Perfusion CHO Cell Culture in a BioBLU® 5p Single-use Packed-bed Vessel

Nick Kohlstrom and Ma Sha, Eppendorf, Inc., Enfield, USA

Abstract

The market for secreted recombinant protein therapeutics, including blockbuster drugs in the form of humanized monoclonal antibodies (hmAbs), has become a multi-billion dollar industry with the expectation of continued growth. One of the most cost-effective methods for the production of secreted proteins is the packed-bed vessel operated under perfusion conditions. The maximum cell density achieved in a packed-bed vessel is typically much higher than suspension cell culture or microcarrier-based adherent cell culture. The protein harvest can be carried out continuously, providing unparalleled product yield. This poster provides an example of using a BioBLU 5p packed-bed single-use vessel to conduct Chinese hamster ovary cell (CHO) perfusion culture producing a secreted hmAb.

The BioBLU 5p vessel (pre-loaded with Fibra-Cel® disks) was controlled by a New Brunswick™ CelliGen® BLU benchtop bioreactor, both from the Eppendorf bioprocess portfolio. The BioBLU 5p vessel was inoculated at an initial cell density of 0.3 x 10^6 cells/mL. Fourteen days of perfusion cell culture were conducted with a working volume of 3.75 L. Glucose, lactate, and hmAb concentrations were monitored daily. The glucose consumption rate was used to estimate the cell density in the packed-bed vessel. After 12 days, the culture reached a peak cell density of approximately 10 x 10^6 cells/mL.

Introduction

Figure 1: New Brunswick CelliGen BLU benchtop bioreactor with a BioBLU 5p Packed-bed Vessel (left) and BioBLU 5c Single-use Vessel (right)

The New Brunswick CelliGen BLU benchtop bioreactor is a versatile, easy-to-use system with built-in controls and monitoring for agitation, temperature, pH, dissolved oxygen (DO), gassing (with air, oxygen, nitrogen and carbon dioxide) and automatic pump control. In addition, the control station can be connected to many other auxiliary devices. The New Brunswick CelliGen BLU benchtop bioreactor is used in conjunction with BioBLU single-use vessels (Eppendorf), as seen in Figure 1, allowing for easy scalability while operating in single-use format. Although the single-use bioreactor market has experienced rapid growth in recent years, packed-bed perfusion bioreactor technology has remained predominantly in the traditional glass and stainless steel formats. The single-use packed-bed vessel contains Fibra-Cel which is a solid support growth matrix that is predominantly used for the production of secreted products from cell culture. Since the cells are attached to the Fibra-Cel, it allows for continuous harvest of secreted products without losing cells over an extended period of time. This makes BioBLU 5p single-use vessels an ideal platform for research and production of secreted proteins or virus from mammalian and insect cell culture.

CHO is a robust cell line that can be cultured to very high cell densities in a packed-bed vessel. Using CHO cells to produce recombinant proteins allows proper protein folding and correct post-translational modifications so that the proteins remain biologically active once injected into humans. The cell line has a proven track record in the biopharmaceutical industry and production in CHO accounts for about 70 % of all recombinant protein therapeutics [1]. The global market for therapeutic monoclonal antibodies (mAbs) is expected to reach US $58 billion in 2016 with a variety of new mAbs in the pipeline [2]. In this experiment, an attachment CHO cell line expressing a hmAb was grown using a New Brunswick CelliGen BLU benchtop bioreactor with a BioBLU 5p single-use packed-bed vessel.

Materials and Methods

The B13-24 CHO cell line (ATCC®, CRL-11397™) was adapted to CD CHO media (Life Technologies®, 10743) supplemented with 8 mM L-glutamine (Life Technologies, 25030), 0.125 % heat-inactivated fetal bovine serum (Life Technologies, 10438-034) and 1X penicillin/streptomycin (Life Technologies, 15140-122). The initial culture was conducted on BioCoat™ collagen-coated T-flasks (Corning®, 354485). Cells were inoculated into the BioBLU 5p single-use packed-bed vessel at 0.3 x 10^6 cells/mL to a total working volume of 3.75 L with the previously described media.

The hardware setup and control loop setpoints used in this study are shown in Table 1. Fresh media was perfused into the vessel as needed to keep the glucose concentration between 1 and 2 g/L. Additional D-(+)-glucose (Sigma-Aldrich®, G5146) was added to the perfusion media as needed to keep the glucose concentration at the desired level without increasing the perfusion rate to a unmanageable level.

Parameter	Setpoint
Agitation	100 rpm
Temperature	37 °C
Dissolved Oxygen (DO)	50 %
pH	7.1 ± 0.05
Volume (Harvest cascaded to pump)	3.75 L
Gas mix	3-gas automatic gas mixing option
Gas flow control	3 Thermal mass flow controllers (TMFCs) with 0 - 1 SLPM flow range

Table 1: Parameters and setpoints used for CHO cell growth in the CelliGen BLU bioreactor

The culture's pH was controlled using automatic CO_2 sparging for acid addition and an automatic pump cascade of 1 M sodium bicarbonate (Fisher Scientific®, S631-3) for base addition. Since the cells were attached to the Fibra-Cel packed-bed, bubbles do not interact with the cells which prevents bubble shear. The layer of medium above the packed-bed allows the dilution and mixing of acid or base before allowing them to come in contact with the cells; therefore higher concentrations of acid or base can be used for pH adjustments without adverse effects. Glucose, lactate and hmAb concentrations were monitored using a Cedex® Bio Analyzer (Roche®).

