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Single-use capacitance measurement of cell viability

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Performance and accuracy of single-use viable biomass measurement

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 single-use 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 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 [2].

This presentation illustrates test results for the first fully integrated online biomass measurement solution for SU systems. Where the electronics are integrated into the local controller and the sensor disc integrated into the gamma irradiated bag.

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

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 [3]. The cultication 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. Biological, chemical and physical tests of integrated sensor discs post gamma irradiation gave excellent compatibility to the relevant pharmacopoeias and guidelines. Sensor discs were welded into Flexsafe® RM optical bags with different volumes (10L, 20L and 50L). The cultivation was controlled using a Biostat® RM optical system and all data was recorded using the Sartorius SCADA software; BioPAT® MFCS [4]. First the influence of the rocking motion on the sensor signal was investigated under various conditions (rocking rate, rocking angle and working volume). With these experiments a former developed filter [5] was optimized and adapted to the broad application range.

Following this, cultivation runs were performed.

Fig. 2: Sensor disc with connector, welded in Flexsafe® RM 20 L

Cell line, Medium and Process Strategy

For the fed-batch process the cell line CHO DG44 (Cellca, Laupheim, Germany) secreting human IgG1 was used. SMD5 medium (Cellca) was prepared for the seed train and PM5 medium (Cellca) 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 3-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 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.

Bioreactor setup

Biostat® RM 50 L with Flexsafe® 50 L optical bag	
Gassing principle	Overlay
Sensors	Single use optical DO and pH patches, temperature, BioPAT® Viamass
Working volume	25 L
Initial volume	10 L

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Parameter setup

Biostat® RM 50L with Flexsafe® 50 L optical bag	
pH-set point	7.15 (controlled by CO_2 headspace)
dO-set point	60 %sat.
dO control	Multi stage cascade comprising N_2, Air, O_2 - gassing
Temperature set point	36.8 C
Agitation (rpm)	25 @ 10 ° rocking angle

Rocking filter

The rocking motion of the Biostat® RM causes signal fluctuations as the liquid level covering the sensor disc varies. At low working volumes, the sensor is not covered with medium during a certain period of rocking. This results in false measurements which have to be filtered out. Figure 3 shows the impact of the rocking filter software as it is turned on and off on the local controller.

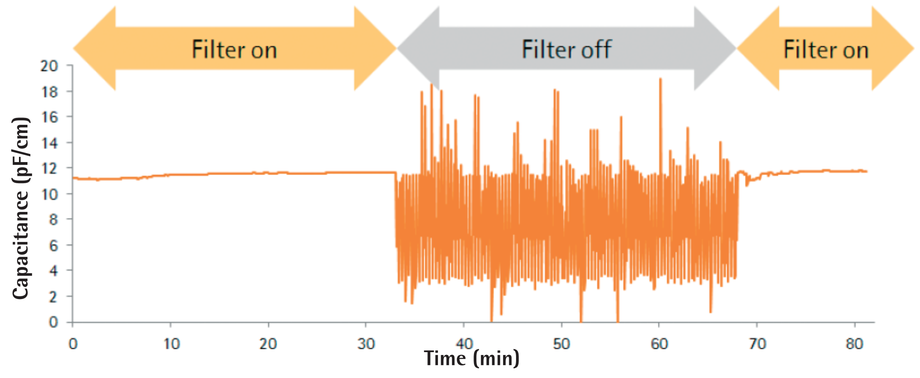


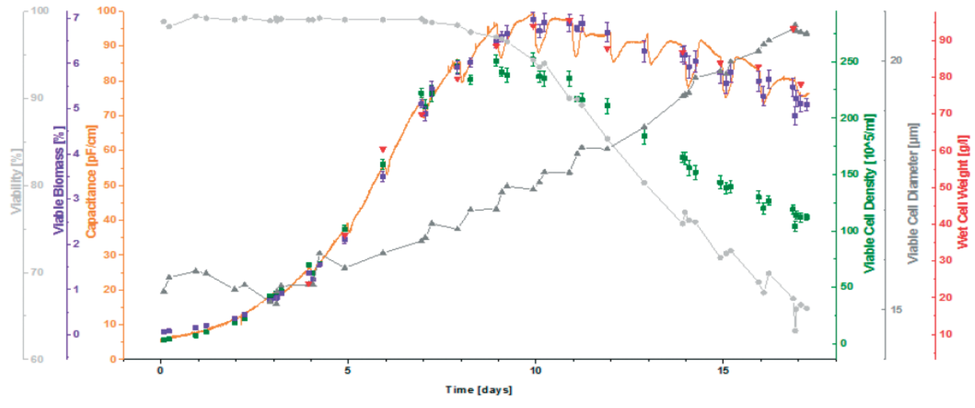
Fig. 3: The effect of the rocking-motion filter: Only representative measurements are recorded

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 (first 6 days) the capacitance signal correlates excellently with both the viable cell density and the viable cell volume. Then, the average cell diameter increases 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 viable cell membranes. This online measurement of the biomass (as percentage of the volume) shows every small effect of biomass change during the cultivation process, e.g. each dilution due to additional feed medium. Furthermore, these effects could be verified with the offline reference measurements, involving additional sampling.

In conclusion, these results show excellent performance of the fully integrated online viable biomass measurement in SU cultivation vessels and equipment.



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