ATF Perfusion Technology: Improved Fed-Batch Throughput and Reduced Seed Train Expansion

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Mario Sinani, Tim Erlandson, Shashi Kudugunti, W. Roy Lin* and James Rusche. Repligen Corporation, R&D, Waltham, MA 02453, USA

Summary

A traditional fed-batch process for biologics production requires multiple stages of seed expansion that begins with thawing of small cryo-vial and progresses to a large scale fed-batch production bioreactor. This entire process can last up to 30 days or longer. In contrast, an ATF perfusion system can significantly reduce the number of seed stages as well as fed-batch production duration, leading to a substantial throughput improvement and operating cost reduction.

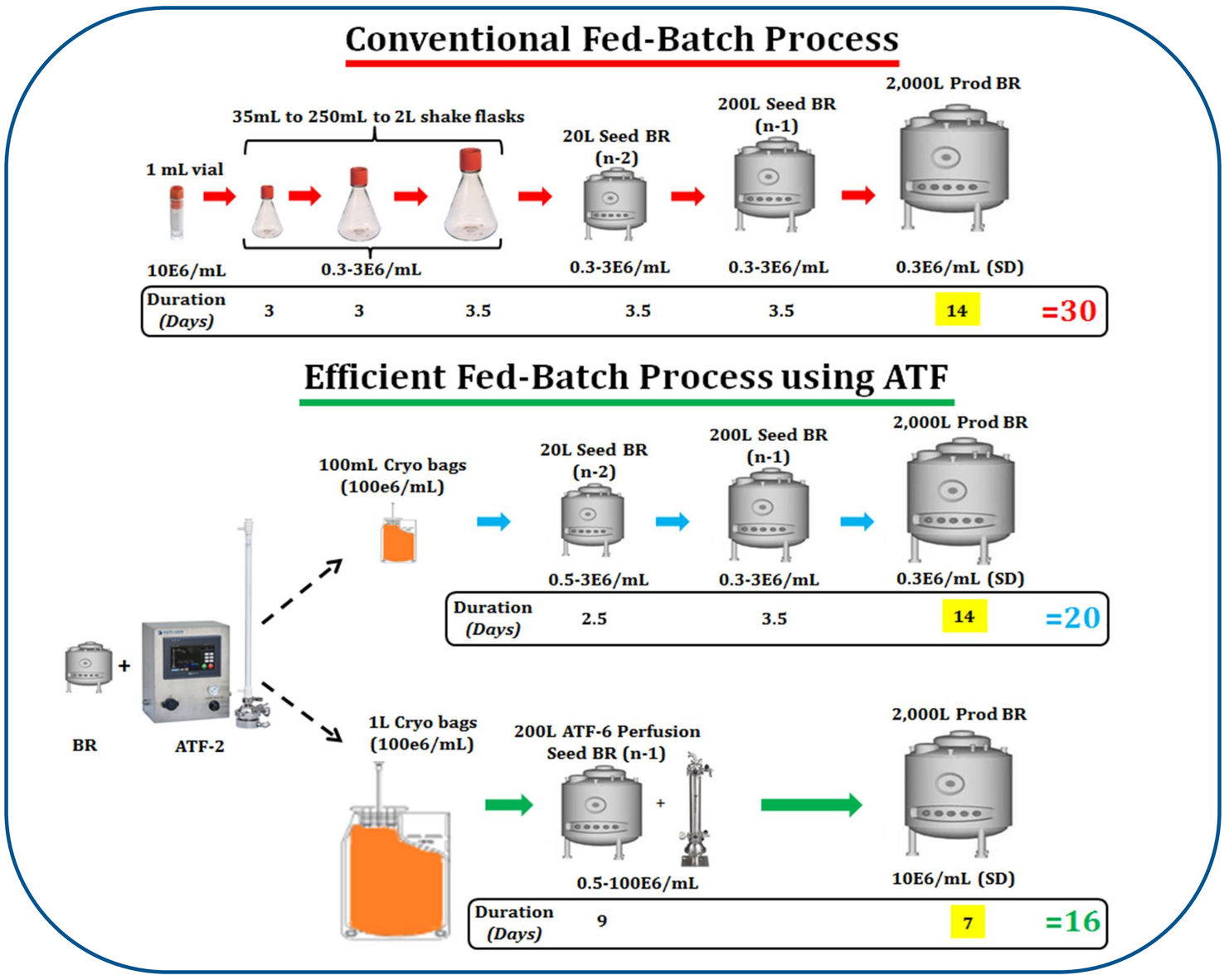
As illustrated below, using an ATF system enabled us to prepare High Density Cryo-Seed-Intermediates (HDCSI) CHO cells in 100mL and 1L cryo-bags as opposed to small vials at low densities in typical cell culture process. Incorporating a pilot-scale ATF-6 perfusion bioreactor (200L) as n-1 in the seed expansion process will allow the seeding of a 2,000L production bioreactor at 10E6 cells/mL, which is greater than 30 times the typical seeding density. This entire process can decrease the duration of seed expansion and production bioreactor culture time by 14 days.

