

Volume 10 / Issue 2 • ISSN 1538-8786

BioProcessing JOURNAL

Trends & Developments in BioProcess Technology

A Production of ISBioTech

Using Pre-Sterilized External Filters in Long-Term Perfusion Cell Culture Applications

By ERIC HUI, JEFF LU, RUBEN BOADO,
KIERON WALSH*, and CHRISTIAN KAISERMAYER

Introduction

Perfusion systems for animal cell cultures are increasingly used for high cell density processes to enhance the productivity of bioreactors. There are four different process modes that can be used in animal cell cultivation: batch, fed batch, continuous culture, and perfusion culture. In both batch and fed batch, the metabolite concentrations cannot be kept constant and the accumulation of catabolites like lactate and ammonium limits the process duration to about ten to 15 days. In contrast, with continuous and perfusion processes, there is a constant influx of fresh cultivation medium and a corresponding removal of recombinant protein and catabolites. The concentrations of metabolites remain relatively constant and the process duration is not limited by the buildup of waste products. This extends the duration of these cultures to several weeks or months.^[1,2] The resulting steady-state conditions for metabolites can enhance cell-specific productivity^[3] and product quality, for example, by improved glycosylation or reduced aggregate formation.^[4,3] In continuous culture, cells are removed in the effluent and this limits the product output per liter of bioreactor volume (volumetric productivity). In contrast, substantially higher cell concentrations are attained in a perfusion system because the cells are

retained in the bioreactor resulting in increased volumetric productivity. A five- to 20-fold improvement over batch cultivation has been reported for perfusion cultures.^[5-7]

A reliable separation device which allows for the retention of cells in the bioreactor but removes spent medium and secreted product is critical for the performance and stability of a perfusion system. Current retention methods either use size-based (membranes, filters) or density-based (centrifuges or settlers) separation techniques.^[8,9]

The increasing sophistication of single-use bioreactors and pre-sterilized filtration equipment has made filtration-based cell retention methods easier to use. Factors encouraging the use of perfusion culture include cost, increased competition in the supply of biologicals as patents on existing products begin to expire, and the use of capital equipment with continuous production techniques. Typical cell concentrations in perfusion cultures have been in the range of 1×10^7 to 3×10^7 cells/mL^[5,10] but concentrations as high as 1×10^8 cells/mL have been reported.^[9] High cell density processes reduce the size of the reactor required for a given product output and this can lead to more than a 40% reduction in the capital investments required for perfusion versus the fed batch process.^[5,11] Finally, the perfusion process protects product stability by allowing for the removal of the target protein

ABOUT THE AUTHORS

Eric Hui, PhD; Jeff Lu, PhD; and Ruben Boado, PhD are with ArmaGen® Technologies, Santa Monica, California USA. Kieron Walsh and Christian Kaisermayer, PhD, are with GE Healthcare Life Sciences.

*Correspondence should be addressed to kieron.walsh@ge.com.

