Intensified Seed Expansion and High Productivity Harvest of Fed-batch Cultures Using the XCell™ ATF System

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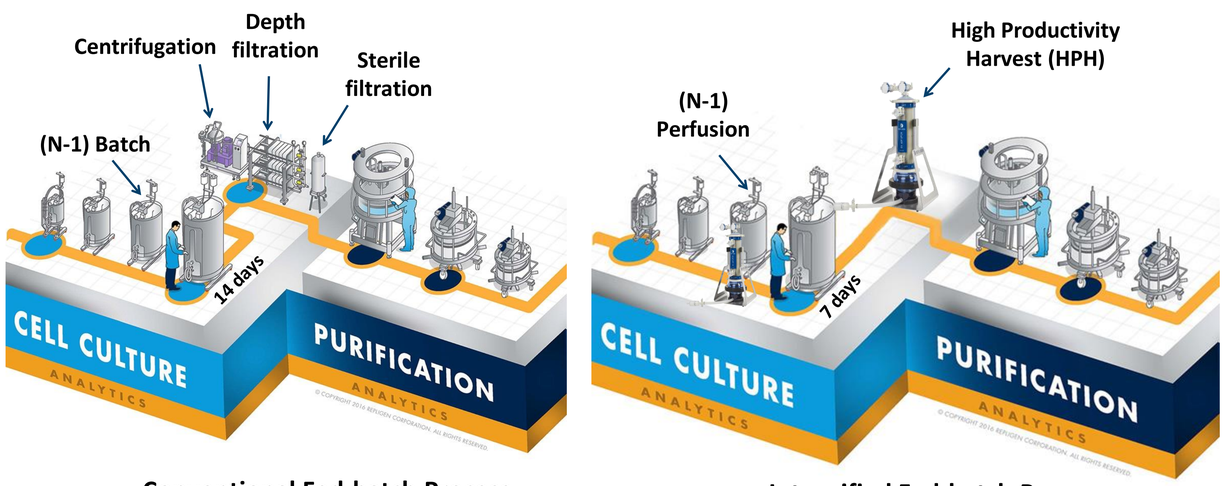
Introduction

A conventional fed-batch (FB) process involves a multi-stage seed expansion and a large footprint of clarification tools to isolate the product of interest from cell culture fluid containing cells and cell debris. The current standard clarification technology of centrifugation and depth filtration often requires open processing and a large footprint. This multi-stage process is cumbersome, difficult to scale, and regularly impacts overall yield. With an increase in demand for production of biotherapeutics, it is essential to simplify the traditional FB process to result in higher yield, shorter process time and smaller footprint.

Repligen developed an intensified FB process using XCell ATF technology. This process involves inoculating the FB bioreactor at high seeding density followed by a novel clarification method, High Productivity Harvest (HPH). This case study presents the N-1 bioreactor in a perfusion mode allowing the FB bioreactor to be inoculated at 20X higher seed density than a traditional process. Increased seed density reduces the FB culture duration by 50%. In addition, implementing the HPH clarification process using the XCell ATF system results in higher VCD and viability due to the replacement of nutrients and removal of toxins. The addition of intensified seed expansion provides a 190% boost in the overall yield in half the process time.

Maintaining high viability throughout the process provides a healthier environment for improved product quality. The entire process is performed as a single step in an integrated, enclosed, bioburden free environment, yielding clarified product ready for downstream purification.

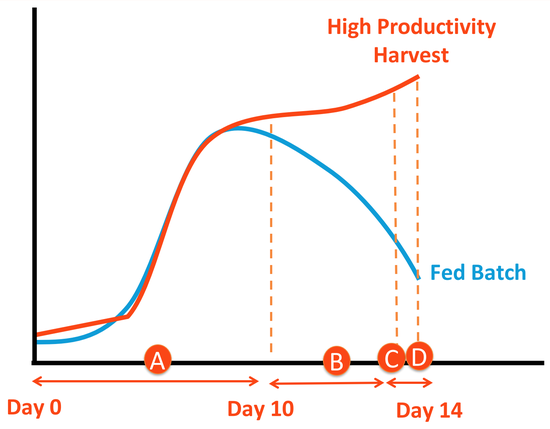
Conventional vs. Intensified Fed-batch Process



