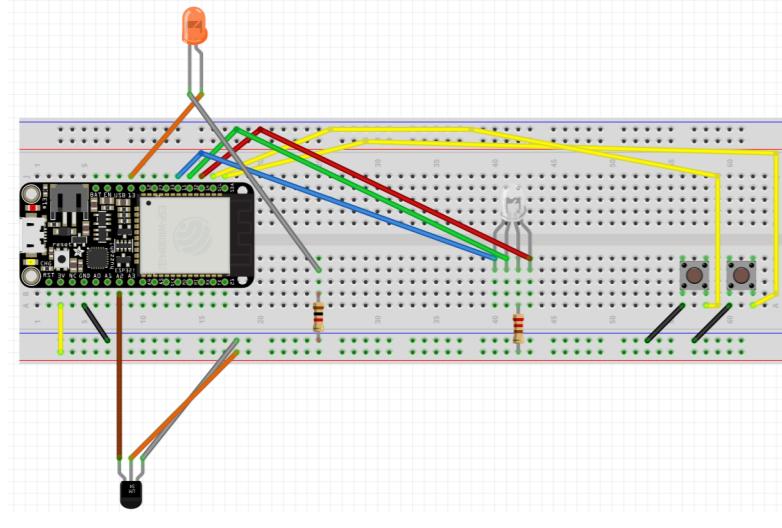


Device for measuring cell growth

Team 30 "Day is always first"
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Overview

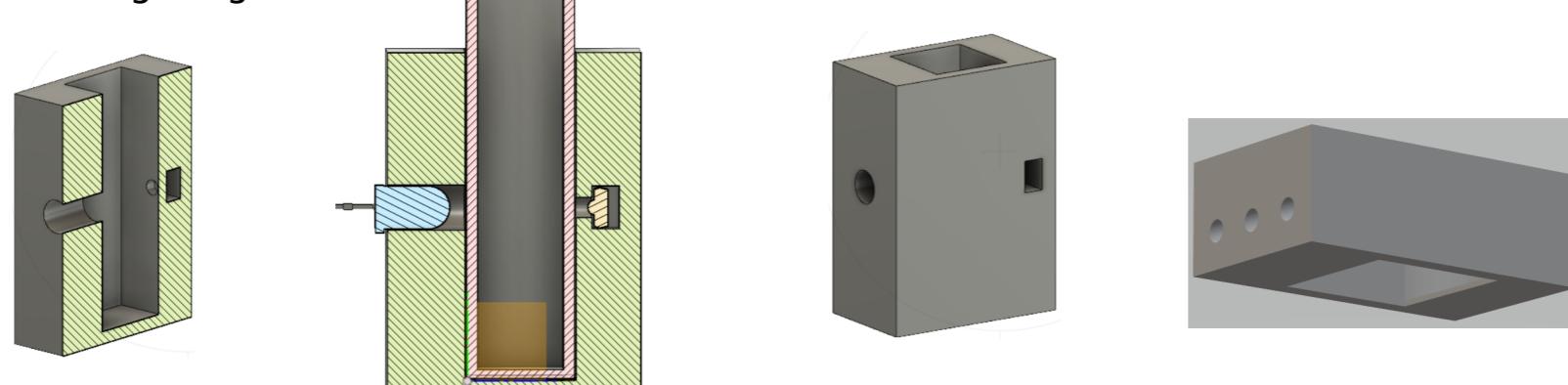
A spectrophotometer is a device used to measure the absorbance of light (600 nm) and based on the optical density (OD) measured, we can estimate the amount of cells in a sample at a time. This project is focused on creating a device like this that can capture the change in OD over time. To achieve this goal we combined our knowledge in 3D printing, software, electronics, lab work, biology and mathematics. The device was tested on two different growth experiments with two types of stock cells (M05 Mead - first cell, M15 Empire Ale - second cell).



Drawing of the breadboard. The black part in the bottom of the picture is representing the light sensor.

The 3D printed cuvette holder is designed so that it meets the following requirements: The holder shall be able to hold the cuvette, LED and sensor so that LED and sensor are in a straight line, placed on each side of the cuvette. The holder must also be light tight but not air tight when the lid is on and the bottom under the cuvette shall not be too thick since the holder is designed to be used on a magnet stirrer.

To let air in without letting light in we made some small holes in the lid that were not horizontal but had an angle of 19 degrees from the horizontal so that the hole was sitting higher in the inside of the lid than on the outside of the lid. After placing the components in the holder we also tested that it was light tight by making the sensor collect data when the LED was turned on, and we found that the holder is light tight.