The approximate amount of glucose consumption per liter per day was calculated by first calculating the average glucose consumption between samples per hour:

$$R = \frac{V(S1 - S2) + \Delta V(PG - \frac{S1 + S2}{2})}{\Delta T}$$

> R = Approximate rate of glucose consumption per hour (g/h)
> S1 = Glucose concentration in media sample 1 (g/L)
> S2 = Glucose concentration in media sample 2 (g/L)
> V = Vessel working volume (L)
> PG = Glucose concentration of fresh perfusate (g/L)
> ΔV = Perfusion volume between samples (L)
> ΔT = Change in time between samples (h)

R was used to calculate the grams of glucose consumption per day by adding the glucose consumed per hour over the 24 hour period. This was then divided by the working volume (3.75 L) to obtain the normalized glucose consumption (g/L/day).

The approximate cell concentration was determined by correlating R with cell growth by obtaining a glucose consumption per cell conversion factor. To obtain the conversion factor, CHO cells were cultured in a T-75 flask until 100 % confluence. A precise amount of fresh medium (8 mL) was then added to the flask, the glucose concentration was measured and the cells were incubated for 7.25 h. After the incubation period, the glucose concentration was measured again, the cells were trypsinized from the T-flask and counted on a Vi-Cell® XR automated cell counter (Beckman Coulter®). This information was then used to calculate the amount of glucose each cell consumed per hour (~3.92 x 10^{-2} ng/cell/h) which was then used to calculate the number of cells in the vessel based on the glucose consumption rate. Please note that the conversion factor may be cell line dependent and may not be applicable to other CHO cells.

Results

Continuous perfusion was used during this experiment to keep glucose levels within a narrow range (Figure 2). The alternative method of fed-batch style non-continuous perfusion can cause large fluctuations in glucose and lactate concentrations which may have an effect on cellular metabolism. Glucose and lactate concentrations were measured multiple times per day during the run. The data were used to adjust the perfusion rate as well as glucose addition rate to keep the glucose level between 1 and 2 g/L where possible.

The cells were attached to the Fibra-Cel and could not be counted directly. Assuming that glucose consumption is proportional to cell growth, glucose consumption was used to calculate the approximate cell number. At the start of the run, glucose consumption steadily increased until day 6 where it began to plateau (Figure 3). Using the conversion factor described previously, approximate cell concentrations were determined throughout the run (Figure 4).

Samples were taken throughout the bioreactor run and the IgG concentrations were determined by Cedex Bio Analyzer (Figure 5). These concentrations were measured from samples taken from the vessel and do not include IgG harvested during perfusion.

The cell line used was the only healthy CHO cell line available from ATCC expressing an hmAb. Although this cell line is useful as a model system, the antibody yield is very low. Given a different cell line, much higher cell numbers and antibody production yields are possible.

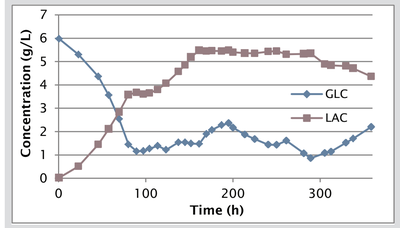

Figure 2: Glucose (GLC) and lactate (LAC) concentrations throughout the culture

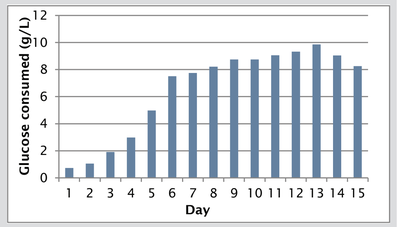

Figure 3: The approximate amount of glucose consumption (g/L/day)

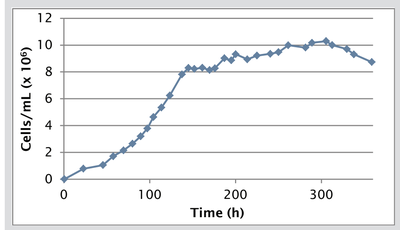

Figure 4: The approximate cell concentration

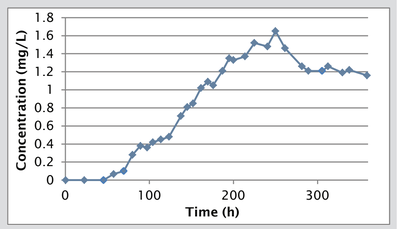

Figure 5: The IgG concentration in the bioreactor over the culture period

Conclusion

The B13-24 CHO cell line (ATCC®, CRL-11397™) was adapted to CD CHO media (Life Technologies®, 10743) supplemented with 8 mM L-glutamine (Life Technologies, 25030), 0.125 % heat-inactivated fetal bovine serum (Life Technologies, 10438-034) and 1X penicillin/streptomycin (Life Technologies, 15140-122). The initial culture was conducted on BioCoat™ collagen-coated T-flasks (Corning®, 354485). Cells were inoculated into the BioBLU 5p single-use packed-bed vessel at 0.3 x 10^6 cells/mL to a total working volume of 3.75 L with the previously described media.

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[1] Jayapal K, Wlaschin K, Hu W-S, Yap M. Recombinant protein therapeutics from CHO cells - 20 years and counting. *CHO Consortium* 2007; SBE Special Section:40-7.
[2] BCC Research. Antibody drugs: Technologies and Global Markets. 2012; Report code BIO016H.