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Parallel Fed-batch CHO Culture on SciVario® twin, the Flexible Controller for All Your Bioprocess Needs.

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Abstract

We performed fed-batch cultures using BioBLU® 1c and 3c Single-Use Vessels controlled in parallel by the new SciVario® twin bioreactor control system. This method highlights the SciVario twin's capabilities to run complex processes simultaneously using various sized vessels. The initial release of SciVario twin can control vessel sizes from 0.7 L to 4 L. Equipped with 14 integrated advanced thermal mass-flow controllers (TMFCs) that have a turndown ratio of 1:12,000 the system is designed to be a future-proof solution. Developed following the Agile principles, future updates will allow users to run vessels from as small as 0.3 L to as large as 40 L, on the same controller. Our 3 L CHO culture reached its highest density of over 17 x 10^6 cells per mL by day 11 with minimal human intervention. The experiments demonstrated both the capability of fed-batch cell culture and the flexibility of the SciVario twin platform.

Introduction

The SciVario twin is the first bioreactor control system developed by Eppendorf that has the capability to control two bioreactors in parallel or individually across a wide range of vessel sizes. It is a dynamic, easy-to-operate bioprocess controller with flexibility that adapts to your needs:

> SciVario twin is designed for future software updates and hardware extension releases, allowing the system to evolve with your processes without requiring additional investments in new control systems.

> The system's wide range of gas flow rates helps to meet oxygen demands ranging from the lows of standard batch runs to the highs of high-density cultures, allowing users to run both simple and complex regimens at the same time.

> As the first bioprocess controller with VisioNize®-onboard, the SciVario twin is equipped with an intuitive user interface already known on other Eppendorf products, ranging from PCR cyclers to incubator shaker to freezers.

> In this application note, we demonstrate the parallel process control of a fed-batch CHO cultures in 1 L & 3 L BioBLU Single-Use Vessels.

Fig. 1 SciVario twin bioreactor control system.

Material and Methods

SciVario twin has the versatility of controlling both glass and BioBLU Single-Use Vessels individually or in parallel. We chose to use single-use vessels for this experiment. Substituting traditional glass bioreactors with single-use equipment can greatly simplify the bioprocess workflow by eliminating the need for cleaning and autoclaving. This reduces the time needed to prepare the bioprocess run and lowers the risk of contamination.

Procedure

Cell line and medium

All experiments used a suspension CHO cell line from TPG Biologics, Inc., expressing an hMAb. We cultivated the cells in Dynamis™ AGT™ Medium (Thermo Fisher Scientific) for both runs. The medium was supplemented with 8 mM L-glutamine and 1 % Gibco® Anti-Clumping Agent (Thermo Fisher Scientific).

Inoculum preparation

We prepared the bioreactor inoculum by cultivating the cells in single-use baffled polycarbonate shake flasks (Corning®) in a New Brunswick™ S41i CO2 incubator shaker set at 125 rpm and 8 % CO_2 with passive humidification. Cells from a cryopreserved stock vial were inoculated at a density of 0.3 x 10^6 cells/mL in a 125 mL flask with a 20 % fill volume. After one week of passaging every other day, we scaled-up the culture volume by increasing the flask size from 125 mL to 250 mL, and finally 1 L, while keeping the inoculation density, percentage fill, and all other parameters constant. Using this method, each bioreactor was inoculated with cells that were at approximately the same passage and duration of culture post-thaw.

Cultures were fed with culture medium prepared as described above with one modification: the glutamine concentration in the perfusion feed media was changed from 8 mM to 2 mM to reduce ammonia production during the run.

Bioreactor control and process parameters

We used BioBLU 1c and 3c Single-Use Vessels for this experiment.