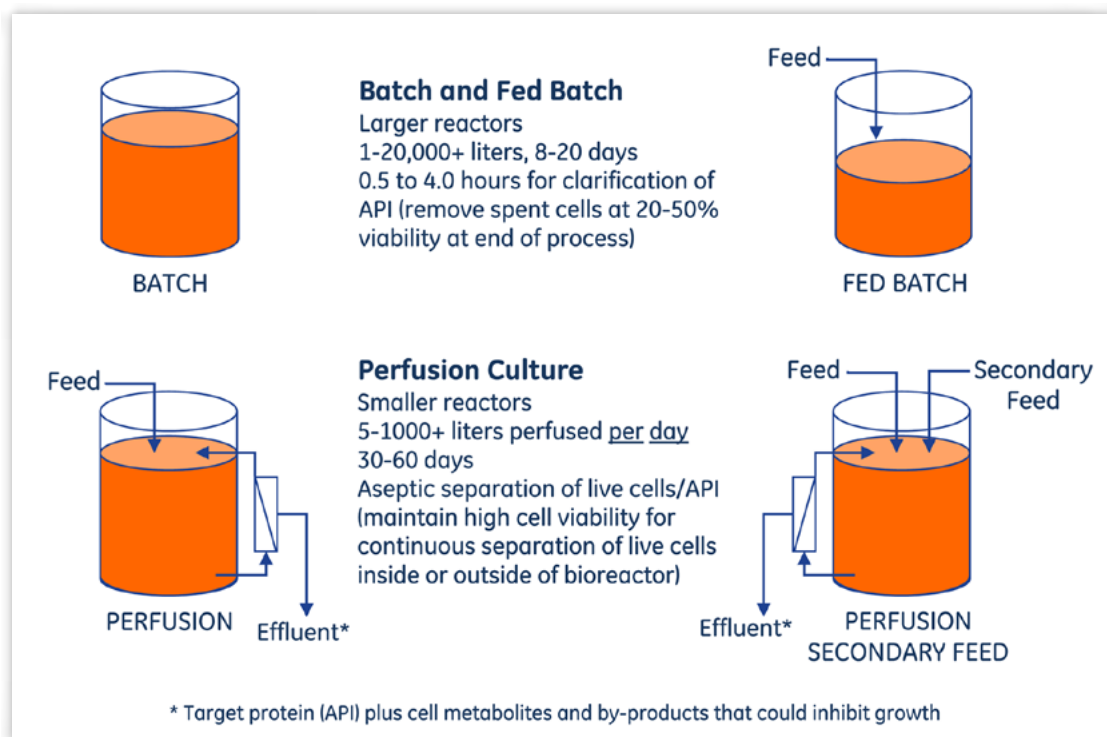


FIGURE 1.
A schematic comparison of different methods for cell culture production of a secreted target protein.

from the culture within 24 to 48 hours rather than at the end of the culture period (Figure 1).

The additional equipment required for continuous separation of the expressed protein from cells and, in addition, the return of the separated cells to the culture inherently makes perfusion cultivation of cells more complex than batch and fed batch cultivations. In general, filters (internal and external) tend to retain cells more efficiently than settling devices. However, filter fouling can have adverse effects on the production process. Internal filters simplify the production process but they cannot be replaced in the course of a production run. Hence, they may be suitable for smaller reactor volumes (< 10 L) and/or shorter production periods (< 20 days). External

filters are widely used on large-scale production facilities. They provide a failsafe mechanism against membrane fouling because they can be easily replaced, and this allows potentially indefinite continuation of perfusion. Hitherto, the relatively complex assemblies requiring autoclaving or switching via steamable connections result in tedious replacement of external filters which are more subject to potential contamination. Recent developments such as thermoplastic tubing welders have made the replacement of autoclaved components easier. In 2010, GE Healthcare Life Sciences introduced components with disposable aseptic connectors so that external cell separation components (hollow fiber microfiltration devices) could be added quickly, as required by the process (Figure 2).

FIGURE 2.
ReadyToProcess™ hollow fiber cartridge with ReadyMate™ aseptic connectors on all four ports. (Both are GE Healthcare products).



A perfusion process incorporating external cell separation using components from GE Healthcare was demonstrated by ArmaGen Technologies. The company tested this cultivation method for production of a recombinant protein. ArmaGen developed a proprietary technology solution that can non-invasively target recombinant proteins, therapeutic monoclonal antibodies (mAb), and small interfering RNA (siRNA) to the brain. Seeking an alternative to the internal perfusion filter that had previously fouled after 17–19 days (Table 1), the external perfusion method was tested in this study for the production of an IgG fusion protein in a mammalian cell line.

TABLE 1. Bioreactor runs using a standard disposable cell culture bag with an internal cell separation filter.		
Cell Line	Bioreactor Run	Internal Filter Clogging and Replacement of Cell Culture Bag
A	I	17 days
A	II	19 days
B*	I	19 days
B	II	18 days**
<p>*Cell line B was also used in the hollow fiber test run.</p> <p>**Cell culture bag was replaced at day 4 due to an internal filter rupture, and again at day 22 due to clogging of the internal filter.</p>		

