One-step seed culture expansion from one vial of high-density cell bank to 2000 L production bioreactor

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY15025-07Jul20-AN

One-step seed culture expansion from one vial of high-density cell bank to 2000 L production bioreactor

This application note describes how perfusion cell culturing can be used to reduce processing time, simplify operations, and maximize equipment utilization in seed culture expansion processes. For creation of high-density cell banks, Chinese hamster ovary (CHO) cells were expanded using the ReadyToProcess WAVE™ 25 bioreactor system operated in perfusion mode. At 50 × 10^6 viable cells/mL, the cultures were terminated by addition of cryopreservation medium in a 1:1 ratio. As culturing was performed in a Cellbag™ perfusion bioreactor, cells could be concentrated back to 50 × 10^6 viable cells/mL using an integrated cell retention filter without the need for a separate centrifugation step. The cell suspension was divided into 4.5 mL aliquots, frozen, and stored in liquid nitrogen. From a high-density cell bank, one vial was thawed and used to inoculate a 1 L ReadyToProcess WAVE 25 bioreactor culture. The culture volume was gradually expanded to 10 L, whereupon culturing was continued in perfusion mode until a cell density of 50 × 10^6 viable cells/mL was reached. The described method generated enough cells to seed a 2000 L production bioreactor in only 10 days. The use of perfusion in seed culture expansion allowed a one-step process, from cryovial to N-1 bioreactor, omitting the need for intermittent bioreactor steps and associated labor.

Introduction

Seed culture expansion is commonly performed in several consecutive batch cultures. Starting from a cryopreserved cell stock, the initial culture expansion is typically performed in shake flasks, while the final steps are performed in bioreactor cultures. Each expansion step commonly takes three to four days and, unless using a culture vessel with a large turn-down ratio, a new culture vessel is needed for each volume increase. Such a procedure is time-consuming and labor intensive, and each manual interaction constitutes a risk of contamination of the culture.

With perfusion culturing, on the other hand, cells can be maintained in exponential growth phase for an extended period of time and reach higher viable cell densities compared with batch and fed-batch culturing. By using perfusion in seed culture applications, seed cultures with split ratios of 1:50, 1:100, or even higher can be achieved, eliminating the need for many of the subsequent intermediate cell expansion steps that are required in a traditional seed train. In addition, minimizing the number of steps that require manual interaction reduces the contamination risk and gives an opportunity for a higher level of automation of the process. A prerequisite for successful high-density perfusion culturing is a robust bioreactor system with accurate control features capable of maintaining culture parameter set points also at high cell densities.

Here, we describe the use of perfusion to simplify operations in cell bank preparation and shorten the overall seed expansion process, from cryovial to the N-1 bioreactor step. High-density cell banks were prepared by culturing CHO cells in the ReadyToProcess WAVE 25 system. Cryoprotectant was added to the culture and cells were cryopreserved in 4.5 mL aliquots. One vial from the high-density cell bank was sufficient for direct inoculation of a bioreactor culture, eliminating the need for several shake flask steps prior to seed culture expansion (Fig 1). The seed culture was expanded in perfusion mode, making subsequent intermediate bioreactor steps redundant. Figure 2 gives an overview of the overall experimental setup and the system setup. The described method can be used to decrease capital and operational expenditures (CAPEX and OPEX), reduce equipment footprint, and simplify the cell expansion phase.