For all experiments, we measured DO using a polarographic sensor (Mettler Toledo®) and controlled it at 50 % by sparging air and/or O_2 at a flow of 0.02 SLPH – 30 SLPH for the 1c and 0.02 SLPH – 90 SLPH for the 3c using a user defined cascade. The pH was measured using a potentiometric sensor that was inserted in a spare PG 13.5 port aseptically in the BioSafety Cabinet after being sterilized separately in an autoclavable pouch. The pH was controlled at 7.0 (dead band = 0.1) via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base). All cultures were inoculated at a final density ranging between 0.25 - 0.27 x 10^6 cells/mL (target = 0.3 x 10^6 cells/mL). We cultivated the cells at 37 °C and held the temperature constant. Table 1 summarizes important process parameters. Inoculation target density, gassing control, DO control, and tip speed were the same for both experiments.

Table 1. Overview of process configurations and setpoints for all cell culture runs.

Parameters	1c Setpoints	3c Setpoints
Starting volume	500 mL	1.5 L
Ending volume	1 L	3 L
Medium Feed Rate	5 % of total volume per day	5 % of total volume per day
Glucose Bolus Feed Target	> 3 g/L	> 3 g/L
Agitation	230 rpm (0.6 tip speed)	174 rpm (0.6 tip speed)
Temperature	37 °C	37 °C
DO Sensor	polarographic sensor	polarographic sensor
DO Setpoint	50 %, (P= 0.1; I= 0.001)	50 %, (P= 0.2; I= 0.002)
pH Sensor	potentiometric sensor	potentiometric sensor
pH Setpoint	7.0 (deadband = 0.1), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)	
Target Inoculation Density	0.3 x 10^6 cells/ mL	0.3 x 10^6 cells/ mL
Gassing range	0.02 SLPH – 30 SLPH	0.02 SLPH – 90 SLPH
Gassing cascade	Set O_2 % at 30 % to 21 % and at 100 % to 100 %. Set flow at 0 % (demand) to 0.02 SLPH, and at 100 % (demand) to 30 SLPH.	Set O_2 % at 30 % to 21 % and at 100 % to 100 %. Set flow at 0 % (demand) to 0.02 SLPH, and at 100 % (demand) to 90 SLPH.

Cascade control of DO

To control DO in both fed-batch runs, a user defined cascade was used. The cascade screen can be found under "DO control" on the SciVario twin interface. An example of the DO cascade screen is shown in Figure 2. For our fed-batch using the BioBLU 1c bioreactor, we chose the following cascade settings:

"Set O_2 % at 30 % to 21 % and at 100 % to 100 %. Set flow at 0 % to 0.02 SLPH and at 100 % to 30 SLPH."

For our fed-batch run using the BioBLU 3c bioreactor, we chose the following cascade:

"Set O_2 % at 30 % to 21 % and at 100 % to 100 %. Set flow at 0 % to 0.02 SLPH and at 100 % to 90 SLPH."

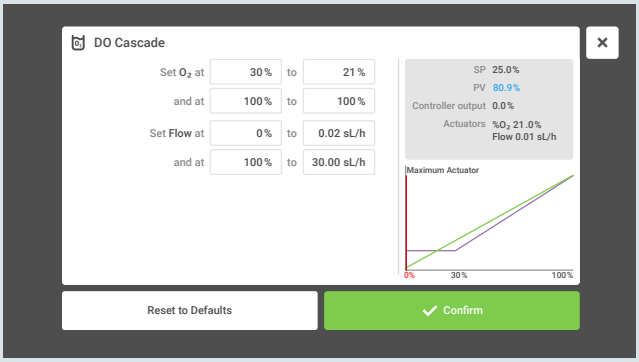

Fig. 2: VisioNize-onboard user interface - DO cascade screen

Sampling and analytics

We took two samples from each bioreactor daily, one in the morning and one in the evening, to check offline values such as cell density, viability, glucose, ammonia (NH3), lactate, and hMAb concentration. To collect the highest quality sample from the growing culture, we connected a sterile 5 mL syringe to the sample port Luer Lock and removed and discarded a dead volume of 3 mL. We then collected a second 3 mL sample in a new syringe to provide a fresh, viable sample for analytics.

