a connector to unify all wires, attention again to voltage.



5- Put the led strips inside each ring. It is a good recommendation to use the ring holes to pass the wires to the outside.



You can see that the light distribution is well uniform along all the reactor:



# BIOREACTOR SENSORS AND ELECTRONICS:

## RASPBERRY PI

You will need a Raspberry pi 3 or 4. Install the raspberry official Operation System on the SD card. Connect it to the web to be able to install all updates and software. Raspberry pi 2 and below or zero are not recommended.

## MYCODO SOFTWARE

We have tested multiple, but the easiest way to go is to install Mycodo according to their instructions.

Mycodo repository: <https://kizniche.github.io/Mycodo/>

After installing it, you will need to open the Raspberry pi IP address on a computer on the same network.

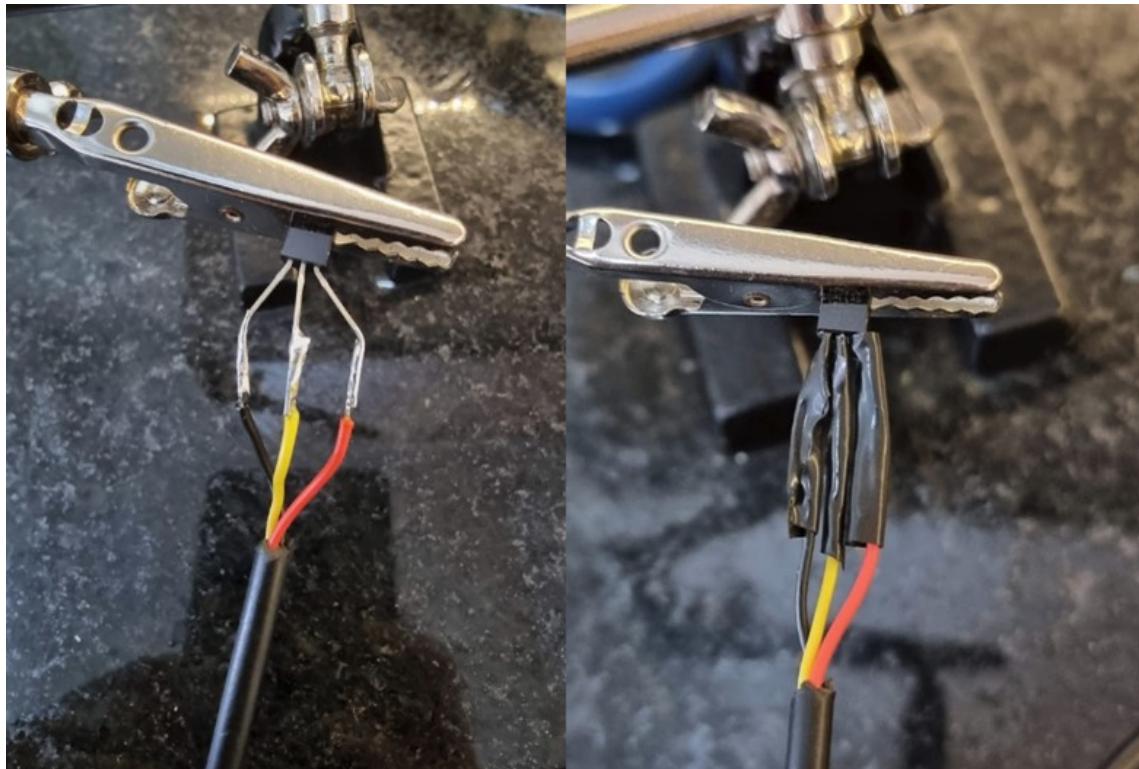
On the Input page, you will select the desired sensor (e.g.: pH: atlas Scientific-I2C, Temperature: DS18B20)

The screenshot shows the Mycodo software interface with the 'Data' tab selected. On the left, a sidebar lists various sensor types and their models. The 'Pressure/Temperature/Humidity: BME 280' section is currently highlighted. On the right, there is a list of configured inputs, each with a 'Deactivate' button and up/down arrows for reordering. At the bottom, there is a row of buttons for adding new inputs (a plus sign icon) and selecting SIGNAL\_PWM Input.

