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Image analysis based realtime-control of glucose concentration

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Concept

Microscopic methods are widely used in biotechnology for offline examinations of cultivations. Samples are taken and brought to the microscope, thus it is not possible to control the process without interfering with it. Cell number and viability are determined with delay. In-situ microscopy is a new method to monitor bioprocesses directly in the cultivation vessel. An image analysis software allows to evaluate acquired images without a time lag and direct cultivation parameters as cell number and biomass are obtained online. A process control unit can use these cultivation parameters to optimize the process.

The in-situ microscope consists of the in-situ microscope hardware, the microcontroller, a compact PC with a frame grabber, an image analysis software and a control software for the microscope itself (Figure 1). In combination with the frame grabber and a progressive scan CCD-camera the in-situ microscope represents the image acquisition unit. The frame grabber digitizes the analog signal from the CCD-camera and the digital data can be evaluated by means of image analysis.

The microscope is based on a standard brightfield microscope. To acquire in-situ images the microscope fits into a bioreactor's standard 25 mm side port. To sterilise the microscope body the interior steel tube with the objective is removed (Figure 2).

Figure 1: Equipment of the in-situ microscopy system.

After sterilization the optical system is reinserted into the exterior steel tube. To monitor yeasts, achromatic objectives with a 20-fold magnification (overall magnification 400-fold) and numerical aperture of 0.4 are used. To monitor mammalian cell cultivations, achromatic objectives with a 4-fold magnification (overall magnification 80-fold) and a numerical aperture of 0.1 are used.

The sampling zone is defined by two sapphire windows that work as slide and cover slip of a standard light microscope (Figure 2).

It is possible to retract the sampling zone of the in-situ microscope into a cleaning chamber. The principle is similar to the Intrac System by Mettler Toledo. Furthermore, the retractable probe housing allows to maintain and exchange the optical parts during the cultivation process.

In contrast to previous types of the in-situ microscope the new flow through sampling zone consists of a fixed slide and a movable cover slip (Figure 2). The steel tubes are attached to stepper motors. To define the sampling zone volume a micro controller gives a signal to a stepper motor attached to the outer tube and the desired height of the sampling zone is adjusted. With a second stepper motor, attached to the interior tube, the objective is automatically focussed.

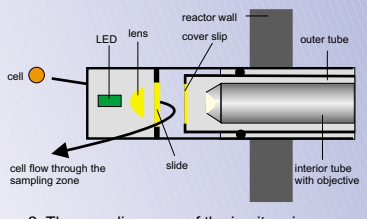

Figure 2: The sampling zone of the in-situ microscope

Online process control

With the in-situ microscope it is possible to control the cultivation process based on direct cultivation parameters (e.g. cell concentration, cell size). For this purpose it is necessary to develop adequate algorithms for each cell type. In the following image analysis for mammalian cells is shown as an example. Furthermore, the integration of the image analysis in the in-situ microscopy system is explained.

Image 1 in Figure 3 shows an image of CHO cells taken during a perfusion cultivation. The magnification is 80-fold. Two main features are visible in image 1: cells and bubbles, created by microsparging. In the first step of image analysis the background is separated from the bubbles and cells by automatic thresholding. Each region is labelled, bubbles and cells are separated. This step results in image 2 that represents cells and image 3 that represents bubbles. The bubble area is substracted from the total field of view. This step is necessary to calculate the cell density. During the next step of image analysis (region growing) each area representing a single cell is evaluated. The outline of each cell is defined and cell size and cell volume are calculated. The results of image analysis allow to calculate the cell concentration.

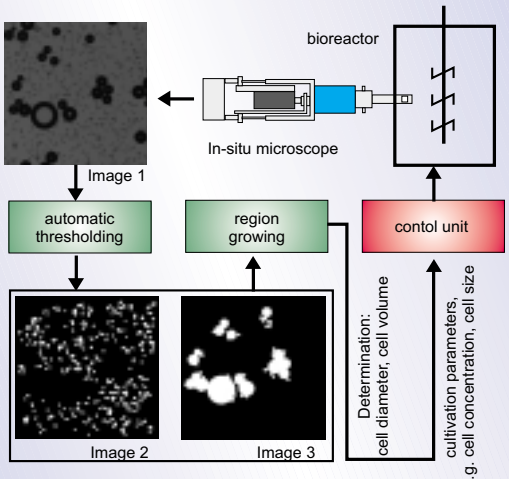

Figure 3: Image analysis embedded in the process control

Based on the results of image analysis it is possible to calculate and adapt the cultivation parameters with a control unit.