We measured cell density and viability (via the trypan blue exclusion method) using a Vi-Cell® XR Viability Analyzer (Beckman-Coulter), and pH using an Orion Star™ 8211 pH-meter (Thermo Fisher Scientific). Using the offline pH value, we restandardized the controller pH calibration daily, if necessary, to prevent any discrepancy between online and offline measurements. Glucose, ammonia, glutamate, lactate, and hMAb were measured using a Cedex® Bio Analyzer (Roche). Using the obtained offline glucose concentration, the target glucose concentration in the culture was achieved by pumping the appropriate amount of 200 g/L sterile glucose solution into the culture as needed.

Feeding

We performed bolus glucose feeding as described above with a final target concentration > 3 g/L in both runs. If the glucose levels at the time of sampling was at or lower than 3 g/L, we bolus fed the bioreactors to ~ 4 g/L.

One major strategy to keep a CHO run healthy is to keep ammonia levels low, at around 3 mmol/L or less, by adjusting the feed rate if necessary, until ammonia falls below a desired level.

Results

Fed Batch run using BioBLU 1c:

Our bioreactor was prepared with media at a starting volume of 500 mL. We inoculated the vessel at 0.27 x10^6 cells/mL. The ammonia levels were monitored daily and feeding was started on day 3, when ammonium levels reached close to 3 mmol/L. Ammonia was targeted for under 3 mmol/L and maintained under 4 mmol/L for the whole run except the decline phase. The bioreactor was fed 5 % of the total volume per day until the feed bottle was empty. The BioBLU 1c reached a peak density of 13.8 x 10^6 cells/mL on day 13 (Figure 3A) and peak antibody production of 682 mg/L on day 15 (Figure 3B).

After collecting our sample and measuring the glucose, we manually fed our vessel glucose if the concentration fell below 3 g/L, as described previously. Lactate remained under 2 g/L for the entire run.

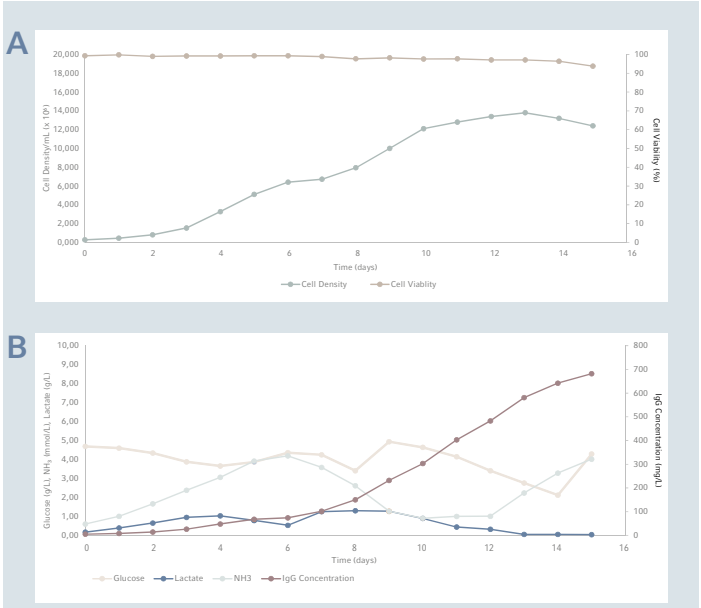

Fig. 3: BioBLU 1c -
Cell growth, antibody production and metabolic profile.
A: Total viable cells/mL throughout the 1c fed-batch run.
B: The antibody production and metabolic profile.