The following figure illustrates the comparison of traditional process with improved shortened process using ATF:

Materials and Method

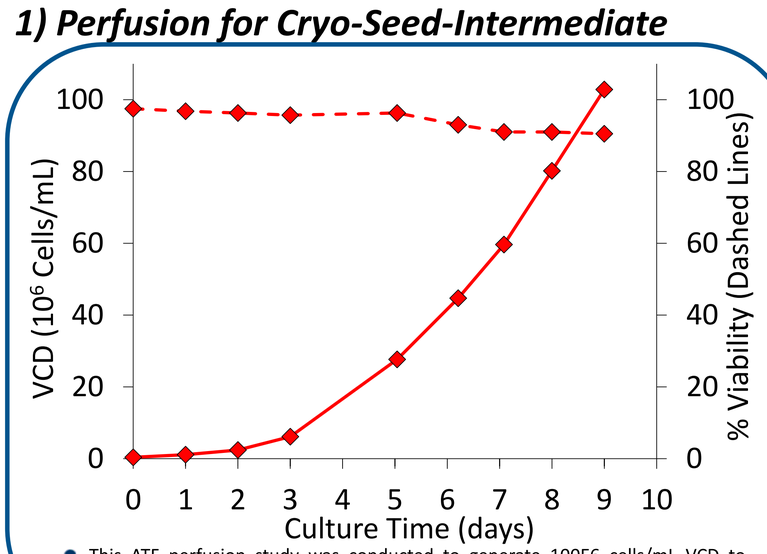
- An industrially relevant mammalian CHO DP12 cell line (ATCC# CRL-12445™) was selected to evaluate the seed train process using an ATF-2 perfusion system. CHO DP12 cells were adapted in house to grow as suspension culture in CD OptiCHO medium supplemented with 100ng/mL LONG®R3IGF-1, and 4mM Glutamax. This cell line is reported to express recombinant human anti-IL-8.
- High density cryo-bags (Charter Medical) were prepared by filling desired volume of conditioned medium at cell density of 100E6 cells/mL. Conditioned medium contains 5% (v/v) DMSO and 0.11% (w/v) carboxymethylcellulose (CMC) (Sigma# C4888) as cryo-protectants. The filled cryo-bags were then placed in freezer cassettes (Custom Biogenics Systems) and the resultant cassettes were positioned in racks. Prior to storing them in liquid nitrogen, these racks were kept in -80°C freezer for 24 hours.
- The ATF-2 system (Repligen) consists of a C24 controller (version 2.5), 0.2µm hallow fiber filter (1mm ID x 60cm L) connected to a diaphragm pump and a vacuum system. All the perfusion runs were operated at 1 LPM ATF rate.
- 1.5L glass bioreactors (Applikon) connected to an ATF-2 perfusion system were used to generate 100E6 cells/mL VCD and also to mimic the n–1 seed-train stage (200-L bioreactor) as mentioned in the figure. 1.5L glass bioreactor (without perfusion) was used to mimic 2,000L production fed-batch bioreactor in the above figure.

Abbreviations: ATF (Alternating Tangential Flow), BR (Bioreactor), CD (Chemically Defined), CMC (carboxymethylcellulose), HDCSI (High Density Cryo-Seed-Intermediates), IVCC (Integral Viable cell Count), LPM (Liter Per Minute), n-1 (Last seed stage before production bioreactor), SD (Seeding Density), and VCD (Viable Cell Density)



