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Application Note

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Automated Glucose Control

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Abstract

Cell cultivation's primary feed component is glucose which is used as a starting point for all growth and energy pathways within the cells. Ensuring that the cells do not have too much or too little, enables them to grow fast and maximize the product secretion. Additionally, it has been noted that an excess in glucose concentration has an effect on the glycosylation rate of secreted soluble proteins. Thus, controlling the available glucose would improve the consistency of glycosylation patterns on final products and may improve the quality.

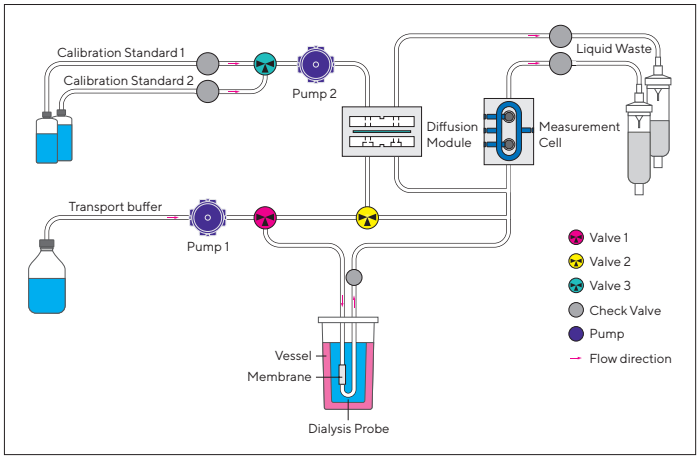
Introduction

One of the goals of mammalian upstream bioprocess development is to handover a process to clinical manufacturing that is robust, safe and in the end produces enough material to meet the studies demand. These process development teams are increasingly being asked to do more and in less time. Thus, automated feeding strategies are becoming more and more popular as a way of reducing the development efforts and as a side benefit have shown to improve process performance and product attribute consistency.

Within this application note a stepwise example method on how to establish glucose feed control is documented. The materials and methods show how this was done using Sartorius Stedim Biotech hardware and equipment. An overview of the results and discussion of the data is given in order to highlight some of the key benefits of applying this method to other processes.

Materials and Methods

A Biostat® B-DCU was used as bioreactor control system. The Biostat® B-DCU is a bioreactor for advanced process optimization and characterization featuring the option of a glucose concentration controller. The integration of the BioPAT® Trace allows a real-time monitoring of the glucose level enabling a user defined glucose setpoint within the Biostat® B-DCU control software. The first two runs, insitu glucose concentration was maintained by discontinuous bolus feeds. Subsequently the third run utilized a glucose setpoint controller using defined PID settings and an internal, speed-controlled peristaltic pump.



The BioPAT® Trace was set up for dialysis mode glucose & lactate measurement at a 20 minutes sampling rate. It was set to a fully automated and self-calibrating protocol as part of the schedule tab function. Each 17 day run used 12 L of transport buffer, two calibration solutions (high: 10 g/L glucose; low: 1 g/L glucose) and a 10 L waste container attached to the tube set. The dialysis probe was prepared, filled and installed into the UniVessel® Glass 5 L prior to sterilization and connected to the BioPAT® Trace tube set and primed for analysis before inoculation.

The UniVessel® Glass 5 L was equipped with two 3-blade segment impellers for low shear stress and good homogenization of the cell broth. The blade angle was 30° and set to down pumping. A ring sparger with holes faced up was used for all trials. Additionally, the vessel was equipped with several ports for feeding, a classical pH sensor, pO_2 sensor, dialysis probe, exhaust cooler and gas filters.

To evaluate the BioPAT® Trace integration in the Biostat® B-DCU a CHO fed-batch process was used. The 17 day cultivation comprises of a 3 day batch phase and a 14 day fed-batch phase. After the inoculation with 0.3×10^6 cells/mL the peak viable cell density (VCD) is typically reached at day 8 with $25 - 30 \times 10^6$ cells/mL and a viability of 99%. After the following 9 day dying phase the VCD should be above 10 + 106 cells/mL with a viability of more than 50 % at the point of harvest.