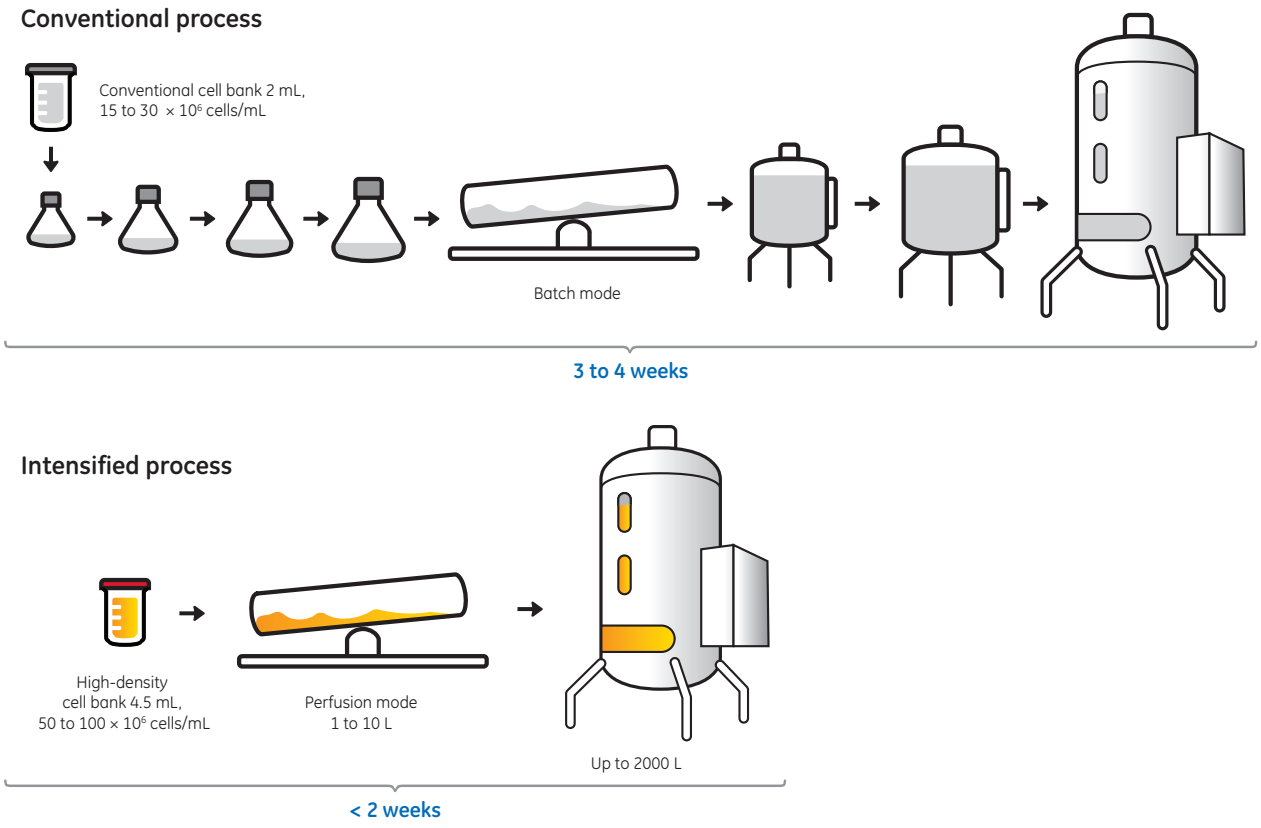

Fig 1. Vials from a high-density cell bank can be used for direct inoculation of a small bioreactor culture, eliminating the need for shake flask operations in the immediate post-thaw expansion phase. The use of perfusion in the bioreactor stage allows cells to stay in exponential growth phase throughout the entire culture, for high final viable cell density and cell viability. By such a procedure, an N-1 culture at 10 L scale can be used to seed a production culture with a final volume of 2000 L.