Materials and Methods

Cell cultivation was performed in a bioreactor with a 50 L disposable cell culture bag (Cellbag™, GE Healthcare) customized to a working volume of about 25 L. The Cellbag was equipped with a pair of 3/8" ID silicon “dip tubes” extending to the center bottom of the bag and configured to remain submerged in the culture during rocking. This prevented air from being drawn into the cell cycling loop. The dip tubes were connected to the external filter apparatus depicted in Figure 3 (below) and 4 (on the following page). A Watson-Marlow Bredel 720S recirculation pump was used. STA-PURE® pump tubing (WL Gore & Assoc., Inc.) was used because of the required

high mechanical resistance. A 3/8" ID tubing was used for the recirculation loop and 1/2" ID tubing was used for the pump tubing in order to obtain a pump speed of less than 50 rpm and minimize the effect of peristalsis on cell viability. Infusion and perfusion pumping was performed using PUMP 20 (GE Healthcare). C-Flex® (Saint-Gobain Performance Plastics) thermoplastic tubing was used to weld the feed (fresh media infusion) and harvest (spent media perfusion) flow paths as required. A pair of four-position manifolds was used to allow multiple connections to the retentate side of replacement cartridges. On the permeate side of the external filter, one port was connected

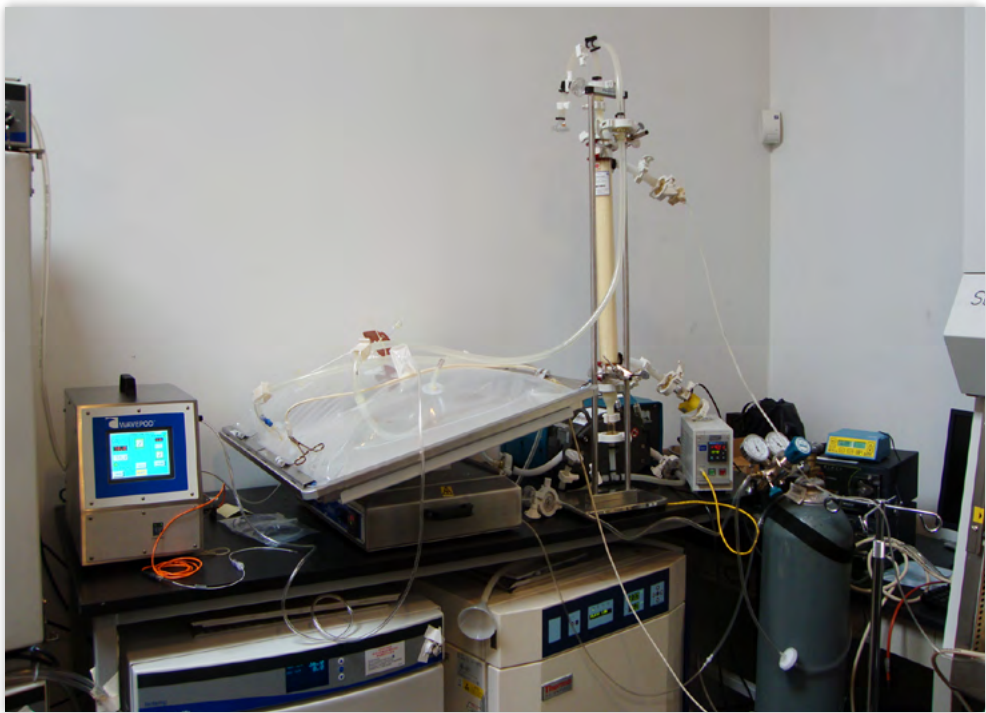


FIGURE 3. External perfusion controller assembly prior to use. The loop was attached to a customized 50 L cell culture bag using aseptic connectors outside of a laminar flow hood.

to a perfusion metering pump which was continuously removing culture supernatant. The perfusion rate was between 0.6 and 0.9 culture volumes per day. It was adjusted as necessary to keep the residual glucose concentration higher than 0.5 g/L. The other permeate port was attached to a SciPres™ (SciLog® Inc.) pressure/vacuum sensor to monitor any buildup of negative pressure—which can be indicative of membrane fouling. Fresh media was automatically added to the Cellbag Bioreactor using the existing perfusion controller modulated by the weight of culture in the reactor in order to keep the culture volume constant at 25 L during the trial.