Input ID	Description	Deactivate	Up	Down
[01] MYCODO_RAM				
[02] RPi				
[03] RPICPUload				
[04] RPISpace				
[05] BMP180				
[06] HTU21D	Activate		Up	Down
[07] SIGNAL_PWM	Activate		Up	Down

## TEMPERATURE SENSOR

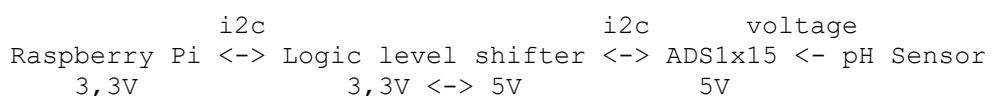
We have chosen the sensor Dallas DS16B20 because it is digital, it is 3,3V compatible, and has an encapsulation TO-92. This allows us to introduce the sensor inside a stainless tube (stainless straw), so we can read the temperature inside the reactor chamber. We add a silicone the one end of the tube and keep the other side open. The closed end is pointing down, it is inserted in the silicone lid. We can autoclave the liquid inside the reactor, without the sensor, and introduce the sensor before running the batch.

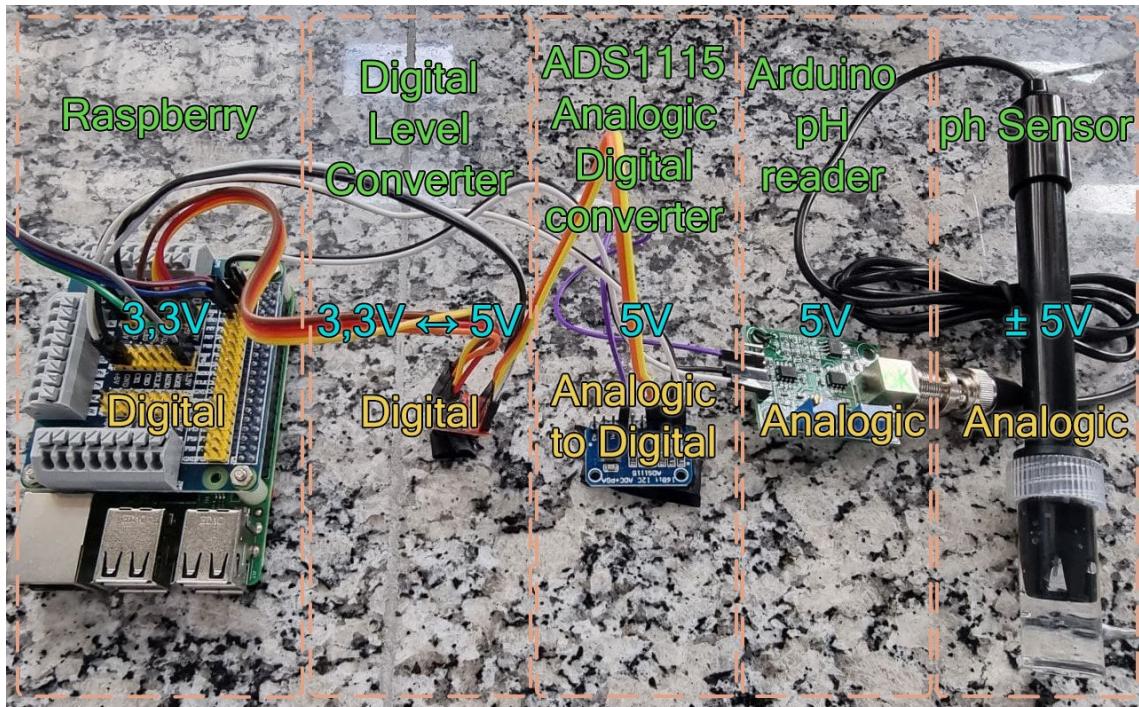


## PH SENSOR

To keep it accessible to everyone, we use a 5V pH sensor for Arduino. This comes with a problem, this sensor uses 5V analog signal and Raspberry pi uses only digital 3,3V. To solve it, we add to the circuit a Level shifter (voltage converter for logic values). We also must solve the lack of analog input on the raspberry, so we use the ADS1115 or ADS1015 analog to digital converter. This can communicate with the raspberry using the I2C protocol.

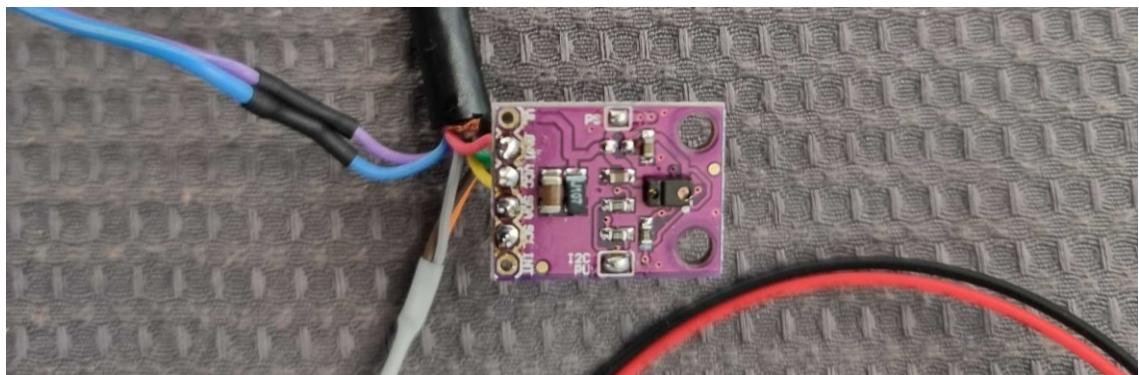
The sequence of steps is:





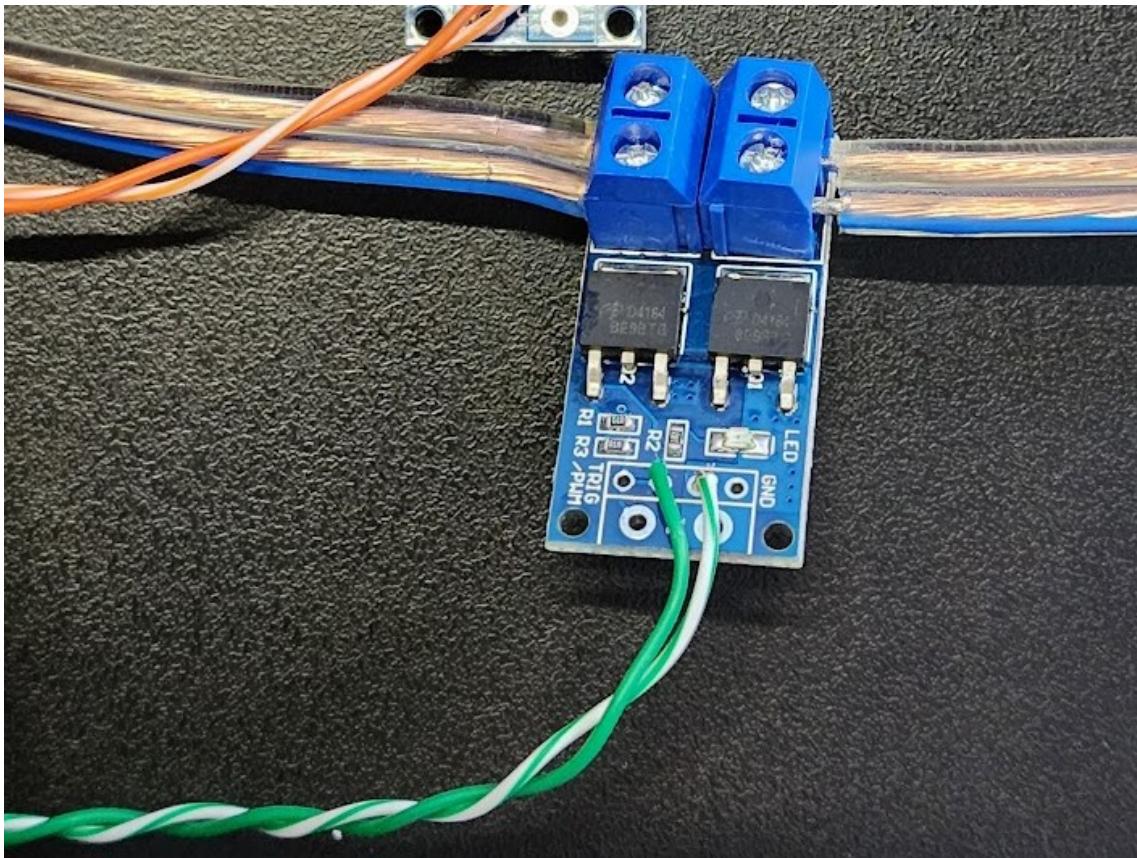
## COLOR SENSOR

We have implemented the color sensor APDS-9960. It is connected to the I2C ports of the raspberry, and we developed a holder to it integrated in the 3D CAD of the LED structure.



## ACTUATORS

To digitally control actuators, we found that the electronic module which can be found by the name 400w 30A Mosfet module D4184 works very well with Raspberry pi. You will need to connect it to the Raspberry PWM pins.



On Mycodo you will need to configure the output page to control the output port with the chosen pin. Mycodo has the capability to trigger an actuator in response to changes, for example, light values changes, and activate the controller.

### **ATTENTION**

Never connect the raspberry pi output to any motor or use it to directly control anything! You will burn the raspberry ports, and there is risk of electrical short circuits and fire. Always use it inside the datasheet specs, and never inject any voltage above 3.3V. Also never use the Raspberry 5V or 3.3V to power anything that is power hungry.

Using the aforementioned MOSFET module, you can also control Peristaltic pumps. Note you will need an adequate power source.

