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Growing CHO Cells in a New Brunswick™ CelliGen® BLU Benchtop, Stirred-Tank Bioreactor Using Single-Use Vessels

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Abstract

The study presents a typical protocol for the setup and operation of the Eppendorf New Brunswick CelliGen BLU single-use, stirred-tank bioreactor, a versatile new benchtop system for the culture of a wide range of mammalian cells. This bioreactor has been designed to provide research and production facilities with a single-use vessel which combines the benefits of both traditional stirred-tank design and single-use technology, capable of seamless process scale-up. The system can be operated in batch, fed-batch or continuous modes. A procedure for culturing Chinese Hamster Ovarian (CHO) cells in a 5.0 L vessel, using CD CHO serum-free medium in a batch culture is described.

Introduction

Historically, stirred-tank fermentors and bioreactors have been the trusted design for culturing all types of submerged cultures including suspension and anchorage-dependent mammalian cells, insect, yeast, plant and microbial cultures. The tried and tested tank design offers scalability and proven reproducibility which is pivotal for cost-saving process development and productivity. In the last decade, there has been an increasing acceptance and use of single-use technologies, due to their convenient operation and low start-up cost. Single-use systems eliminate the need for cleaning and sterilization, reduce validation requirements, provide rapid turn-around between runs, and significantly reduce the risk of cross contamination and microbial contamination because the culture vessel is only used once and then discarded. Although single-use, stirred-tank systems in the 75 – 2000 L scale have been on the market for some time, as have small-scale single-use bags that are gently rocked rather than stirred, until now there has been no single-use stirred-tank system for small-scale work. The new Eppendorf New Brunswick CelliGen BLU fills that void, offering a proven stirred-tank design as well as the benefits of single-use technology in a benchtop system.

Materials and Methods

Single-Use Vessels
BioBLU® single-use vessels are offered in 5.0, 14.0 and 50.0 L total volume capacities. The vessels are delivered preassembled with pitched-blade impeller, porous microsparge, and all the necessary tubing, filters, and connectors; and come sterilized, ready for use right out of the package. All components in product contact are made of materials that meet USP Class VI standards and have been tested for leachables and extractables, making these vessels appropriate for cGMP environments. In this protocol, we describe use of a CelliGen BLU with 5.0 L vessel.

Rapid set up, easy operation, and elimination of autoclaving and cleaning between runs. These are a few of the many advantages of the new BioBLU 5.0, 14.0 and 50.0 L stirred-tank bioreactors for growth of mammalian cultures.

Controller

CelliGen BLU's compact control station is designed to provide advanced process management and monitoring capability, ranging from three fixed-speed pumps for additions and harvesting, to a powerful controller with 15 in. industrial color touchscreen monitor. Multiple options, including gas flow control, a weight scale, validation packages and more, enable customization to your needs.

The control station used in this protocol was configured with one 2 – 100 cubic centimeters per minute (ccm) Thermal Mass Flow Controller (TMFC) for direct sparging of gases and an integrated gas overlay with 0.1 – 3.0 Standard Liters Per Minute (SLPM) flow rate also regulated by a TMFC. Both the gas flow and gas overlay are capable of 4-gas mixing for automatic pH and Dissolved Oxygen (DO) control. Pumps, temperature control, agitation, as well as all of the other process loops, were controlled and monitored through the powerful Reactor Process Controller (RPC) firmware installed in the controller. DO was monitored using a noninvasive reusable polarographic DO probe; and pH was monitored using a non-invasive optical pH probe and fluorescence sensor.

Inoculum Preparation

One 2.5 mL vial of CHO cells was thawed and used to inoculate a 125 mL shake flask which contained 25 mL of serum-free CD CHO medium (Life Technologies® 10743-029) which was pre-warmed to 37 °C.

On day 4, when the viable cell density reached 1.5 x 10^6 cells/mL, the cells were transferred into a 500 mL shake flask which contained 100 mL of freshly made, pre-warmed medium and allowed to incubate for 3 additional days at the same conditions as earlier. The cells were then transferred to two 1 L shake flasks, each containing 250 mL of the freshly made medium. The inoculum was grown in the shake flasks until cell density reached 2.0 – 3.0 x 10^5 cells/mL, with greater than 90 % cell viability, sufficient for the bioreactor inoculation.

Bioreactor Set-Up and Inoculation

One day before the cells reached inoculation density, the growth medium was warmed to 37 °C and the DO probe was polarized. For this study, 3.0 L of sterile CD CHO serum-free medium was prepared by pre-warming at 37 °C for 24 hours in a CO_2 incubator. During this time, the DO probe was connected to the controller for at least 6 hours to enable polarization, as per the manufacturer's recommendation.

Once the medium was warmed and the inoculum grown to sufficient starting density, the CelliGen BLU bioreactor vessel was removed from its sterile packaging and the heat blanket supplied with the unit was wrapped around the outside of the vessel. Next, the vessel containing the cell culture medium was connected to one of the bioreactor vessel's inlet lines using a tube welder. (A tube welder is offered as an optional accessory to the CelliGen BLU. A pre-sterilized medium filter with an attached quick connect or Luer connection can also be used if a tube welder is not available). Since this was a batch process, all of the medium was pumped into the bioreactor vessel. All additional connections to the controller including sparge, overlay, RTD, pH, and agitation were also made.

pH and DO were calibrated through the touchscreen controller, and all process setpoints were entered on the touchscreen using the Control Setpoint values shown on the next page. Once the parameters were at their setpoints, the inoculum flasks were connected to the addition line in a sterile manner using a tube welder and contents were pumped into the bioreactor vessel.

