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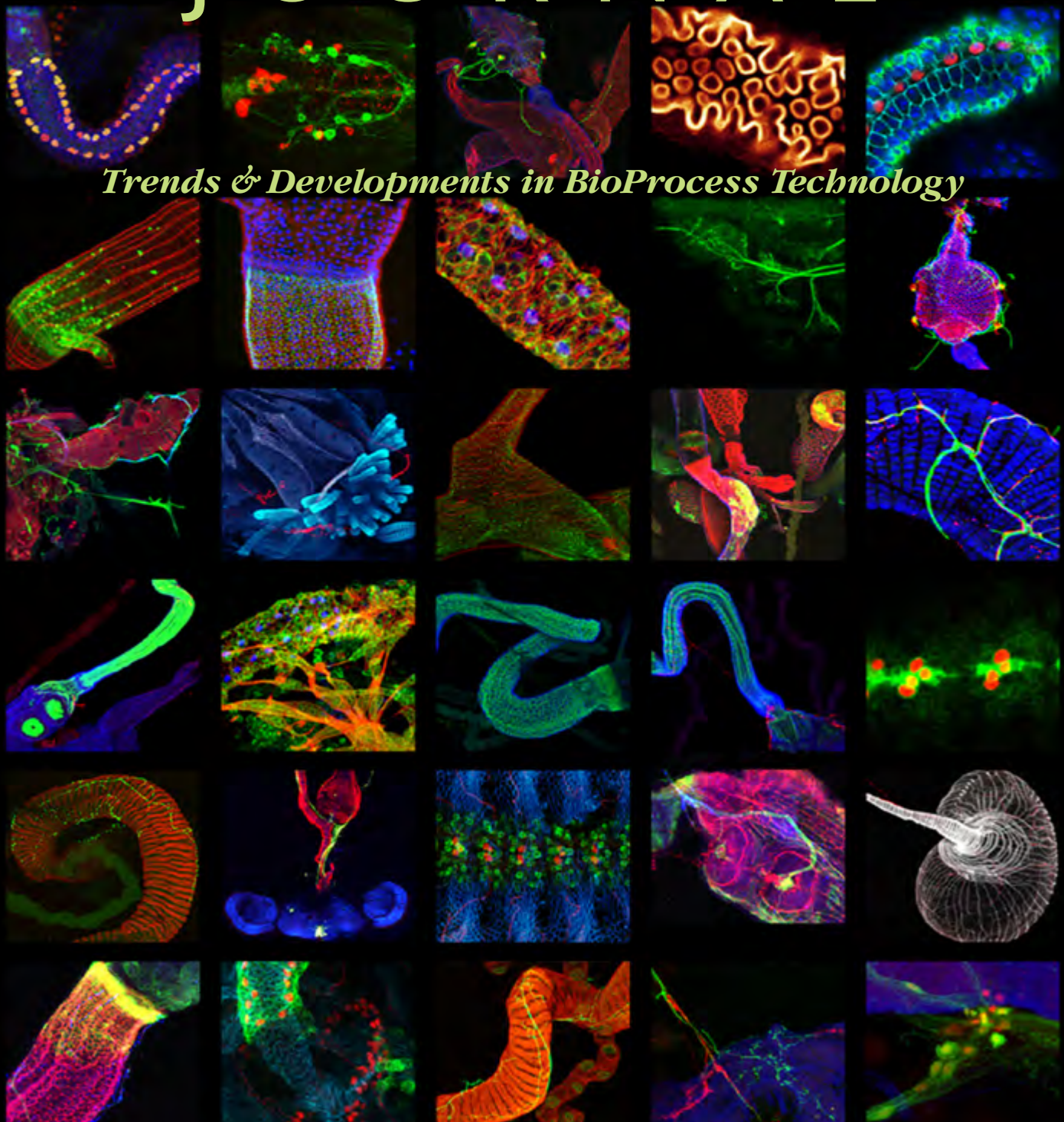

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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

A Comparative Bioreactor Vessel Study: Conventional Reusable Glass and Single-Use Disposables for the Production of Alkaline Phosphatase

By TAYLOR HATTON, SHAUN BARNETT, MA SHA, and KAMAL RASHID

Abstract

Single-use, stirred-tank bioreactor systems have been used in large-scale production for a number of years. Bench-scale, stirred-tank bioreactors have not been commercially-available for single-use until recently. The New Brunswick™ CelliGen® BLU pitched-blade bioreactor was introduced in 2009, and the CelliGen BLU packed-bed bioreactor, in 2012.

