Evaluation of a new glucose control strategy using CITSens Bio APC (Automated Process Control) for CHO Fed-Batch application

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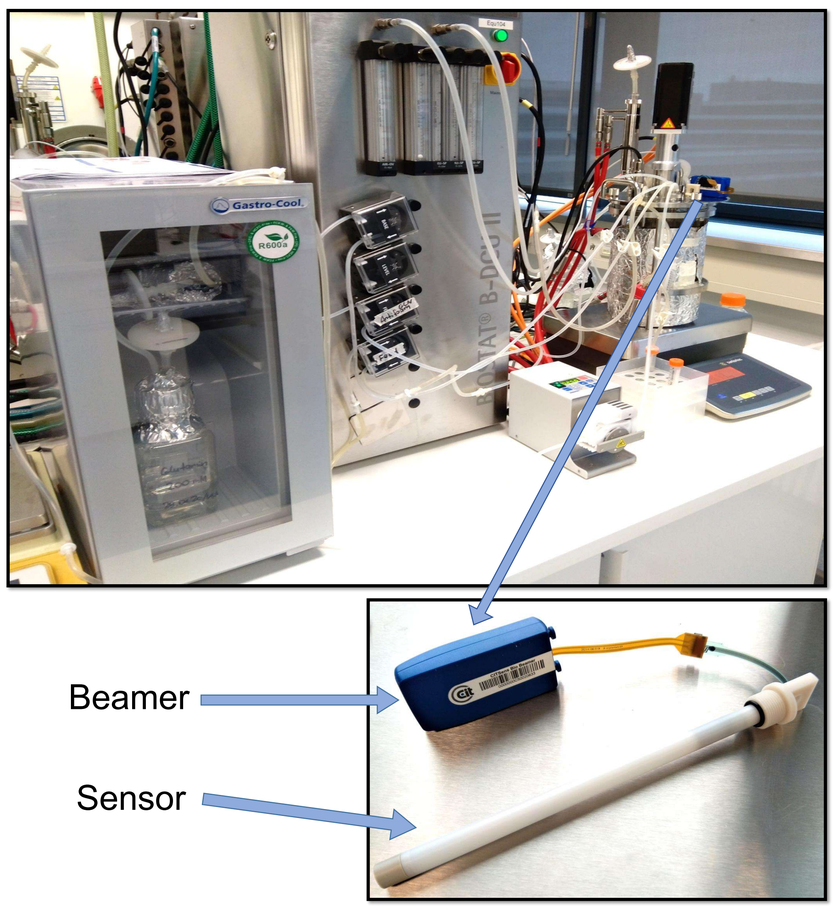
Introduction

For the cultivation of cells in research and development, high cell densities and reproducibility are fundamental aims. For that goal a high degree of automation is favourable to reduce the risk of contamination and deviation between batches.

As glucose is one of the main energy sources for cells, it is crucial to maintain a constant and controlled automated glucose supply. Therefore, the CITSens Bio APC a combined system to measure and control the glucose concentration in cell culture applications was used (CITSens Bio, C-CIT Sensors AG, Switzerland).

To test the CITSens Bio APC multiple Fed-Batch-cultivations with CHO K1-cells were executed. Different cultivation parameters and glucose concentration-thresholds were applied.

Material & Methods



Material & Methods

Fed-Batch
- 2 L bioreactor (BioStat, Sartorius) with 1 L start volume
- 14 days cultivation time
- HighClone medium (Cytiva)
- GlycanTune feed (Thermo Fisher Scientific)

Glucose sensor
- CITSens Bio APC (C-CIT Sensors AG) for in-situ glucose monitoring and controlling

Setpoints
- pH = 7,1
- pO2 = 40 %

Setpoints	Fed-Batch	1 Fed-Batch	2 Fed-Batch
Glucose	2 g L^{-1}	0,5 g L^{-1}	0,5 g L^{-1}
Glutamine	1x per day on demand	1x per day on demand	6 pulses over 24 h on a lower level

Fig. 1: *Table of the different setpoints of glucose and the feeding strategy of glutamine.*

