Single-use technologies

Comparison of the HyPerforma GXCore and G3Lab Bioprocess Controllers for CHO cultivations

A user's study

Keywords
GXCore, bioprocess controller, automation, cell culture, fed-batch, CHO, IgG antibody, glass bioreactor

Summary
Here we describe the cultivation of an immunoglobulin G (IgG)-expressing CHO cell line using the new Thermo Scientific™ HyPerforma™ GXCore™ Bioprocess Controller in conjunction with a Thermo Scientific™ HyPerforma™ Glass Bioreactor, featuring a maximum working volume of 2 L. The same process was also carried out using a Thermo Scientific™ HyPerforma™ G3Lab™ Bioprocess Controller for comparison. Two fed-batch cultivations were performed with each system, resulting in highly similar growth and IgG production profiles. At the end of the 14-day process, IgG titers of 2.79 ± 0.045 g/L were reached.

Introduction

The cultivation of mammalian suspension cells demands tight control of the process conditions. For this, a capable bioprocess controller that can regulate pH, dissolved oxygen concentration (DO), and temperature as well as handle feed and antifoam addition is required. The HyPerforma GXCore controller is such a bioprocess controller—it is operated using Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ Distributed Control Platform and allows the execution and automation of complex cultivation processes. With a small footprint, the HyPerforma GXCore Bioprocess Controller has two built-in peristaltic pumps and allows connection of a variety of analog and digital sensors as well as peripheral equipment, including mass flow controllers, scales, pumps, and temperature control units.

This study focuses on the cultivation of IgG-producing CHO cells in fed-batch mode using serum-free, chemically defined media. The cells were cultivated in batch mode for the first 3 days before daily feeding was started. When required, a glucose solution was added to ensure that the glucose concentration exceeded 2 g/L at all times. When the cultures were harvested, average titers of 2.79 g/L were measured while the viable cell density (VCD) was approximately 8.07 x 10^6 cells/mL with 83% viability. During the whole process, the DO could be held above 40% saturation by oxygen sparging without causing foam buildup.

The aim of this study was to compare the newly developed HyPerforma GXCore Bioprocess Controller with its predecessor, the HyPerforma G3Lab Bioprocess Controller. Two HyPerforma glass 3 L bioreactors were operated in parallel and the experiment was conducted twice to demonstrate repeatability, equating to a total of four cultivations.

Materials and methods

Cell line

A stably transfected Gibco™ ExpiCHO-S™ cell line was used in this study. The cells constitutively express a full-length IgG antibody. Since the IgG gene is coupled with a dihydrofolate reductase gene, methotrexate can be used to maintain selective pressure for product formation.

Media and feed

The cells were cultivated in serum-free Gibco™ ExpiCHO™ Stable Production Medium (SPM) supplemented with 4 mM alanyl-L-glutamine (AlaGln). The corresponding Gibco™ EfficientFeed™ C+ 2X Supplement was used for feeding. A glucose solution at a concentration of 450 g/L was used to supply the cells with additional glucose when needed. To prevent excessive foam buildup, a bottle of 1:1,000 diluted Antifoam C Emulsion (MilliporeSigma) was attached to the reactor. However, no antifoam addition was required since no foam buildup was observed.

Hardware and software

The cultivations were carried out in HyPerforma 3 L glass bioreactors featuring a maximum working volume of 2 L. A Rushton-like turbine and a segment blade impeller were installed as recommended by the manufacturer. A micro-sparger positioned beneath the stirrer was used for sparging. The digital Thermo Scientific™ TruFlow™ mass flow controller (MFC) assembly was used with the HyPerforma G3Lab controller, whereas a newly designed MFC assembly was utilized in conjunction with the HyPerforma GXCore controller. An operator PC with the DeltaV (v13) automation software in conjunction with TruBio 5.0 software and the add-ons for the corresponding bioprocess controllers was used for data acquisition and control.