Little information is currently available on the utility of these bioreactors for bench-scale production of recombinant products. Thus, we designed this study to perform multiple comparisons with these single-use bioreactors and their traditional glass vessel counterparts. The data comparisons included: (1) CelliGen BLU pitched-blade vs. glass pitched-blade; and (2) CelliGen BLU pitched blade in batch mode vs. CelliGen BLU packed-bed in perfusion mode. Chinese hamster ovary (CHO) cells were used to measure alkaline phosphatase (ALKP) production in each bioreactor. The final measured concentration of ALKP, after eight days of batch-mode culture in the single-use, pitched-blade bioreactor, was 1.6 U/mL compared to 2.1 U/mL in the reusable bioreactor. After six perfusion harvests in the single-use, packed-bed bioreactor, the combined ALKP production was 16.2 U/mL compared to 17.4 U/mL in the reusable bioreactor in batch mode. Multiple batch culture runs in the pitched-blade bioreactor would be required to match the output of a single run in the packed-bed bioreactor in perfusion mode.

Results demonstrate that there are no significant differences between the reusable and single-use systems for bench-scale production of recombinant proteins. Our results also suggest that the CelliGen BLU packed-bed bioreactor, when operated in perfusion mode, is superior to the CelliGen BLU pitched-blade bioreactor when operated in batch mode, confirming our studies from 2012.[1]

Introduction

Stainless steel, stirred-tank bioreactors have been the trusted and dominant design for decades in scale-up of animal cells. Along with proven reproducibility of these bioreactors come some minor disadvantages, namely, cleaning and maintenance.[2-4] When reusable bioreactors are used for production of biopharmaceuticals, the cleaning process needs to be validated which increases cost in economic terms. In a survey conducted by BioPlan Associates, the primary reason for biopharmaceutical developers increasing their utilization of single-use systems was to eliminate cleaning requirements (90.2 % of respondents).[5]

The New Brunswick CelliGen BLU single-use bioreactor line, available in the pitched-blade and now in packed-bed design, has helped to make single-use, stirred-tank bioreactors readily available commercially to the bench-scale community.

The CelliGen BLU pitched-blade bioreactor incorporates one large plastic pitched-blade impeller which efficiently mixes the media while disrupting the sparger bubbles. The CelliGen BLU packed-bed bioreactor incorporates two horizontal perforated plastic sheets which entrap Fibra-Cel® disks, creating a bed for cells.[6] This design allows for media exchange while eliminating the need for filtration, sedimentation, or centrifugation of the cells, as is the case with other bioreactor types.[7] The packed-bed bioreactor, combined with a perfusion mode of operation, is a very useful means of increasing cell growth and productivity of recombinant proteins. Cells cultured in

packed-bed bioreactors are not exposed to hydrodynamic forces which allows for maximum cell growth and protein expression.[8]

Reusable packed-bed bioreactors in perfusion mode have been compared to reusable pitched-blade bioreactors in batch mode[1] and perfusion systems have been compared to fed-batch systems.[9] However, to date, no published studies have compared the productivity of protein-secreting cells in reusable packed-bed and pitched-blade bioreactors to single-use, packed-bed and pitched-blade bioreactors. Therefore, the objective of this study was to perform a multi-comparative study between single-use and reusable bioreactors. We expect the results from these experiments will help aid in the introduction of the single-use (CelliGen BLU) bioreactors as an alternative to reusable packed-bed bioreactors while also highlighting the advantages of the CelliGen BLU packed-bed bioreactor operated in perfusion mode.

Perfusion modes of operation offer many advantages over batch or even fed-batch modes of operation. Systems operated under perfusion mode do not accumulate toxic byproducts, as seen in the batch operations, because the media is removed on a regular basis. Perfusion systems can often be operated at smaller scales. These systems have the ability to increase cell concentrations up to 30× more than batch systems. Long run times allowed with the packed-bed bioreactor decrease the constant need for re-seeding cells and re-establishing seed cultures. This dramatically reduces setup time and labor over batch systems, advantages that will lead to reduced cost of operation[10, 11] A study performed by Biopharm Services showed that conventional stainless steel bioreactor facilities were the slowest to become cash positive. This was due to the increased capital investment required up front and the longer process of building a stainless steel bioreactor facility.[12]

Materials and Methods

Culture Procedures

In order to evaluate the impact of these bioreactor systems on protein secretion by cultured cells, we employed a recombinant ALKP-secreting CHO cell line (rCHO), generously provided by CDI Bioscience, Inc. The rCHO cells were engineered with their IPTG-regulated RP Shift® vector so that the rCHO cells stop replicating and shift to protein production when induced with isopropyl β-D-1-thiogalactopyranoside (IPTG). 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 (Gibco, Life Technologies). Frozen rCHO cells were thawed and transferred to T-75 flasks with CD-CHO serum-free medium and allowed to expand. Once a sufficient number of cells were achieved, sterile disposable spinner flasks were utilized to further expand the cells. Cell subculturing continued until a sufficient number of viable cells was achieved for use as a seed culture at a density of approximately 5×10^5 cells/mL. Two New Brunswick CelliGen 310 advanced bench-top, stirred-tank bioreactors incorporating two single-use CelliGen BLU bioreactors were utilized to grow the rCHO cells. A solution of $NaHCO_3$, 8 % w/v (8 g/100 mL), was used to help control the pH inside the bioreactor systems.

