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Trends & Developments in BioProcess Technology

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APPLICATION NOTE

CHO Cell Culture with Eppendorf CelliGen® BLU Single-Use Packed-Bed Fibra-Cel® Basket

Introduction

The packed-bed basket technology, developed by Eppendorf, provides a shear-free environment for the production of animal cells. At present, little information is available on the utility of the New Brunswick CelliGen BLU single-use bioreactor system for the production of secreted proteins, especially in perfusion mode of operation. Thus, this study was conducted to measure the growth and productivity of alkaline phosphatase (ALKP)-secreting recombinant CHO cells.

Two Eppendorf packed-bed bioreactor types were used: (1) 5 L CelliGen BLU single-use vessel; and (2) 2.5 L autoclavable glass vessel—both operated by an Eppendorf CelliGen 310 console in perfusion mode. The perfusion process provides a homeostatic environment for optimal cell growth similar to that experienced by cells *in vivo* where waste products are constantly removed and fresh nutrients are replenished.

Cells cultured in packed-bed bioreactors are not exposed to hydrodynamic forces, thus allowing for maximum cell growth and protein expression.[1] The objective of this study was to compare the two types of bioreactors to determine if any differences would be observed between the productivity of the two bioreactors.

Eppendorf CelliGen 310 bioreactor system, packed-bed Fibra-Cel basket (top inset photo), and CelliGen BLU single-use bioreactor Fibra-Cel basket (bottom inset photo).

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Materials and Methods

Culture Procedures

In order to evaluate the impact of these bioreactor systems on protein production, we utilized an alkaline phosphatase-secreting recombinant CHO cell line (rCHO), a proprietary cell line provided by CDI Bioscience, Inc. The rCHO cells were engineered with the isopropyl β-D-1-thiogalactopyranoside (IPTG)-regulated RP shift vector so that the rCHO cells stop replicating and shift to protein production when induced with IPTG. Serum-free CD-CHO medium (Gibco, Life Technologies) was used throughout these experiments. The media contained 6.3 g/L glucose and was supplemented with 8 mM L-glutamine and 100 µg/mL of an antibiotic/antimycotic solution (Invitrogen, Life Technologies). Frozen rCHO cells were thawed and transferred to T-75 flasks with CD-CHO medium and allowed to expand.

Once a sufficient number of cells were achieved, sterile disposable spinner flasks were utilized to further expand the cells. Subculture of the cells continued until a sufficient number of viable cells was achieved for use as a seed culture at the density of 5×10^5 cells/mL. Two CelliGen 310 advanced bench-top stirred-tank bioreactors were utilized to grow the rCHO cells. One of the consoles was connected to an adaptor kit (available from Eppendorf) for use of the CelliGen BLU single-use vessel.

Packed-Bed Basket Impeller Operated in Perfusion Mode

Two experimental trials were performed using the packed-bed vessels in perfusion mode: 2.5 L total volume autoclavable vessel (1.7 L working volume) and a 5 L total volume single-use vessel (3.5 L working volume, pre-loaded with 150 g of Fibra-Cel disks). The perfusion process was initiated once the cells reached the exponential growth phase as shown in Table 1. Both experimental trials had the following parameters shown in Table 2.

Biomarkers of Cell Growth and Productivity

Cell productivity was assessed by measuring activity of the secreted ALKP protein using an enzyme assay (AnaSpec, Inc.) according to the manufacturer's protocol. For simplicity, unit measurements were used in this study. One unit (U) of ALKP activity was defined as the amount of enzyme that hydrolyzes 1 µmol of p-nitrophenylphosphate to p-nitrophenol in a total reaction volume of 1 mL in 1 minute at 37 °C. The YSI 2700 Select Biochemistry Analyzer (YSI, Inc.) was utilized to monitor the glucose and lactate levels in the culture media every 24 hours for the duration of each trial.

TABLE 1. **Comparison of perfusion volumes.**

Perfusion	Volume	
	Glass	BLU
Day 1	0.5 L	1.0 L
Day 2	1.0 L	2.0 L
Days 3–15*	2.0 L	4.0 L
**NOTE: Perfusion occurred every other day.*		

TABLE 2. **Bioreactor parameters.**

Parameter	Setpoint	
	Glass	BLU
Temperature	37°C (±0.1°C)	37°C (±0.1°C)
Agitation	120 rpm (±5 rpm)	120 rpm (±5 rpm)
Dissolved O_2	35% (±1%)	35% (±1%)
pH	7.1 (±0.01)	7.1 (±0.01)
Gas flow	0.5 slpm	1.5 slpm

Results and Discussion

Glucose Utilization and Lactate Production

Glucose is the main energy source for cell proliferation and ALKP production. Thus, glucose levels were expected to directly correlate with ALKP production in each experiment. Because lactate is a secondary energy source, lactate levels were expected to decline following this initial increase and the utilization of glucose in the media. Lactate metabolism is beneficial to the system by reducing a major metabolic by-product from the system.[2, 3] Glucose levels measured at the time of induction (day 3) were

nearly 0 g/L in both experiments (Figure 1). Media lactate concentrations increased in response to decreasing glucose availability. The use of lactate as a secondary energy source can also be observed as levels decrease at each 2 L perfusion.