Operational Parameters

Cultivation of animal cells in an environment optimal for manufacture of desired end products require monitoring and control of a substantial number of physical and chemical parameters. Physical parameters include temperature, fluid flow (gas flow and liquid flow) rates and agitation rates. Chemical parameters include the dissolved oxygen (DO) concentration and pH.

Control Setpoints

Temperature	37 °C
pH	7.0
DO	40 %
Agitation	80 rpm

pH Control Parameters

pH control was set to Auto mode, which automatically adds base solution or CO_2 gas to the system based on culture demands.

Dead-band	0.10
PID values	Factory set default values
Base	Sodium bicarbonate, 7.5 % solution
Base Solution Transfer tubing	Narrow bore silicone tubing with Luer-connection (1/18 in. ID & 1/4 in. OD)
Vessel inlet	1/8 in. inlet tubing in the vessel headplate

Dissolved Oxygen (DO) Control
DO control was set to Auto mode, which automatically regulates gas mixing based on culture demand. PID values: factory set default values.

Gas Control
The gas control was set to 4-gas mode, which automatically maintains DO and pH. The gas flow rate was based on the vessel size.

Up until day 3, gases were introduced into the vessel headspace only through the overlay port at a rate of 0.30 L/min using 4-gas mixing to maintain pH and DO. On day 3, and for the remainder of the run, 5 – 10 ccm of gas were directly sparged into the system using a porous sparger and automatic 4-gas mixing. The overlay gas flow in the vessel headspace was kept at the previous settings.

A built-in sampling device enabled sterile sampling. Daily offline measurements of glucose and lactate concentration were read using a YSI® 2700, and cell density and cell viability was measured using an Automated Cell Counting System (New Brunswick NucleoCounter®).

All data was logged via BioCommand® Batch Control PC-compatible Supervisory Control and Data Acquisition (SCADA) software (New Brunswick).

Results and Discussion

As shown in Figure 1, the CHO cells in this study grew steadily, reaching a maximum viable cell density of 5.55 x 10^6 cells/mL on day 5.

Cell viability, shown in Figure 2, ranged between 97.1 and 97.9 % through Day 5, until the nutrient source, glucose, was depleted from the medium, as shown in Figure 3.

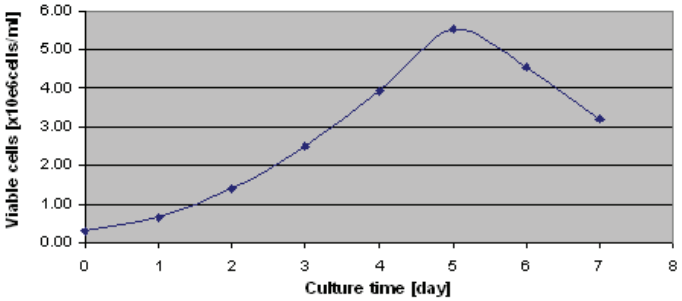

Figure 1. Cell growth over the 7-day run.

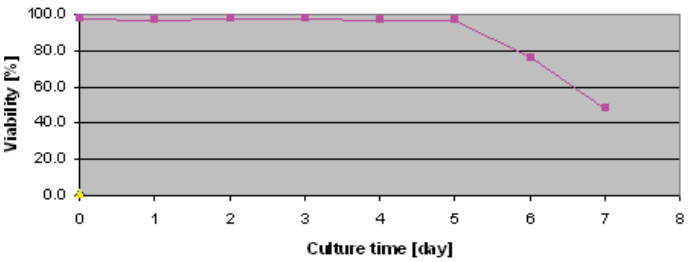

Figure 2. Cell viability remained high through day 5.

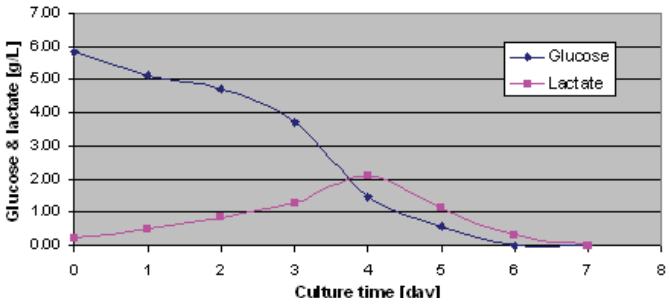

Figure 3. Glucose consumption and lactate production.

As expected, lactate production steadily increased as the available glucose in the medium was consumed. As glucose in the medium became exhausted, consumption of lactate as a secondary carbon source also declined[1].

This data presented here, and in Table 1, demonstrates that the CelliGen BLU bioreactor is an easy-to-use, efficient system for the culture of CHO cells. No effort was made to optimize either the medium or the cell culture process control parameters. This study was only intended to document a general guide to bioreactor setup and operation, and present typical results you could expect to achieve with your mammalian cell line. For protocols on other cell lines, or for additional information on the CelliGen BLU, see eppendorf.com.

Day	Total [10^6 cells/mL]	Viable [10^6 cells/mL]	Viability [%]	Glucose [g/L]	Lactate [g/L]
0	0.31	0.30	97.9	5.83	0.23
1	0.69	0.68	97.1	5.14	0.52
2	1.42	1.39	97.6	4.711	0.87
3	2.57	2.51	97.6	3.74	1.27
4	4.02	3.92	97.5	1.47	2.10
5	5.70	5.55	97.3	0.59	1.12
6	5.98	4.52	76.6	0.00	0.32
7	6.71	3.21	47.8	0.00	0.01

Table 1.

References

[1] **A single nutrient feed supports both chemically defined NS0 and CHO fed-batch processes: Improved productivity and lactate metabolism.** Ma N, Ellet J, Okediadi C, Hermes P, McCormick E, Casnocha S. *Biotechnol Prog.* 2009; 25 (5): 1353-63.

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