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SciVario® twin Self-Scale-up of CHO Culture-Based Antibody Production from BioBLU® 3c to BioBLU® 50c Single-Use Bioreactor

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Abstract

The complexity of developing new generations of therapeutics, enabling increasingly personalized treatments, is growing rapidly and faces major challenges from a manufacturing perspective. That is why an effective scale-up strategy is required to implement a reliable process and ensure reproducible yield at large working volumes without additional optimization. This study details the use of a combination of a single SciVario® twin bioreactor control system and BioBLU® 3c and BioBLU 50c Single-Use Bioreactors as a model platform for efficient bioprocess scale-up.

Specifically, we cultured Chinese hamster ovary (CHO) cells and produced antibodies in a BioBLU 3c Single-Use Bioreactor (3 L working volume) for 3 days and used its content as inoculum to scale up the CHO culture to a BioBLU 50c Single-Use Bioreactor with 30 L working volume, while both culture runs were controlled by the same SciVario twin bioreactor control system. We monitored and analyzed metabolites, cell density and viability twice daily, and achieved a robust cell growth (peak density around 15×10^6 cells/mL) in line with previous applications [1].

Introduction

Manufacturing the next generation antibody therapeutics requires flexible and rapid pilot scale adaptation which always presents a challenge for companies seeking to meet product demand. In this sense, a predictable scale-up process is a good strategy to increase the production volume on a commercial scale [2].

BioBLU Single-Use Bioreactors combine all the advantages of single-use technology with the trusted performance and scalability of a stirred-tank design, eliminating labor-intensive cleaning and enhancing product safety by reducing the contamination risk.

Furthermore, the combination of BioBLU Single-Use Bioreactors and the SciVario twin bioreactor control system creates an ideal toolset for bench to pilot process scale-up with one system, reducing time and investment needs for additional bioprocess equipment [3–5].

By controlling two bioreactors sequentially or in parallel, it is possible on the one hand to obtain a healthy and robust inoculum in a smaller scale bioreactor (using one of the two sides of the SciVario twin unit) and on the other hand to inoculate another bioreactor (located on the other side of the controller unit).

With the SciVario twin, such a self-scale-up process is possible in a range from 0.2 L to 40 L and has the advantage of precisely controlling and comparing the process parameters amongst varying scales.

It is possible to maintain parameters such as pH, DO, temperature at the desired setpoints during the inoculation process, avoiding any cell culture lag phase after inoculating a new bioreactor and achieving high cell density and viability.

In this study, our main goal was to demonstrate the feasibility of scaling up 3 L of CHO culture to 30 L, using the BioBLU 3c and 50c Single-Use Bioreactors without using multiple controllers or managing turnover with multiple runs. We analyzed the cell growth, viability, and metabolic activity (levels of glucose, lactate, and ammonia in the medium) in both bioreactors.

Material and Methods

SciVario twin and BioBLU Single-Use Bioreactors
In these experiments, the SciVario twin bioreactor control system was employed to scale up from 3 L to 30 L using BioBLU 3c and BioBLU 50c Single-Use Bioreactors equipped with a single pitched-blade impeller. Each bioreactor control system is equipped with three universal port connectors for pH and Dissolved Oxygen (DO) sensors, a heat blanket, agitation control and a gas module that includes a TMFC (Thermal Mass Flow Controller) with standard gas flow rates of 0.1 – 1,200 sL/h (resulting in an ultra-high turndown ratio of 1:12,000), as well as four solenoid valves (Figure 1).

Fig. 1: The SciVario twin bioreactor control system allows the control two glass or single-use bioreactors, either individually or in parallel, at the same time across a wide range of vessel sizes from small- to bench-scale. It was developed for both cell culture and microbial fermentation applications.

To learn more about the possibilities of the SciVario twin bioreactor controller, please visit www.eppendorf.group/sci-vario

Cell line and medium
For all experiments, a proprietary suspension CHO cell line (capable to produce a human monoclonal antibody (hmAb) from TPG Biologics, Inc., was culture in Dynamis AGT Medium (Thermo Fisher Scientific®). The medium was supplemented with 8 mM Gibco® GlutaMAX (Thermo Fisher Scientific), 1 % Antibiotic Antimycotic solution (Thermo Fisher Scientific), and 1 % Gibco Anti-Clumping Agent (Thermo Fisher Scientific) for a complete medium.

