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

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Abstract

The objective of this study was to compare Eppendorf BioBLU single-use packed-bed bioreactor vessel and the traditional glass vessel counterpart used in New Brunswick™ CelliGen® 310. Alkaline phosphatase (ALKP)-secreting Chinese Hamster ovary (CHO) cells were used to measure ALKP production in each bioreactor. Overall, the results from these comparisons suggest that there is no significant difference between the reusable and single-use FibraCel basket systems for bench-scale production of recombinant proteins. Productivity of cells and collection of secreted proteins will not be hindered by the implementation of single-use bioreactor systems.

Introduction

The packed-bed basket technology, developed by Eppendorf, provides a shear free environment for production of animal cells. At present, little information is available on the utility of the Eppendorf BioBLU 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 rCHO. Two packed-bed bioreactor types were used: an Eppendorf BioBLU 5p single-use vessel (3.75 L working volume) and a 3 L autoclavable glass vessel (1.25 - 3.75 L working volume), both operated by New Brunswick 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 are observed between the productivity of the two bioreactors.

Materials and methods

Culture procedures
In order to evaluate the impact of these bioreactor systems on protein production, we utilized a recombinant alkaline phosphatase-secreting CHO cell line (rCHO), a proprietary cell line provided by CDI Bioscience, Inc. (Madison, WI). The rCHO cells were engineered with the 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®, Grand Island, NY) was used throughout these experiments. The media contains 6.3g/L glucose and was supplemented with 8 mM L-glutamine and 100µg/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 x 10^5 cells/ml. Two New Brunswick CelliGen 310 advanced bench-top stirred-tank bioreactors were utilized to grow the rCHO cells. One of the New Brunswick CelliGen consoles was connected to an adaptor kit (available from Eppendorf) for use of the Eppendorf BioBLU single-use vessel.

Table 1: Comparison of perfusion volumes

Perfusion	Glass	BioBLU
Day 1	0.5 L	1 L
Day 2	1 L	2 L
Days 3 - 15*	2 L	4 L

* Perfusion occurred every other day.

Packed-bed basket impeller operated in perfusion mode

Two experimental trials were performed using the packed-bed vessels in perfusion mode: 3 L autoclavable vessel (1.25 - 3.75 L working volume) and a BioBLU 5p single-use vessel (3.75 L working volume, pre-loaded with 150g 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.

Table 2: Bioreactor parameters (setpoints)

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

Biomarkers of cell growth and productivity

Cell productivity was assessed by measuring activity of the secreted ALKP protein using an enzyme assay (AnaSpec, Freemont, CA) according to the manufacturer's protocol. For simplicity unit measurements were used in this study. A unit (U) of ALKP activity was defined as the amount of enzyme that *hydrolyzes 1µmol* 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., Yellow Springs, OH) was utilized to monitor the glucose and lactate levels in the culture media every 24 hr for the duration of each trial.

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 (Fig. 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 lactate levels decrease at each 2 L perfusion.

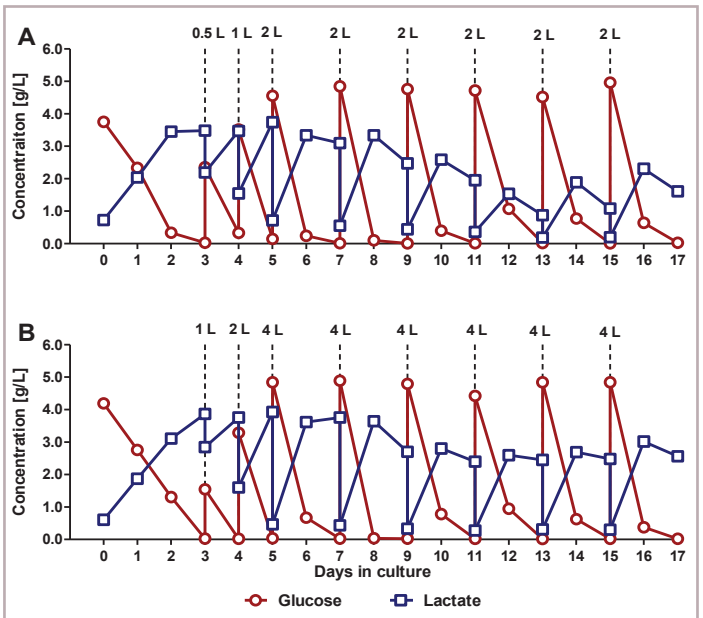

Figure 1. Glucose consumption and lactate production by rCHO cells cultured in two packed-bed bioreactor system. Values shown are the amounts of glucose and lactate measured in the culture media at each media exchange. The time and volume of the media exchange is indicated at each dashed line. Induction 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).

Comparison of bioreactor systems for ALKP production

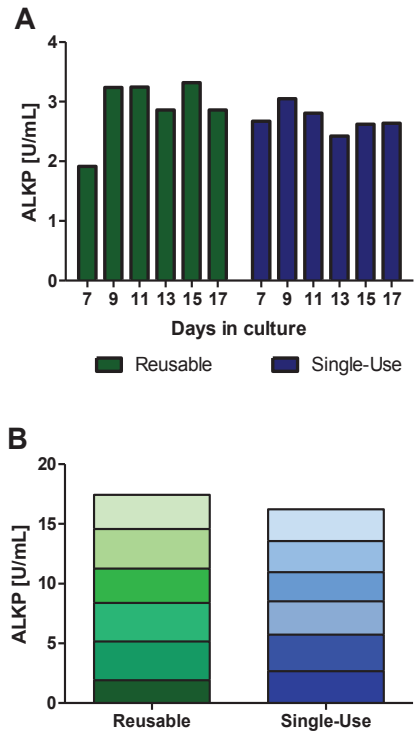

Figure 2. ALKP production by rCHO cells cultured in two packed-bed bioreactor system. (A) ALKP concentration in culture media measured each day of each experimental trial. IPTG induction of ALKP began on culture day five and continued every two days for the remainder of the experiment. (B) Stacked bar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

The average total ALKP production per experiment 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 five media exchanges in the reusable vessel was 17.44 U/mL and 16.22 U/mL in the single-use vessel.

In summary, these results demonstrated comparable yields in ALKP production (within the usual biological fluctuations) between the two packed-bed bioreactor systems when operated in perfusion. 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 Eppendorf BioBLU packed-bed, single-use bioreactor system.

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References

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3. Hu, W.S., T.C. Dodge, K.K. Frame, and V.B. Himes (1987) Effect of glucose on the cultivation of mammalian cells. Dev Biol Stand 66: p. 279-90.

Ordering information	Order no.
New Brunswick™ CelliGen® 310	
Master Control Station	M1287-2110
Utility Station (no display)	M1287-2112
Perfusion Kit	
3 L Perfusion Kit	M1287-1186
BioBLU® Single-Use Vessel	
BioBLU 5p, Microsparger, Packed bed, 1-pack	M1363-0119
BioBLU 5p, Microsparger, Packed bed, 4-pack	M1363-0120
BioBLU 5p, Macrosparger, Packed bed, 1-pack	M1363-0133
BioBLU 5p, Macrosparger, Packed bed, 4-pack	M1363-0134
New Brunswick™ CelliGen® 310 Vessel Kit	
3 L, Water Jacket, Magnetic Drive	M1287-0311
3 L, Water Jacket, without Drive	M1287-0331
Adaptor Kit: BioBLU® Single-Use Vessel & New Brunswick™ CelliGen® 310	
100-120 V, BioBLU® 5c	M1376-9927
200-240 V, BioBLU® 5c	M1376-9928

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