PAT Tools For Accelerated Process Development and Improvement: Application of Cells on Microcarriers With Continuous Control of Metabolite Concentrations Using the BioProfile® FLEX Sampling and Analysis System

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Abstract

Current initiatives within the biopharmaceutical industry to adopt PAT (Process Analytical Technology) approaches in manufacturing, include activities in early process development. With adherent cell lines, online sampling for real-time process monitoring and process adjustment is challenging due to the viscosity of the cell culture and potential blockages to sampling systems caused by microcarriers. In this study, we have tested the BioProfile® FLEX analyser and online autosampler with OPC (Open Productivity & Connectivity) for continuous feedback control of metabolite concentrations in lab-scale bioreactors.

The first part of the study demonstrated that glutamine, glucose and glutamate consumption was dependent on cell growth phase. In the second part of the study, metabolite concentrations were analysed and controlled in the bioreactor using the OPC enabled BioProfile® FLEX analyser. Sampling was performed via sterile online sampling; allowing automated nutrient feed control. Once established on non-viral infected cells, the system was further applied to a model of a virus (rabies) production process for 14 days. Cell culture experiments were carried out with automated sampling and analysis performed every hour to control and monitor the viral production process at Sanofi Pasteur in Lyon, France.

This study demonstrates that it is possible to effectively control and adjust (in real-time) the required concentration of metabolites to sustain controlled cell metabolism, as viral infection and production progresses. The BioProfile® FLEX online autosampler and analyser, in conjunction with an OPC enabled bioreactor controller, provided reliable results and improved process control for development activities.

Introduction

Nova Biomedical's BioProfile line of chemistry, gas and cell density analysers are used extensively in the biopharmaceutical industry. Uses include cell line optimization, media development, process development, bioreactor characterization, and commercial product process monitoring. With the ability to measure pH, gases, nutrients, metabolites, electrolytes, osmolality, IgG, phosphate, cell density and viability. The array of cell culture analytical systems available by Nova Biomedical provide single-system solutions for almost any cell culture application.

Materials & Methods

Figure 1. BioProfile® FLEX with Online Autosampler

Materials

- Vero cell lines in serum free medium culture
- Microcarriers (1 g/L at 3 g/L)
- Medium added continuously by peristaltic pump
- Nova BioProfile FLEX analyser and online autosampler
- Ceramic sampling probes with porosity at 0.2 µm and 20 µm

Methods

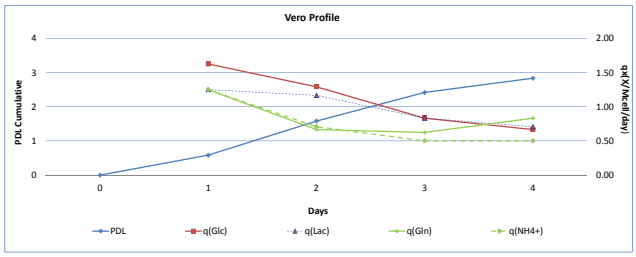
Vero cells were grown in serum free medium on cytodex 1 microcarriers. A fed-batch process was applied with continuous injection of media at different glucose and glutamine concentrations to control metabolism.

Custom OPC software from Nova Biomedical was connected to the MFCS controller from Sartorius to control and track glutamine and glucose concentration. Three conditions were established. The first condition was without regulation of metabolites. The second condition was a Vero cell culture with OPC control, and the last condition was virus productivity with continuous feedback control in response to monitoring of metabolite consumption profiles with the Nova Biomedical analysis system.

Results

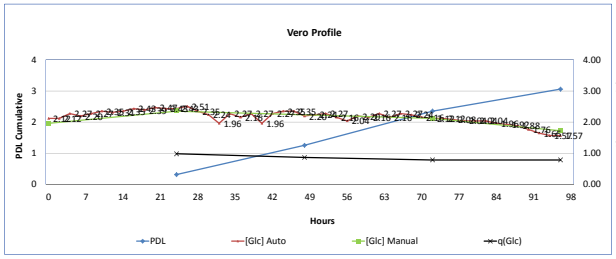
At the beginning of the study, a profile of Vero cells in serum-free medium was tracked by metabolite consumption. The qGlutamine and qGlucose were at the maximum level at the beginning of the culture when metabolite concentrations were at highest levels. Cell growth reached the maximum on day 2 and 3 (PDL cumulative). On day 4, the cell growth decreased, which is linked to a lower concentrations of key metabolites.

Figure 2. Vero Cell Culture Profile



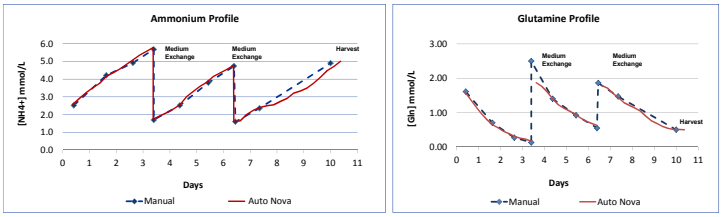
PDL: Population Doubling Time
q(Glc); q(Gln): Metabolite consumption by million cells/day
q(Lac); q(NH4+): Metabolite production by million cells/day

Figure 3. Vero Cell Culture Profile, Automated Sampling

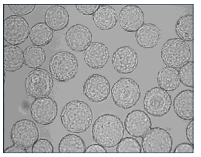


To maintain the glucose and glutamine concentration at a low level, an OPC enabled BioProfile® FLEX analytical system was used with a feed-addition of specific glucose and glutamine concentrations. Metabolite concentrations were tested every hour to control the automated feed-back loop. In this study, a ceramic sampling probe with 0.2 µm porosity was used to avoid blocking the autosampler. The data obtained with manual control was comparable to automated analysis. At the end of the process, the glucose concentration decreased due to medium addition in the bioreactor.

Figure 4. Ammonium and Glutamine Profiles



Profiles of glutamine and glucose were generated in viral infected cells for 14 days. The sampling system was adjusted to limit flow restrictions by increasing the porosity of the filtration probe to 20 µm. During the study, cell lysis caused by the viral production restricted the sampling flow of the system at lower probe porosity. Figure 5. shows the accumulation of debris in the cell culture. At 20 µm, a very high sample correlation between manual and automated sampling was achieved.



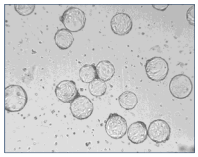


Figure 5. Vero Cells on Microcarriers

The image on the left shows Vero cells on microcarriers before the infection by rabies virus. The image on the right shows Vero cells after two weeks of infection.

At an hourly sample frequency, no failures were observed or experienced after 20 days of cell culture with the BioProfile® FLEX analytical system. Glutamine and glucose concentration can be managed in cell culture or virus production by an OPC enabled bioreactor controller in conjunction with the BioProfile® FLEX online autosampler.

Summary

The Nova BioProfile® FLEX and autosampler can be implemented to provide an online process control feedback loop for metabolite concentrations (glucose, glutamine, glutamate, lactate and ammonium) continuously in cell culture process development. The flexible platform allows the use microcarrier technology, without flow restrictions.

In harmony with PAT initiatives, the complete automated system is able to increase data density and process efficiency with reduced in-process operational steps.