Pictures of the 3D drawing (from left) 1st picture: Cut through cuvette holder. 2nd picture: Cut through cuvette holder with the LED, sensor and cuvette. 3rd picture: The cuvette holder from the outside. 4th picture: The lid with the air holes.



Dilutions 3, 4 and 5 on agar plates

Dilution number	OD	Number of cell colonies	Expected number of cell colonies on agar plate	Cells/mL
0	0.1	-	50,000	500×10^3
1	0.1×10^{-1}	-	5000	500×10^2
2	0.1×10^{-2}	-	500	500×10^1
3	0.1×10^{-3}	50	50	500
4	0.1×10^{-4}	7	5	50
5	0.1×10^{-5}	4	0.5	5

CFU analysis

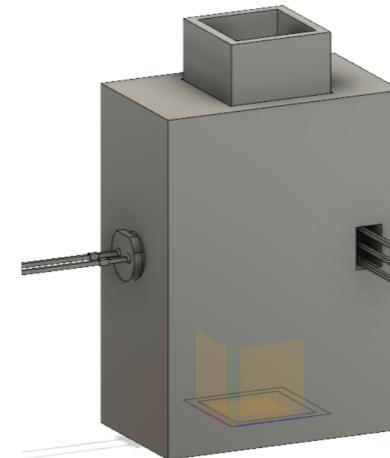
To estimate the number of cells/OD/mL we did a colony forming units (CFU) analysis.

For the analysis we made a dilution series starting with a dilution of OD 0.1 (dilution 0) with Empire Ale yeast. The rest of the dilutions series (dilution 1-5) were 100µL of sample and 900µL of medium.

All of our results can be seen in the table on the left. The OD of dilution 1-5 is calculated from the OD of dilution 0 and both the expected number of cell colonies on agar plate and cell/mL are calculated from the number of cell colonies on the dilution 3 plate.

Hardware design

The electronics of the device were designed to fulfill the requirements set by the project and to support the user friendly features of our design (RGB LED and buttons). The orange LED and the light sensor are used for executing the experiment and taking measurements of OD. The RGB LED acts as an indicator of the active mode of the device at a given time and the buttons are used to trigger mode change and individual action for each mode.



The 3D model of the cuvette holder with the LED, sensor and the cuvette.

The program was written in MicroPython and implemented on a Huzzah 32 board. It starts by configuring the pins, initializing the sensor and the LED. The program then checks the current mode and whether the mode button or action button is clicked. A RGB LED is used to indicate which mode the program is currently in. The different modes perform specific functions and check for certain conditions before running, as shown in diagram. Whenever the action button is clicked in the reference setup mode, a single reference point is collected. In the measurement mode, the action button first checks if reference data is captured and then starts collecting a data point every 5th minute.

The calibrated OD data is calculated in the program using the previously measured reference point and then a regression function is applied to translate it to OD.