Pitched-Blade Impeller Operated in Batch Mode

The first bioreactor system utilized the pitched-blade impeller. The CelliGen BLU pitched-blade bioreactor comes pre-packaged and sterilized with the pitched-blade impeller, tubing, sparger, and filters for easy setup. The vessels are made of materials that meet USP Class VI standards and have been tested for leachables.[13] The blades on the pitched-blade impeller are flat and set at a 45° angle. This blade orientation provides good axial and radial mixture of the media while also increasing the oxygen mass transfer rate and disruption of bubbles released from the sparger. The pitched-blade impeller is designed to minimize the stress of mixing on shear-sensitive cells.[5]

Two experimental trials were performed utilizing the pitched-blade bioreactor. One trial was performed in a reusable (glass) 2.2 L total volume vessel (1.75 L working volume) and a second trial was performed utilizing a single-use CelliGen BLU 5.0 L total volume vessel (3.5 L working volume). For each trial, the bioreactor was allowed to operate until the cell concentration reached approximately 2×10^6 cells/mL at which time the cells were induced with IPTG. Both experimental trials had the following parameters, as shown in Table 1.

Packed-Bed Basket Impeller Operated in Perfusion Mode

The second bioreactor system utilized the packed-bed basket impeller. The CelliGen BLU packed-bed bioreactor

TABLE 1. Bioreactor parameters.

Parameter	Setpoint	
	Glass 2.2 L	CelliGen BLU 5.0 L
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

comes pre-assembled and sterilized with the impeller pre-packed with 150 g of Fibra-Cel disks. The head-plate is equipped with all necessary tubing, filters, sparger, and connectors for easy setup. The single-use packed-bed bioreactors meet the same USP Class VI standards as the pitched-blade bioreactor. This bioreactor system is suitable for both anchorage-dependent and suspension cells, and this system does not require the adaptation of anchorage-dependent cells to suspension culture. The packed-bed basket impeller is commonly used in the production and collection of extracellular proteins.[14] This system incorporates a basket with two horizontally positioned perforated screens. Fibra-Cel disks are placed in between the screens creating a bed to entrap suspension cells or provide a surface for attachment of anchorage-dependent cells. The Fibra-Cel disk bed provides a culture environment that allows freshly oxygenated media to slowly pass over the cells while also providing protection from external shear forces.[1] The rotation of the impeller creates a negative pressure that draws media up through the hollow center shaft where the sparger introduces oxygen to the media. The packed-bed bioreactor is the ideal system to use when a product is secreted out of the cell. Because cells are immobilized in the Fibra-Cel bed, samples of media can easily be removed without cell loss or culture disruption.

Two experimental trials were performed utilizing the packed-bed bioreactor. One trial was performed in a 2.2 L reusable (glass) vessel and a second trial was performed utilizing a single-use CelliGen BLU 5.0 L vessel. Both vessels were equipped with a basket (as described above) containing 85 g of Fibra-Cel disks in the reusable bioreactor and 150 g in the single-use bioreactor. The perfusion process was initiated once the cells reached the exponential growth phase as shown in Table 2. All experimental trials had the same growth conditions (temperature, oxygen, and pH) as the batch process (Table 1).

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.

TABLE 2. Comparison of perfusion volumes.

Perfusion	Volume	
	Glass 2.2 L	CelliGen BLU 5.0 L
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.*

For simplicity, unit measurements were used in this study. A 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 one 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.

