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Optical online analysis of synchronized yeast cultivations

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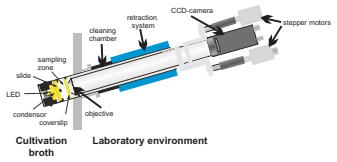
Introduction

The technical use of yeast has been known or many years e.g. in bakeries and breweries. Until the beginning of the 19th century it has been unknown that yeast is a micro organism. Nowa days about 5 million tons of yeast are produced each year. The most commonly known yeast strain is *Saccharomyces cerevisiae*. Due to the fact that cultivating these cells is easy and cheap they are often used in research as model organism. Continuous yeast cultures show the ability to have an oscillating growth rate, the have an synchroniyed cell cycle. Although this is has often been investigated in different studies, the mechanism has not yet been fully understood. First theories, like the one from von Meyenburg (1966) indicates that the pH value and the sodium concentration play an significant role. In the early 1990's experiments showed that the oscillating can be started by a glucose pulse. But also a pulse of Ethanol, Glycerol, Acetate and Pyruvat are able to start the process .

In situ microscopy

For our experiments we use an continuos oscillating yeast culture. After a batch culture of 21 hours, the continuous culture starts.

Two different optical online analytic systemswere used to observe a yeast cultivation. Firstly the in situ microscope shows directly the biomass concentration, cell density and cell size. With the in situ microscope the cell size has been observed. During the G1 Phase the highest concentration of single cells have been detected. During the S Phase this concentration decreases and more doublets can be observed which inclines the beginning of the yeast sprouting .

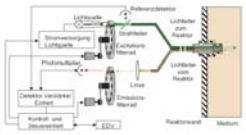


Instrumentation

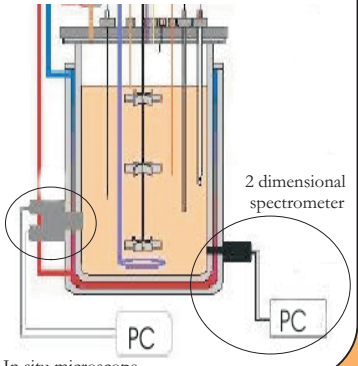
Yeast strain
Saccharomyces cerevisiae wild type H620
Reactor
2 L steel tank
Cultivation parameters
1.8 L Schatzmann media, 30 °C, 5L/min air rate, stirrer 1200 rpm
Cytometer
Coulter Epics XL
BD FACS Vantage SE with Macro Sort option
Fluorescent dye
Sytox Green S-7020

2 dimensional spectroscopy

The 2 dimensional spectroscopy is a direct, non invasive method to measure the relative concentration of fluorophoric compounds. In the case of yeast and other eucariontic cells one can detect NADH, pyridoxin, tryptophan and flavines, which play an important role in the metabolism. The fluorophores can be differed by their excitation- and emmision- maxima.

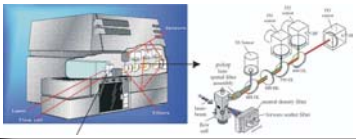


Setup of the 2 litre steel tank reactor



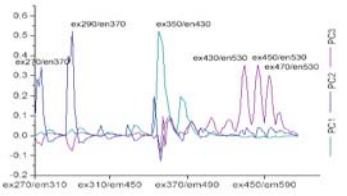
Flow cytometry

With flow cytometric analysis cells or particles in suspension are measured. The particles are focused hydrodynamically by the sheath flow stream and are excited by a Laser. In our case an Argon Ion Laser (488 nm, 15 mW). Measurement rate of up to 4000 cells per minute and the ability measure different parameters simultaneously give fast answer about the statistic distribution of the measurands.

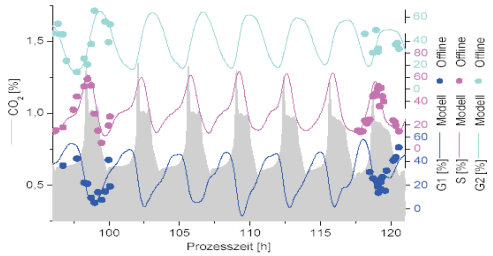


Prediction of the oscillating yeast cultivation by appling chemometric modells

The 2 dimensional spectrometer delivers almost unmanageable amounts of data. To evaluate this data we use multi variant data methods like the Principal Compound Regression (PCR) and generate the chemometric models with the Partial Least Squares method. With these models we are able to predict more complicated process values indirectly.



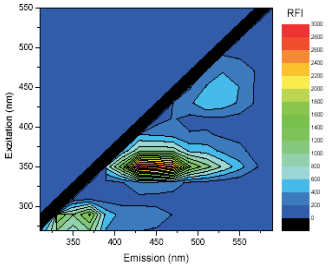
Loading vectors after Princble compound analysis. The peaks are labelled with the corresponding wavelengths.



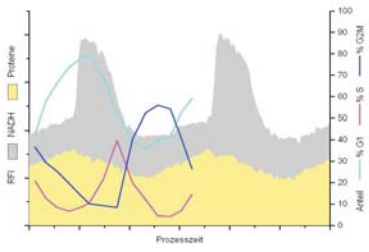
Prediction of the partial cell cycles of the whole population. And validating with offline data. Models are created with 2 dimensional spectra data and flow cytometry results.

2 dimensional spectroscopy vs. Flow cytometry

In our experiments we determine the cell cycle by flow cytometry. After correlating 2 - dimensional spectra with the cytometric results it is possible to predict the periodic change of the cell cycle in the cultures. For a more precise prediction chemometric modells has to be applied.



Contour plot of an 2 dimensional spectra of a continous *Saccharomyces cerevisiae* cultivation



Comparing the results of the 2 dimensional spectra and flow cytometry analysis

Summary

The experiments have shown that it is possible to predict complicated process values based on online optical sensor systems. In further experiments the gene expression of synchronious *Saccharomyces cerevisiae* will be analysed, to achieve an additional understanding of the nature of the synchronous behavior of the cells. Therefore the cells have to be sorted with an FACS without destroying the RNA. One sorting indicator can be the cell size allthough up to now the sorting results only to an maximum of 90% cells in the G1 phase and 70 % cells in the G2 phase.