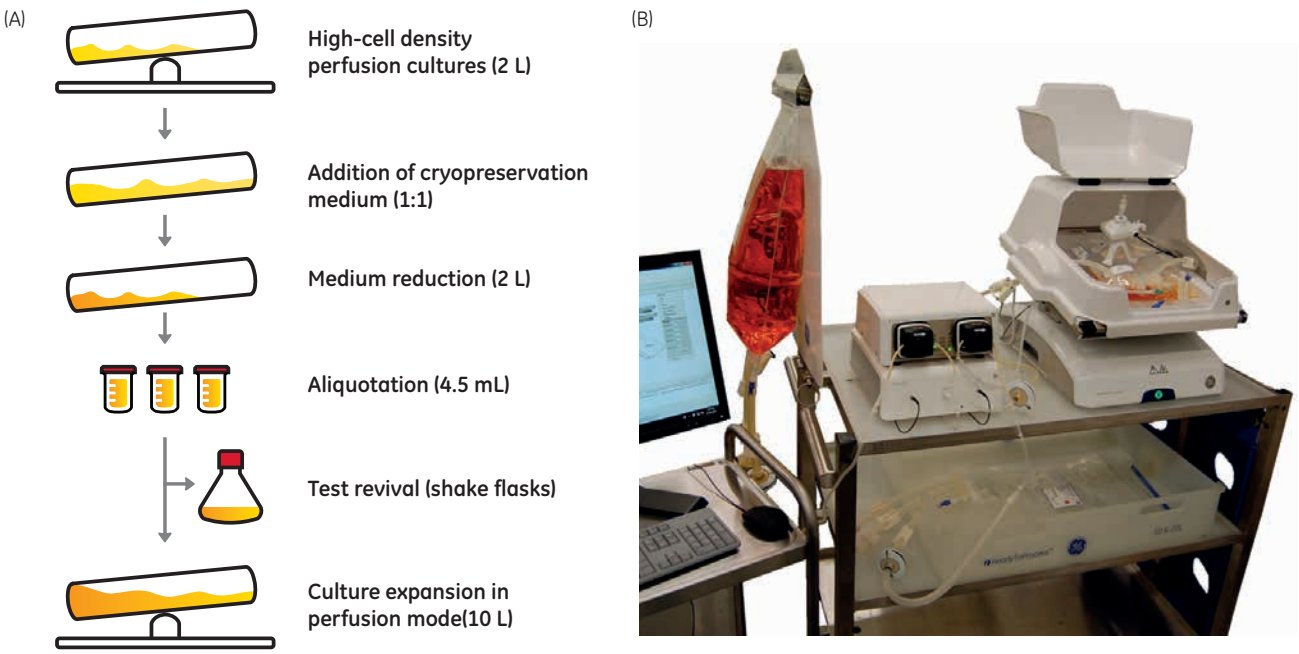

Fig 2. (A) Overall experimental setup. (B) System setup.

Materials and methods

Cell bank generation

CHO DG44 cells (licensed from Cellca GmbH) and CHO-S cells (licensed from Cobra Biologics Ltd.) were seeded from exponentially growing shake flask cultures into 2 L ActiCHO™ P medium in 10 L Cellbag perfusion bioreactors at a density of 0.4 × 10^6 cells/mL. Cells were cultured using the ReadyToProcess WAVE 25 system at a target cell-specific perfusion rate of ~ 137.5 pL/cell/d. Adjustments to the perfusion rate were made every two hours based on assumed exponential growth rate, as programmed using the ***Method Editor*** function. The cultures were controlled at pH 7.1, 40% dissolved oxygen (DO), and a temperature of 36.8°C. Target end criteria were 50–100 × 10^6 viable cells/mL at a viability of > 95%. For feed and perfusate, 20 L ReadyToProcess™ bags were used. Tubing of 0.8 mm i.d. was used for perfusion, whereas tubing of 3.2 mm i.d. was used for fill and drain.

Cryopreservation

For preparation of cells for cryopreservation after reaching the target cell concentration, chilled, fresh medium supplemented with 15% DMSO was added to the bioreactor at a 1:1 ratio and agitation was lowered from 20 to 15 rpm. Culture volume was reduced back to original cell concentration and volume (2 L) by pumping out culture medium through the cell retention filter. Cell suspension was drained, aliquoted into 4.5 mL cryovials, and frozed using standard methods. After storage for one week in liquid nitrogen, viable cell density was determined in a test revival in shaker flasks before use in seed culture generation. Target criterion was a cell viability of > 90% at all stages.