Cell line
- CHO-K1 producing recombinant human MAb

Results

Glucose controlling

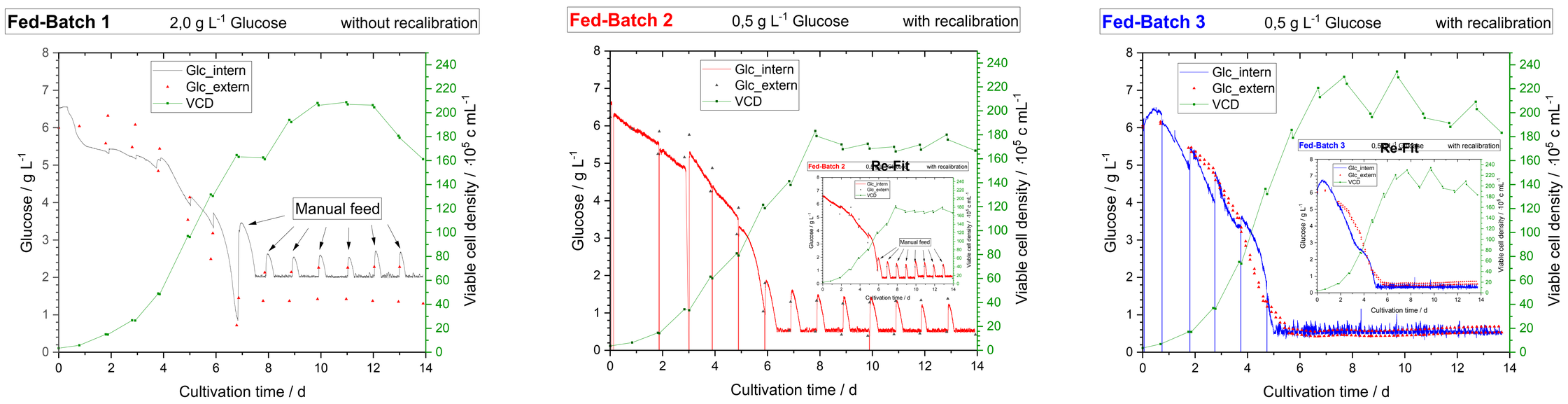


Fig. 2: *Online and external glucose measurement and viable cell density of the three Fed-Batches is shown.* ***Fed-Batch 1*** *has a setpoint of 2,0*g L^{-1} *glucose without recalibration of the sensor and manual feeding.* ***Fed-Batch 2*** *has a setpoint of 0,5* g L^{-1} *glucose with recalibrated sensor and manual feeding.* ***Fed-Batch 3*** *has a setpoint of 0,5*g L^{-1} *glucose with recalibrated sensor and the feed was splitted in six pulses in 24 h. A second graph of Fed-Batch 2 and 3 shows a re-fit of the online values without recalibration.*

Discussion

This study has demonstrated the ability of the CITSens APC-system to maintain a constant glucose concentration at various thresholds. Furthermore, it could be shown that online glucose measurement generates comparable results to the established measurement via a Biochemistry Analyzer. Throughout all cultivations the cells has shown a high viability over 95 % except on the last 2 days when it decreased to 90 %. The viable cell density reached a high level up to 2,3·10^{7} cells mL^{-1}.

The first Fed-Batch shows how the sensor responses and works in general at a level of 2,0 g L^{-1} glucose without recalibration. In the second Fed-Batch a level of 0,5 g L^{-1} was chosen to test the sensor at a lower level. The sensor is calibrated due to the deviation of online and external values in the first run. In the third Fed-Batch the feed strategy was changed from a daily feed to a continuous feed to stabilize the glucose level even better.
As pictured in Figure 2 (Fed-Batch 3) the variation in the measured glucose concentration could be minimized by splitting the feed into six feeds. A re-fit of the online values shows that a recalibration was not absolutely necessary.

Conclusion

The measurement and controlling of the glucose level works reliable even at lower glucose concentration of 0,5 g L^{-1} with the CITSens Bio APC.

The sensor was recalibrated during the second and third Fed-Batch. A re-fit shows that a recalibration was not required necessarily.

The provided user interface is easily comprehensible. A real time representation of the in process glucose concentration is possible over all the process phases.

The CITSens Bio APC system is a good tool to reach a higher level of automation even wireless for a better process and consistent batches.

Different process parameters

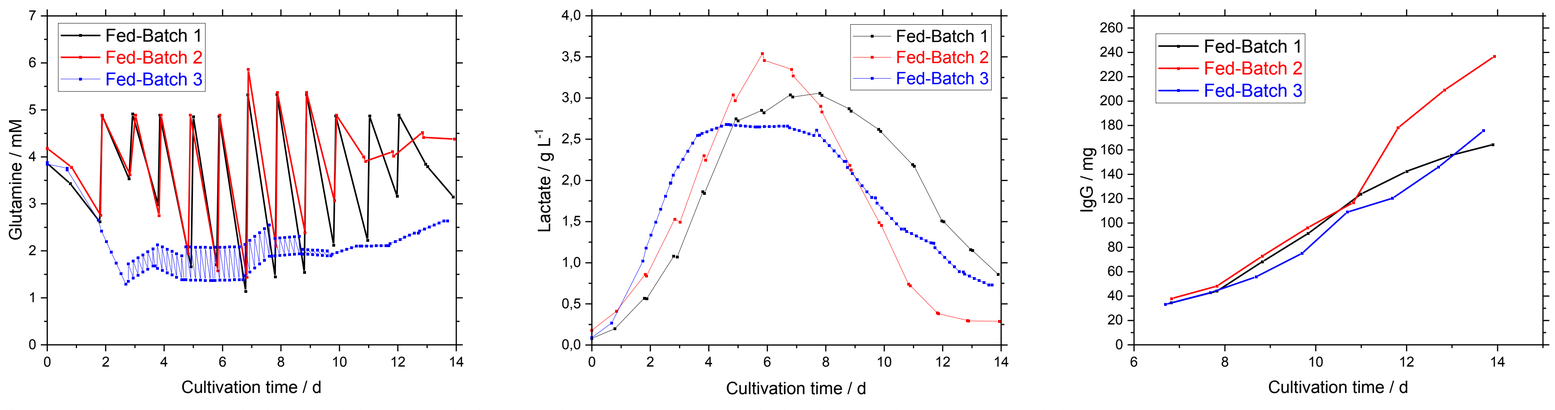


Fig. 3: *The glutamine concentration, lactate concentration and the produced amount of antibody of all three Fed-Batches is shown.*