Results

Pitched-Blade Bioreactor Operated in Batch Mode

Cell Density and Viability

Figure 1 shows the cell growth and viability of two independent experimental trials in the pitched-blade bioreactor. The seeding density in the single-use (CelliGen BLU) bioreactor was 6.1×10^5 cells/mL (Figure 1A) while the seeding density in the reusable (glass) bioreactor was 5.7×10^5 cells/mL (Figure 1B) as calculated by trypan

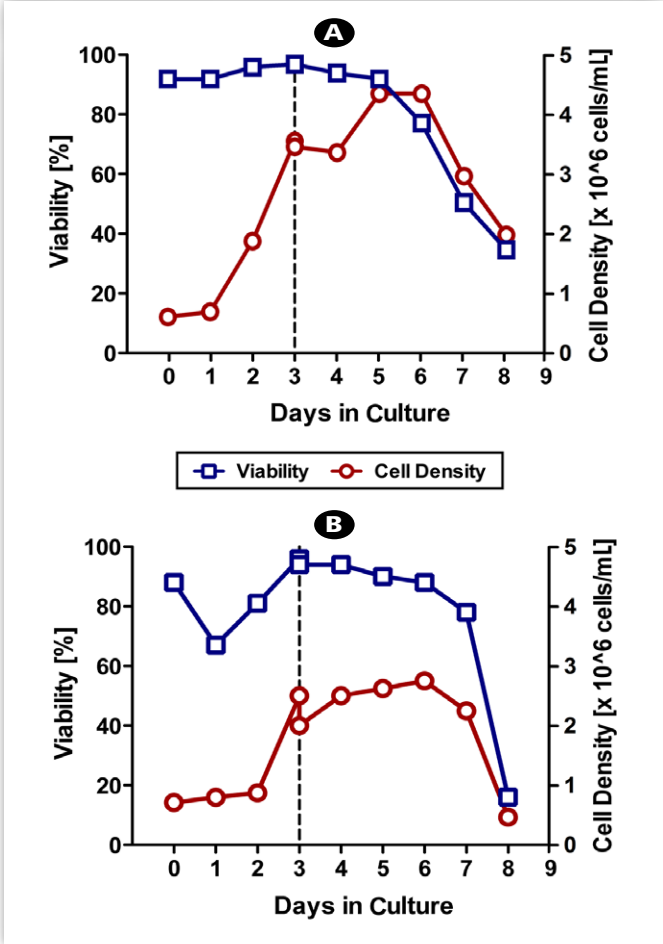

FIGURE 1. Growth of rCHO cells in the pitched-blade bioreactor system. Values shown are the cell density and viability on each day of culture. Each panel represents an independent experimental trial: **(A)** single-use [CelliGen BLU]; and **(B)** reusable [glass]. The dashed line indicates the time of induction of ALKP production by IPTG.

blue staining utilizing the Countess® cell counter. The maximum cell density observed in the single-use bioreactor (4.4×10^6 cells/mL) was significantly higher than in the reusable bioreactor (2.2×10^6 cells/mL). Cell viability was greater than 90 % for the majority of the experimental trial in the single-use bioreactor while the viability in the reusable bioreactor recovered to greater than 90 % after a few days in culture.

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.[15,16] Glucose levels measured at the time of induction (day 3) were nearly 0 g/L in both experiments (Figure 2). Media lactate concentrations increased in response to decreasing glucose availability. The observed gradual decrease of lactate near the end of each trial indicates its use as a secondary energy source.

ALKP Production

Figure 3 shows the concentrations of ALKP measured daily in two independent experimental trials in the pitched-blade bioreactor. ALKP concentrations increased over the six culture days post-induction. However, a decrease in ALKP activity was observed at the time of harvest in both trials, possibly due to degradation of the ALKP protein at the end of the experiment.[17] Serum-free media was utilized for growth of rCHO cells in this study. Thus, ALKP was susceptible to the action of proteases made by the rCHO cells.[18] As reported above, cell density in the single-use bioreactor was higher than the reusable bioreactor, suggesting that cell proliferation was dominant over ALKP production. This observation likely accounts for the slightly lower amount of ALKP detected in the single-use bioreactor.

Packed-Bed Bioreactor Operated in Perfusion Mode

Cell Density and Viability: The packed-bed bioreactors in both experimental trials were seeded with 5.0×10^5 cells/mL. However, because of the presence of the Fibra-Cel discs, it was not possible to sample the cells directly during culture to determine cell yield and viability. Therefore, cell density and viability were not monitored on a daily basis; rather, the rate of glucose consumption was used as a surrogate to approximate changes in cell density.[19] The growth of cells in the packed-bed bioreactor was estimated using the average glucose consumption