CHO cell scale-up procedure
After thawing, the cells were subjected to routine passaging and seed train or flask culture scale-up (Figure 2). Hereafter, the bioreactor process using the SciVario twin bioreactor control system in combination with the BioBLU Single-Use Bioreactors was initiated (Figure 2). For this study, a batch run was performed. Each step of the process is further described in more detail below.

Sensor calibration
Prior to the preparation of the BioBLU 3c and 50c Single-Use Bioreactors, the ISM® gel-filled pH sensors (MettlerToledo®) were connected to the SciVario twin bioreactor control system through one of the universal ports where they were automatically detected by the software. Calibration was performed according to the operations manual using known buffer solutions of pH 7 as “zero” and pH 4 as “span” respectively. Thereafter, the pH sensors were disconnected from the controller and sterilized in an autoclavable pouch.

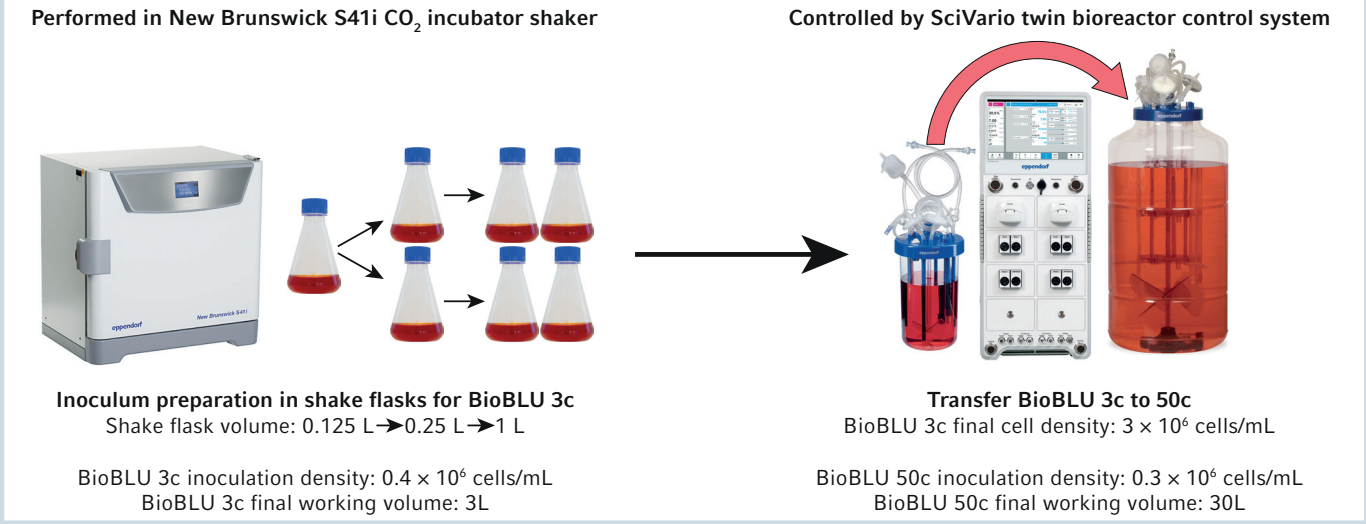

Fig. 2: Overview of CHO cells scale-up process.

BioBLU 3c and BioBLU 50c Single-Use Bioreactors preparation and process parameters

The BioBLU 3c and BioBLU 50c Single-Use Bioreactors were equipped with magnetic drives. The sterilized pH sensors were inserted into a spare PG 13.5 port under aseptic conditions in a biosafety cabinet. In addition, the polarographic DO sensors (Mettler Toledo®) were inserted into a non-invasive sensor sleeve inside the bioreactors. DASGIP® Peltier exhaust condensers were connected to each bioreactor and the sparge line of the bioprocess controller was connected to the sparge filter. Three liquid addition ports were used on each bioreactor: one for inoculation/medium addition, one for base addition and another for the addition of the 0.1 % of antifoam reagent (Sigma-Aldrich® Antifoam C Emulsion, Merck). Then, the heating blankets were fitted tightly around the BioBLU Single-Use Bioreactors to ensure uniform heat supply. Finally, each bioreactor was filled with Dynamis AGT complete medium and conditioned for at least 24 hours applying the process parameters and setpoints listed in Table 1.

