Multisensor adapter for Disposable Bioreactors

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Introduction
Commonly most bioprocesses are performed in stainless steel tank reactors. For the past years pharmaceutical and bioprocess industry established new production lines based on single-use technology. There are several advantages for the integration of disposable reactors (Fig. 1) into the process chain. The main factors are time and cost savings followed by the need of less validation and maintenance efforts. The amount of cost savings is estimated to range between 20 - 40 %. Even flexibility in production can be enhanced with respect to the market situation.

A major drawback of this single-use technology is the lack of appropriate process analytics and monitoring technology. In contrast to commonly used sensor systems for steel bioreactors, sensors for disposable units have to be disposable as well. These sensor units should be low priced, reliable and sterilizable. To achieve these goals a viable method is to construct a modular sensor, with separate disposable sensor adapter and non-disposable control/detection unit.

Selection of sensor elements
Possible sensor technologies were reviewed with respect to availability and applicability in disposable systems. Most common sensor techniques are not miniaturizable, thus an alternative has to be considered. For measurements of pH and dissolved oxygen a fluorescence based technology was chosen. The fluorescent dye is immobilized on a silica membrane and adjusted to the bioreactor's inner wall. This enables a modular design of the sensor (Fig. 2). An optical fiber guides the excitation light to the measuring unit. Both, optical fiber and measurement unit are reused for further experiments. Therefore only the patch material with the immobilized dye is in direct contact with the cultivation medium and disposed afterwards. Additionally, Pt 1000 thermistors and thickfilm electrode assemblies for conductivity measurements can also be constructed from single-use components. All these sensor elements were unified into a single sensor adapter.

Monitoring of pH and patch lifetime
The measurement of pH is performed by ratiometric measurement of the intensities of two fluorescence emissions excited by a single wavelength. This enables a higher accuracy in measurement.

An important factor in using fluorescence measurements is the time span in which reliable data can be obtained by the patch. A main factor limiting its lifetime is photo-bleaching, which happens due to intensive light irradiation onto the fluorescence dye. To determine the bleaching effect onto the patch material we performed a long-term test using 50 mM PBS pH 7.4 with an overall ionic strength of 150 mM at 37 °C. For this test a measurement interval of 2 min was chosen. While temperature and reference pH remain constant over the duration of the experiment, there is an obvious change on the pH measured by the sensor patch material. Over the first 5,000 illuminations (day 1 to day 7) good correlation with an overall deviation below 0.1 pH units could be observed, but with further measurements a linear deviation with a constant slope of $2.27 \times 10^{-5}$ pH-units/measurement was determined. To guarantee an accurate measurement, a daily recalibration is suggested.

Testing of the sensor module prototype
As mentioned, it is possible to obtain reliable cultivation data by choosing an adequate measuring interval. After the final construction of the multisensor module prototype a test cultivation of in TB medium was performed. Over the whole process conductivity, dissolved oxygen, pH and temperature were measured with the prototype and compared with reference sensors. Furthermore optical density was monitored by offline measurements to control the cultivation progress.

Fig. 4 only shows some selected data measured from the cultivation. It is possible to see that the sensor patches for dissolved oxygen show reliable data and the integrated Pt 1000 shows a good correlation to the temperature measured by dissolved oxygen sensor as well. Conductivity and pH measurements show similar correlations but are not shown here for reasons of clarity.

Conclusions
Even though there is a growing failure during the measurement due to photo-bleaching effects of the pH patch, it could be well used for cultivation, as the measurement interval can be chosen depending on the user demand, especially when performing daily recalibrations. In combination with temperature, conductivity and dissolved oxygen measurement the multisensor adapter is a very useful tool for bioprocess monitoring in the single-use process equipment. The sensor adapter shows good prospects to overcome the actual limitations in use of this technology due to restricted process monitoring possibilities.

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