Automation of feeding strategy through the real-time measurement of glucose in fed-batch cultivation

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Introduction

Cell culture is sensitive to changing physiological conditions during the cultivation time. With the fundamental aim of reproducibility a high degree of automation is favourable and can reduce the risk of deviation between batches and contamination.

Glucose is well known as the main energy and carbon source for cells. Therefore real-time measurement during cell culture cultivation can be helpful for the automation of feeding strategy in fed-batch cultivations. For this purpose, 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).

The CITSens Bio APC was tested in multiple Fed-Batch-cultivations with different CHO cell lines, and various cultivation parameters were executed.

Material & Methods

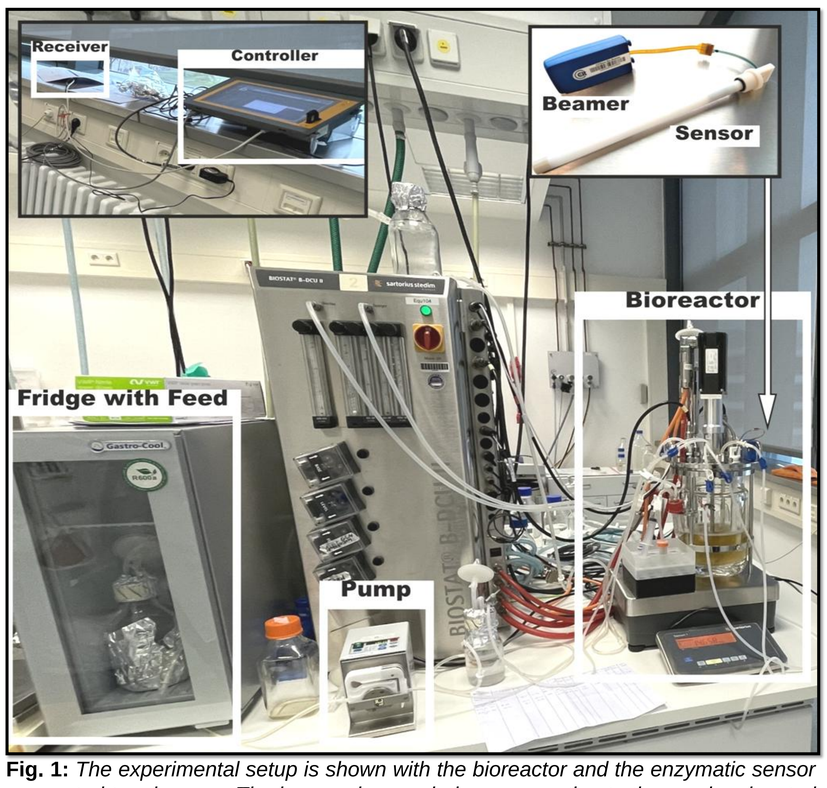

Fig. 1: *The experimental setup is shown with the bioreactor and the enzymatic sensor connected to a beamer. The beamer has a wireless connection to the receiver located in the same room. Finally, the controller gets the electric signal, and calculates it into a concentration. If the concentration drops below the setpoint, the pump will start to hold the glucose concentration.*

Fed-Batch
- 2 L bioreactor (BioStat, Sartorius, Germany) with 1 L start volume
- 14 days cultivation time

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

Setpoints
- pH = 7.1
- pO_2 = 40 %
- Temperature = 37°C

Parameters	Fed-Batch 1	Fed-Batch 2
Cell line	CHO-1	CHO-2
Medium	HyClone (Cytiva)	CHOlean w/o glucose (Xell AG, Sartorius)
Feed	GlycanTune (Thermo Fisher Scientific)	TCX7D (Xell AG, Sartorius)
Glucose-Setpoint	0.5 g L^{-1}	1.5 g L^{-1}
Glutamine	6 pulses over 24 h on a lower level	1x per day on demand