Overview of procedure setup

- Day –7: Thawing of CHO cell aliquot, cultivation in shaker flasks
- Day –4: Subcultivation of inoculum
- Day –2: Subcultivation of inoculum
- Day –1: Preparation and sterilization of bioreactor
- Day 0: Inoculation with 0.3 x 10^6 cells/mL in 1.64 L ExpiCHO SPM
- Days 3–13: Daily feed with EfficientFeed C+ 2X Supplement at a feed volume corresponding to 2% of the current working volume
- Day 4: Start of optional daily glucose addition based on metabolism
- Day 14: Termination and harvest of bioreactor contents

Inoculum and bioreactor preparation

The inoculum was incubated in Corning shake flasks (SF) without baffles at 37°C, 8% CO_2, and 80% relative humidity, with shaking at 120 rpm with an orbital diameter of 25 mm. The inoculum preparation was carried out according to Table 1. To maintain selective pressure towards IgG production, 400 nM methotrexate (Sigma-Aldrich) was added to the N-2 culture medium.

The two HyPerforma 3 L glass bioreactors were assembled identically, making sure that the sample tube, the sparger, the thermowell, and the probes for foam, pH, and DO were at the same locations in both vessels. Conventional DO and pH probes were used. One day before inoculation, the bioreactors were filled with PBS and autoclaved at 121°C for 30 min.

Subsequently, the PBS was aspirated and replaced with 1 L SPM. The control loops were started to adjust the process conditions according to the setpoints listed in Table 2. Afterwards, the pH and DO probes were recalibrated. During inoculation, cells and additional SPM were added to reach an initial VCD of 0.3 x 10^6 cells/mL and a starting volume of 1,640 mL. The pH was controlled using CO_2 sparging only, and no base was added. The DO was maintained at ≥40% saturation by oxygen sparging.

Table 1. Overview of inoculum preparation.

Day	Event	Step	Culture system	Volume (mL)	Inoculation density (x10^6 cells/mL)	Number of vessels
–7	Thawing	N-3	125 mL SF	40	~0.35	1
–4	Subcultivation	N-2	250 mL SF	80	0.5	2
–2	Subcultivation	N-1	500 mL SF	160	0.5	4
0	Inoculation	N	3 L bioreactor	1,640	0.3	2

Table 2. Process parameters.

Parameter	Value
Temperature	37°C
pH	≤7.2
DO	≥40% saturation
Stirrer speed	200 rpm
Stirrer tip speed	0.58 m/s
Overlay gassing (air)	0.2 L/min
Working volume	1.64—1.93 L
Inoculation density	0.3 x 10^6 cells/mL
Process duration	14 days

Feeding strategy

Daily bolus feeding with EfficientFeed C+ 2X Supplement was started on day 3 and continued until day 13 for a total of 11 feed additions. The reactors were fed with 2% of their current working volume, resulting in an exponential feeding profile. Additionally, a 450 g/L glucose solution was added gravimetrically based on the calculated glucose consumption to ensure that a glucose concentration above 2 g/L could be maintained at all times.

Sampling and analysis

Samples were taken daily prior to feed addition via a sterile sampling valve attached to the reactor using sterile 10 mL syringes. Cell density and viability were measured using a Cedex™ HiRes Analyzer (Roche Diagnostics). Substrate and metabolite concentrations (glucose, lactate, IgG, alanyl-glutamine, and ammonia) were measured using a Cedex™ Bio Analyzer (Roche Diagnostics). Additionally, pH was measured off-line, and the on-line pH value shown by the controllers was recalibrated if the deviation was more than 0.05 pH units. No sampling was carried out post-feed; instead, the substrate and metabolite concentrations after the feed were calculated based on the feed volume.

Results

The growth curves were similar for all cultivations (Figure 1). The exponential growth phase lasted until approximately day 4. Peak VCDs of 13 to 14 x 10^6 cells/mL were reached between day 7 and 8 before the cell count slowly decreased to around 9 x 10^6 cells/mL at the end of the cultivation. The cell viability remained high throughout the process, but it started to slowly decrease after the maximal VCDs were reached.

The production of IgG was very similar in all cultivations, and the highest cell-specific IgG production rates of 35–40 pg/cell/day were determined in the later phase of the cultivation after day 10 (Figure 2). Final titers of 2,790 ± 45 mg/L were reached. Based on the IgG concentration trend, it is likely that even higher titers could be reached by extending the feeding phase.