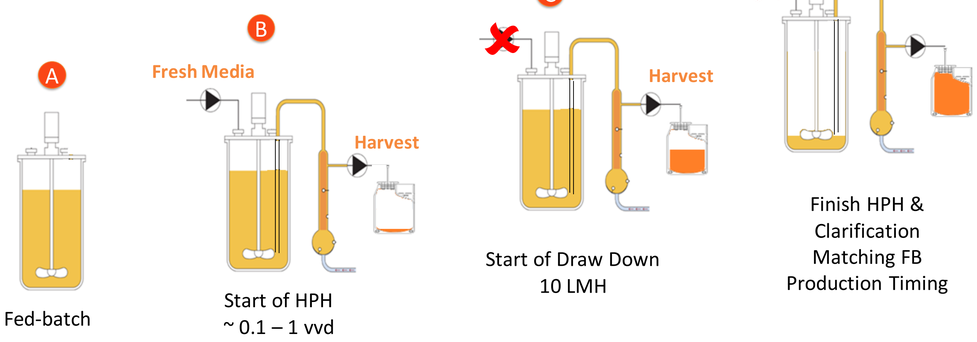
Conventional Fed-batch Process

Intensified Fed-batch Process

Novel High Productivity Harvest Process using XCell™ ATF



The fed-batch process is started per typical procedure (Step A). Once the peak cell density is achieved, Day 10 in this example, fresh media is pumped in at a set flow rate (Step B). As fresh media is pumped in, product harvesting is initiated. On the last day of the run, media addition ends and draw down of the volume in the reactor begins at 10 LMH (Step C, ~4-8 hours). The HPH process is completed to match the original fed-batch production timing.



Materials & Methods

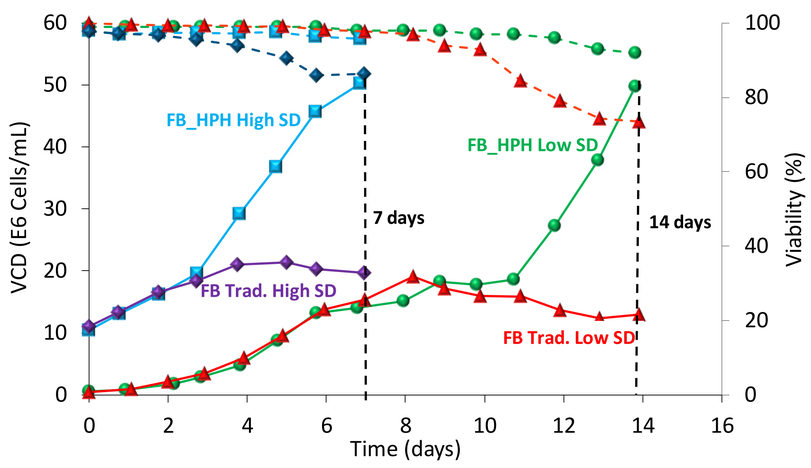
High expressing mammalian CHO-GS, was selected to evaluate the HPH process. These cells were grown in Ex-CELL FB medium supplemented with 100 ng/mL LONG®R^3 IGF-I, 4g/L Poloxamer, and 4 mM Glutamax. This cell line is reported to express IgG, trastuzumab. All cultures were conducted using 3L (*w.v* 1.2L) Applikon glass bioreactors. The cell line and the method for traditional FB process (including feeding strategy) is provided by ATUM[1] & Horizon Discovery[2].

Abbreviations: DD Clarification (Drawdown Clarification, FB (Fed-Batch), IGF-1 (Insulin like Growth Factor 1), HPH (High Productivity Harvest), HCP (Host Cell Protein), LMH (L/m^2*H), SD (Seeding Density), VCD (Viable Cell Density), VVD (Vessel Volume/day)