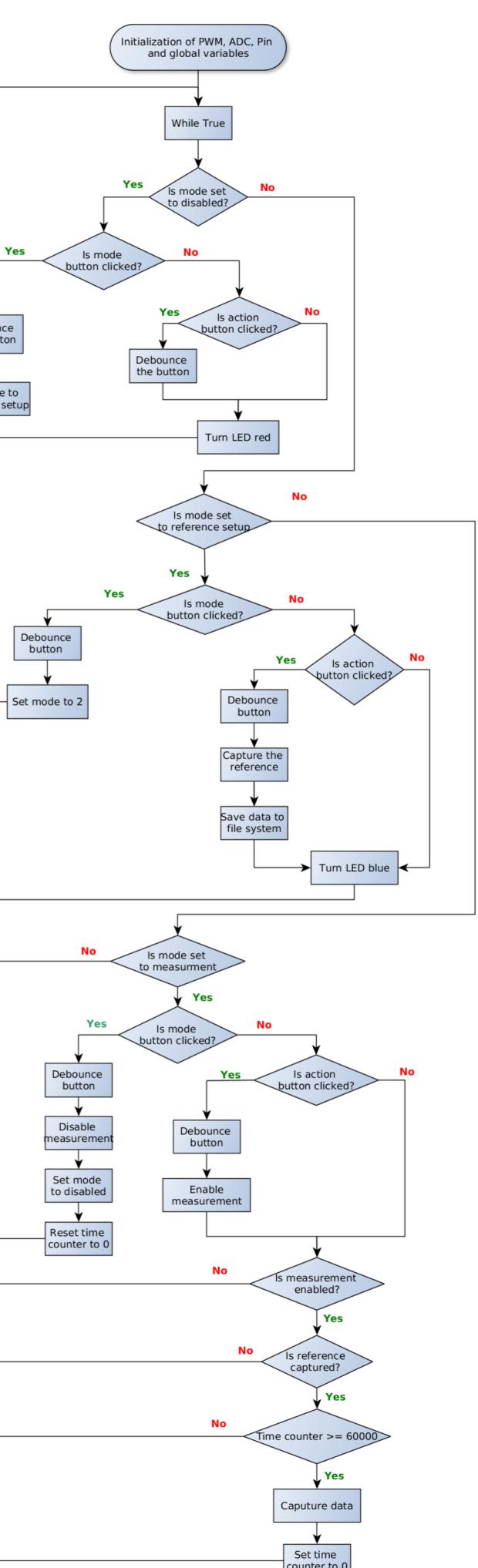
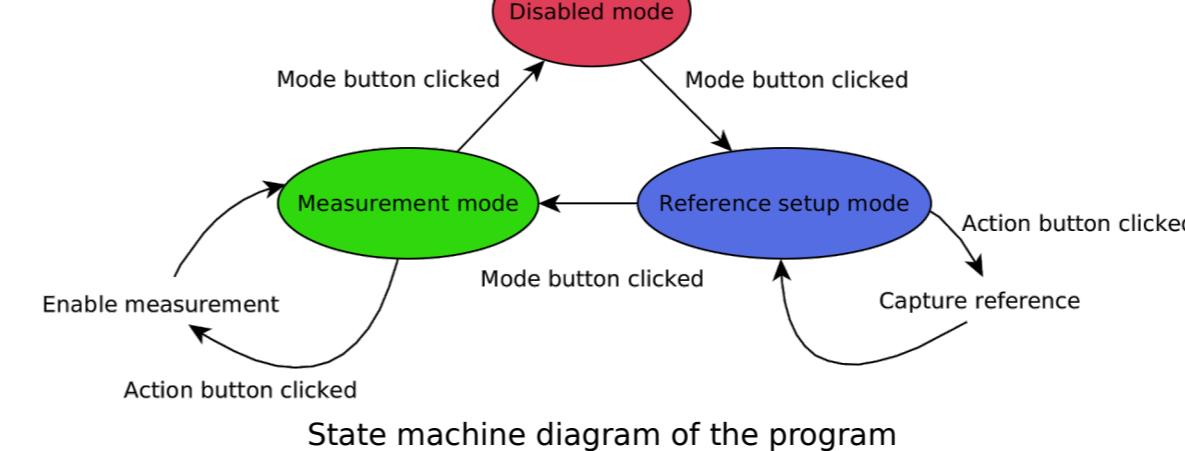
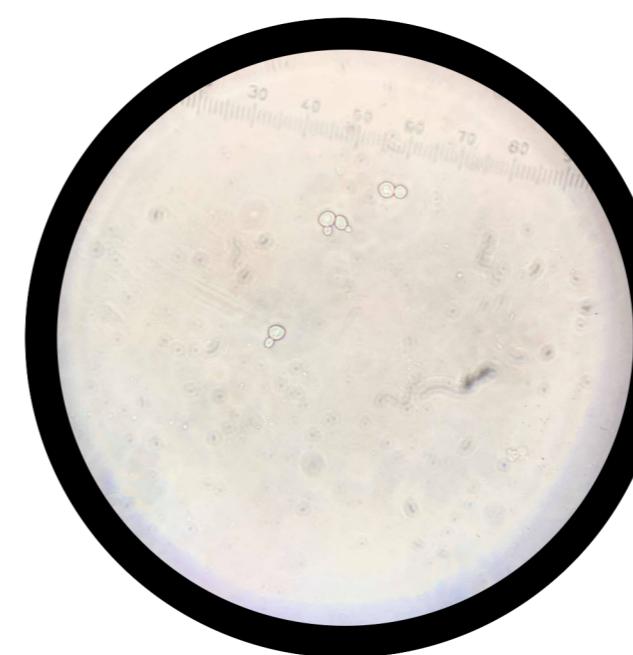


Diagram of the program

Microscopic observations

The yeast cells from the second overnight experiment, were observed under an Olympus BX40 microscope. The microscope lens was set to 20x and 40x (see the figure). Living and dead yeast cells as well as few bacteria cells were observed in the sample.



