Fall 11-2-2015

Tools for process intensification upstream and continuous processing downstream

James Rusche
Repligen Corporation

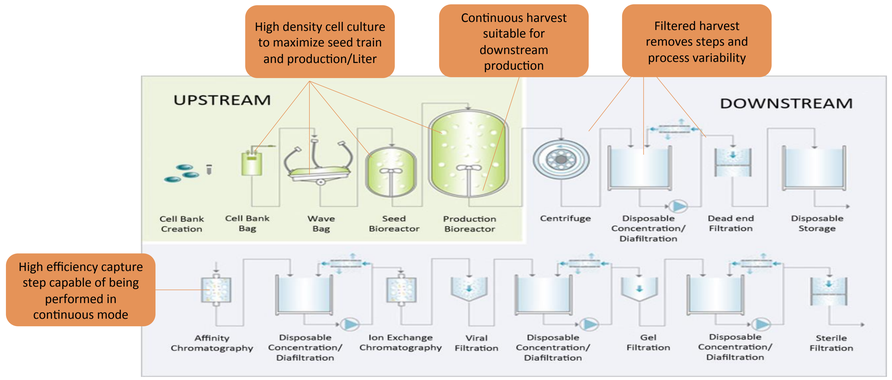
Tools for intensification of cell culture production and continuous downstream processing

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

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

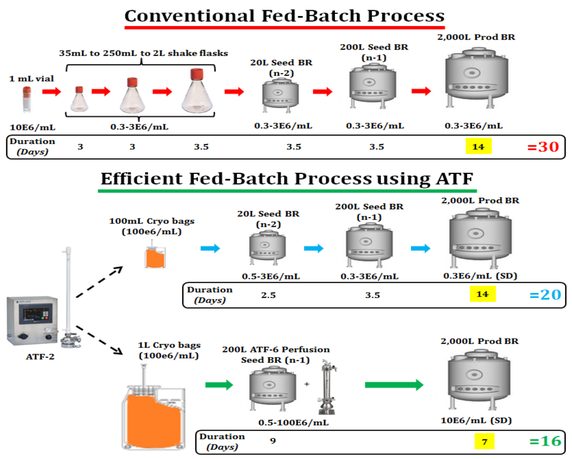
Summary

The intensification of cell culture production and continuous downstream processing are two important features of many new biomanufacturing schemes. Additionally, perfusion of cell culture bioreactors using an alternating tangential flow (ATF) device can increase viable cell density 5-10 fold and can be used to continuously harvest product suitable for immediate downstream processing. This ATF process is used in current commercial processes. More recently, perfusion has been applied to high density cell banking (Tao 2011 Biotechnol.Prog., 27: 824–829) and preparation of N-1 cell inoculum (Yang 2014 Biotechnol. Prog., 30:616–625) to greatly accelerate production turnover in fed batch processes. The capture of a continuous process feedstream is facilitated by use of prepacked columns of uniform performance. Process intensification may include rapid load using low aspect ratio columns. To maintain an aseptic environment we are developing gamma irradiated proteinA affinity media that can operate for long periods with low bioburden. Together these tools of perfusion and aseptic capture can facilitate the engineering of a more productive manufacturing process.

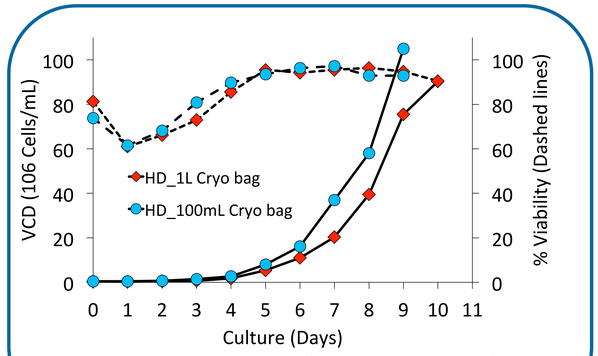


Upstream: ATF Perfusion Technology to Intensify Fed Batch

- 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 with 0.2 µm hollow fiber microfilter (Repligen). 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% DMSO and 0.11% (w/v) carboxymethylcellulose (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.
- 1.5L glass bioreactors (Applikon) equipped with an ATF-2 perfusion system (Repligen) 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 bioreactor was used to mimic 2,000L production fed-batch bioreactor in the figure.

