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BioPAT® Trace Glucose PID control

Application Note

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turning science into solutions

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1. Introduction

Current industry practices for large-scale mammalian cell cultures typically employ a standard platform fed-batch process with fixed volume bolus feeding. Although widely used, these processes are unable to respond to actual nutrient consumption demands from the culture, which can result in accumulation of by-products and depletion of other certain nutrients. This work demonstrates the application of automated glucose control via dynamic feeding with BioPAT® MFCS PID controller. The method is based upon automated glucose measurements obtained from The BioPAT® Trace where cultures were fed to maintain a previously manual achieved target glucose level of 6.0 g/L.

2. Material and Methods:

The automated feeding trials were performed in CHO fed batch mode cultivation with following batch parameters:

Batch Parameters

Cultivation vessel	Biostat® B-DCU II UniVessel® 1L
Temperature set point	36.8°C
pO_2	Set point 60% (controlled with Air and oxygen)
pH	Set point 7.20 (controlled with CO_2 and 1 M (Na_2CO_3, sodium carbonate)
Cultivation media	ActiCHO PM
Feeding quantity	ActiCHO Feed A – 40 mL/L daily on 3rd day onwards ActiCHO Feed B – 4 mL/L on 3rd day onwards
Batch volume	700 mL
Initial cell concentration	0.32×10^6 Cells/mL
Speed	200 rpm – 250 rpm

The BioPAT® Trace system consists of a measurement unit, single-use fluidic system, calibration solutions & transport buffer. The fluidic system assembled with measurement unit was used for calibration and online measurements. The software 'trace_mon' version 1.3.03 was used to control the device. Calibration solutions with10 g/L glucose & 5 g/L lactate and 0.5 g/L glucose & 0.25 g/L lactate were used. The BioPAT® Trace system was connected to the bioreactors with a dialysis probe.

The glucose & lactate measurement frequency was set to 20 minutes. Offline reference samples were taken from the bioreactor daily. An internal measurement correction was performed, if there was a deviation from external reference method. The external referencing was performed using spectrophotometric assay methods. For glucose, a test kit from Agappe Diagnostics Ltd. based on glucose oxidase was used. For lactate a test kit from Centronic GmbH based on lactate oxidase was used.

Initial setup trials with water & glucose solution were carried out to optimize the BioPAT® MFCS PID values with different reactor volumes. The optimized PID set up for automatic feeding shown in Table 1.

Batch Volume	XP	TI	TD
5 L	200	1000	0
2.5 L	100	1200	0
1 L	100	1200	0

Table 1: BioPAT® MFCS PID parameters used for feed control with 20 minute glucose sample frequency

3. Experiments and Results:

Three replicate CHO cell cultivations in fed batch mode were performed where glucose of 6.0 g/L was maintained with PID & lactate concentrations were monitored with a BioPAT® Trace. During the setup of glucose control loop, feed line was primed and the pump was evaluated for its total flow rate (rpm) capabilities. The starting settings were adjusted to ensure no initial over feeding. An overview of the achieved cell densities and viabilities are presented in figure 2.

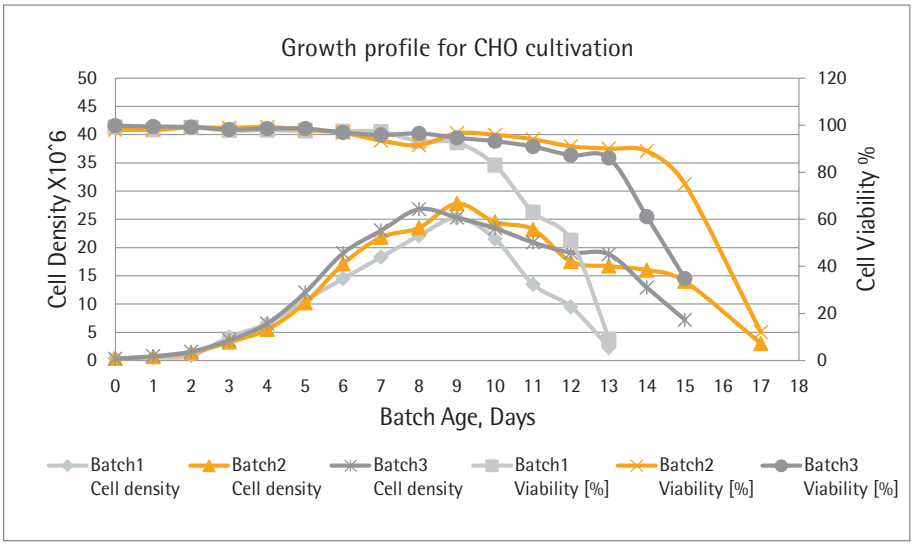

Figure 2: Overview of the growth patterns of the performed CHO cell cultivations.