Tab. 1: *Table of the different cultivation parameters .*

Results

Glucose controlling

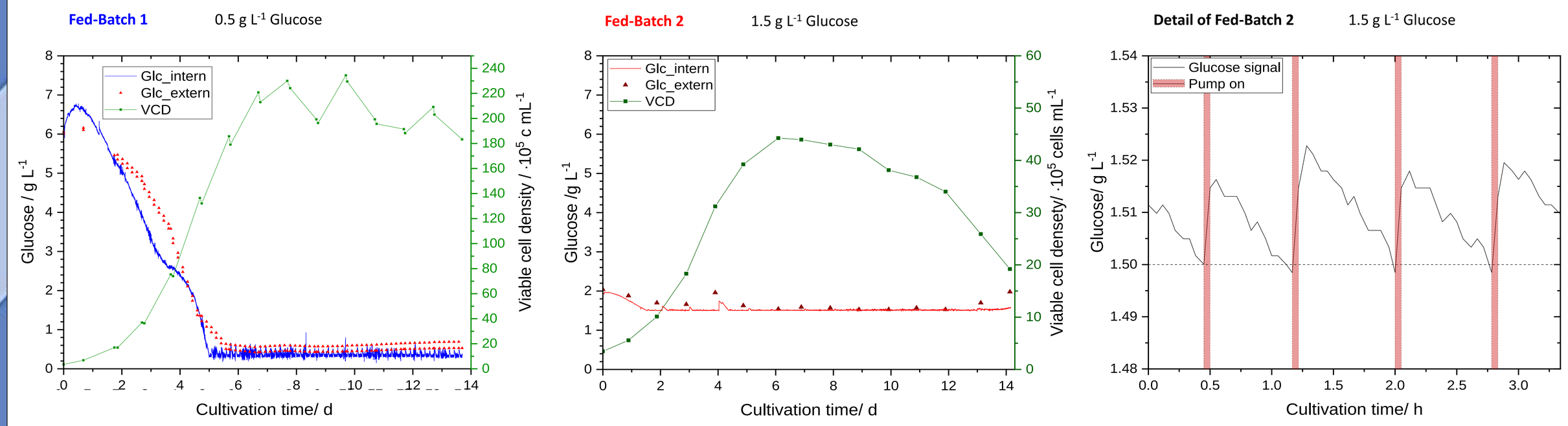

Fig. 2: Online and offline measured glucose concentrations are shown with the viable cell density of the two Fed-Batches. The setpoints for the glucose control were 0.5 g L^{-1} in **Fed-Batch 1** and 1.5 g L^{-1} in **Fed-Batch 2**. Additionally, **FedBatch1** was fed six times in 24 h with the GlycanTune feed solution and the glucose concentration was controlled with a 200 g L^{-1} glucose stock solution. **Detail of Fed-Batch 2** shows the glucose signal change within three hours of the cultivation. If the concentration drops below 1.5 g L^{-1}, the pump will start to hold the glucose concentration with the glucose rich TCX7D feed solution.

Discussion

The results demonstrate the ability of the CITSens APC-system to maintain a constant glucose concentration at various thresholds over the cultivation time of 14 days. Furthermore, it could be shown that online glucose measurement generates comparable results to the established measurement via a Biochemistry Analyzer.

The first Fed-Batch shows how the sensor responds and works at a low level of 0.5 g L^{-1} glucose. With this automated feeding system, it is possible to keep a constant low glucose concentration reliable over a long period.

In the second Fed-Batch, a different CHO cell in another medium without glucose was cultivated. The starting glucose concentration was 2.0 g L^{-1}, and after one day, the CITSens APC-system maintained a constant glucose concentration of 1.5 g L^{-1} till the end of the cultivation after 14 days.

Conclusion

The measurement and controlling of the glucose level works reliable even at a lower glucose concentration of 0.5 g L^{-1} with the CITSens Bio APC. With this system experiments with low glucose concentration over a longer time period are performable. Beside facilitating present processes, the system could also lead to new process development opportunities since such low glucose concentrations can just hardly be maintained with manual sampling.

With the right medium the glucose concentration can be held constant over the whole cultivation time and provide a high level of consistency. Through the connection of feeding and glucose concentration, robust processes can be achieved and lower variances between batches can improve the quality of the product and batch to batch reproducibility.

Furthermore automatic online control systems decrease the risk of contamination through human interaction. Because of the less human interaction at one bioreactor through measurement and manual feeding a higher parallelisation is possibly and working hours at weekends can be reduced.

The CITSens Bio APC system is an innovative tool to reach a higher level of automation, intensify processes and increase the consistency of batches.