Seed culture expansion

The content of one vial from the cryopreserved CHO DG44 cell bank was thawed and suspended in 100 mL prewarmed, fresh culture medium (ActiCHO P medium supplemented with 6 mM L-glutamine and 2.8 g/L bicarbonate) in a sterile transfer bottle. The suspended cells were transferred to a 20 L Cellbag perfusion bioreactor filled with 900 mL culture medium at 0.225 × 10^6 viable cells/mL. The volume was stepwise increased to a final working volume of 10 L using the ***Medium Addition*** function of the ReadyToProcess WAVE 25 system. The culture was continued at a perfusion rate of ~ 140 pL/cell/d, programmed using the ***Method Editor*** function of the system. The culture was controlled at pH 7.1, 40% DO, and a temperature of 36.8°C. Target criteria were 50–100 × 10^6 viable cells/mL at a viability of > 95%. For feed and perfusate, 100 L ReadyToProcess bags were used. Tubing of 3.2 mm i.d. was used for feed and harvest.

Results and discussion

High-density cell bank

CHO cell suspensions for preparation of the high-density cell banks were generated using the ReadyToProcess WAVE 25 system operated in perfusion mode. Total medium perfused over the culture period was 20 L.

For CHO DG44 cells, the average specific growth rate was 0.838 day^{-1} (doubling time ~ 19.9 h). The cells were harvested after six days at 55.8 × 10^6 viable cells/mL and a cell viability of > 95%.

For CHO-S cells, the average specific growth rate was 0.797 day^{-1} (doubling time ~ 20.9 h. Also the CHO-S cells were harvested after six days at 48.8 × 10^6 viable cells/mL and a cell viability of > 95%.

The results are shown in Figure 3.

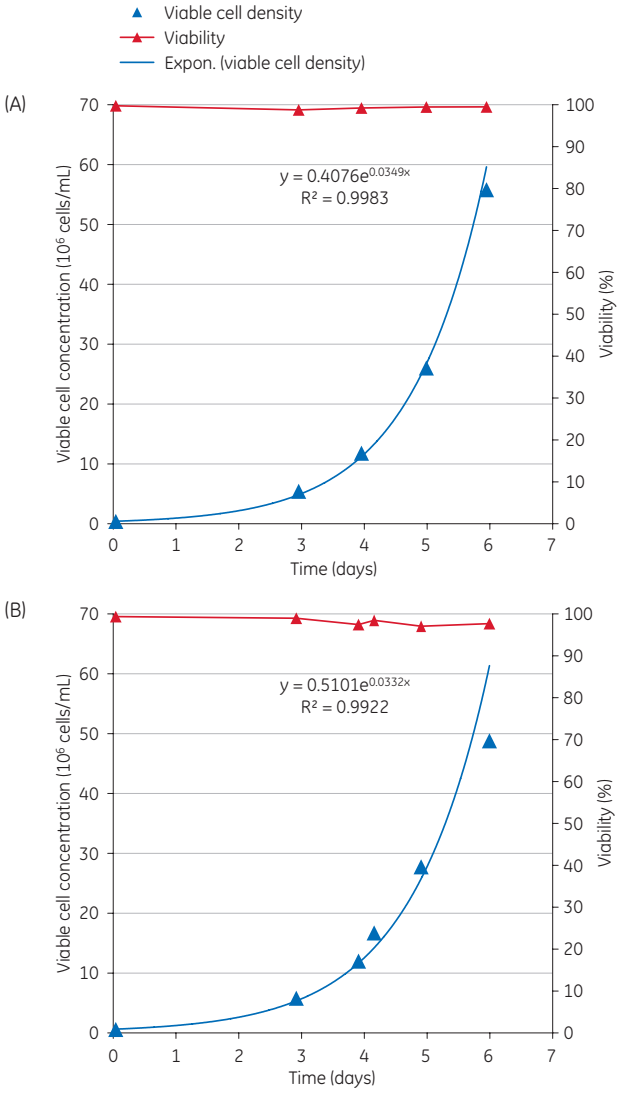

Fig 3. High-density cell bank generation from (A) CHO DG44 cells and (B) CHO-S cells.