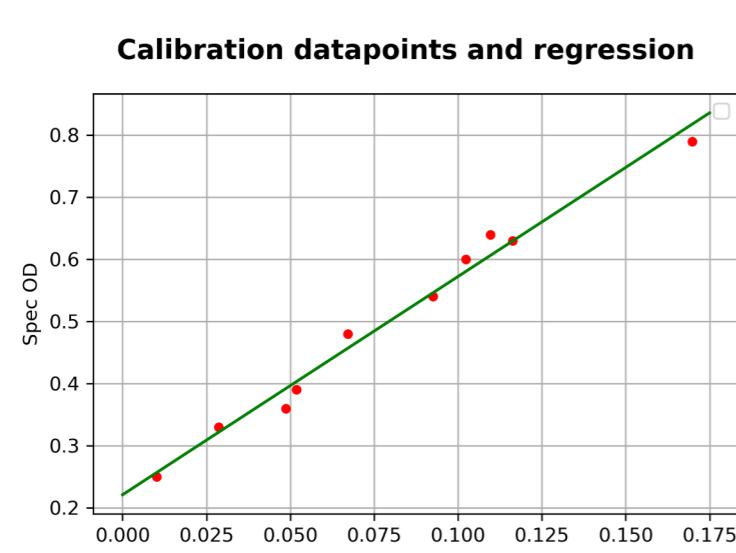
Picture captured with 40x lens

Device Calibration

To find the relationship between the OD measured with the spectrophotometer and the OD measured with the device, we made a calibration of the device. We samples with OD in the linear range of the spectrophotometer, measured the two different OD values and made a linear regression of the relationship between the OD measurements. We had to do the calibration several times before we found our final regression. The device OD is calculated from the ADC (I) and the reference ADC (I_0).

$$OD_{device} = -\log_{10}\left(\frac{I}{I_0}\right)$$

Calibration datapoints and regression

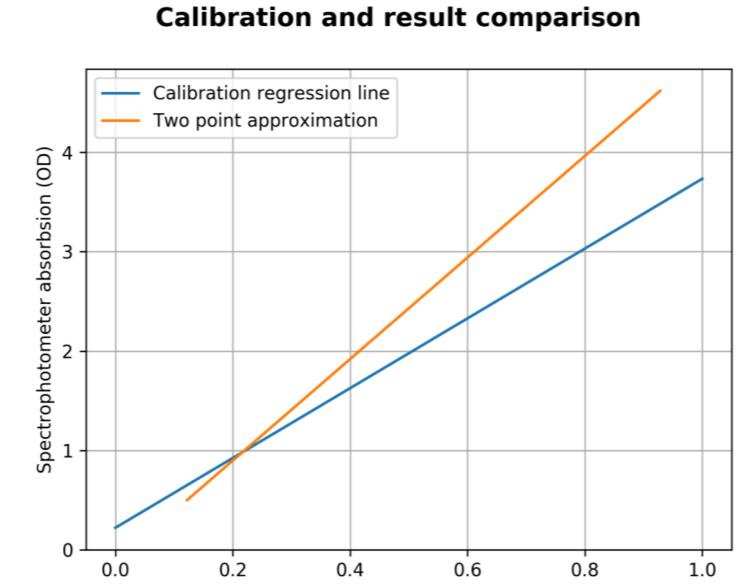


The calibration data points (red dots) and the linear regression (green):

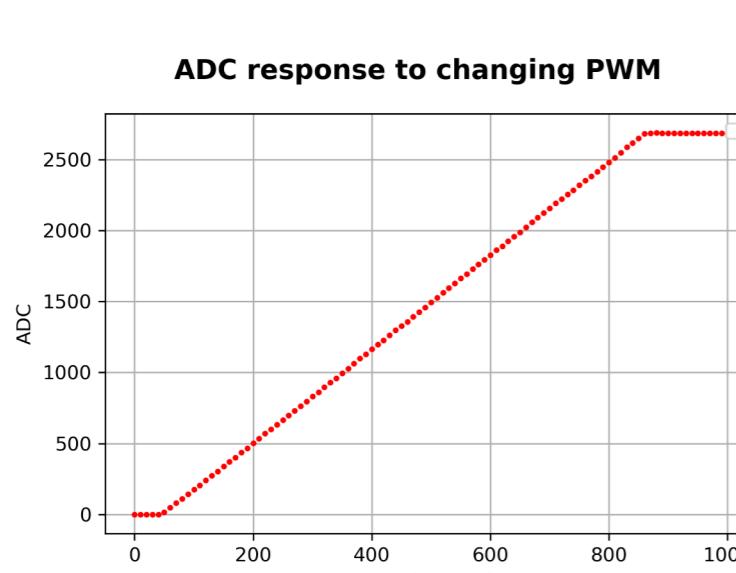
$$3.51358227885507 * x + 0.220979790461345$$

In the plot we can see that our device cannot measure OD values under approximately 0.2 OD.