The average total ALKP production per experimental trial is shown in Figure 2. Overall, there is not a significant difference in ALKP production between the two bioreactor systems. The total amount of ALKP measured after six media exchanges in the reusable vessel was 17.44 U/mL and 16.22 U/mL in the single-use vessel.

In summary, the results of this study show that there are no significant differences in ALKP production between the two packed-bed bioreactor systems when operated in perfusion mode. Given the greater productivity of cells cultured in the packed-bed bioreactor and the multitude of advantages of this system operated in perfusion mode, researchers desiring to scale up mammalian cell culture for protein production should strongly consider utilization of the New Brunswick CelliGen BLU packed-bed, single-use bioreactor system.

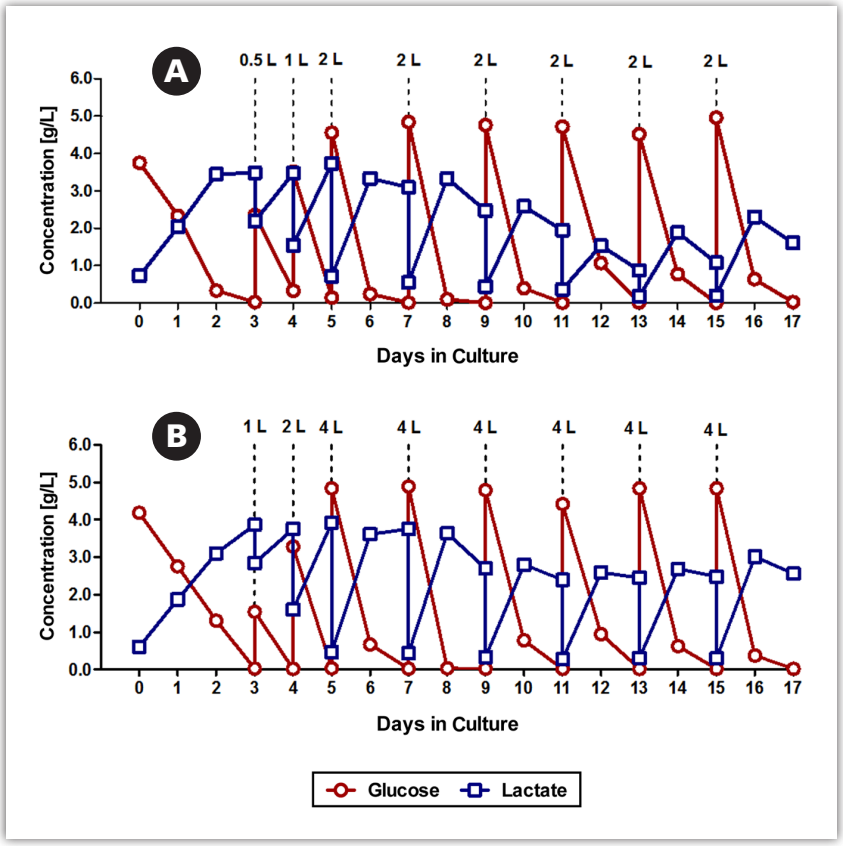

FIGURE 1. **Glucose consumption and lactate production by rCHO cells cultured in the packed-bed bioreactor systems.** Values shown are the amounts of glucose and lactate measured in the culture media at each media exchange. The time and volume of the media exchange is indicated at each dashed line. Induction of ALKP activity by IPTG began on culture day 5 and continued every two days throughout the remainder of the experiment. Results of two experimental trials are shown [(A): reusable; (B): single-use].

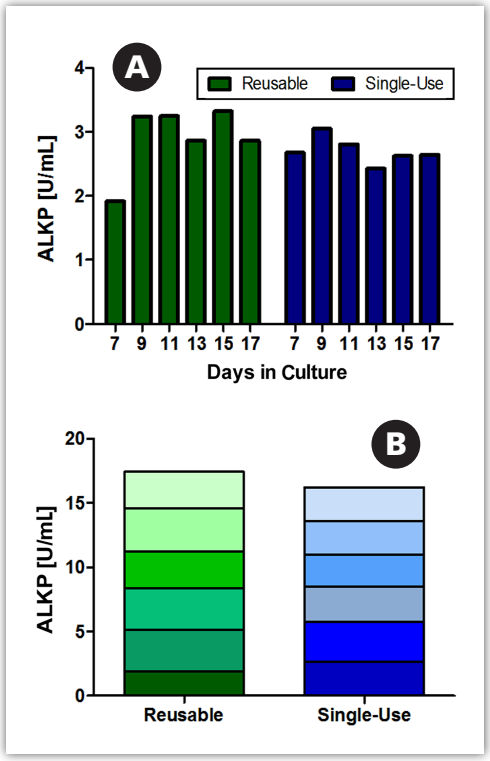

FIGURE 2. **ALKP production by rCHO cells cultured in two packed-bed bioreactor systems.** (A): ALKP concentration in culture media measured each day of each experimental trial. IPTG induction of ALKP began on culture day 5 and continued every two days for the remainder of the experiment; (B): stacked bar charts show the cumulative production of ALKP throughout the experiment, with each bar representing a perfusion.

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NOTE

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