BioBLU 3c Single-Use Bioreactor inoculum preparation

Initial cell expansion was carried out in single-use, baffled bottom shake flasks (Corning®) with 20 % maximum fill volume. For that, the cells were thawed from a cryopreserved stock vial and seeded into a 125 mL flask at a seeding density of 0.3 × 10⁶ cells/mL. The cells were passaged every other day. After monitoring cell growth and viability (determined to be >95 %), the culture volume was stepwise increased from 125 mL to 250 mL, and finally 1 L shake flasks. During this scale-up step, the cells were cultured in a New Brunswick S41i CO_2 incubator shaker at 37 °C, 8 % CO_2 and 125 rpm agitation speed. Some parameters like flask inoculation density, percentage fill among others remained constant. More than 1.5 × 10⁹ cells were obtained from the 1 L shake flasks. 1.2 × 10⁹ of these cells suspended in 200 mL Dynamis AGT complete medium were subsequently used to inoculate the BioBLU 3c Single-Use Bioreactor for a final working volume of 3 L (initial inoculation cell density: 0.3 × 10⁶ cells/mL).

Table 1: Process parameters and setpoints of the batch culture experiments.

	BioBLU 3c	BioBLU 50c
Working volume	3 L	30 L
Agitation	174 rpm (tip speed 0.6 m/s)	69 rpm (tip speed 0.6 m/s)
Temperature	37 °C	
Inoculation density	0.3-0.4 × 10⁶ cells/mL	
Cell culture medium	Dynamis AGT complete medium	
DO setpoint	50 % (P = 0.1; I = 3.6/h)	
pH setpoint	7.0 (deadband = 0.1), cascade to CO_2 (acid) cascade to 0.45 M sodium bicarbonate (base)	
Gassing range	0.1 SLPH – 60 SLPH	0.1 SLPH – 450 SLPH
Gassing cascade	Set O_2 % at 30 % controller output to 21 % and at 100 % controller output to 100 %. Set flow at 0 % controller output to 0.1 SLPH, and at 100 % controller output to 60 SLPH	Same as BioBLU 3c, except the maximum gassing limited to 450 SLPH

BioBLU 50c Single-Use Bioreactor inoculum preparation in the BioBLU 3c Single-Use Bioreactor

In order to obtain the inoculum for the larger 30 L run within the BioBLU 50c Single-Use Bioreactor, a 3 L CHO cell culture was grown in a BioBLU 3c Single-Use Bioreactor as described in "BioBLU 3c Single-Use Bioreactor inoculum preparation". Once the cell density in that culture set-up reached approximately 3 × 10^6 cells/mL (cell viability >95%), the harvest line of the BioBLU 3c Single-Use Bioreactor was welded onto the harvest line of the BioBLU 50c Single-Use Bioreactor to facilitate inoculation of the larger bioreactor (initial inoculation cell density: 0.3 × 10^6 cells/mL). Incubation parameters were maintained as described in Table 1 and 0.1% Sigma-Aldrich® Antifoam C Emulsion (Merck) was added as needed.

Sampling and analytics

Samples were collected twice a day from each bioreactor to determine cell viability, cell density, and the concentration of the metabolites glucose, lactate, and ammonia (NH_3). For that, a sterile 5 mL syringe was connected to the Luer Lock sample port. 3 mL of dead volume were discarded before collecting 3 mL of the culture for analysis in a new sterile 5 mL syringe. Cell density and viability were measured in a Vi-CELL® XR Viability Analyzer (Beckman Coulter®) *via* the trypan blue exclusion method. pH values were analyzed offline by using an Orion Star 8211 pH-meter (Thermo Fisher Scientific). The resulting pH value was used to re-standardize the pH calibration on the controller daily in order to prevent any discrepancy between online and offline measurements. Glucose, lactate, and ammonia levels were identified by using a CEDEX® Bio Analyzer (Roche Diagnostics®).

Results and Discussion

The purpose of this application was to demonstrate the feasibility of the SciVario twin bioreactor control system and BioBLU Single-Use Bioreactors for CHO cell self-scale-up. The versatility of the SciVario twin allows for the operation of two processes using bioreactors of different sizes from small scale (1 L) to bench/pilot scale (up to 40 L).