Components /Conditions	FB Traditional Low SD	FB_HPH Low SD	FB Traditional High SD	FB_HPH High SD
(N-1) Process	(N-1) Batch Duration: 4 days Achieved VCD: 6E6 cells/mL		(N-1) Perfusion Duration: 11 days Achieved VCD: 100E6 cells/mL	
FB Seeding Density	0.5E6 cells/mL		10E6 cells/mL	
FB Duration	14 days		7 days	
XCell™ ATF System	N/A	XCell™ ATF2 PES 0.2µm	N/A	XCell™ ATF2 PES 0.2µm
Feed/ Perfusion Rate	7.5% Feed: Day 3, 5, 7, 9, 11 & 13 ↓ Day 14: Clarification	7.5% Feed: Day 3, 5, 7, 9, 11 & 13 ↓ Day 10: 1 VVD HPH Day 14: DD Clarification	7.5% Feed: Day 0, 1, 2 3, 4, 5, & 6 ↓ Day 7: Clarification	7.5% Feed: Day 0, 1, 2 3, 4, 5, & 6 ↓ Day 3: 1 VVD HPH Day 7: DD Clarification
Drawdown (Flux)	N/A	10LMH	N/A	10LMH
Shear Rate	N/A	2000s^{-1}	N/A	2000s^{-1}

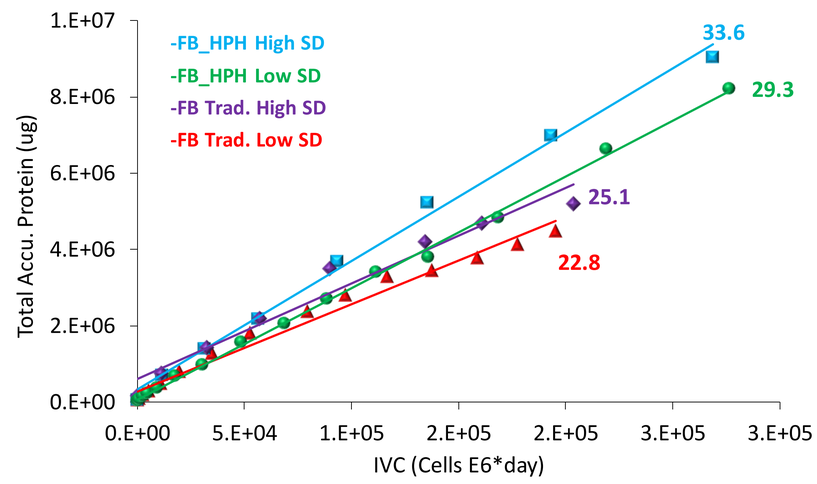
Results

Viable Cell Density & Viability



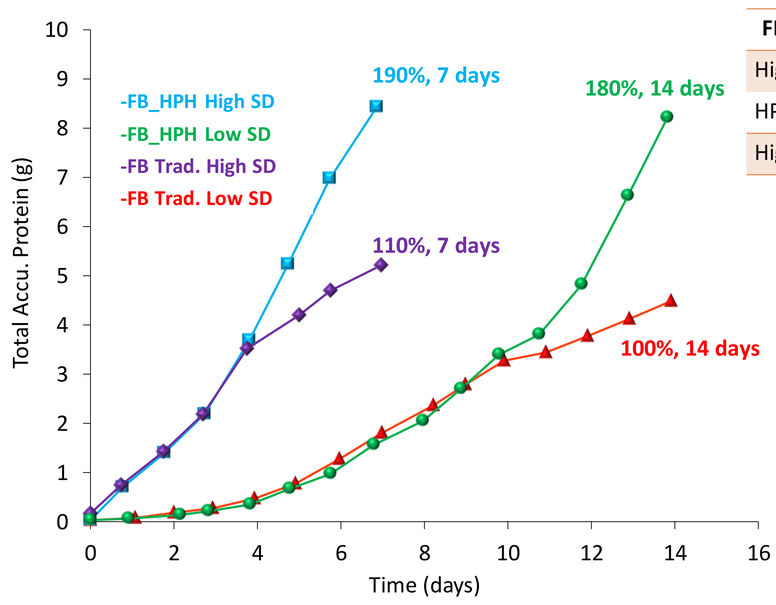
- Enabling the (N-1) bioreactor with perfusion technology achieved a VCD of 100e6 cells/mL in 11 days (Data not shown). This intensified seeding process allowed the FB cultures to be inoculated with 10e6 cells/mL (purple & blue) – 20X higher seed density than FB traditional (green & red)
- Inoculating with a higher SD shortened the culture duration by 50% for both the FB traditional and HPH processes
- Implementing HPH method increased the VCD 3 fold (blue & green) compared to the FB traditional cultures (purple & red)