For CHO DG44 cells, the glucose concentration gradually decreased from 6 g/L at the start of the culture to approximately 1 g/L on Day 6 when the culture was stopped. Lactate increased to approximately 1.5 g/L on Day 3, and then remained relatively constant until the end of the culture. For CHO-S cells, the glucose and lactate concentrations remained fairly constant at ~ 2.5 g/L and 1.5 g/L, respectively, over the last days of the culture period. The results show that the perfusion rate was sufficiently high to maintain nutrient and metabolite concentrations at levels that allowed keeping cells in exponential growth phase throughout the culture (Fig 4).

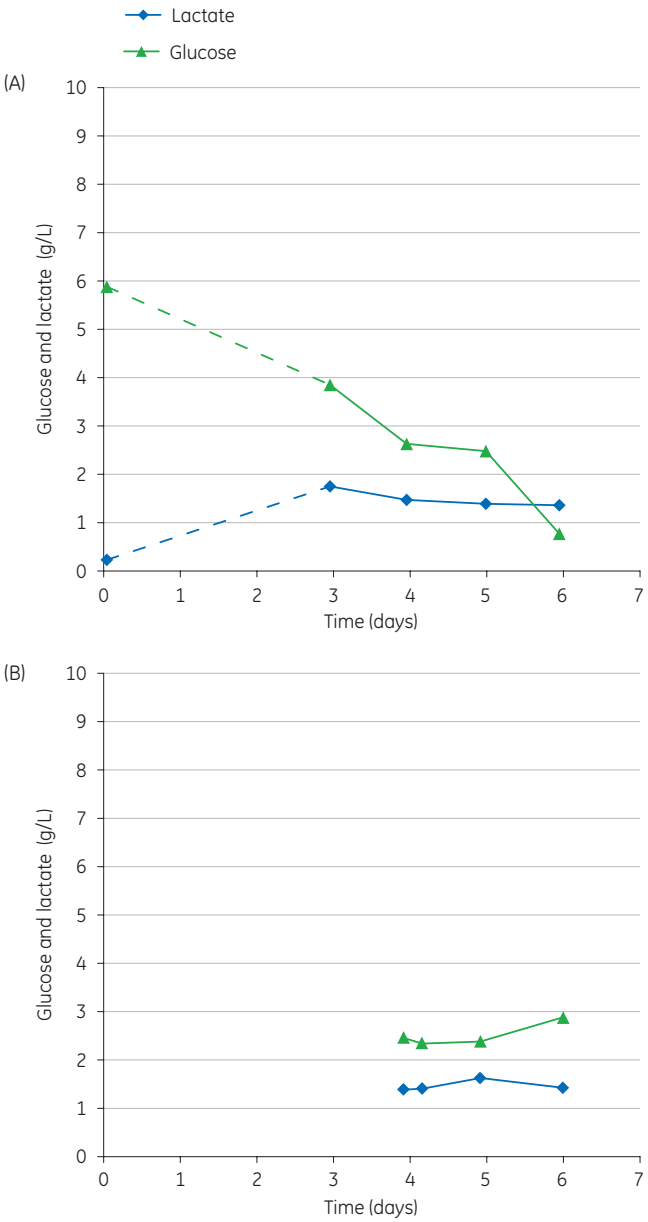

Fig 4. Glucose and lactate concentrations for (A) the CHO DG44 cell culture and (B) the CHO-S cell culture.