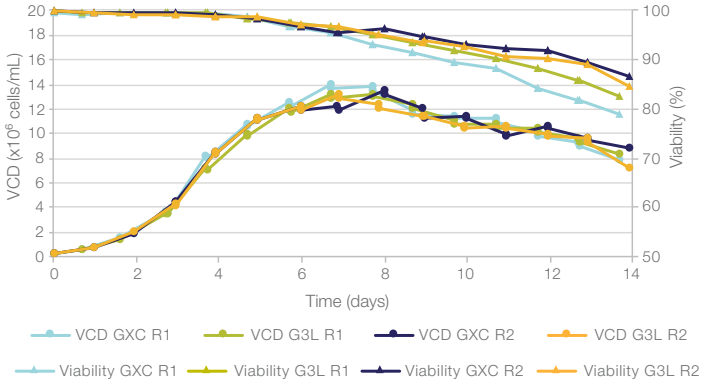

Figure 1. VCD and viability for all four cultivations.
GXC = HyPerforma GXCore Bioprocess Controller, G3L = HyPerforma G3Lab Bioprocess Controller, R1 = first cultivation run, R2 = second cultivation run.

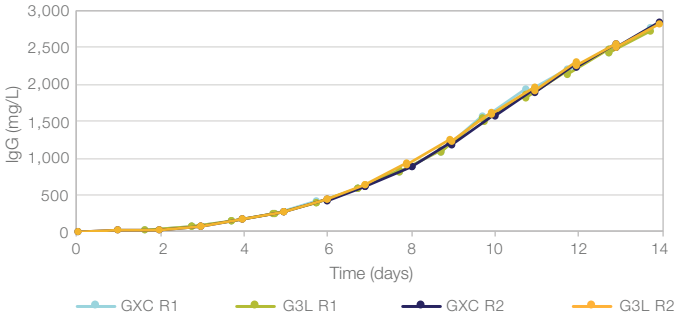

Figure 2. IgG concentration during the cultivations.
GXC = HyPerforma GXCore Bioprocess Controller, G3L = HyPerforma G3Lab Bioprocess Controller, R1 = first cultivation run, R2 = second cultivation run.

The glucose concentration fell below the target concentration of 2 g/L on some occasions (Figure 3). However, no glucose limitation occured at any time of the cultivation. Up to day 4, lactate accumulated to a concentration of 1.39 ± 0.04 g/L. Subsequently, lactate consumption started to exceed production, and the concentrations decreased again.

The ammonia concentration increased to about 13.4 ± 0.8 mM during the cultivation (Figure 4). Starting approximately on day 5, the cell diameter increased from around 13 µm to 18 µm until the end of the cultivation. Again, no noticeable differences between the four cultivations were found.

In the first two days of the cultivation, the pH was tightly controlled at 7.2 by CO_2 addition (Figure 5). However, as soon as the oxygen demand of the culture required O_2 sparging, the controllers started to oscillate as both gases were added through the same micro-sparger, and the dead volume of the tubing caused delays in the controller feedback. As soon as the pH fell below 7.2 and no CO_2 was added anymore, the fluctuations disappeared. However, it is noticeable that the pH often started to rise several hours before feeding, while feed addition caused it to quickly fall again. The rise in pH may be due to the metabolism of previously formed acidic compounds by the cells. The pH trend was very similar in all cultivations, and the overall behavior of the cultures was the same regardless of the controller used.

The DO first fell below the set point of 40% saturation after approximately 1.5 days. The substantial fluctuations visible until day 3 were caused by the previously mentioned interactions between the pH and the DO control loop. Even though different mass flow controllers were used with the two process controllers, the fluctuations were comparable. It was found that the DO control could be improved by adjusting the PID parameters on day 4 of the second experiment (black arrows in Figure 5). This resulted in lower oscillations, but additional tuning of the PID parameters is required to further improve the pH and DO control.

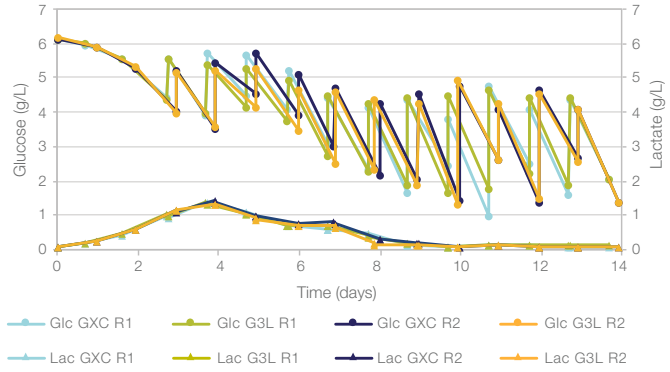

Figure 3. Glucose (Glc) and lactate (Lac) concentration during the cultivations. GXC = HyPerforma GXCore Bioprocess Controller, G3L = HyPerforma G3Lab Bioprocess Controller, R1 = first cultivation run, R2 = second cultivation run.

