

A Quality-by-design Approach to Upstream Bioprocess Interrogation and Intensification



Efficient biopharmaceutical process development relies on the quality-by-design (QbD) paradigm. QbD is a scientific, risk-based proactive approach to drug development that aims to have a full understanding of how the process and product are related. This knowledge is gained by process analytical technology (PAT). In this case study the Applied Process Company (APC) integrated external PAT and an APC-developed controller with a parallel bioreactor system. Online PAT measurement and control of the identified critical process parameters led to greater understanding and the streamlined optimisation of a mammalian cell bioprocess. The study exemplifies the value of flexible bioreactor systems which allow ease of integration of third-party technology.

Introduction

Biopharmaceuticals represent a significant and growing sector of the pharmaceutical industry. The global biopharmaceutical industry is currently worth over 107 billion euros, according to research conducted by BioPlan Associates¹. However, a lack of understanding of the process and product interdependencies potentially results in manufacturing challenges. Optimisation of protein production based on empirical experimentation and quality testing of the end product often results in bioprocesses operating under suboptimal conditions and hence in extended cycle times, excessive raw material and utility requirements, and elevated numbers of process or product failures, which together culminate in a high cost of manufacturing.

To ensure consistent drug quality and reduce batch-to-batch variability, pharmaceutical companies and regulatory authorities aim to replace the "quality-by-testing" with a "quality-by-design" strategy. In a QbD approach, critical quality attributes of the end product are defined and subsequently a production process is developed which aims at meeting those attributes. This involves identification of a design space, a range of experimental conditions within which variations in process parameters do not affect product quality.

Prerequisite is the identification of critical process parameters by PAT that in the course of protein manufacturing are then monitored and, if needed, controlled. The PAT required to support a QbD product may be simple or complex, depending on the nature of the product and process used for its manufacture. Generally speaking, mammalian cell culture processes and the products which they produce are complex and hence, there are many challenges to implementing a PAT/QbD strategy. However, the potential benefits coupled with regulatory pressures are driving a strong interest in and an increased level of adoption in the biopharmaceutical industry. Biological processes are known to have a much higher level of variability than their chemical counterparts. PAT and QbD offer an avenue to better understand and control this variability, which has implications for the process economics, control and final product quality. Traditionally, temperature, pH, and dissolved oxygen are the main parameters measured and controlled online due to the availability of traditional, robust sensors. However, as technology advances, other process parameters such as cell density, cell viability, substrates concentrations, product and by-product concentration, dissolved carbon dioxide and biomarkers can now be measured and analysed in real time in an automated manner, although not yet routinely. The availability of real-time process information is of particular value in addressing the variability and unpredictability of animal cell cultures as it opens up the possibility of implementing advanced control strategies capable of directly impacting critical process parameters and critical quality attributes.

APC delivers chemical and bioprocess engineering solutions and technologies to enable streamlined development, optimisation, and supply of new and existing chemical and biological entities. APC's process development technology platform (BioACHIEVE™) combines PAT technology, multivariate data analysis, process modelling, and advanced control strategies².

This article describes the successful integration of external PAT with a parallel bioreactor system using an object linking and embedding for process control (OPC) communication protocol. The aim was to optimise the performance of a Chinese hamster ovary (CHO) mammalian cell bioprocess. In a previous study the glucose concentration was identified as a critical process parameter³. The objective of the following case study was to improve process performance by optimising the glucose feed profile. By moving from the traditional bolus fed-batch to a continuous feeding fed-batch strategy, nutrient depletion should be prevented and a stable macro-environment for the cells should be established.

Material and Methods

Cell Culture

CHO cells were cultivated in a glucose-free formulation of EX-CELL® CHO DHFR-medium (Sigma-Aldrich® Co., LLC, USA) supplemented with 20 mM glucose, 4 mM glutamine, 1 µM methotrexate, 0.1% (v/v) Pluronic® F-68 (Sigma-Aldrich Co., LLC, USA) and 10 mL/L penicillin-streptomycin (Sigma-Aldrich Co., LCC, USA). Cells were cultivated for 7 to 9 days at 37°C using a four-fold DASGIP® Parallel Bioreactor System for



Fig. 1: Eppendorf DASGIP Parallel Bioreactor System for cell culture.

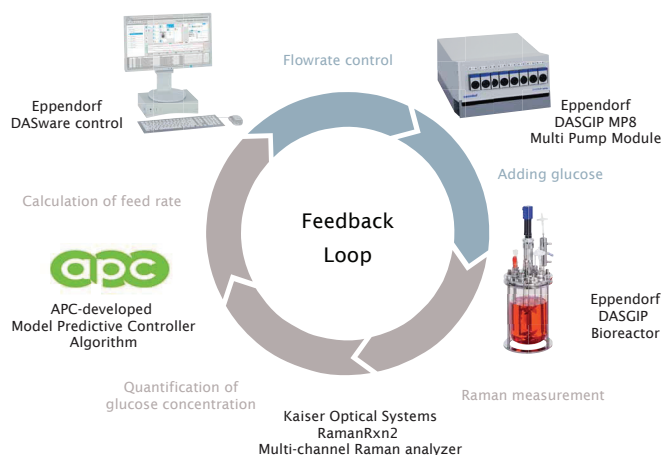


Fig. 2: Integration of external PAT with an Eppendorf DASGIP Parallel Bioreactor System.

cell culture equipped with 2.5 L (working volume) DASGIP® Benchtop Bioreactors (Eppendorf AG, Germany) (Figure 1). Cultures were agitated at 120 rpm. The pH was controlled at 7.2 using 1 M NaOH and CO₂ gas, respectively. Air saturation was set to 50 % and controlled using air and oxygen supplied via a sparger.

