Tools for intensification of cell culture production and continuous downstream processing

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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 seeding (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 use immediate downstream processing. This ATF process is used in current commercial processes. More recently, perfusion 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 at suspension culture in CD OptiCHO medium supplemented with 100mg/mL LONG®33G-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 containing 5% DMSO and 0.11% (w/v) carboxymethylcellulose (Dignam 4888) 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 2.00L production fed-batch bioreactor in the figure.

Production Fed-Batch (VCD and Viability)

Production Fed-Batch (MAB Titer)

Downstream: pre-packed columns for aseptic chromatography

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

Packed Column Performance

Separation performance at low aspect ratio

Procedures to preserve performance of antibody affinity agarose resin

- Protectant additive to column buffer identified
- Low leaching
- Stable to multicyle use
- Minimal loss of resin capacity

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

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