Bioreactor cultures of 2 L were sufficient for generation of high-density cell banks, comprising 400 vials of 4.5 mL each, with cell concentrations of 50 × 10^6 cells/mL. All procedures up until aliquotation could be performed in the bioreactor (Fig 5). No separate centrifugation step was required. From the generated cell banks, cells were successfully revived and cultured in shake flasks with viabilities of > 90% (Fig 6), which is similar to the performance of conventional cell banks.

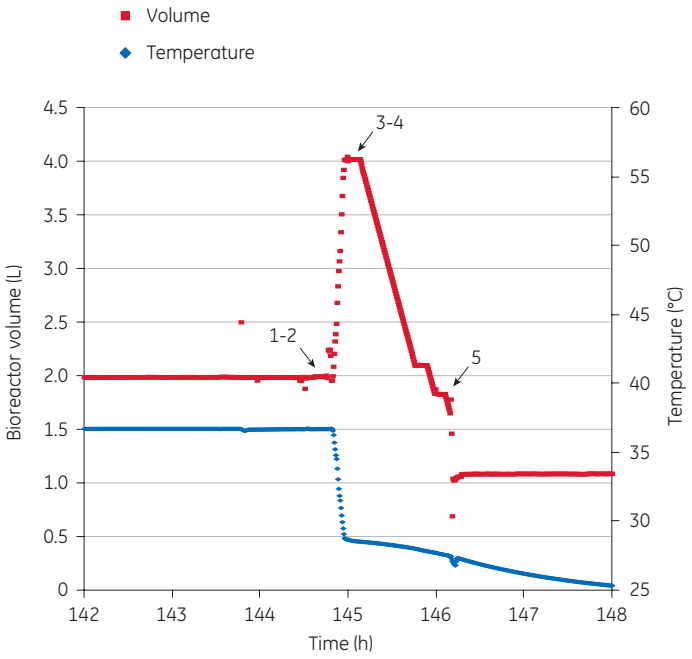

Fig 5. Cell bank generation. Perfusion and temperature control was stopped at ~ 50 × 10^6 cells/mL, after which chilled (4°C to 8°C), fresh medium supplemented with 15% DMSO was added at a 1:1 ratio (1,2). Agitation was lowered from 20 to 15 rpm and culture volume was reduced back to the original volume (2 L) using the cell retantion filter (3,4). The cell suspension was drained, aliquoted into 4.5 mL cryovials, and frozen (5).