Cell Specific Protein Productivity



- Employing a high seed density culture (purple & blue) resulted in 10-15% higher protein productivity compared to low seed density FB processes (red & green)
- Implementing the HPH process increased the cell specific protein productivity by 30% (blue & green)
- Sustaining high protein productivity translates to better product quality

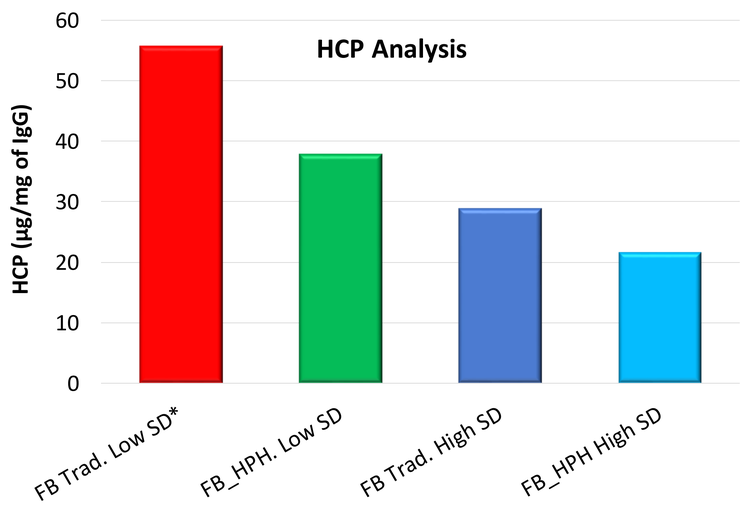
Total Accumulated Protein



FB Conditions	Benefit Achieved
High SD	↓ FB duration by 50%
HPH	↑ Protein yield by 90%
High SD & HPH	↓ FB duration + ↑ Protein yield

- High SD shortens the production FB culture duration and allows facilities to process more batches/year
- Combining both applications (High SD and HPH) enables increased efficiency and productivity of an upstream facility
- A 4-day HPH process at 1VVD increases the total clarified volume 5X as compared to a traditional FB process . Performing UF to concentrate the harvest material OR continuous capture chromatography can mitigate this concern

Product Quality



- HCP values are lower for cultures where HPH is implemented
- Cultures with high viability produced less HCP, indicating less process related impurities for further downstream applications

Notes:

- For all samples, HCP analysis is performed on harvest pool at the end of the clarification
- * FB Trad. Low SD sample is clarified using centrifugation

Conclusions

A production bioreactor seeded with a high cell density and operated with the HPH process results in significant increase in product yield, improved process efficiency and smaller operational footprint. The productivity and process benefits include:

- Up to 190% boost in protein production
- 50% reduction in production culture duration
- Eliminate centrifugation and depth filtration equipment and operations
- Healthier culture translates to lower HCP and lower process related impurities
- Closed system and single-step process
- Harvested material is 0.2µm filtered and ready for purification

1: ATUM, formerly DNA2.0, offers an integrated pipeline of tools including gene design, optimization and synthesis, expression vectors, and platforms for protein and strain engineering and production. ATUM exploits the dependence of biological activity on well-designed sequences. ATUM's tools and solutions are fueling the transformation of biology from a discovery science to an engineering discipline. By collaborating with clients, ATUM accelerates breakthroughs and moves research further faster. The company is privately held and is headquartered in Newark, California. For more information, please visit https://www.atum.bio.
2: Horizon Discovery Group plc (LSE: HZD) ("Horizon") is a world leader in gene editing and gene modulation technologies. Horizon designs and engineers' cells using its translational genomics platform, a highly precise and flexible suite of DNA editing tools (rAAV, ZFN, CRISPR and Transposon) and, following the acquisition of Dharmacon, Inc., its functional genomics platform comprising gene knockdown (RNAi) and gene expression (cDNA, ORF) tools, for research and clinical applications that advance human health. Horizon's platforms and capabilities enable researchers to alter almost any gene or modulate its function in human or mammalian cell lines. Horizon is headquartered in Cambridge, UK, and is listed on the London Stock Exchange's AIM market under the ticker "HZD." http://www.horizondiscovery.com