The bolus feeding from day 3 comprises feed medium A (FMA), feed medium B (FMB) and a highly concentrated glucose solution (400 g/L). The fed amount of FMA and FMB is constant throughout the complete fed-batch phase. Typically on day 7, additional glucose is needed to maintain a glucose concentration of at least 3 g/L in the cell broth. The feeding process is automated using balances and pumps connected to the digital control unit (DCU) and S88 recipe in the SCADA software BioPAT® MFCS.

Analytics were an essential part of this evaluation. Among other things, offline glucose and lactate measurements were performed with the Radiometer ABL 800 basic. VCD and viability were analyzed with a Cedex HiRes.

A total of three different trials were performed using the following characteristics:

1. Monitoring
Trace technology was solely used to monitor the online glucose trend throughout the cultivation.

2. Online value replaces offline measurement
During the CHO culture a daily sample is taken. The offline glucose value is used to fill to a certain glucose concentration in the bioreactor. In trial 2 the glucose online value is used instead of the offline value for this feeding procedure.

3. Glucose control
During the third trial glucose control at 6 g/L was activated after 6 days. The usual bolus feed of FMA was modified to continuous feeding to reduce glucose concentration peaks.

Fee ding scheme for trial 1 and 2:

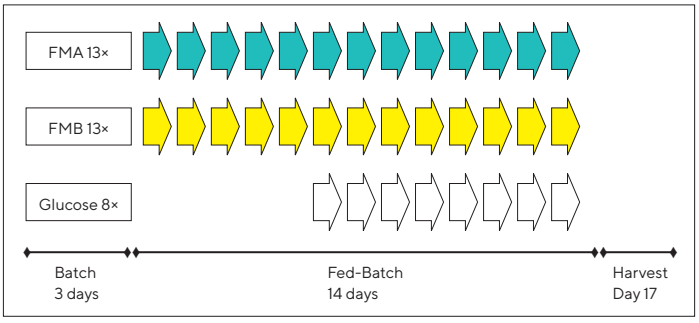


Fig. 1: Feeding scheme 1 and 2

For trials 1 and 2 the feeding scheme was identical. During the batch phase no medium was fed to the culture. On day 3 a daily bolus feed of FMA and FMB started. After glucose fell below 6 g/L an additional feed of glucose (400 g/L) started.

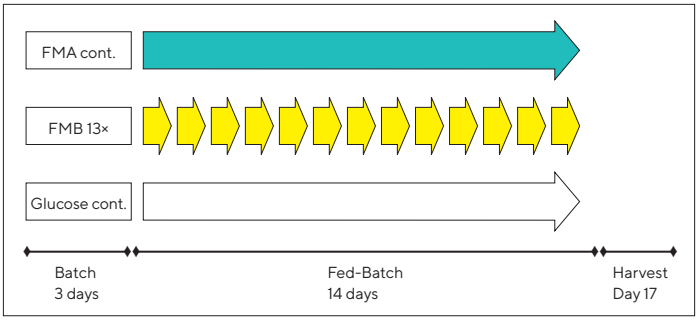


Fig. 2: Feeding scheme 3

In trial 3 the bolus feeding of FMA was modified to continuous feeding to remove daily peaks in the glucose concentration. After glucose reached a concentration below 6 g/L, a controlled feed of glucose solution (400 g/L) was initiated to maintain the glucose concentration at 6 g/L.

Results & Discussion

The batches ran sequentially on the same Biostat® B-DCU and BioPAT® Trace systems. The results are shown in figure 3 and 4 to demonstrate the reproducibility and conformance of the process batches (compared to the historical golden batch). The glucose monitoring of the BioPAT® Trace and subsequent glucose controller are shown in figures 5–7.

