A multifunctional at-line analysis system for monitoring of cell cultivations

Andreas Prediger\textsuperscript{1}, Tim Höpfner\textsuperscript{1}, Patrick Lindner\textsuperscript{1}, Mehriban Akin\textsuperscript{2}, Merve Yuksel\textsuperscript{1}, Sascha Beutel\textsuperscript{1}, Suna Timur\textsuperscript{3}, Thomas Scheper\textsuperscript{1}

\textsuperscript{1} Leibniz University Hannover, Institute of Technical Chemistry, Callinstr. 5, D-30167 Hannover
\textsuperscript{2} Ege University, Faculty of Science, Biochemistry Department, 35100 Bornova-Izmir, Turkey

Introduction

In biotechnology lots of interesting products are produced in suspension cell cultures. Process security and a high quality of the final product are essential in this field. This is why continuous monitoring of the bioprocesses is essential. A multifunctional at-line analysis system was developed to allow the automatic acquisition of process data from different sensor systems. Fig. 1 shows a simplified schematic setup of the device. A flow microscope (Fig. 2) allows the monitoring of cell density and cell viability. Dead cells are stained using dyes (e.g. Methylene blue) and the acquired images are analyzed with an image processing software. The development of glucose and ethanol concentrations during a cultivation can be monitored using a dual biosensor. The pH-value is determined via fibreoptical measurements. A mixing chamber that is attached to an injection system allows the automatic dilution of samples and thus the monitoring of broad cell density and concentration ranges.

Biosensor

A flow electrode with a dual biosensor is used for the detection of glucose and ethanol concentrations. Pyranoseoxidase and ethanoloxidase are immobilized separately on two gold electrodes using PAMAM (Polyamidoamine). They catalyze the following reactions:

\[
\text{Glucose} + \text{O}_2 \rightarrow \text{Glucolactone} + \text{H}_2\text{O},
\]

\[
\text{Ethanol} + \text{O}_2 \rightarrow \text{Acetaldehyde} + \text{H}_2\text{O}.
\]

The substrate concentration is measured indirectly via the oxygen consumption. Oxygen is detected at a potential of -700 mV and thus no interference with media ingredients occurs. The linear range of both biosensors is between 0 and 0.5 mM. A stability test showed that after 11h 98% (Pyranoseoxidase) and 94% (Ethanoloxidase) of the initial enzyme activity were retained.

Control software

The device is controlled with the software FIA-Master. Via a RS232 serial interface all pumps, valves and the illumination system can be controlled. All actions are determined with a script that is easily created and modified for different applications.

Control software

The device is controlled with the software FIA-Master. Via a RS232 serial interface all pumps, valves and the illumination system can be controlled. All actions are determined with a script that is easily created and modified for different applications.

Perspectives

An integration of further sensors into the system is desired. A fibre optical detection of the pH-value could be carried out analogously to the pO\textsubscript{2}-value detection. The used enzymes in the biosensor could be exchanged to allow the detection of other relevant biological substances such as lactate or organic acids. New image processing algorithms are constantly developed. An aim for the future is the cell viiability determination via optical properties of the cell without the need to stain them. One approach for this is the implementation of neuronal networks. A new flow microscope prototype for fluorescence measurements is under development. It will be used for at-line monitoring of micro algae cultivations and could be integrated into the multisensor system as well.