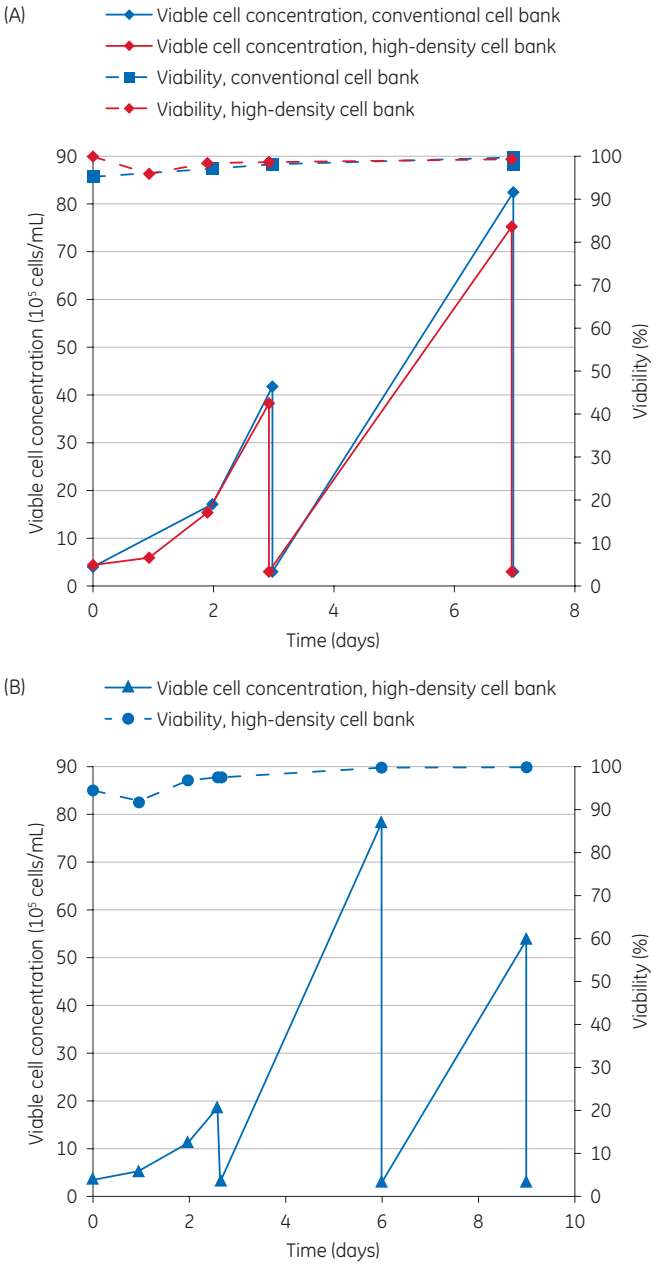

Fig 6. Cells from the high-density cell bank were revived with a viability of (A) > 95% for CHO DG44 cells (subcultured on Day 3 and 7) and (B) > 90% for CHO S-cells (subcultured on Day 3, 6, and 9). Growth after revival in shake flasks was similar to conventional cell banks.

One-step seed culture process

One vial of the high-density CHO DG44 cell bank was used to inoculate a 20 L Cellbag perfusion bioreactor at a starting volume of 1 L. The culture volume was further expanded to 10 L, whereupon perfusion was started. Cells were maintained in rapid exponential growth throughout the 10 day culture period (Fig 7).

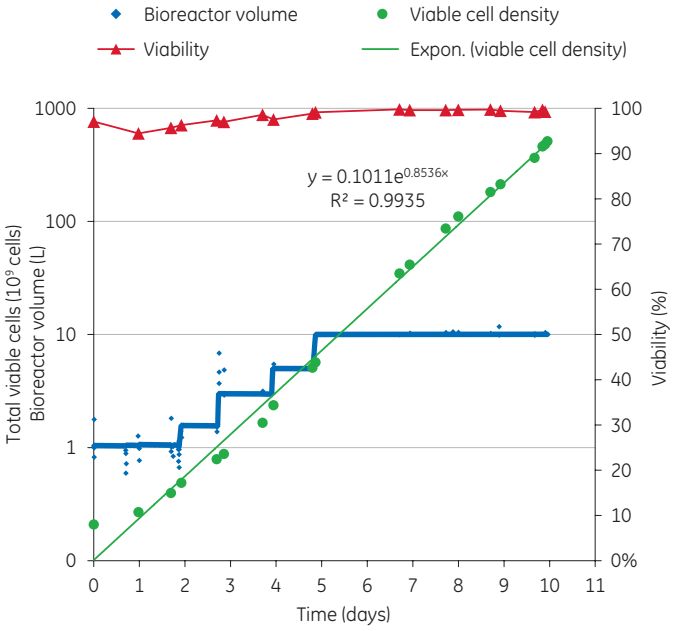

Fig 7. One-step seed culture expansion from one vial of CHO DG44 cell bank.

The average specific growth rate was 0.853 day^{-1} (doubling time ~ 19.5 h). Total medium perfused over the culture period was 90 L (Fig 8). CHO DG44 cells were harvested at 51.2 × 10^6 viable cells/mL and a cell viability of > 95%.