In a first attempt yeasts were used as a test organism because of the simple handling compared to mammalian cells. The image analysis for yeasts works similar but due to different cultivation conditions no bubbles occur. For the cultivation control a mathematical model has been developed based on four differential equations that describe the process by modelling biomass concentration (1), glucose concentration (2), specific growth rate (3) and working volume (4).

(1) $\frac{dX(t)}{dt} \quad \mu_{max} \frac{S(t)}{K_M \quad S(t)} X(t) \quad \frac{V(t)_{feed}}{V(t)} X(t) \quad \frac{V_{sample}}{V(t)} X(t)$

(2) $\frac{dS(t)}{dt} \quad \mu_{max} \frac{S(t)}{K_M \quad S(t)} \frac{X(t)}{Y \frac{x}{s}} \quad (S_0 \quad S(t)) \frac{V(t)_{feed}}{V(t)}$

(3) $\frac{d\mu_{max}(t)}{dt} \quad 0$ (4) $\frac{dV(t)}{dt} \quad V(t)_{feed} \quad V_{sample}$

Results

For online process control of cultivation processes by in-situ microscopy it is necessary to monitor a wide range of cell concentration in the cultivation vessel without the possibility of dilution. To test the new flow through sampling zone the yeast concentration in a 3 L bioreactor was permanently increased. The results are shown in Figure 4. There is a linear dependency between the cellular dry weight and single cells calculated with the image analysis software. Up to a cell concentration of 4 x 10e8 cells/mL it was possible to monitor the accumulation of yeasts in the bioreactor without recalibration. Concerning mammalian cell cultivations, the in-situ microscope was testet in a perfusion system with 5 L working volume. The test organisms were CHO cells. The sampling zone height was adjusted from 100 µm at low cell densities to 50 µm at high cell densities. Image 1 in Figure 3 shows an image taken in-situ at the end of the cultivation (9.5 x 10e6 cells/mL). The results of image analysis (in-situ microscopy) were compared to an automated cell counting system, Cedex (Innovatis, Bielefeld, Germany), see Figure 5.

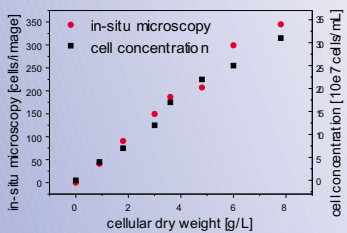

Figure 4: Linear dependency of hemocytometer and in-situ microscopy with cellular dry weight

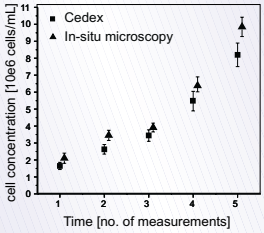

Figure 5: Cell density during the cell accumulation phase as determined by Cedex and In-situ microscope

The results shown in Figures 4 und 5 allow for the embedding of the in-situ microscopy system in a cultivation regulating system. In a first step yeasts were used to test a process control based on in-situ microscopy. The aim of the yeast cultivation was to minimize the production of ethanol and to maximize the growth rate by control of glucose concentration. The optimal glucose concentration for this purpose is below 0,1 g/L. To run the complete cultivation at the optimal glucose concentration a regulating system based on the CO_2 concentration in the exhaust air and the biomass calculated by the in-situ microscope was developed. The mathematical model that calculates the pump rate for the glucose is based on four differental equations (see above). The cultivation of the strain *S. cerevisiae* H620 was carried out in a 1,5 L bioreactor. A profile for the glucose concentration was driven with a maximum of 0.08 g/L and a minimum of 0.05 g/L. Images were taken and biomass was calculated every minute. During the cultivation no ethanol was produced. Unfortunately, the system ran very unstable. However, these very early results of bioprocess control by direct cell parameters are very promising.