Online monitoring of viable cell concentrations in small bioreactors

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INTRODUCTION

The production of monoclonal antibodies with a mammalian cell culture process is a complex and challenging step in modern medicine to achieve final pharmaceutical products such as vaccines. Viable cell concentration (VCC) is one of the most important key performance indicator during mammalian cell cultivation mainly measured offline. According to FDA´s PAT initiative, process monitoring and control should be applied to gain process understanding and improve control of process parameters leading to high product quality [1].
Thus, the implementation of an online capacitance probe in a small scale bioreactor is demonstrated. Capacitance sensors using one frequency are frequently used to monitor biomass [2]. However, the capacitance signal is dependent on cell diameter changes and correlates with the viable cell volume instead of the VCC [3]. In this work a multivariate data analysis (MVDA) based on frequency scans is used to investigate the ability to directly predict the online VCC based on capacitance measurements at different frequencies. To achieve a robust model five standard cultivations were carried out, followed by robustness trials. with voluntary induced process changes to test the model´s predictability.

MATERIAL & METHODS

A CHO cell culture fed-batch process producing a monoclonal antibody was used for the experiments. Cultivations were conducted in a single-use, small scale bioreactor (0.25 L). A capacitance sensor was integrated into the bioreactor. Based on the frequency scans the multivariate model was created using the orthogonal partial least square regression (OPLS) method. Multiple standard cultivations were carried out to validate the model. Model robustness trials were executed including a process with dilution steps and two processes with a changed feeding strategy. The feeding was reduced compared to the standard fed-batch process.

Integration of online capacitance sensor

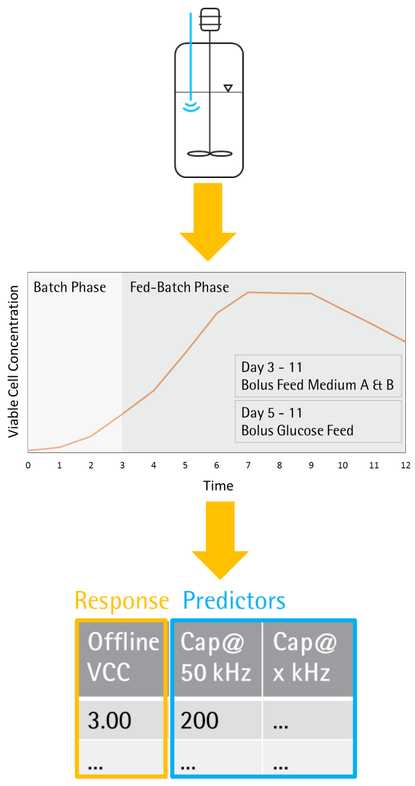
- Single-use bioreactor (0.25 L)
- 5 standard cultivations (FB#1-FB#5)
- 1 dilution trial (FB#6)
- 2 feed variations (FB#7 – FB#8)

Measurements

- In-line sensor with 25 frequencies (50 kHz – 20000 kHz), including single-frequency at 607kHz
- Offline reference: automated trypan blue measurement

Data treatment and MVDA analysis

- OPLS method used for MVDA model
- Linear regression method used for single-frequency analysis (VCC values included until a diameter change > 0.5 µm)
- Smoothing with Savitzky-Golay filter (moving average of 30 values)
- One-point calibration was applied based on first offline value



RESULTS & DISCUSSION

Comparison of single-frequency measurements and frequency scanning with MVDA

For the comparison of single-frequency (SF) analysis and the frequency scanning approach the Leave-One-Batch-Out (LOB) method was applied on the standard cultivations. Each cultivation was predicted based on a model containing all other fed-batches. Table 1 summarizes the results of the Root Mean Square Error of Prediction (RMSEP) and the Relative Error (RE) of the two measurement methods.

Table 1: Comparison of single-frequency analysis of the capacitance and the MVDA of the frequency scans.

Included Fed-Batches into the model	Predicted Fed-Batch	RMSEP [10E6 cells/mL] SF	RE [%] SF	RMSEP [10E6 cells/mL] MVDA	RE[%] MVDA
FB#2-FB#5	FB#1	4.0	22.7	1.2	6.6
FB#1, FB#3-5	FB#2	3.5	22.7	1.3	8.3
FB#1-FB#2, FB#4-FB#5	FB#3	3.9	21.4	1.0	5.5
FB#1-3, FB#5	FB#4	3.4	18.6	1.0	5.7
FB#1-4	FB#5	3.2	15.8	2.2	11.0

RESULTS & DISCUSSION

The results for the frequency scanning combined with MVDA showed strong improvements with REs between 5.5% and 11% compared to single-frequency measurements with REs between 15.8% and 22.7% (Table 1). Thus, frequency scanning enabled predictions comparable to the off-line reference that was experienced with a system error of 10%. Figure 1 demonstrates the online trajectory of one standard fed-batch. Especially in the death phase the prediction was strongly improved with the presented novel approach. Thus, the presented method is proposed to be used for future online monitoring of the VCC that reduces manual sampling and contamination risks.

