Standardized online biomass measurement in single-use fermentation

PAT Tools for Bioprocess Monitoring & Control

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Introduction

According to a number surveys, the monitoring of viable biomass is the most requested parameter in industrial cell cultivation [1][2]. Viable biomass progress during a cultivation process is a key performance indicator and yields deeper process knowledge and the ability to improve the by defining harvest or infection points.

Offline sampling methods, such as Trypan blue systems still lead the biomass monitoring in bioproduction. However, these offline methods are manual and based on representative sample removal, sample preparation and independent data generation. Typically, they are subject to operator errors, operator availability and a risk of contamination of the cultivation.

Biomass monitoring

The capacitance method for in-situ detection of viable biomass is already well established in the biotech industry. Here, it uses traditional stainless steel equipment. Progressively, industrial cell cultivation has tended more to single-use (SU) vessels and equipment.

This presentation illustrates test results for the first fully integrated online biomass measurement solution for SU systems.

Experimental Approach

BioPAT ViaMass sensor discs were integrated into Flexsafe® RM bags and used for the cultivations using rocking motion agitation. These systems utilize capacitance technology from ABER Instruments Ltd. to determine the viable biomass [2]. The cultivation experiments were performed at 580 kHz with polarization correction applied. The SU sensor disc consists of HDPE with platinum electrodes. The sensor fulfills FDA and USP class VI requirements and has been qualified using Sartorius Stedim Biotech validation protocols.

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Fig. 1: BioPAT ViaMass system – electronics with SU sensor patch

CHO Cultivation

Figure 4 shows the results of a CHO cultivation in Flexsafe® RM 50 L. The capacitance signal from BioPAT® ViaMass was compared with the viable cell density measurement from the Cedex HiRes and the wet cell weight as a reference. In addition, the Cedex offers the average cell diameter. With this, the average cell volume was calculated then multiplied with the viable cell density. This results in the viable cell volume (cm^3/mL), which represents the viable biomass as percentage of the total volume. The error bars of viable cell density in figure 4 are the standard deviations given by the Cedex results, the error bars of viable cell volume are derived from these.

During the exponential phase (the first 6 days) the capacitance signal correlates excellently with both the viable cell density and the viable cell volume. Then, the average cell diameter increase and the deviation from cell density measurement occurred. However, the correlation with the calculated biomass volume is maintained to the end of the cultivation run. This is as expected, because the capacitance is proportional to the volume covert by the rocking filter software as it is turned on and off on the local controller.

Fig. 2: The effect of the rocking-motion filter: Only representative measurements are recorded

Cell line, Medium and Process Strategy

For the fed-batch process the cell line CHO DG44 (Cellica, Laupheim, Germany) secreting human IgG1 was used. SM05 medium (Cellica) was prepared for the seed train and PM5 medium (Cellica) as a basal medium for the fed-batch culture. The feeding strategy comprised of three different feeds; A, B and 40% concentrated glucose. After a 2-day batch phase, an 8 day fed-batch phase started. From day 11, the discontinuous bolus feed of A and B was supplemented by the 40% glucose feed to maintain a 3 g/L glucose concentration.

Within the daily sampling regime, metabolites like glucose and lactate were analyzed by the Radiometer ABL800 basic (Radiometer, Germany). Viable cell density, average cell diameter and viability were determined by the Cedex HiRes (Roche Diagnostics, Germany). For downstream purification testing, the harvest point was defined to 80% minimal cell viability.

Fig. 3: Sensor patch with connector, welded in CultiBag RM 20 L

Bioreactor setup

BioPAT® RM 50 L with Flexsafe® 50 L optical bag

Gassing principle

<table>
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<tr>
<th>Sensor</th>
<th>D.O. and pH patches, temperature, BioPAT® ViaMass</th>
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<tr>
<td>Working volume</td>
<td>25 L</td>
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<tr>
<td>Initial volume</td>
<td>10 L</td>
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References


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