UTRECHT SCIENCE PARK
INNOVATIONLAB
LIFE SCIENCES & CHEMISTRY

University of Applied Sciences
Research Centre Life Sciences & Chemistry

HOGESCHOOL UTRECHT

Optimal growth conditions of *Saccharomyces cerevisiae* in bioreactors

QVQ Quality in Antibodies

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Introduction

The company QVQ has invested in fermentation equipment (bioreactors) and now can profit from years of knowledge to obtain high quantities of VHH's in a fermentation process. QVQ has got two standard protocols that are available and aligned to produce well VHH's.
QVQ would like to further optimize its fermentation protocols in collaboration with Hogeschool Utrecht. To achieve the optimization, we first have to calibrate and validate the HU bioreactor system. The yeast *Saccharomyces cerevisiae* is used to calibrate, validate and optimize the protocols. For the optimization of the fermentation process, we would investigate under which condition (pH, glucose concentration and temperature) the yeast would double the most in the less of time.

Plan of action

Period A:
- Plan of action
- Literature report
- Making protocols

Period B:
- Calibration
- Validation
- Optimum conditions *Saccharomyces cerevisiae*
- Final report/presentation

Plan	
Date	**Experiment**
29 & 30 October	Testing multiple yeast concentration + validation
6 & 7 November	Testing 5 g/l glucose
14 & 15 November	Validation dagy1
21 & 22 November	Validation day 2
28 & 29 November	Higher glucose / lower yeast concentration
5 & 6 December	Testing pH 4 & 6
10 & 11 December	Testing glucose 44g/l
12 & 13 December	Testing temperature 25°C
19 & 20 December	Test ingtemperature 21°C

Materials & Method

Before we could investigate the uptimum conditions in which the yeast doubles, we first have to calibrate and validate

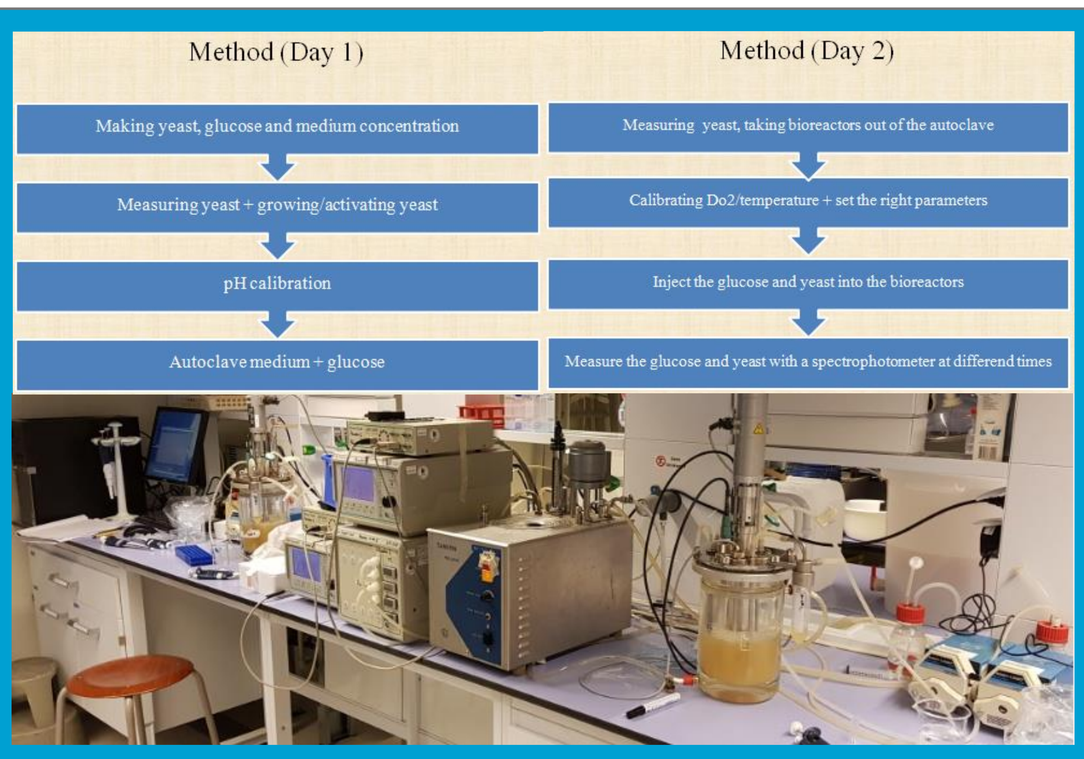
Calibration
The pH is calibrated with 2 buffers pH 4 and 7.
The Temperure is calibrated by extern thermometer.
The DO_2 is calibrated by testing and setting the setpoint at 100% oxygen and 0% oxygen.