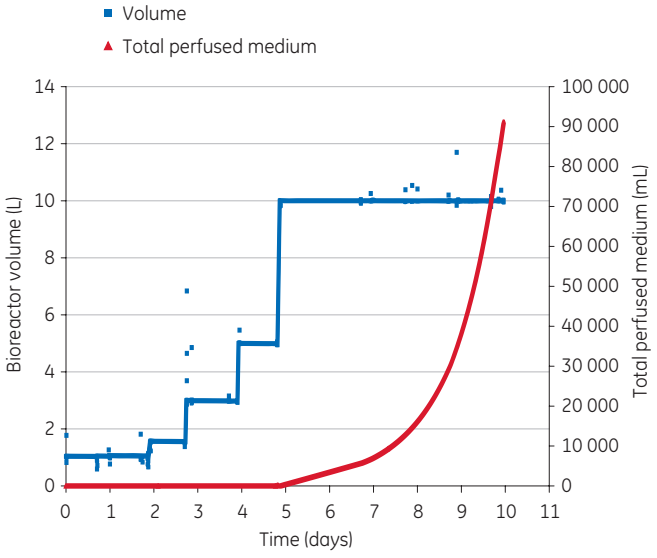

Fig 8. Bioreactor volume and perfused medium over the culture period.

The ReadyToProcess WAVE 25 bioreactor system allowed real-time monitoring and control of culture parameters (Fig 9). For example, dissolved oxygen was shown to be well-controlled in the culture, even at high cell density and large working volume.

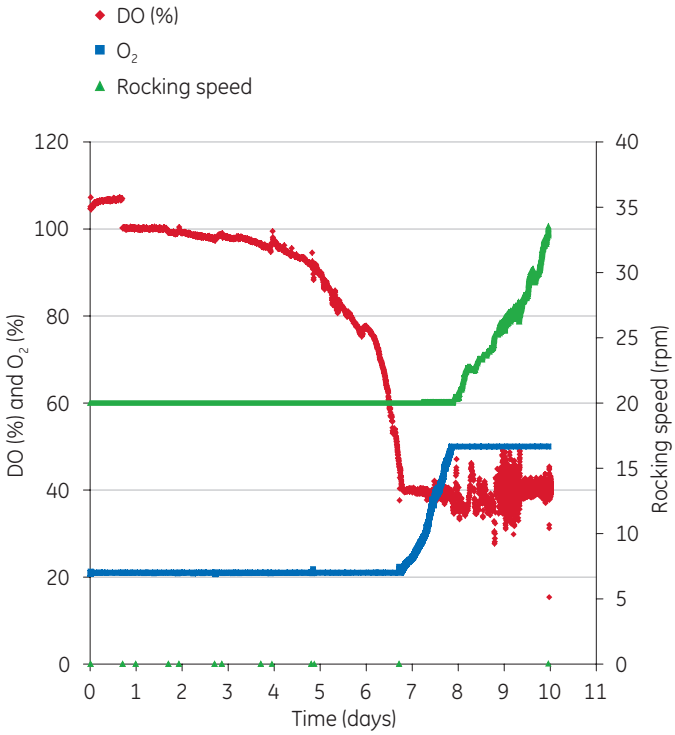

Fig 9. Real-time monitoring and control of culture parameters.

Conclusions

Here, we show the benefits of a perfusion-based strategy in seed culture applications, from the creation of high-density cell banks to seed culture generation. Culturing in a perfusion bioreactor with an integrated cell retention filter enabled cell propagation for cell bank generation to be performed in one, closed system. A separate centrifugation step for cell concentration was not required. The created cell bank exhibited high cell viability when thawed. One vial of cryopreserved cells was sufficient for direct inoculation of a bioreactor culture at 1 L without the need for prior cell expansion in shake flasks. Using perfusion in the bioreactor culture expansion step allowed an efficient one-step seed culture process from a 4.5 mL cryovial to a 10 L culture of 50×10^6 viable cells/mL in only 10 days. The achieved cell density is sufficient, for example, for seeding of a 2000 L production bioreactor at a starting volume of 1700 L and a cell density of 0.3×10^6 cells/mL.

The described method allowed culturing of CHO cells to high cell densities for simplified cell bank generation and efficient seed culture expansion.

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/upstream

29160932 AA 06/2015