Offline Measurement of Process Parameters

Cell density and cell viability were determined offline using a Cedex® HiRes system (Roche Diagnostics® GmbH, Germany). Offline measurements of glucose concentrations were performed using an enzymatic assay kit (Megazyme® International Ireland Ltd).

Integration of External PAT and In-house Developed Controllers

Figure 2 illustrates the integration of external PAT and an in-house developed controller with a parallel bioreactor system. Online measurements of glucose concentration were performed by Raman spectroscopy. A RamanRxn2 Multi-channel Raman analyser (Kaiser Optical Systems, Inc., USA) was integrated with the bioreactor system using DASware analyze software. DASware analyze utilises an OPC communication protocol, an industry standard which facilitates the communication of devices from different manufacturers. To translate the Raman spectra into concentration information, chemometric partial least squared calibration models were developed using the SIMCA® multivariate data analysis package (MKS Umetrics AB, Sweden).

To maintain the glucose concentration

close to the set-point, an APC-developed model predictive controller (MPC) algorithm was integrated via bidirectional OPC communication. The essence of MPC is to optimise forecasts of process behaviour. This forecasting is accomplished with a process model, and, therefore, the model is an essential element of an MPC controller.

Based on online readings of the identified critical process parameters, the model is used to calculate optimal feed rates for set-point maintenance. MPC facilitates the ability to proactively counteract deviations from the set-point and to simultaneously control multiple process parameters, and is hence especially suited to ensure constant culture conditions in multivariate bioprocesses.

The MPC actuated a DASGIP MP8 multi-pump module to execute continuous glucose feeding.

Bolus Fed-batch and Continuous Fed-batch Glucose Feeding

The glucose concentration was adjusted using a feed medium consisting of 653.6 mM glucose, 58.8 mM glutamine and 58.8 g/L soy protein hydrolysate dissolved in glucose-free EX-CELL CHO DHFR- medium.

Two fed-batch feeding strategies were investigated in the study. The first was a fed-batch culture manually fed with bolus additions, at 24 h intervals, the volume of which was proportional to offline integral viable cell density measurements for the previous 24 h interval. The second regime was a continuous feed, the rate of which was determined and adjusted automatically using a model predictive controller and Raman-determined glucose values to keep the glucose concentration in the bioreactor at a set-point of 11 mM throughout the culture.

Results and Discussion

Using the DASware analyze software a RamanRxn2 Multi-channel Raman analyser and an APC-developed MPC algorithm were successfully integrated

with an Eppendorf DASGIP Parallel Bioreactor System. The glucose concentration in the CHO mammalian cell culture was determined online using Raman spectroscopy coupled with chemometric partial least squared modelling. The offline results determined via an enzymatic assay were highly comparable to the results obtained online by Raman spectroscopy (Figures 3A, 3B). Overall, the continuous feeding fed-batch bioprocess resulted in an increase in peak viable cell density and the integral of the viable cell density (Figures 3C, 3D) which is directly related to increased titre.

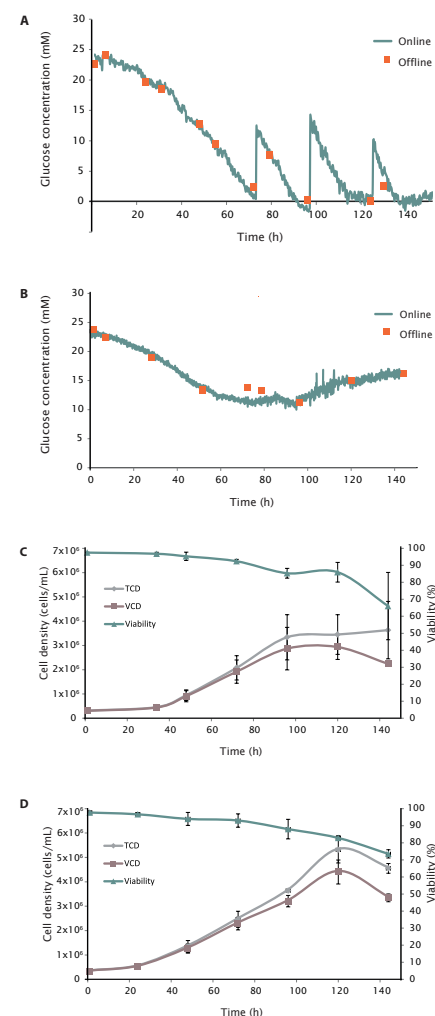


Fig. 3: Glucose set-point control via a model predictive controller algorithm resulted in an increase in cell densities.

A, B: Glucose concentration was measured online and offline, respectively. (A) Glucose concentration was adjusted via a bolus fed-batch feeding strategy. (B) The glucose set-point was maintained in a continuous feeding fed-batch strategy using MPC.

C, D: Total cell densities (TCD), viable cell densities (VCD), and viability were determined for the bolus fed-batch culture (C) and the continuous feeding fed-batch culture (D).

Conclusion

The parallel bioreactor system used was a suitable host for APC's BIOACHIEVE process development technology platform, which APC applies to upstream bioprocesses. The DASGIP system facilitated the ease of integration of external PAT and transfer of this information to and from APC-developed controllers. This allowed optimisation of the process performance based on greater process understanding, ultimately delivering improvements in cell growth. This case study exemplifies the value of increased process understanding and control in biopharmaceutical manufacturing. Advancements in sensor technology and data analysis facilitate the ability to control the identified critical process parameters to their respective target levels and thus optimise the critical quality attribute design space. To apply the QbD approach to upstream bioprocess development, bioreactor systems which allow smooth integration of external sensors and controllers are crucial.

References

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