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Design of a flow microscope with FIA based sampling for monitoring of cell parameters in shake flask fermentations

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Introduction

As the importance of fermentation processes has been growing over the last years the analysis of cell parameters is still often performed by labour intensive offline sampling. A promising alternative to these methods is the In-situ microscopy, with a fully autoclaveable light microscope placed directly into a bioreactor for inline image acquisition.

As the In-situ microscope is limited to large-scale cultivations in a bioreactor, in this work the system is redesigned into an atline flow-microscope which is suitable for the measurement in small-scale shake flask fermentations, which are particularly important for parameter screening and optimisation of new cultivations in research and development.

A flow cell was designed, fitting and enclosing the sampling zone of the In-situ microscope and using its well established image acquisition and analysis software for cell parameter analysis. The flow-microscope was connected to a FIA system which enabled automated online sampling. The system was applied in cell density monitoring in shake flask fermentations of the model organism *saccharomyces cerevisiae* (Baker`s yeast).

Setup of the flow-microscope

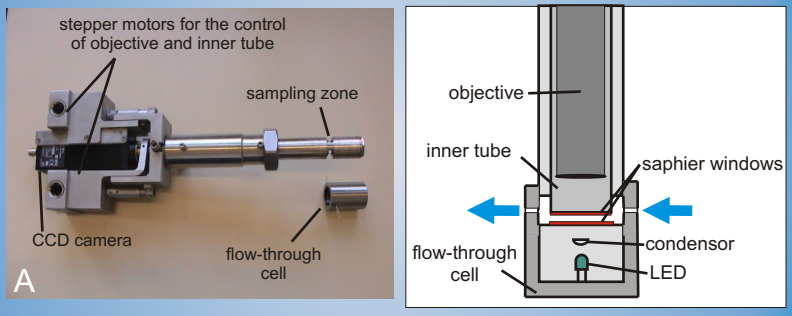

Figure 1: Photo (A) and schematical drawing (B) of the In-situ microscope and the flow-through cell covering the sampling zone.

Setup of the FIA sampling system

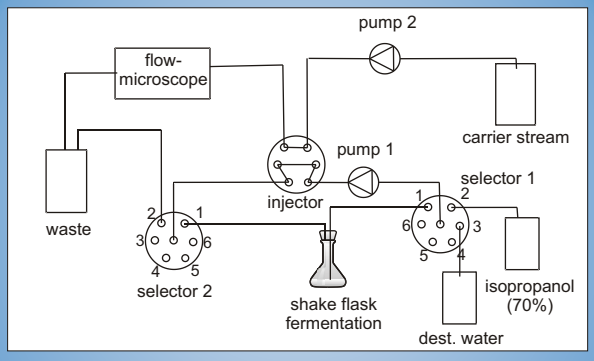

Figure 2: Automated FIA system used to inject a sample (500 µL) from a shake flask fermentation into a carrier stream, which is transported to the flow-microscope for image analysis. It also enables automated sterilization and cleaning.

Calibration of the FIA system

The calibration was performed by injecting solutions of *saccharomyces cerevisiae* with known cell density. Over a certain time interval (80 sec.) after sample injection images were recorded by the microscope (one image per sec.) and the cell density was calculated for each image (for details see " image analysis system"). Plotting this cell density against the time after injection, lead to a FIA result peak (Figure 3). To get a correlation between the result peaks and the deployed cell density the area below the FIA result peaks was determined by integration. Plotting these integrals against the deployed cell density, lead to a linear correlation (Figure 4).

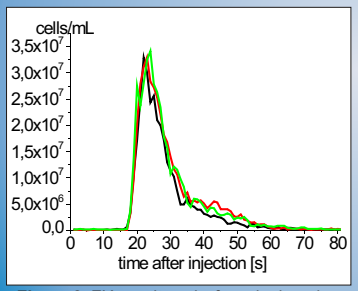

Figure 3: FIA result peaks for a deployed cell density of $1.5 \cdot 10^8$ cells/mL

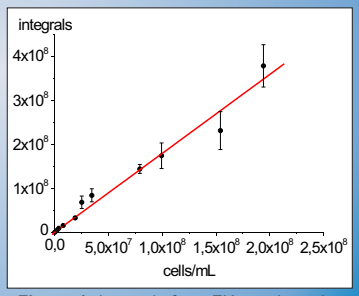

Figure 4: Integrals from FIA result peaks plotted against deployed cell density

Image analysis system

The images were acquired with a monochromatic CCD camera and saved as bitmap-files. The grey value histogram of the images was used to differentiate objects from the background. A border trapping algorithm is applied to determine the shape of the detected objects allowing an analysis of cell morphology and cell size.

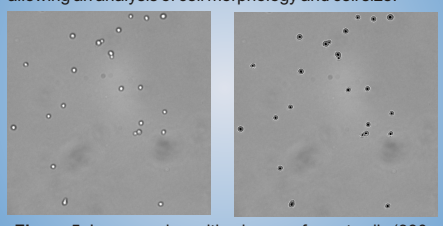

Figure 5: Image and resulting image of yeast cells (390x magnification). The image analysis system detected 24 kernels making 18 single cells and 3 double cells. The cell density is determined from the number of kernels divided by the sampling zone volume (0.0101 mm^3) resulting in $2.4 \cdot 10^6$ cells/mL.

Monitoring of cell growth in shake flask fermentations

Saccharomyces cerevisiae was cultivated in a shake flask at 30°C and 150 rpm, while samples were taken and analyzed every 15 min over 24 h. The resulting cell density was verified by offline sampling after 1 h and 24 h.

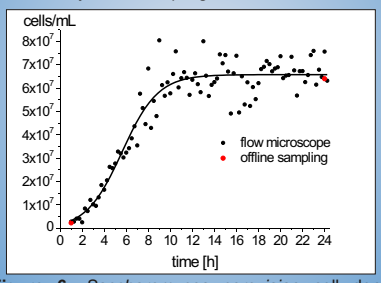

Figure 6: *Saccharomyces cerevisiae* cell density recorded by the flow-microscope and verified by offline sampling

Summary

In this work an established In-situ microscope was redesigned into a flo- microscope and was connected to an automated FIA sampling system. This system was calibrated with samples of *saccharomyces cerevisiae* leading to a linear correlation between deployed cell density and FIA result peaks. Based on this calibration monitoring of cell density in shake flask fermentations of this organism was successfully achieved.

As future work optical sensors for the monitoring of dissolved oxygen and pH will be integrated into the flow-through cell enhancing it into a system for multi-parameter analysis. In addition the FIA based sampling system allows the simultaneous analysis of several parallel shake flask fermentations.