During the cultivations, peak cell densities of 25 – 27 million cells/mL were achieved on 8 – 9th day of cultivation. During that period the cultivations maintained a viability of over 95%. Afterwards both viability and viable cell density decreased until the end of the cultivation.

Throughout the cultivations, the performance of the batches was monitored by off-line sampling of the IgG media concentration. This data is plotted in figure 3 and shows consistent protein production.

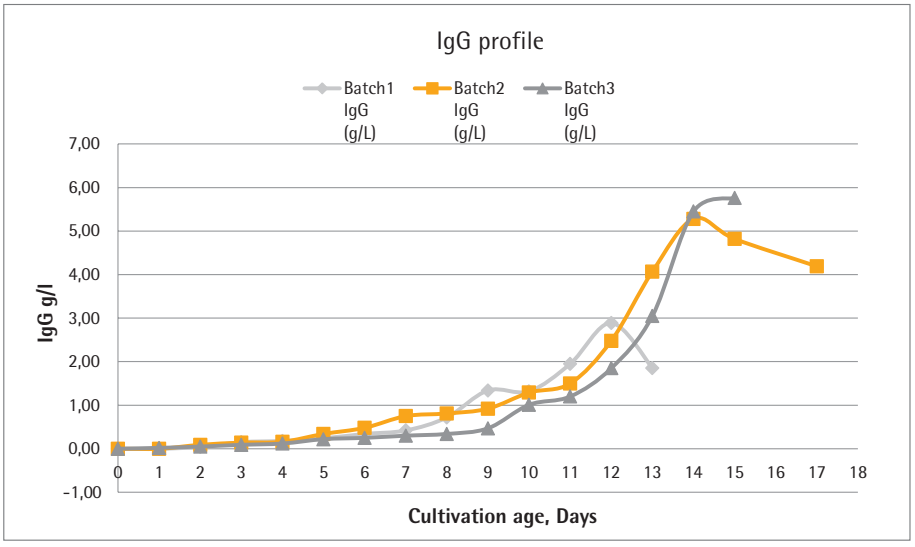

Figure 3: Overview of the IgG production of the performed CHO cultivations.

Two different feeding strategies were used for the cultivations and are described below.

Feeding strategy for batch 1 & 2 was

- A constant glucose level of 6.0 g/L was maintained with PID controller from 3rd day (end of lag phase) to 9th day (peak cell density) by Feed A solution. From 9th day onwards, 40% glucose solution replaces feed A in PID controller & 40 mL/L of feed A was added as bolus dosage.

Feeding strategy for batch 3 changed to

- A constant glucose level of 6.0 g/L was maintained with PID controller from 3rd day (end of lag phase) to 9th day (peak cell density) by Feed A solution. From 9th day onwards 40% glucose solution replaces feed A in PID controller & 40 mL/L of feed A was added over a time period of 24 hrs.

In summary, a guided data plot from the BioPAT® Trace and off-line reference measurement points is shown in figure 4.