Cell cultivation was also performed in standard GE 25/50

Cellbag disposable bioreactors (perfusion version) with an internal cell separation filter. The working volume was about 25 L, rocking speed was 25 rpm, and rocking angle was 8°. Perfusion rates and residual glucose concentrations were adjusted as described above.

Serum-free media supplemented with an additional 2–2.5 g/L of glucose was prepared in 100 L batches. The cultivation temperature was 36–37°C, and CO₂ was used to keep the pH at 7.0 ± 0.15. The culture was aerated via the headspace at a flow rate of 0.3–0.5 Lpm, and O₂ was mixed into the gas flow to keep the dissolved oxygen concentration above 60%. Daily harvests were pooled to volumes of 200 L and then processed with MabSelect Protein A resin packed in a BPG™ column (GE Healthcare).

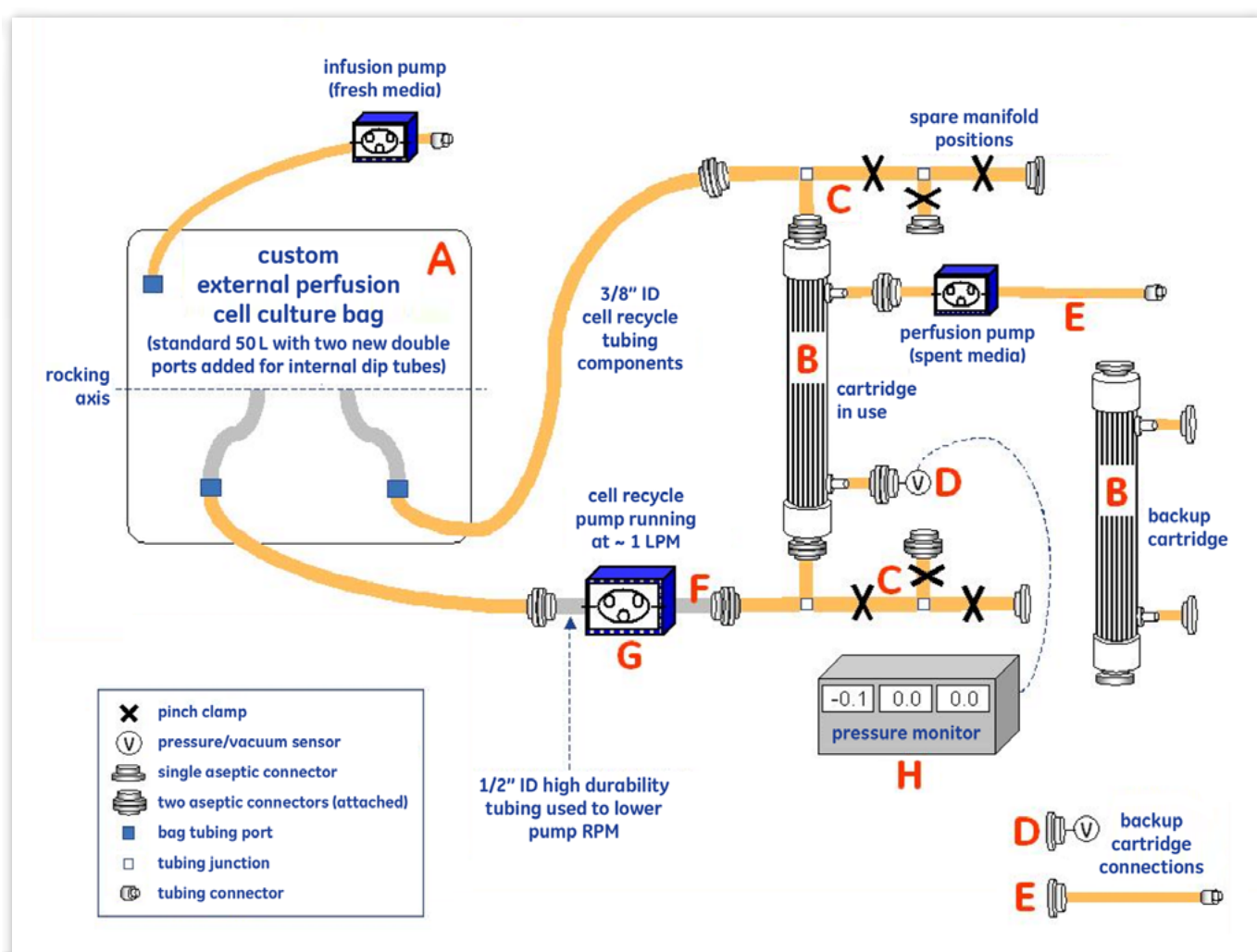


FIGURE 4. WAVE Cellbag with ReadyCircuit™ (GE Healthcare) external perfusion components. Schematic representation of an external perfusion filter assembly using disposable/single-use components and a failsafe strategy for the aseptical addition of backup filters. A) 50 L cell culture bag with two internal dip tubes for cell recycling; B) gamma-sterilized hollow fiber cartridge with genderless aseptic connectors; C) manifold with aseptic connection points; D) sensor to indicate negative pressure denoting membrane fouling; E) tubing segment suitable for welding into infusion or perfusion connections; F) cell recycle pump; G) long-life tubing segment; and H) pressure monitor.