Inoculum preparation for the 30 L run was performed for three days in a BioBLU 3c Single-Use Bioreactor with a working volume of 3 L. During this time cell viability remained >95% and a steady increase in cell density was observed, reaching 3 × 10^6 cells/mL on the third day of incubation (Figure 3). Subsequently, the whole culture

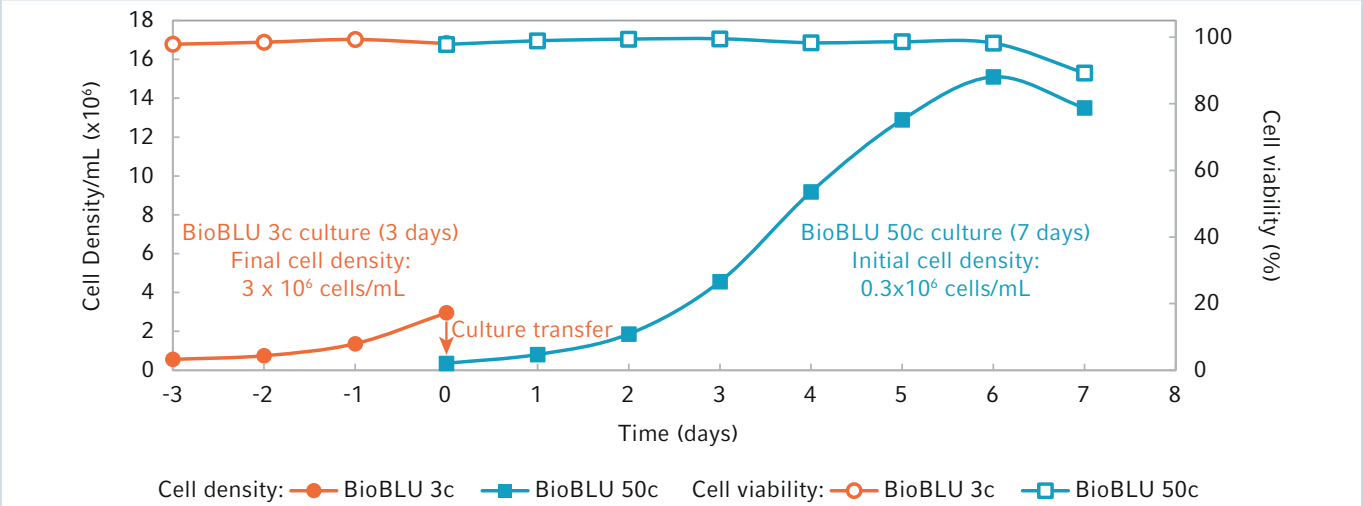

Fig. 3: CHO cell density and viability during the BioBLU 3c and 50c Single-Use Bioreactor batch runs.

volume was used to inoculate a BioBLU 50 c Single-Use Bioreactor to a final working volume of 30 L with a starting cell density of 0.3 × 10^6 cells/mL. With the inoculum being within the logarithmic growing phase, cell density within the BioBLU 50c Single-Use Bioreactor increased steadily, peaking at 15 × 10^6 cells/mL on day 6 of incubation while maintaining viability levels of >95% (Figure 3). This peak was followed by a decrease in cell density and viability on day 7.

Throughout our experiments, metabolite concentration was monitored twice a day. Lactate levels remained under 2 g/L during the run. However, the actively growing culture depleted the initially supplied glucose by day 6 of incubation while ammonia concentration rose to toxic levels of up to 11 mmol/L on day 7 (Figure 4B). Thus, the halt of growth between day 6 and day 7 of incubation can probably be attributed to nutrient deprivation and toxic by-product accumulation, both typical phenomena in elongated batch cultivation. Similar tendencies towards decreasing glucose and increasing ammonia levels were also visible during the inoculum preparation within the BioBLU 3c Single-Use Bioreactor (Figure 4A). However, as this bioprocess run was terminated earlier to use the culture for the BioBLU 50c Single-Use Bioreactor inoculation, the effect on cell density did not manifest in this setting.

Despite the natural limitations of batch cultivation, a steady increase of IgG antibody expression was shown for both bioreactor cultures over the course of the experiment (Figure 4A/B). We would like to highlight that the metabolic concentration levels in the BioBLU 3c Single-Use Bioreactor inoculum were optimal to ensure a perfect inoculation and a successful run (Figure 4A).