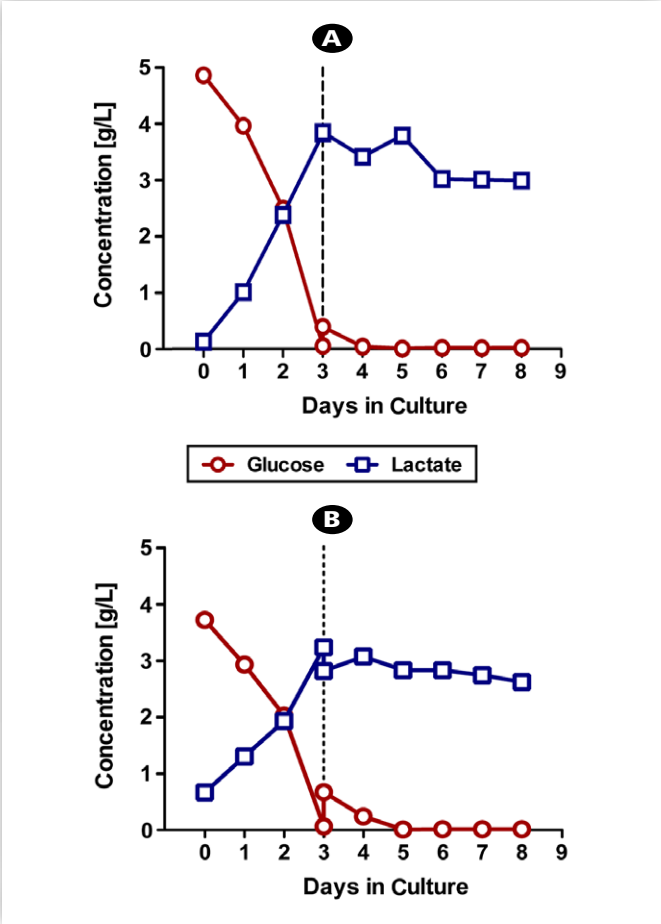

FIGURE 2. Glucose consumption and lactate production by rCHO cells cultured in the pitched-blade bioreactor system. Values shown are the concentrations of glucose and lactate in the culture media measured daily. Each panel represents an independent experimental trial: **(A)** single-use [CelliGen BLU]; and **(B)** reusable [glass]. The dashed line indicates the time of induction of ALKP production by IPTG.

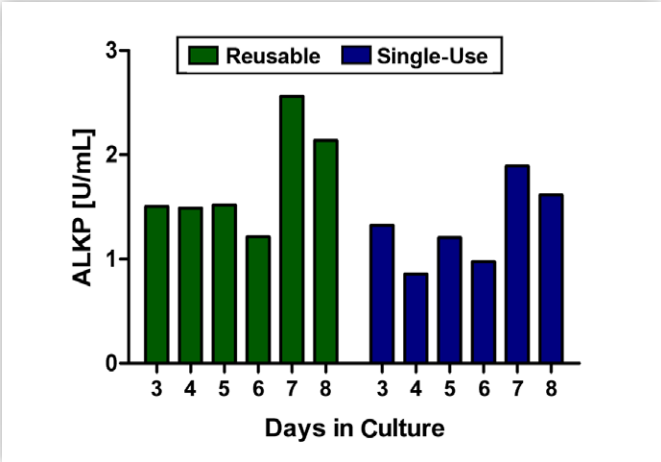

FIGURE 3. ALKP production by rCHO cells cultured in the pitched-blade bioreactor system. ALKP concentration in the culture media was measured each day after induction. IPTG induction of ALKP occurred on culture day 3.

rate. Glucose consumption rates were similar in both the pitched-blade and packed-bed bioreactor systems up to day four (Figure 4). However, after day four of culture, glucose utilization in the packed-bed bioreactor continued to increase exponentially, while the trend for glucose consumption in the pitched-blade bioreactor increased linearly. Increased glucose consumption observed on culture day five suggests that cell density in the packed-bed bioreactor had likely increased.

Glucose Utilization and Lactate Production: Glucose consumption was very similar in both the single-use (Figure 5A) and reusable (Figure 5B) experimental trials. As previously observed with the pitched-blade bioreactor system, media lactate concentrations increased in response to decreasing glucose availability in both trials. The use of lactate as a secondary energy source can also be observed as lactate levels decrease at each 2 and 4 L perfusion.