Validation
The validation consisted of 2 duplo experiments using two different bioractors. The standard condiitions are 30°C, DO_2 (30%), 22 g/l glucose and pH 5.
The bioreactors are validated when the results correspond for 90%.

Growing yeast
$6x10^{-2}$ g/l yeast was activated in medium for 24 hours in 30°C. Medium consisted of 2% glucose, 2% yeast extract and 1% peptone.

Yeast media and glucose concentration
The media consisted 10 g/l yeast extract and 10 g/l pepton. The glucose concentration consisted 22 g/l glucose. Both concentrations must have been in a autoclave before using it in bioreactors.

Sampling and analysis
The yeast and glucose are first being added to the bioreactor (that already contrains media). Second a sample is taking and measured to see it's starting point. Both the density and glucose are being measured to find the doubling time when the yeast still has it's carbonsource (sugar).The OD600 method is used to measure the doubling time at 600nm and the glucose concentration is measured using the GOD-POD method by 503nm. To find the doubling time, the sample is diluted for 100 times. To find the sugar concentration, a standard glucose curve has been made. The samples to find the glucose concentration has been diluted for 20 times.



Results

A standard glucose curve has been made with the formula: y = 0,419x + 0,052
The coefficient of determination is 0,989.
Most of the validations results correspond with each other. The average of the validation results has been made to compare to the results of the other conditions. The validation (standard conditions) has a doubling time of 2 hours and 57 minutes.

5 g/l glucose concentration was started as the first experiment, this turns out to be too little to see the exponential phase.

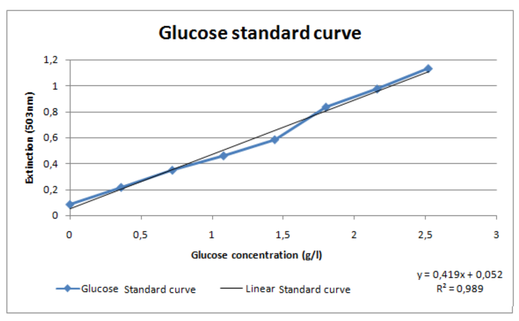


Figure 1 This figure shows the glucose standard curve. The concentrations used, are 0,00-, 0,36-, 0,72-, 1,08-, 1,44-, 1,80-, 2,16- en 2,52 g/l glucose. The coefficient of determination is 0,989 and the formula y = 0,419 + 0,052.

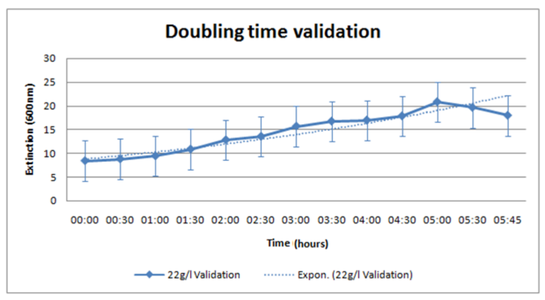


Figure 2 This figure shows the doubling time of the validation from the average of the results. The doubling time is 2 hours and 57 minutes. An average of the standard condition test has been used (N=4)

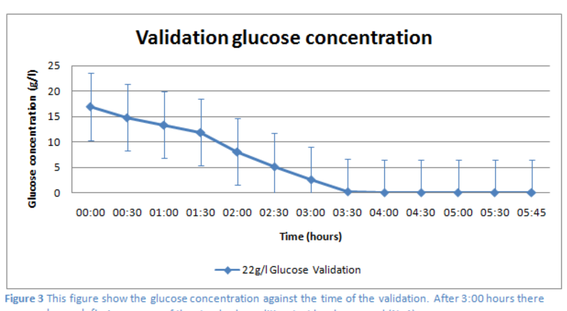


Figure 3 This figure show the glucose concentration against the time of the validation. After 3:00 hours there was no glucose left. An average of the standard condition test has been used (N=4)