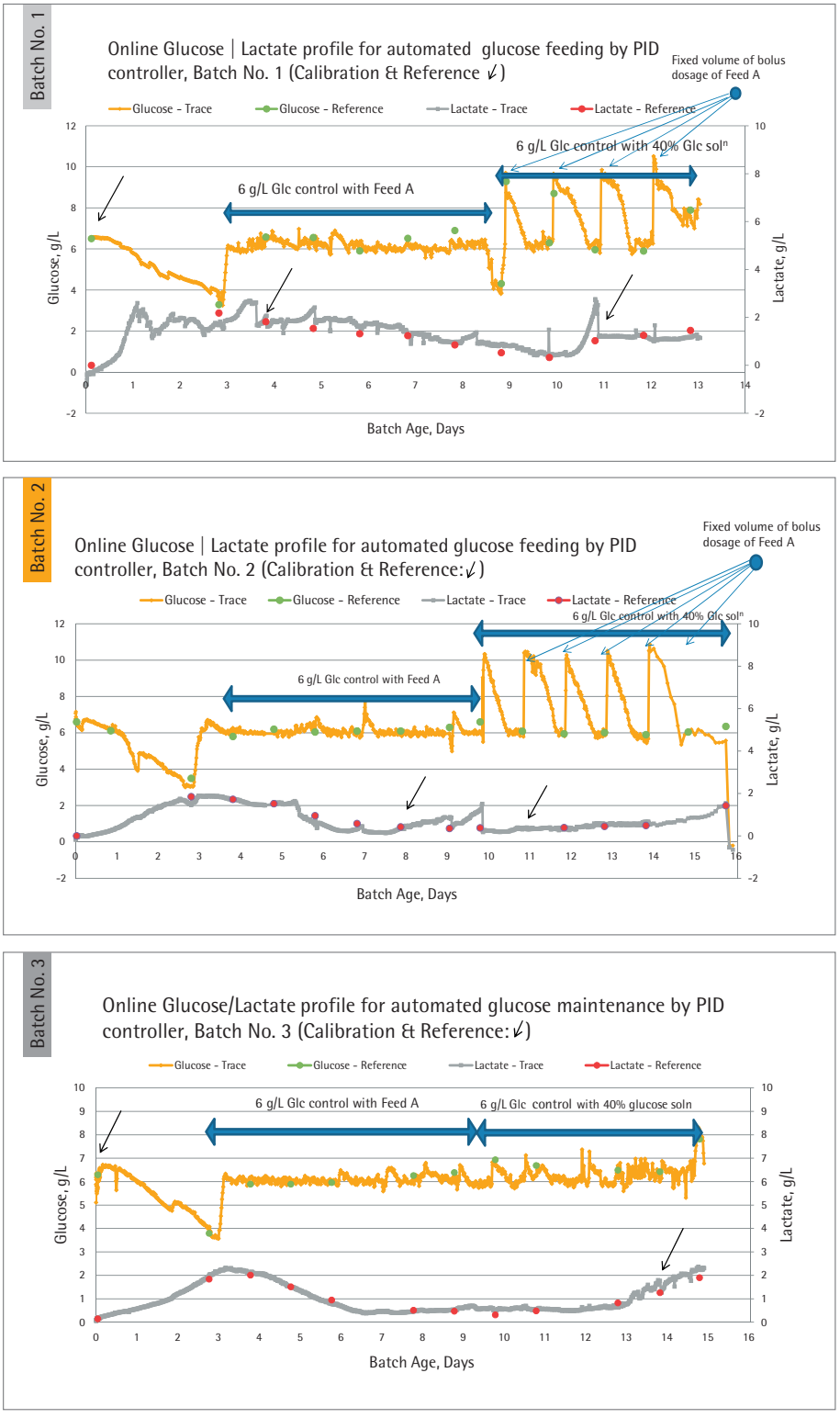

Figure 4: Graph of online values for glucose & lactate for 3 batches & external reference values

With the optimized PID values, the constant glucose level was maintained with feed A up to 9th day (Peak cell density day). The average consumption of the feed A per day was 27 – 30 mL for 0.75 L of working volume to maintain this constant glucose level. Afterwards 40% glucose solution replaced feed A in PID to maintain the glucose level. Approximately 4 – 5 mL of 40% glucose solution was consumed daily to maintain the set-point.

A calibration and referencing procedure of the BioPAT® Trace was performed at the start of the cultivations and during cultivation when the estimation of the device deviated from the reference measurements (indicated with black arrows in figure 4). Any referencing is needed if any basic process parameters are changed like temperature, feeding profiles or measurement ranges of the system. In figure 5 the measured concentration values from the BioPAT® Trace are compared to the reference values and the accuracy displayed.