ALKP Production: Concentrations of ALKP in the two

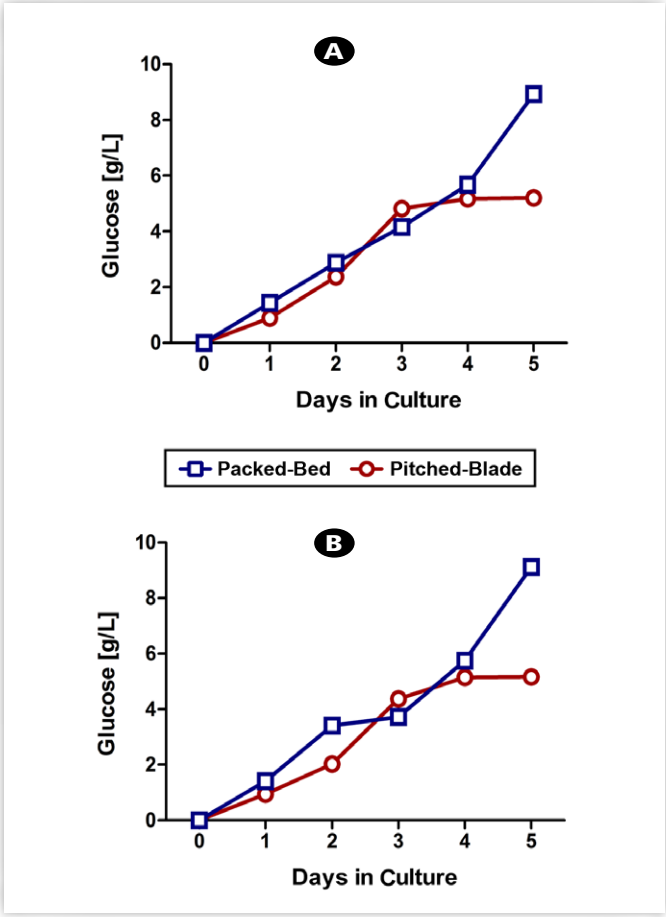

FIGURE 4 (right). Comparison of glucose uptake by rCHO cells in the pitched-blade and packed-bed bioreactor systems. Values shown are the daily glucose media concentrations consumed in two independent experimental trials: **(A)** single-use [CelliGen BLU]; and **(B)** reusable [glass].

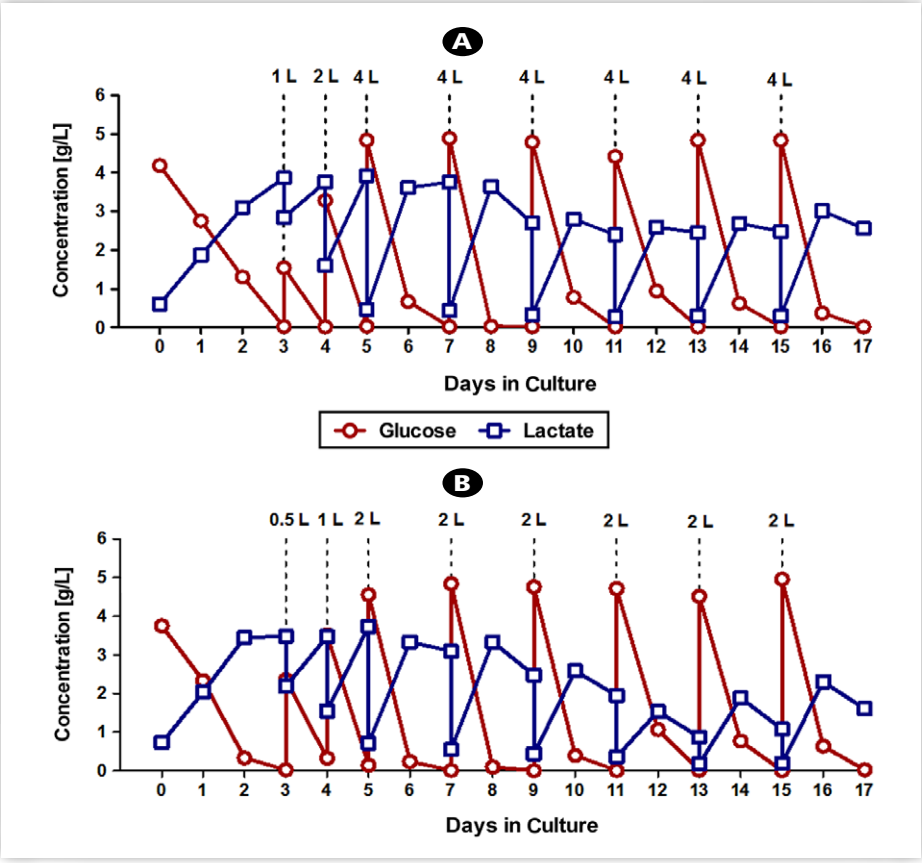

FIGURE 5 (left). Glucose consumption and lactate production by rCHO cells cultured in the: **(A)** pitched-blade; and **(B)** packed-bed bioreactor systems. Values shown are the amounts of glucose and lactate measured in the culture media at each media exchange. The time and volume of the media exchange 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.

independent experiments utilizing the packed-bed bioreactor are shown in Figure 6. Following initial expansion culture of rCHO cells for five days, ALKP production was induced every two days with a media exchange containing IPTG. We determined previously that continuous culture with IPTG in the media yielded greater production of ALKP as compared to a transient exposure to the inducing agent (data not shown). A modest increase in ALKP production was observed at each media exchange, although the level of ALKP varied by induction day and by experiment trial (Figure 6). The correlation between glucose utilization and ALKP production, as previously observed, supports the inference that the rate of glucose consumption in both experiments was conducive to the production of large amounts of ALKP. The rapid exhaustion of glucose and a presumed high cell density were likely contributing factors to the large amounts of ALKP observed.