Calibration and result comparison



The red line is between the start and the end point before and after one of the experiments. This graph shows that there is still some deviation from the regression we made.



This graph shows the relationship between the ADC measured while the PWM rate of the LED was changed. The sensor saturates when the LED intensity is around 850. This relationship was reached by testing the sensor response to the light passed through cuvette with water.

Data analysis

During the development of the device, we have conducted three experiments. However we have agreed to exclude the data from the second experiment as it was terminated before the growth rate saturated. The two experiments we ended up using were the experiments with the Mead yeast and the Empire Ale yeast. The absorbance over time (cyan) is shown on the plots on the left.

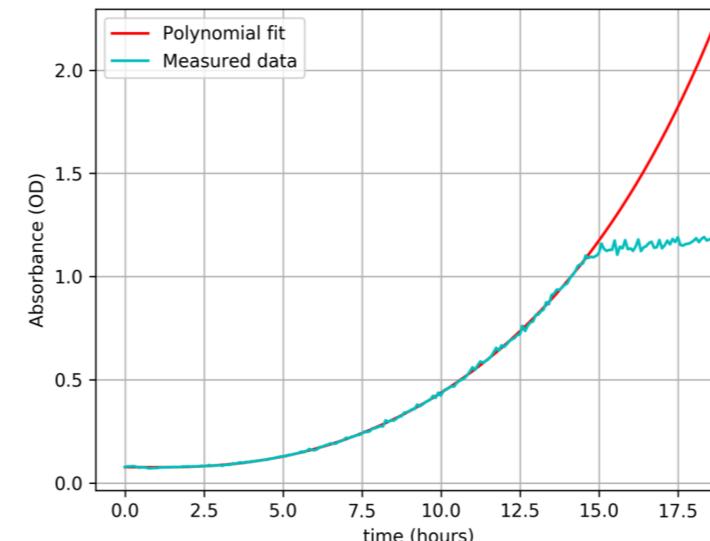
To calculate the growth rate for each of the strains, we needed to find out which approach will work in our case. Since we have not achieved a complete calibration, we could not use the logarithmic approach as it expects the data to be calibrated with the spectrophotometer. We have decided to use the biomass approach, where we first need to fit a polynomial (red) to the data points that are following the exponential part of the growth and then calculate the growth rate using:

$$\mu = \frac{dB}{dt}$$

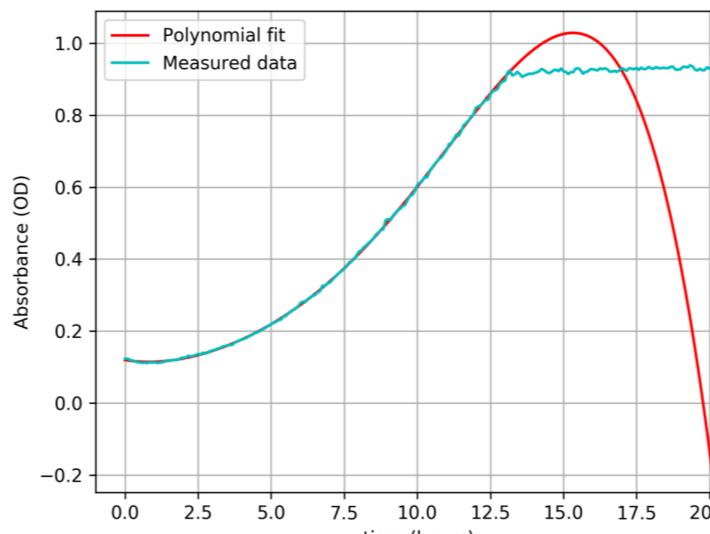
This way we can get a growth rate that is insensitive to background signal.

The growth rate of the Mead yeast was measured to be higher than the one of Empire Ale yeast, however we can see that the EA yeast reached saturation sooner than the M yeast. The reason is that when taking a starting sample from the petri dish, we used more cells in the case of EA. Another thing to notice is the negative growth rate in the beginning of the experiments that is caused by a drop in the OD that was measured, which is probably caused by light leakage, therefore we should not really take this data points in consideration.

Change of absorbance over time (Mead)



Change of absorbance over time (Empire Ale)



Growth rate μ of both strains