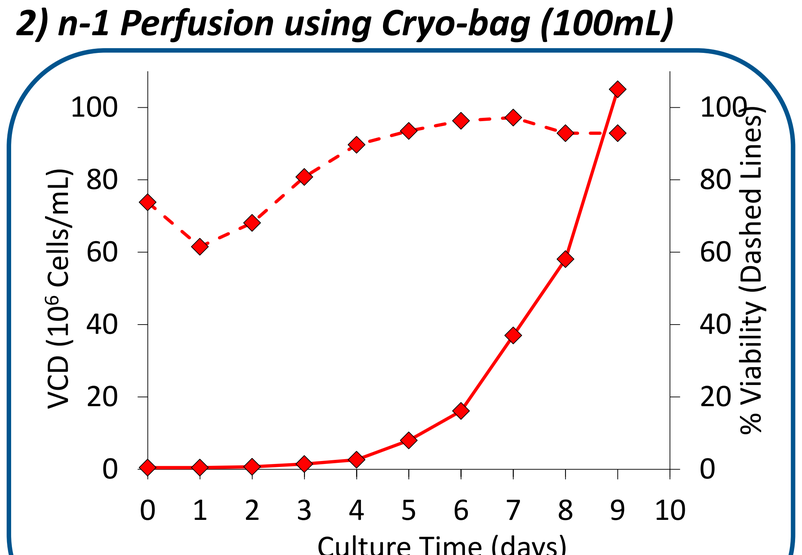
Results

1) Perfusion for Cryo-Seed-Intermediate



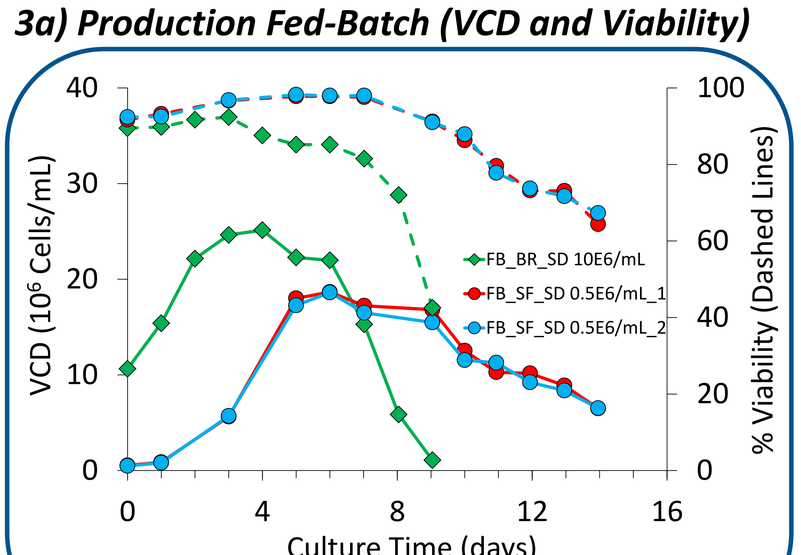
- This ATF perfusion study was conducted to generate 100E6 cells/mL VCD to prepare HDCSI in cryo-bags (5% DMSO and 0.11% CMC were used as in conditioned media as cryo-protectants)

2) n-1 Perfusion using Cryo-bag (100mL)



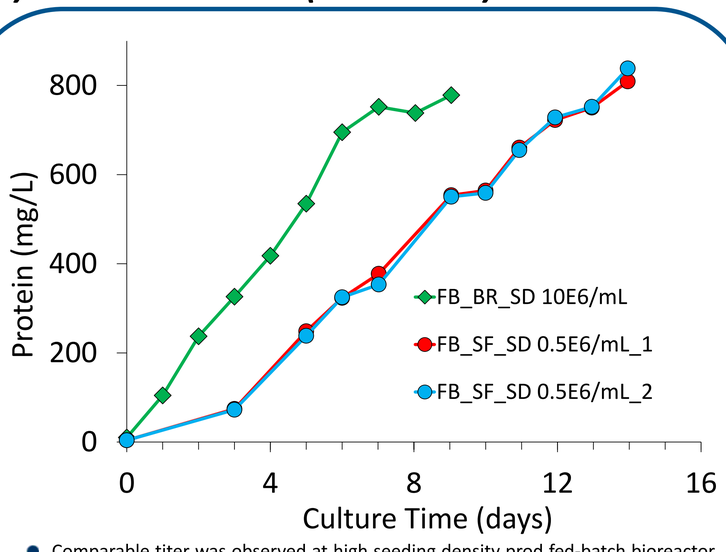
- This is a n-1 perfusion bioreactor and cells inoculated in the reactor were from a high density (100E6 cells/mL VCD) cryo-bag.