Comparison of the Reusable and Single-Use Bioreactor Systems for ALKP Production

A major objective of this study was to compare the single-use to the reusable pitched-blade bioreactor, the single-use to the reusable packed-bed bioreactor, and finally the pitched-blade bioreactor to the packed-bed bioreactor in both vessel types. The total ALKP production per experimental trial is shown in Figure 7. Figure 7A shows the comparison between the two trials performed in the pitched-blade bioreactor. Final ALKP concentrations are very similar in both trials. Figure 7B shows the comparison between the two trials performed in the packed-bed bioreactor. Resembling the pitched-blade bioreactor, final ALKP concentrations in both packed-bed bioreactor types were markedly similar. The total ALKP production in the CelliGen BLU pitched-blade vs. the CelliGen BLU packed-bed bioreactor is shown in Figure 7C. Overall, the packed-bed bioreactor system produced ALKP to a much greater extent (nearly 9-fold greater) compared to the pitched-blade system.

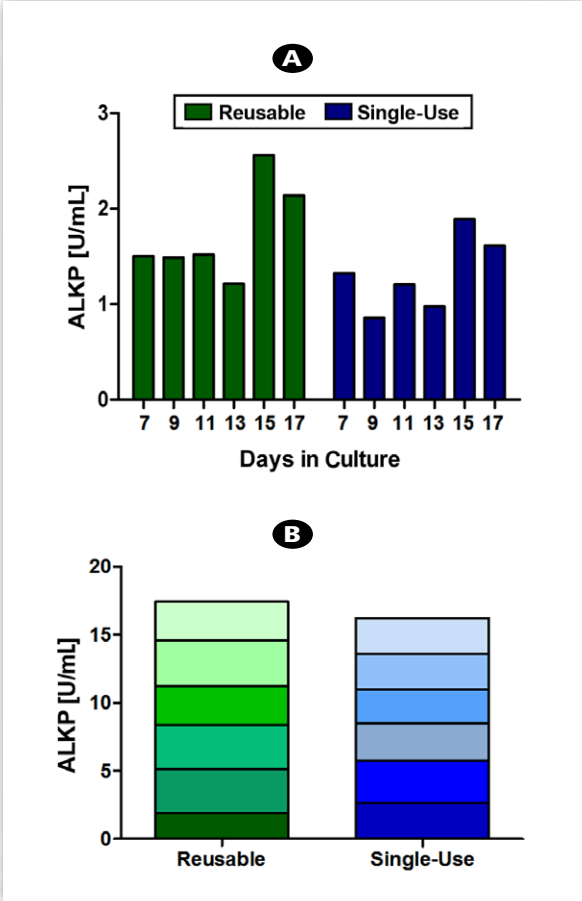

FIGURE 6. ALKP production by rCHO cells cultured in the packed-bed bioreactor system: **(A)** ALKP concentrations in culture media measured each day in two independent experimental trials using the packed-bed bioreactor. IPTG induction of ALKP began on culture day 5 and continued every two days for the remainder of each experiment; and **(B)** cumulative production of ALKP throughout each experiment with each bar representing a perfusion.