Results and Discussion

Perfusion was started on day 3 of the process and the cell concentration increased to a maximum of 2.2×10^7 cells/mL on day 35. Cell viability at this point was about 70% (Figure 5). There was an accumulation of cell debris and subsequent fouling of the filters in the absence of a bleed process. The hollow fiber cartridges were exchanged twice during the run on days 17 and 28 using aseptic connectors on the installed manifold. Cell growth continued with no evidence of contamination. IgG passage through the $0.2 \mu\text{m}$ membrane dropped to $\sim 25\%$ on days 28 and 44. Interestingly, the passage of

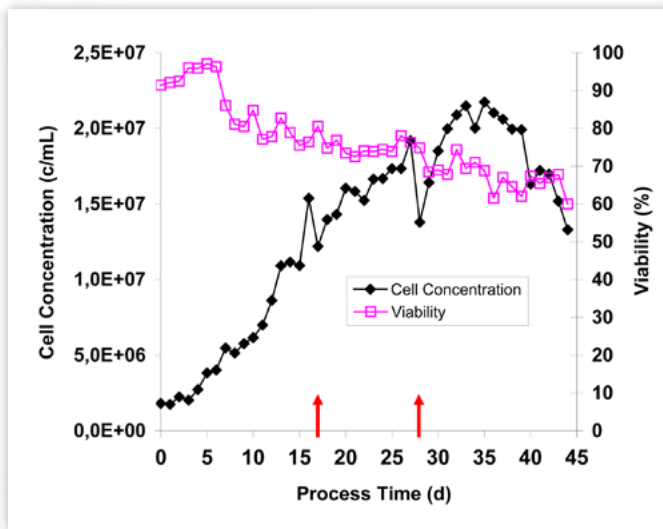


FIGURE 5. Cell concentration and viability during external perfusion using the Cellbag Bioreactor. Red arrows indicate the points at which the external perfusion filter was replaced.

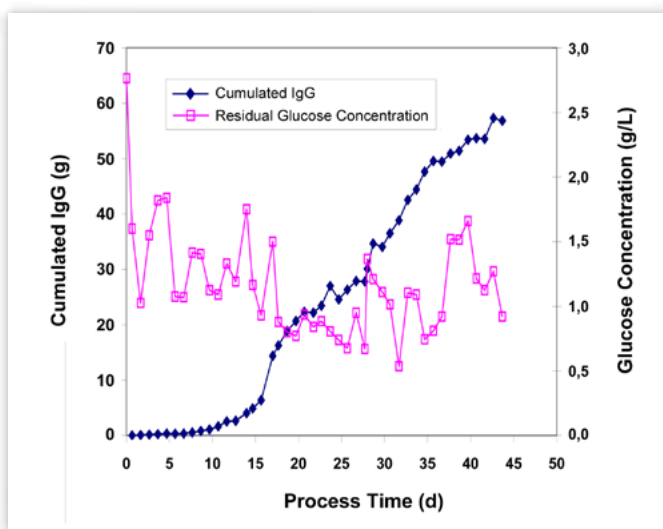


FIGURE 6. Development of IgG concentration and residual glucose concentration. The culture medium was supplemented with glucose from day 10 onward. Perfusion was begun on day 3 and the dilution rate was between 0.6 and 0.9 culture volumes per day.