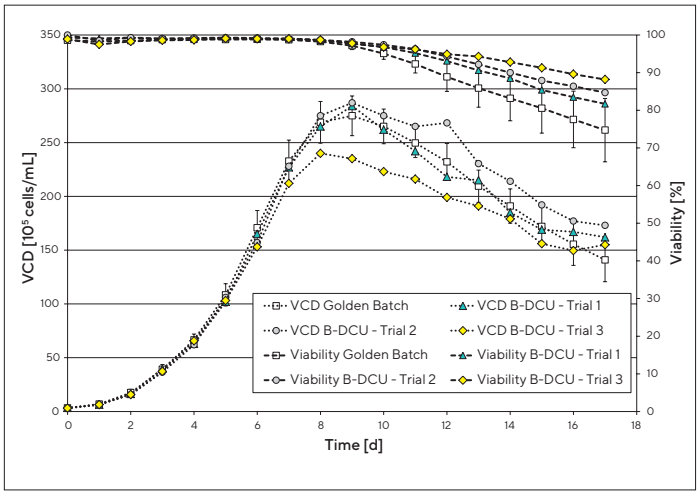


Fig. 3: VCD trend trial 1–3

Trial 1 and 2 fit the golden batch trend well. Due to a general process modification (continuous FMA feed) the VCD trend of trial 3 has a slightly reduced peak VCD. Final VCD and viability fits well of exceeds golden batch values for all trials.

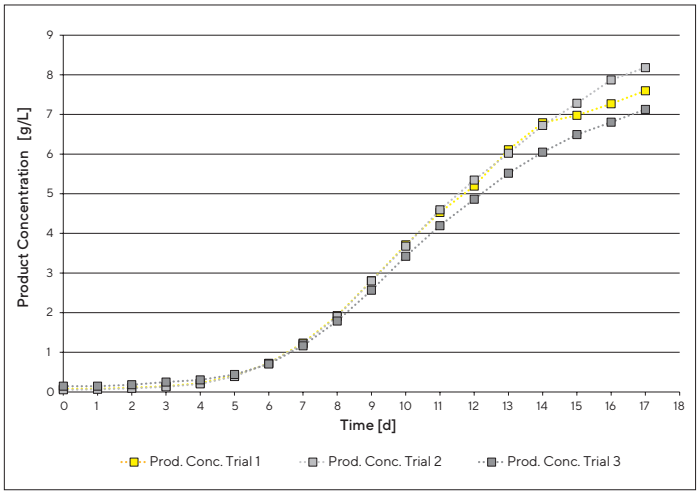


Fig. 4: IgG trend

Comparable productivity in all three trials was demonstrated by daily measurements of the product concentration (IgG concentration, fig. 4). Due to a lower cell growth in trial three a slightly lower product concentration was achieved in this run.

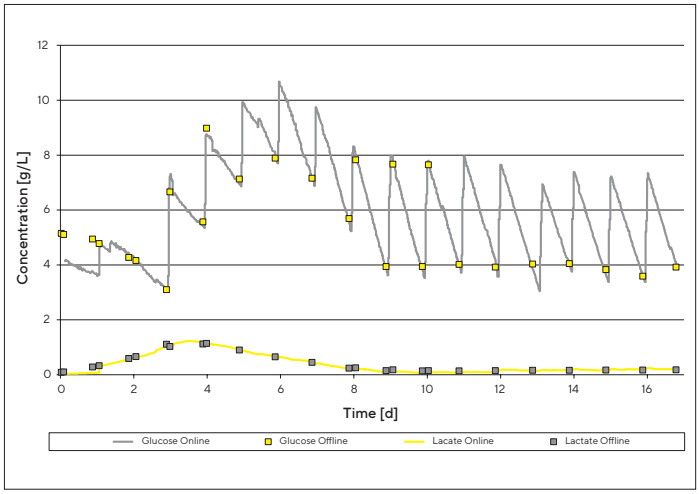


Fig. 5: Glucose and lactate trend trial 1

Despite of a small offset in the first 24 hours the online and offline measurements of glucose and lactate fit well during the cultivation.