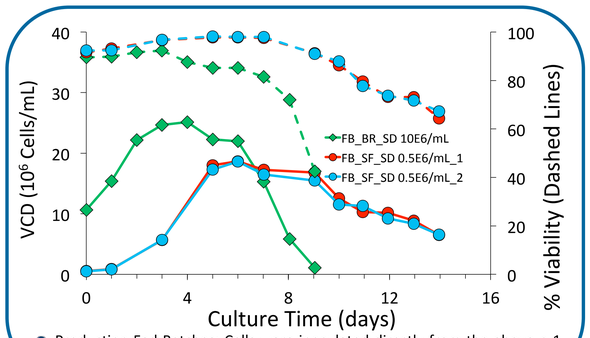


n-1 Perfusion with Large Cryo-bag (100mL & 1L)



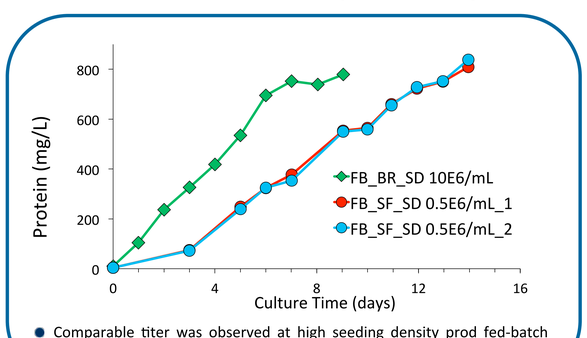
- The recovery and growth curve profiles are similar between 100mL cryo-bag thaw compared to 1L cryo-bag thaw.

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.

Production Fed-Batch (MAb Titer)

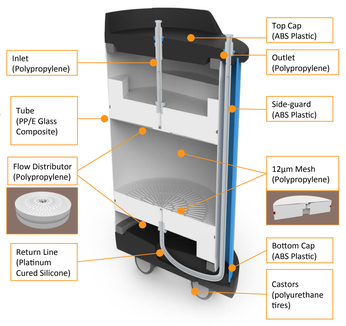


- Comparable titer was observed at high seeding density prod fed-batch bioreactor, in shorter time. Cell specific productivity was unchanged

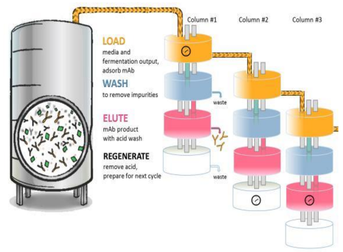
Downstream: pre-packed columns for aseptic chromatography

OPUS

Critical Features:
- Robust, reproducible separation
- GMP ready documentation
- Scalable from 5-60 cm diameter



Separation performance at low aspect ratio



Performance at low bed height

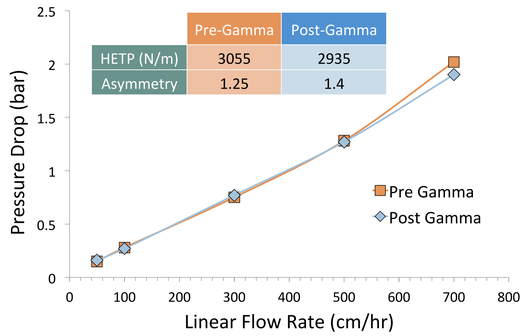
Diameter (cm)	10		20		30	
Bed Height (cm)	5	10	5	10	5	10
HETP (N/m)	3847	2258	3313	2580	2489	3100
Asymmetry	1.28	1.43	1.4	1.2	1.24	1.21

OPUS columns suitable for continuous antibody harvest

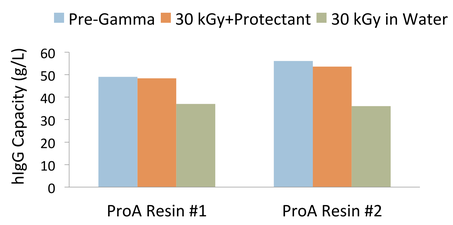
OPUS materials stable to 25 KGy Gamma radiation
- Tensile strength and elasticity unchanged
- Pressure tolerance > 5 bar
- No bioburden for 60 d of operations

Procedures to preserve performance of antibody affinity agarose resin
- Protectant additive to column buffer identified
- Low leaching
- Stable to multicycle use
- Minimal loss of resin capacity

Packed Column Performance



- Protein A Agarose packed in a 10 X 20cm column : Comparable HETP, asymmetry and pressure drop was observed following gamma irradiation



	% Yield	Log HCP	Log DNA	Residual ProA
Pre-Gamma	100%	2.9	3.7	1.2
30kGy - Gamma	97%	2.6	3.6	3.0

Summary and Conclusions

- **Perfusion with ATF can eliminate many steps in seed train and accelerate fed batch production**
- **Perfusion with ATF can produce a continuous harvest feedstream suitable for direct chromatography separation**
- **Prepacked columns can perform efficiently in low aspect ratio multi-column formats**
- **Prepacked prA chromatography media can be prepared gamma irradiated and maintain column and resin performance**

Repligen Corporation | 41 Seyon Street, Building 1, Suite 100 | Waltham, MA 02453 | www.repligen.com