Fed-Batch run using BioBLU 3c:

The BioBLU 3c was prepared with a starting vessel volume of 1.5 L of media. We inoculated the bioreactor at 0.25 x10^6 cells/mL. The ammonia levels were targeted for 3 mmol/L and monitored daily. Feeding was started on day 3, when

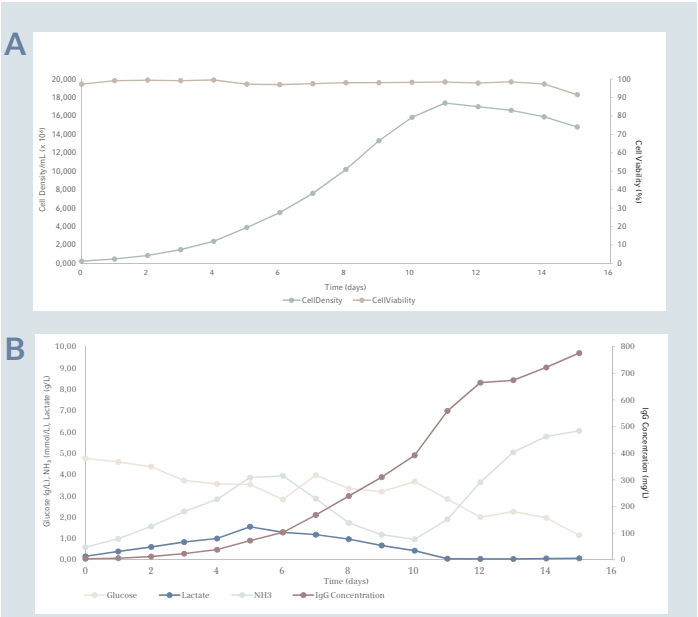

Fig. 4: BioBLU 3c -
Cell growth, antibody production and metabolic profile.
A: Total viable cells/mL throughout the 1c fed-batch run.
B: The antibody production and metabolic profile.

those levels reached close to 3 mmol/L. We fed our bioreactor 5 % of total volume per day (i.e. 150 mL) until the feed bottle was empty. The BioBLU 3c reached a peak density of 17.4 x 10^6 cells/mL on day 11 (Figure 4A), and peak antibody production of 776 mg/L on day 15 (Figure 4B). Ammonia was maintained under 4 mmol/L for the whole run except the decline phase. Lactate was maintained under 2 g/L. The metabolic profile and antibody production for the 3c fed-batch is shown in Figure 4B.

Discussion and Conclusions

With the SciVario twin, we were able to achieve high yields running fed-batch CHO cultures using 1 L and 3 L vessels in parallel, with our highest yields reaching almost 18 x 10^6 cells/ mL.

The SciVario twin has the capabilities to operate multiple processes using different sized vessels at the same time, making it an extremely versatile bioprocess controller. Its ability to operate both glass and single-use bioreactors makes it easy to switch between single-use and autoclavable equipment depending on process needs.

With the integrated VisioNize-onboard software, the controller enables the easy monitoring and control of both processes at the same time. And the easy and reproducible execution of error prone procedures like calibrations or setting up control logics is ensured by the provision of intelligent wizards to mitigate the risk of failure during the process.

At initial release, SciVario twin will support up to 4 L cultures. With future Agile release train updates, processes up to 40 L will be supported. It is the first bioprocess controller to consolidate the operation of Eppendorf small-scale and bench-scale vessels, with parallel operations delivering unparalleled value to the customers. The range of vessel sizes supported will continue to expand and evolve with customer's needs, ensuring that SciVario twin will continue to deliver value far into the future.

Ordering information

Description	Order no. international
SciVario® twin Fermenter/Bioreactor Control System, base unit, 100 – 240 V/50/60 Hz, for 2 vessels	7600100001
BioBLU® 1c Single-Use Vessel, cell culture, open pipe, 2 pitched-blade impellers, optical pH, sterile, 4 pieces	1386110500
BioBLU® 3c Single-Use Vessel, cell culture, microsparger, 1 pitched-blade impeller, optical pH, sterile, 1 piece	1386000100
Heat blanket, for SciVario® twin, for glass and single-use vessels, 2.4 – 3.8 L	7600230201

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