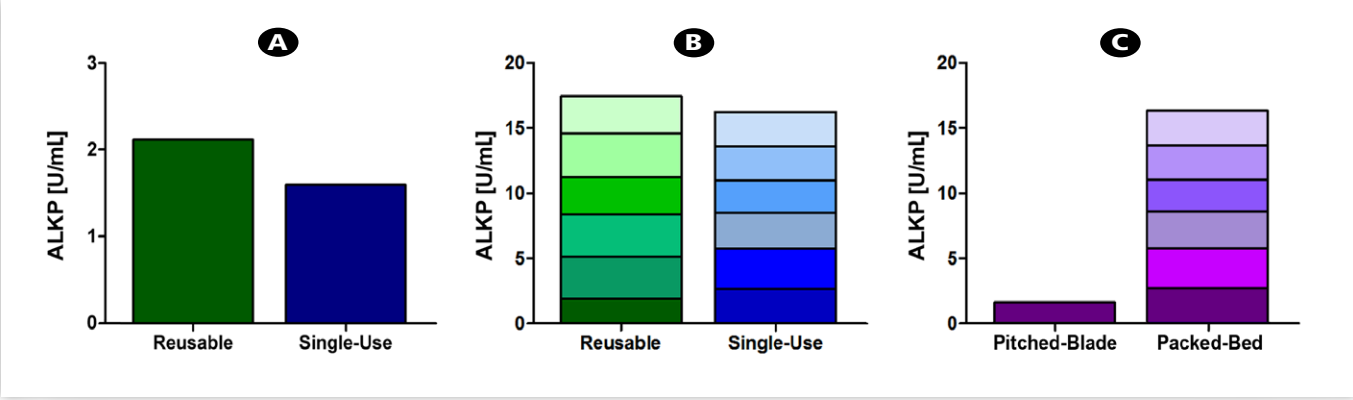

FIGURE 7. Comparison of ALKP production by rCHO cells cultured in the pitched-blade and packed-bed bioreactor systems: **(A)** final ALKP concentrations in the culture media measured in two independent experimental trials using the pitched-blade bioreactor; **(B)** cumulative ALKP concentrations in the culture media measured in two independent experimental trials using the packed-bed bioreactor; and **(C)** ALKP concentrations for two independent experimental trials using either the CelliGen BLU pitched-blade or packed-bed bioreactor systems.

Discussion

Small-scale bioprocessing has been regularly performed in reusable, stirred-tank bioreactors. Cleaning and sterilization of reusable bioreactors is mandatory between runs. Regular maintenance, careful handling, and storage of the glass vessel are also required. A significant monetary investment in sterilization equipment and manpower is required to maintain and clean reusable bioreactors. Cleaning and maintenance of single-use (disposable) bioreactors is not necessary and therefore implementation of these bioreactors can potentially lower required initial investments. Internal analysis conducted by Eppendorf Inc. indicates that the overall cost of using CelliGen 310 vs. CelliGen BLU is nearly identical (data not shown). The cost of operating the CelliGen 310 is driven by upfront investments including control cabinet, autoclavable vessel(s), probes, and autoclave sterilization hardware, as well as the additional labor costs in vessel set-up, break-down, cleaning, and validation. Conversely, the cost of operating the CelliGen BLU is mostly driven by the investment of the control cabinet and the single-use consumable vessels. The reduction in labor hours, cleaning, and validation are major driving forces behind single-use products in bioprocessing.

Process performance by each impeller type were similar in both the reusable and single-use bioreactors, indicating that single-use bioreactors can perform all manufacturing operations analogous to reusable bioreactors.

By virtue of its design, when operated in perfusion mode, the packed-bed bioreactor may be used continuously for months. Cell line maintenance is reduced since preparation of new seed cultures is not required. Moreover, initial setup efforts associated with the packed-bed bioreactor is substantially less than would be required using the pitched-blade system which requires multiple, shorter cultures to equal the production of a single culture with the packed-bed system. Although the pitched-blade bioreactor requires less monitoring over the duration of an experiment, as cells are cultured until nutrient depletion has occurred, this approach does require significant initial input in terms of labor and resources. Multiple culture runs, and thus, multiple seed cultures and system preparations, are required to match the output of the packed-bed system.

In summary, the single-use CelliGen BLU bench-top bioreactor performance was markedly similar to their respective reusable bioreactors. Therefore, significant consideration of single-use bioreactors should be taken into account when small-scale manufacturing of biologics is needed. In both vessel types the packed-bed bioreactor operated in perfusion mode is superior to the pitched-blade bioreactor operated in batch mode for growth of rCHO cells secreting ALKP. However, if a simple batch process is preferred then use of the CelliGen BLU pitched-blade bioreactor should be considered.

Acknowledgements

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Three additional articles published in *BioProcessing Journal* by USU's Center for Integrated Biosystems incorporating Eppendorf's New Brunswick CelliGen products:

Hatton T, Barnett S, Rashid K. CHO cell culture with New Brunswick CelliGen BLU single-use packed-bed Fibra-Cel basket. *BioProcess J*, 2012; 11(2): 50-52.

Hatton T, Barnett S, Benninghoff AD, Rashid K. Productivity studies utilizing recombinant CHO cells in stirred-tank bioreactors: a comparative study between pitched-blade and packed-bed bioreactor systems. *BioProcess J*, 2012; 11(2): 29-36.

Parasar P, Barnett S, Wilhelm A, Rashid K, Davies CJ. Large-scale growth of mouse P815 cells expressing a bovine non-classical major histocompatibility complex class I protein utilizing a pitched-blade bioreactor. *BioProcess J*, 2012; 11(3): 27-34.

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