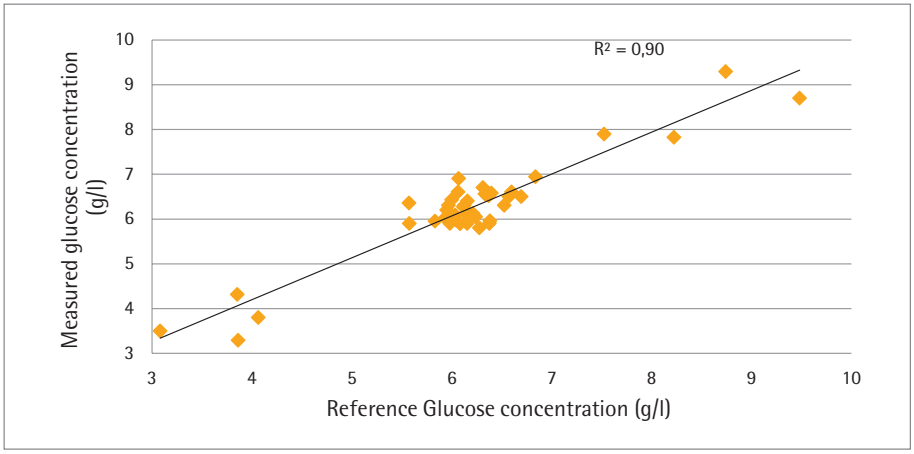

Figure 5: Measured values from BioPAT® Trace vs values from reference method.

Shown are only values inside the calibration range between 1 – 10 g/L. It shows that the values generated by the BioPAT® Trace correlate well with the off-line glucose concentration measurement. A histogram of the observed deviations of the BioPAT® Trace measurements from the reference values is presented in figure 6.

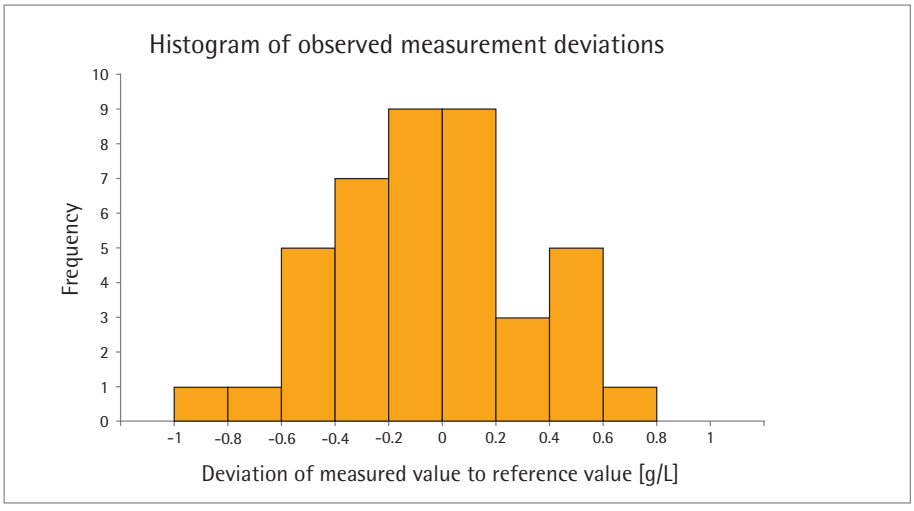

Figure 6: Histogram of the observed deviations of the BioPAT® Trace measurements from the reference values.

It can be seen that the observed deviations are normally distributed. The standard deviation of the observed deviations is 0.36 g/L and all deviations are within a range of ±1 g/L.

4. Conclusion

The BioPAT® Trace ensures a high degree of accurate results for online glucose measurements and dynamic feed control. The standard deviation of the observed error of the BioPAT® Trace values in comparison to the reference values is 0.36 g/L.

BioPAT® MFCS PID controls the glucose level of 6.0 g/L throughout the cultivation very efficiently. The dynamic feeding has the ability to automatically adjust feed rates according to cultures dynamic behavior.

High levels of glucose can result in high levels of lactate through glycolysis. Lactate accumulation can reduce the pH and the low pH can be detrimental to cell viability and productivity.

Traditionally, the control of glucose is being done by taking samples and measuring the sample with an external system such as spectrophotometry. This is a time consuming and operator dependent task that has inherent manual inconsistency.

The BioPAT® Trace is automated handle free equipment after installing the consumables, which provides actual monitoring of glucose level & 100% direct control on the entire cell cultivation process.

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