3a) Production Fed-Batch (VCD and Viability)



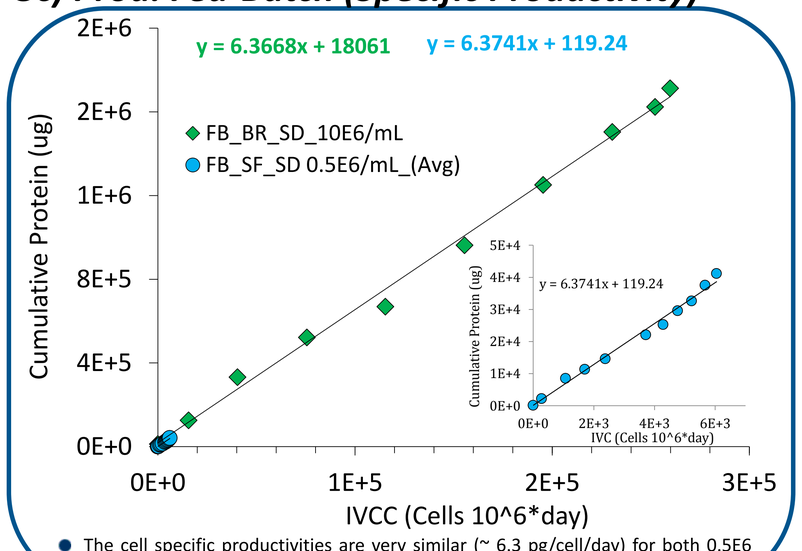
- Production Fed-Batches: Cells were inoculated directly from the above n-1 perfusion bioreactor at 0.5E6 and 10E6 cells/mL seeding densities.

3b) Prod. Fed-Batch (MAb Titer)



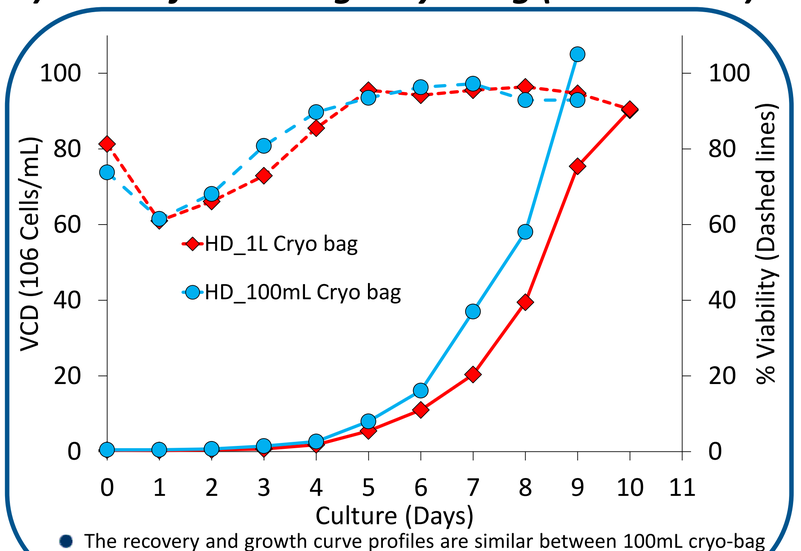
- Comparable titer was observed at high seeding density prod fed-batch bioreactor, in shorter time.

3c) Prod. Fed-Batch (Specific Productivity)



- The cell specific productivities are very similar (~ 6.3 pg/cell/day) for both 0.5E6 and 10E6 cells/mL seeding densities in production fed-batches.

4) n-1 Perfusion Large Cryo-bag (100mL & 1L)



- The recovery and growth curve profiles are similar between 100mL cryo-bag thaw compared to 1L cryo-bag thaw.

Conclusions

- An efficient fed-batch process with significantly reduced seed train and production duration was successfully demonstrated using ATF perfusion technology. The improved process involved the preparation of High Density Cryo-Seed-Intermediates (HDCSI) and n-1 seed expansion using ATF, and high seeding-density fed-batch production bioreactor. This method not only reduced the overall process time by 14 days (including 7-day reduction in production stage), but also decreased the number of seed expansion, which could provide a substantial throughput improvement and operating/equipment cost saving.
- In 50% shorter time (7 vs. 14 days), a comparable protein titer and cell specific productivity was observed at high seeding density fed-batch bioreactor compared to that at regular/low seeding density.
- Cryo-preservation conditions were screened: 5% (v/v) DMSO + 0.11% (w/v) CMC was selected based on post-thaw recovery and growth profiles (data not shown). The post-thaw recovery and growth profiles were similar between 100mL & 1L cryo-bags.