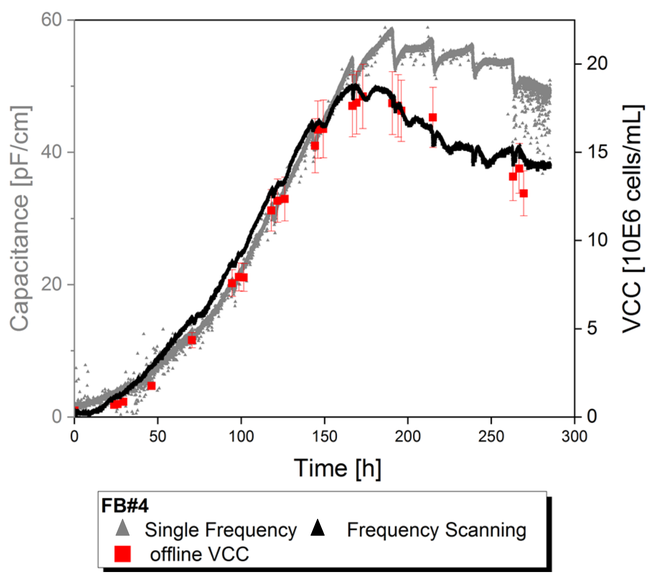


Fig. 1 Predictions of a standard fed-batch applying MVDA on a frequency scan in comparison to the capacitance signal of a single-frequency (607 kHz).

Robustness trials to investigate the predictability of the MVDA model

In future applications, the model will be trained with every new cultivation. Therefore, the robustness trials were predicted based on the MVDA model containing all 5 standard fed-batches. The coefficient of determination of this model resulted to be 95.4% and 1+2 (predictive + orthogonal) principle components were used.

Figure 2 displays the results for a dilution trial (FB#6). The red arrows indicate the timings of a 30% dilution of the cell broth with process media. The RMSEP was calculated to be 1.3 million cells/mL resulting in a RE of 6.7%. The predictions of the MVDA model were within the accepted error range of 10% for the complete cultivation even though the model itself contained only standard fed-batches.

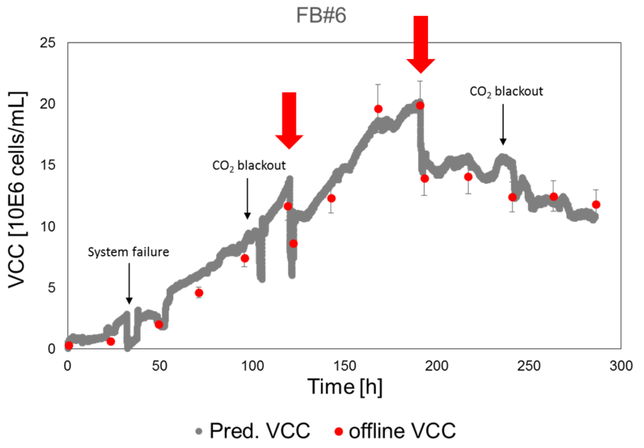


Fig. 2 Prediction based on the MVDA model for a fed-batch process with voluntary induced dilution steps.

Figure 3 shows the online trajectory of two fed-batches (FB#7, FB#8) with a changed feeding strategy within a Batch Evolution Model based on the frequency scan of the standard cultivations. Clear differences were detected for both runs once the feeding started. The difference was not detectable in the VCC offline reference (data not shown).
To summarize, the novel approach can lead to a future monitoring and control with active alarms to keep the batch within an accepted trajectory.

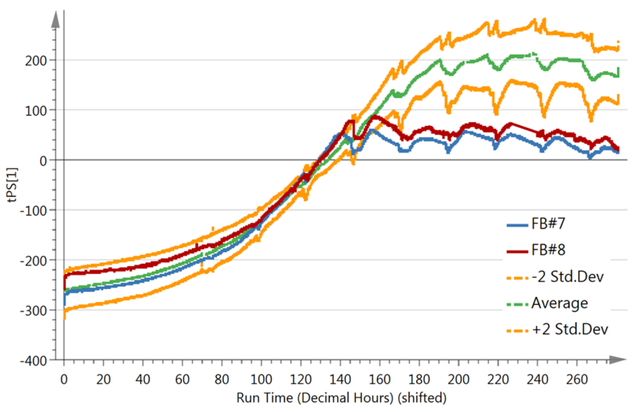


Fig. 3 The Golden Batch trajectory based on a Batch Evolution Model containing the frequency scan of all standard fed-batches (FB#1-FB#5) used to compare the trajectory of the robustness trials.

CONCLUSION

- Combining MVDA and frequency scanning enabled online predictions of VCC values over the complete cultivation time comparable to the offline reference.
- Robustness trials showed the great potential of the novel approach to monitor and control the VCC as a key performance indicator.
- The method is proposed to be used for process understanding, process optimization and process control.

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