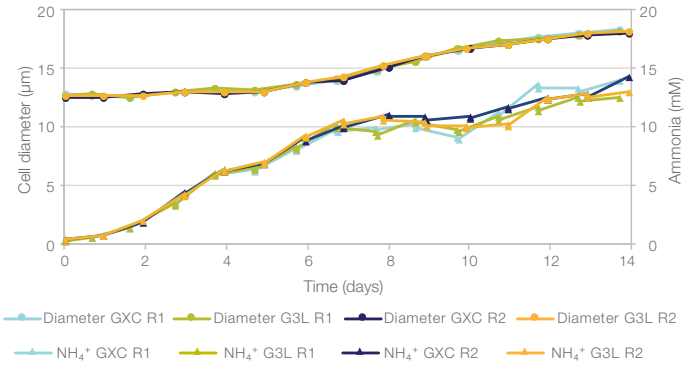

Figure 4. Cell diameter and ammonia levels during the cultivations. GXC = HyPerforma GXCore Bioprocess Controller, G3L = HyPerforma G3Lab Bioprocess Controller, R1 = first cultivation run, R2 = second cultivation run.

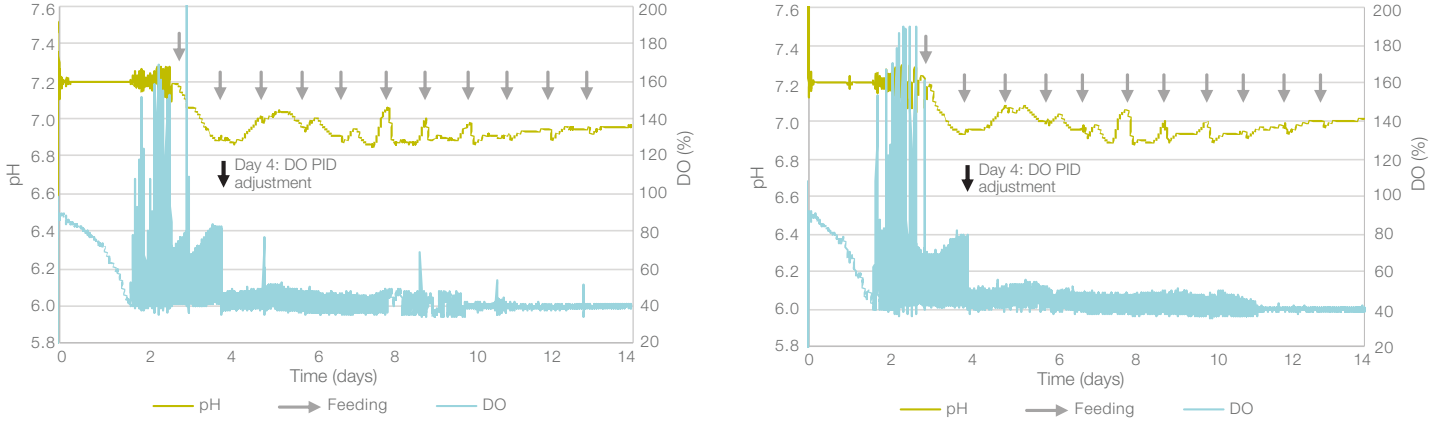

Figure 5. pH and DO for the second cultivation run with the (A) HyPerforma GXCore controller and (B) HyPerforma G3Lab controller.

Conclusion

In summary, it was demonstrated that CHO cultivations performed in the 2 L HyPerforma Glass Bioreactor in conjunction with the TruBio control software platform are highly reproducible. Additionally, it was shown that there is no discernible difference between data acquired using the HyPerforma GXCore and G3Lab Bioprocess Controllers.

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