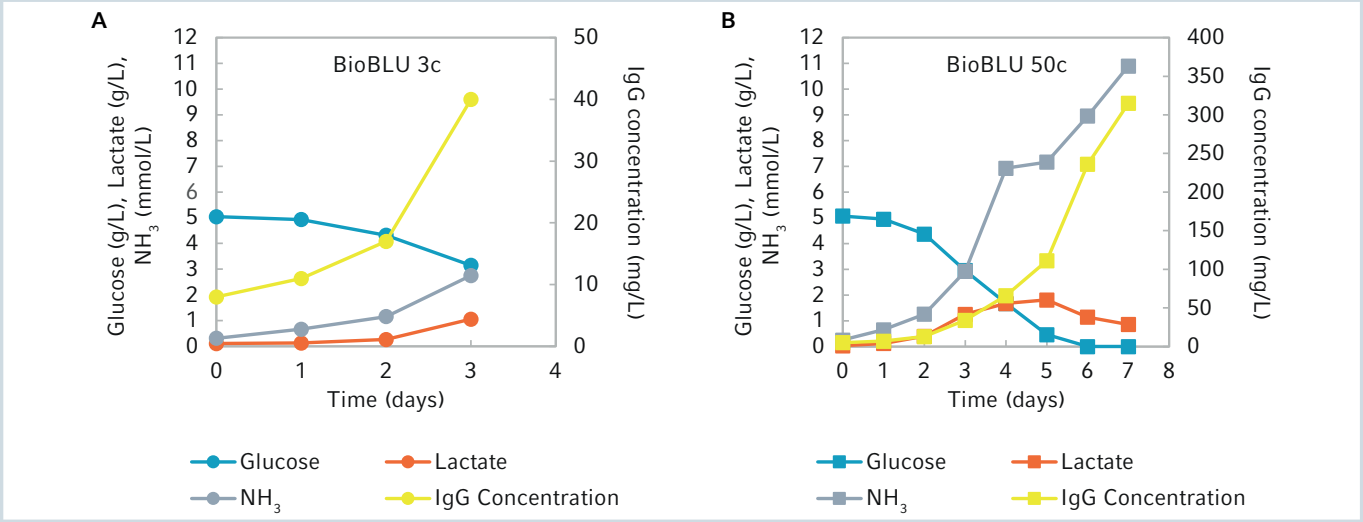

Fig. 4: Metabolic profile and antibody production. A: BioBLU 3c Single-Use Bioreactor. B: BioBLU 50c Single-Use Bioreactor.

Conclusion

In this study, we demonstrated a scale-up process using a single SciVario twin bioreactor control system connected to two different BioBLU Single-Use Bioreactors. The SciVario software allows users to run small to bench/pilot scale bioreactors with volumes ranging from 0.2 to up to 40 L. The set-up used here with two bioreactors simultaneously connected to one SciVario twin bioreactor control system enabled efficient preparation of a 3 L inoculum in one vessel (BioBLU 3c Single-Use Bioreactor) and transfer of that inoculum under sterile conditions to another larger vessel (BioBLU 50c Single-Use Bioreactor) with a working volume of 30 L.

The direct inoculum-transfer from one bioreactor to the next by connecting their harvest ports strongly decreases

the contamination risk and the use of one control system for two reactors reduces equipment requirements. The efficient and simple configuration of SciVario twin bioprocess control system allows precise control of the inoculum environment during the inoculation process leading to a rapid CHO cell growth for 6 days. These experiments were conducted primarily for feasibility demonstration. Still, we consider this approach as a helpful starting point for future scale-up applications using the SciVario twin bioreactor control system.

Literature

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Ordering information

Description	Order no.
SciVario® twin fermenter/bioreactor control system, base unit for 2 bioreactors	7600 100 001
BioBLU® 3c Single-Use Bioreactor, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile	1386 000 300
BioBLU® 50c Single-Use Bioreactor, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile	M1363-0129
Heat blanket, for SciVario® twin, for glass and single-use vessels, 2.4-3.8 L	7600 230 201
Heat blanket, for SciVario® twin, for single-use vessels, 50 L	7600 230 203
DASGIP® Peltier Exhaust Condenser Adaptor, for BioBLU® 3c/5c/5p Single-Use Bioreactors	7820 132 6
New Brunswick S41i, 170 L, CO_2 incubator shaker with inner shelf and touch screen control, stackable	S41I 120 010

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