water and small molecules was not affected, as shown by the residual glucose concentration remaining stable at about 1 g/L (Figure 6). The process was stopped on day 44 as cell viability dropped to 60%. Figure 6 shows the IgG accumulated during the process. Daily productivity was 100–200 mg during the initial phase of cultivation. On day 10, the cultivation medium was supplemented with glucose to limit the perfusion rate to a maximum of 0.9 culture volumes per day while maintaining a residual glucose concentration of ~ 1 g/L. This enhanced cell-specific production and, in conjunction with a high cell concentration ($> 1 \times 10^7$ c/mL), helped the daily protein output of the bioreactor to increase substantially. IgG productivity remained constant with an average daily output of 2.6 g between days 17 and 35 (Figure 6) and then declined because of lower cell concentration and viability (Figure 5). Total IgG production was about 57 g. An average daily output of 1.3 g of antibody was obtained during the 44 days of process time.

Inhibition of antibody passage through the filter membrane was observed in this study. This was probably caused by a buildup of cell debris due to decreasing cell viability. Figure 7 shows the residual cell debris after the

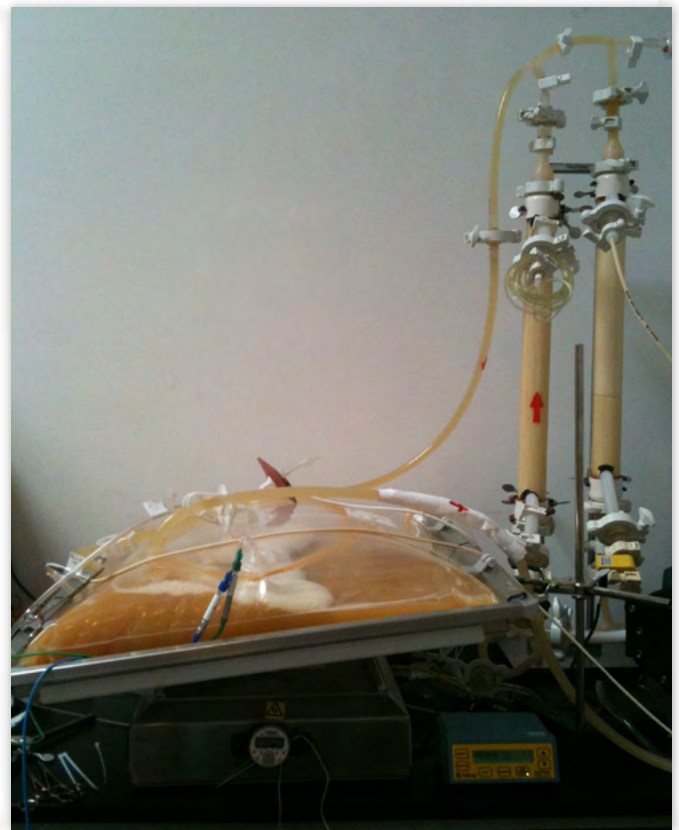


FIGURE 7. External perfusion of cell culture bioreactor on day 18 of culture period showing backup filter cartridge in operation.

first filter exchange on day 18. Perhaps the use of a cell-specific perfusion rate higher than the 0.6–0.9 volume exchanges that were maintained throughout the trial could improve cell viability. Other improvements that could increase overall yields include the reduction of cell debris by the periodic removal of biomass.

The study demonstrated that disposable single-use filter units can be used for the long-term perfusion cultures in disposable Cellbag Bioreactors. During the process described, more than 700 L of cultivation media were perfused through a bioreactor with a working volume of 25 L and the culture was maintained for more than six weeks by exchanging the external cell separation filters.