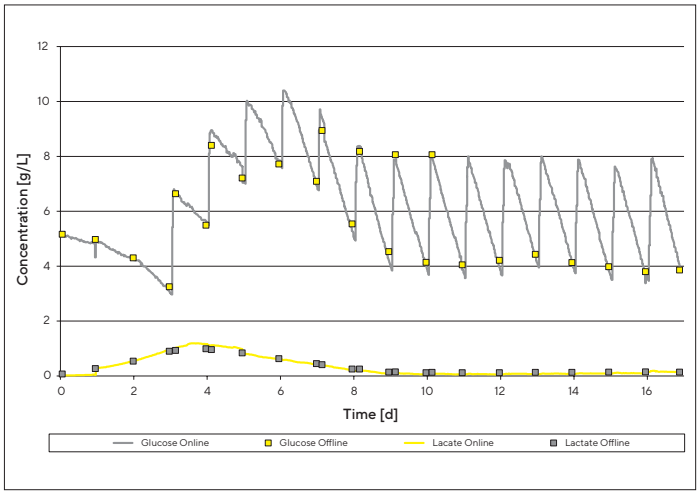


Fig. 6: Glucose and lactate trend trial 2

For trial 2 the online and offline glucose and lactate measurements showed good comparability.

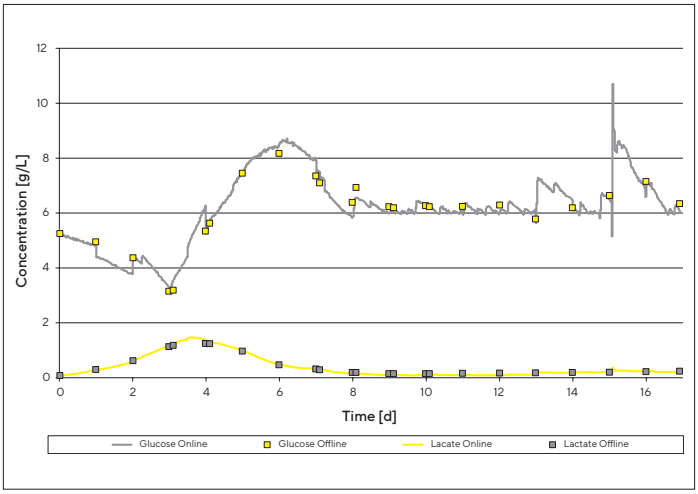


Fig. 7: Glucose and lactate trend trial 3

Also in trial 3 the online and offline trends fit well for glucose and lactate. At day 13 and 15 an unnecessary recalibration was performed (operational mistake).

The transition from glucose monitoring to glucose control requires a change of the process. The effects of tighter glucose control are known to be beneficial to the process performance and product quality (reference An Zhang et al). Considering this, changing a process using non tried and tested technology is undesired. Thus, learning the system and building confidence around the measurement output (comparing to offline sampling) and ensuring the device's robustness is generally needed before making that change. The first two process runs show the high resolution monitoring capabilities of the integrated Biostat® B-DCU and BioPAT® Trace whereas the third run engages the glucose controller. The overall user operational interactions with the bioreactor related to sampling glucose are removed. This means the normal hours and out of normal working hours needed to maintain the Biostat® B-DCU system in a glucose controlled state are reduced and more manageable. In table 1 a summary of the time savings are presented, in addition all user interaction work with the system was done within the initial stages of the Biostat® B-DCU setup.

Process Step	Hours
Setup of trace probe and electronics	2
Saved time for sampling	9 (18 × 0.5)
Saved time	7

Table 1: FTE resource balance per batch when using the BioPAT® Trace for glucose

Summary & Conclusion

The stepwise integration of the glucose control technology to a CHO fed-batch process was successful and maintained an inprocess steady-state glucose concentration of 6 (+/– 0.25) g/L.

The direct integration of the BioPAT® Trace technology into the Biostat® B-DCU is fully functional and the utilization was user friendly, easy to establish and dynamically variable when required. As the system package is coming from Sartorius Stedim Biotech completely, the lifecycle of the glucose controller is managed by us through all scales of development and commercial manufacturing. This ensures the benefits uncovered in development can be transferred to commercial production for the complete product lifecycle.

Outlook

Further trials with varying, automatically controlled glucose concentrations will be performed in order to improve product concentration and product quality attributes. Additionally, the operator time saving (7 hours per batch) and manageable working hours makes steps towards a walk away bioreactor.

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