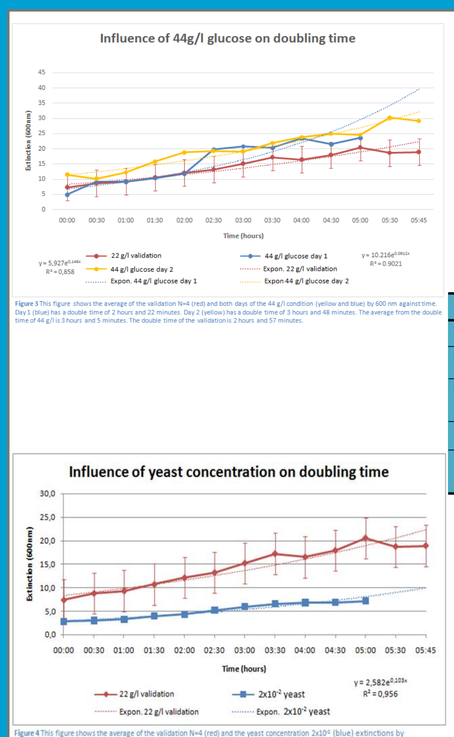


Figure 3 This figure shows the average of the validation N=4 (red) and both days of the 44 g/l condition (yellow and blue) by 600 nm against time. Day 1 (blue) has a double time of 2 hours and 22 minutes. Day 2 (yellow) has a double time of 3 hours and 48 minutes. The average from the double time of 44 g/l is 3 hours and 5 minutes. The double time of the validation is 2 hours and 57 minutes.

Doubling time at different conditions for *Saccharomyces cerevisiae*		
Condition	Concentration/ value	Doubling time
Glucose	22 g/l	02:57 hours
	44 g/l	03:05 hours
pH	4	04:22 hours
	5	02:57 hours
	6	04:34 hours
Yeast	$6x10^{-2}$ g/l	02:57 hours
	$2x10^{-2}$ g/l	03:21 hours
Temperature	21°C	05:51 hours
	25°C	06:05 hours
	30°C	02:57 hours

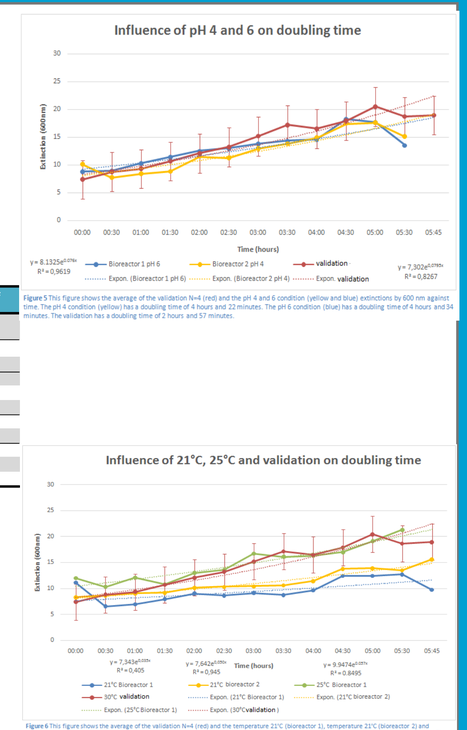


Figure 5 This figure shows the average of the validation N=4 (red) and the pH 4 and 6 condition (yellow and blue) extinctions by 600 nm against time. The pH 4 condition (yellow) has a doubling time of 4 hours and 22 minutes. The pH 6 condition (blue) has a doubling time of 4 hours and 34 minutes. The validation has a doubling time of 2 hours and 57 minutes.

Figure 4 This figure shows the average of the validation N=4 (red) and the yeast concentration $2x10^{-2}$ (blue) extinctions by 600nm against time. The $2x10^{-2}$ yeast concentration has a doubling time of 3 hours and 21 minutes. The doubling time from the validation was 2 hours and 57 minutes.

Figure 6 This figure shows the average of the validation N=4 (red) and the temperature 21°C (bioreactor 1), temperature 21°C (bioreactor 2) and temperature 25°C extinctions by 600 nm against time. The temperature 21°C in bioreactor 1 (blue) has a doubling time of 4 hours and 53 minutes. The temperature 21°C in bioreactor 2 (yellow) has a doubling time of 6 hours and 49 minutes. The average of both 21°C experiments is 5 hours and 51 minutes. The temperature 25°C has a doubling time of 6 hours and 5 minutes. The average of the validation has a doubling time of 2 hours and 57 minutes.

Conclusion

Both of the bioreactors have been validated with the standard conditions: 30°C, DO_2 of 30%, 22 g/l glucose and pH 5. Most of the validation results correspond for 90% with each other, resulting in a doubling time of 2 hours and 57 minutes.

The results reveal that the best growth conditions for *Saccharomyces cerevisiae* are: 30°C, pH 5,0, a higher concentration yeast than $2x10^{-2}$ g/l and a glucose concentration of 22 g/l gave the best doubling time of 2 hours and 57 minutes.

Future work

- Testing the same yeast strain as QVQ
- Testing protein production
- Testing other conditions (for instance ethanol)