In previous experiments, perfusion processes used

a 50 L Cellbag with an internal cell separation filter (GE product code CB0050L-10-04 REV AF) which fouled within 17 to 19 days (Table 1). To extend the culture duration and increase production of the IgG fusion protein, an external cell recycling filter apparatus was connected to a modified 50 L Cellbag that was assembled using single-use disposable components. This resulted in a three-fold increase in process duration and a significant improvement in overall productivity without the need to replace the bioreactor cell culture bag.

REFERENCES

- [1] Chen ZL, Ben-Chuan WU, Liu H, Liu XM, Huang PT. 2004. Temperature shift as a process optimization step for the production of pro-urokinase by a recombinant Chinese hamster ovary cell line in high-density perfusion culture. *Journal of Bioscience and Bioengineering* 97(4):239-243.
- [2] Jardin BA, Zhao Y, Selvaraj M, Montes J, Tran R, Prakash S, Elias CB. 2008. Expression of SEAP (secreted alkaline phosphatase) by baculovirus mediated transduction of HEK 293 cells in a hollow fiber bioreactor system. *Journal of Biotechnology* 135(3):272-280.
- [3] Spearman M, Rodriguez J, Huzel N, Butler M. 2005. Production and glycosylation of recombinant β -interferon in suspension and Cytopore microcarrier cultures of CHO cells. *Biotechnology Progress* 21(1):31-39.
- [4] Lipscomb ML, Palomares LA, Hernandez V, Ramirez OT, Kompala DS. 2005. Effect of production method and gene amplification on the glycosylation pattern of a secreted reporter protein in CHO cells. *Biotechnology Progress* 21(1):40-49.
- [5] Jardin BA, Montes J, Lanthier S, Tran R, Elias C. 2007. High cell density fed batch and perfusion processes for stable non-viral expression of secreted alkaline phosphatase (SEAP) using insect cells: Comparison to a batch Sf-9-BEV system. *Biotechnology and Bioengineering* 97(2):332-345.
- [6] Lipscomb ML, Mowry MC, Kompala DS. 2004. Production of a secreted glycoprotein from an inducible promoter system in a perfusion bioreactor. *Biotechnology Progress* 20(5):1402-1407.
- [7] Yang JD, Angelillo Y, Chaudhry M, Goldenberg C, Goldenberg DM. 2000. Achievement of high cell density and high antibody productivity by a controlled-fed perfusion bioreactor process. *Biotechnology and Bioengineering* 69(1):74-82.
- [8] Pinto RCV, Medronho RA, Castilho LR. 2008. Separation of CHO cells using hydrocyclones. *Cytotechnology* 56(1):57-67.
- [9] Voisard D, Meuwly F, Ruffieux PA, Baer G, Kadouri A. 2003. Potential of cell retention techniques for large-scale high-density perfusion culture of suspended mammalian cells. *Biotechnology and Bioengineering* 82(7):751-765.
- [10] Yoon SK, Ahn YH, Jeong MH. 2007. Effect of culture temperature on follicle-stimulating hormone production by Chinese hamster ovary cells in a perfusion bioreactor. *Applied Microbiology and Biotechnology* 76(1):83-89.
- [11] Lim AC, Washbrook J, Titchener-Hooker NJ, Farid SS. 2006. A computer-aided approach to compare the production economics of fed-batch and perfusion culture under uncertainty. *Biotechnology and Bioengineering* 93(4):687-697.

NOTES

GE is a trademark of General Electric Company.

BPG, Cellbag, MabSelect, ReadyCircuit, ReadyMate, ReadyToProcess, WAVE Bioreactor are trademarks of GE Healthcare companies.

ReadyMate is covered by US patent number 6,679,529 B2 owned by Johnson & Boley Holdings, LLC and licensed to GE Healthcare companies.

© 2011 General Electric Company – All rights reserved.

We Want Your Next Article

Your data and methods are needed more than ever as the industry struggles to recover from one of its toughest periods. We want to make your work available to those who really appreciate it.

Please let us know what you are working on, and if you aren't sure how your material will fit, let me know so I can work with you on the best approach for publishing.

The *BioProcessing Journal* is in its 10th year as a unique and highly respected source of bioprocess technology. Help us continue this tradition with the latest technological developments and best practices needed to develop and produce safe and effective biologics.



Keith L. Carson, Executive Editor

kcarson@bioprocessingjournal.com

