Mitigation of Protein Retention in Perfusion Process and Effect of Filter Pore Size

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Abstract

Membrane based perfusion devices are the most widely used technology for performing high cell density (HD) perfusion processes with efficient cell retention. However, one potential complication encountered with filter technology is protein retention. It has been reported that XCell™ Alternating Tangential Flow (ATF) demonstrates less protein retention compared to TFF-based perfusion systems, due to XCell™ ATF's uninterrupted pressure and exhaust cycles which create a continuous self-cleaning process (backflush) through filter pores (Karst, et al. 2016; Clincke, et al. 2013, Part I & Part II). The continuous backflush in XCell™ ATF is assumed to mitigate rate of filter fouling followed by protein retention. Several parameters, such as antifoam, cell debris, media components and protein related issues either alone or in combination, could lead to protein retention in an HD perfusion culture. Despite the various levels of protein retention frequently observed in perfusion processes, the potential root cause might be different from one process to another. The objective of this project is to investigate the root cause of protein retention and provide troubleshooting guidance.

Performing a series of perfusion bioreactor and shake flask experiments identified cell lysis as the most important parameter influencing protein retention. Furthermore, particle size analysis on HD perfusion cell culture samples revealed a special attention, as the size of cell debris is similar to pores of 0.2µm filter. To gather further evidence, HD perfusion cultures were conducted using hollow fibers with different pore sizes of 750kD MWCO and 0.65µm and results indicated that 0.65µm hollow fiber demonstrated slight improvement in protein retention at end of perfusion culture whereas, 750kD performed similar to a 0.2µm hollow fiber.

Material & Methods

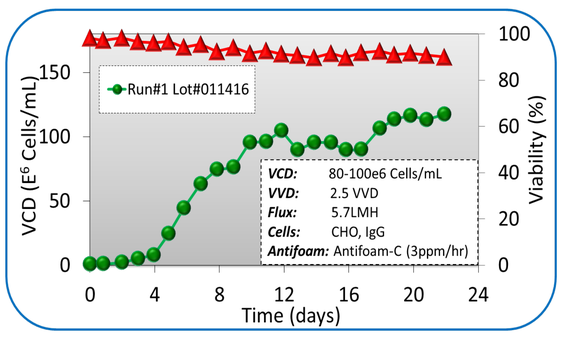
- An industrially relevant mammalian CHO DP12 cell line (ATCC# CRL-12445™) was selected to evaluate and troubleshoot protein retention concerns. These cells were adapted to grow as suspension culture in CD OptiCHO medium supplemented with 100 ng/mL LONG®R^3 IGF-I, and 4 mM Glutamax. This cell line is reported to express recombinant human anti-IL-8 antibody.
- All perfusion cell culture processes were conducted using 1.5 L glass bioreactors (Applikon) equipped with an XCell™ ATF-2 perfusion system containing a hollow fiber cartridge. The ATF 2 system was operated by a C24 controller (version 2.5), with a perfusion rate of 2.5 vvd, shear rate of 2000s^{-1} and a flux of 5.7 LMH throughout the run.

Abbreviations: BR (Bioreactor), CD (Chemically Defined), IGF-1 (Insulin like Growth Factor 1), LDH (Lactate Dehydrogenase), LMH (L/m^2*H), LPM (Liter/Minute), VCD (Viable Cell Density), VVD (Vessel Volume/day)

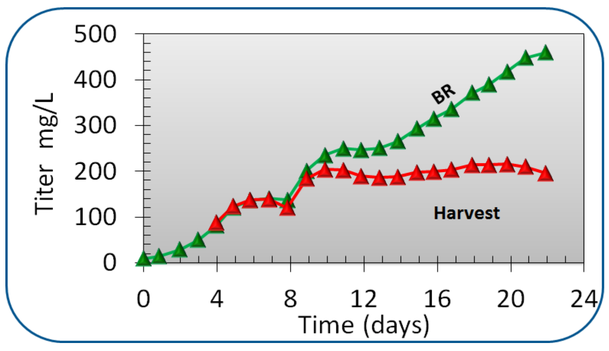
Results

Protein Retention vs Protein Recovery

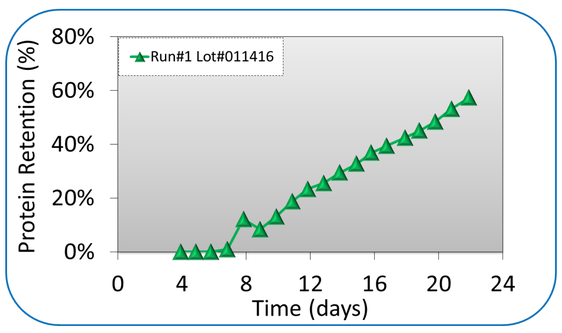
VCD and Viability



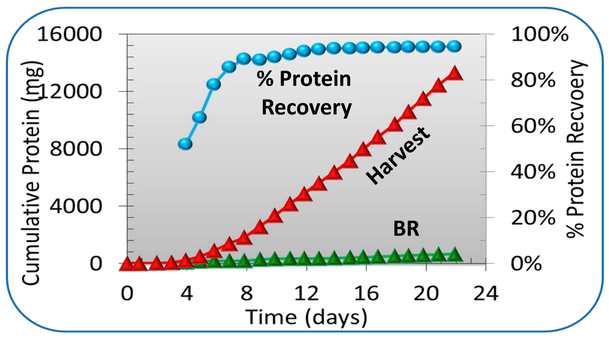
Protein Concentration



% Protein Retention



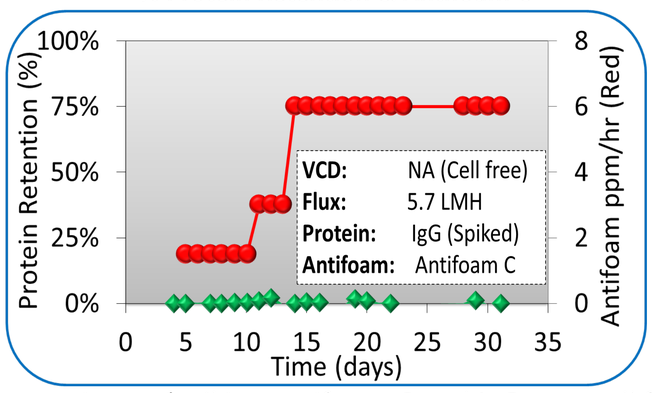
% Protein Recovery



Even at 60% protein retention, the total protein recovered in the harvest is > 95% (blue circles) and the total protein retained in BR is negligible

Effects of Antifoam C on Protein Retention

% Protein Retention & Antifoam C



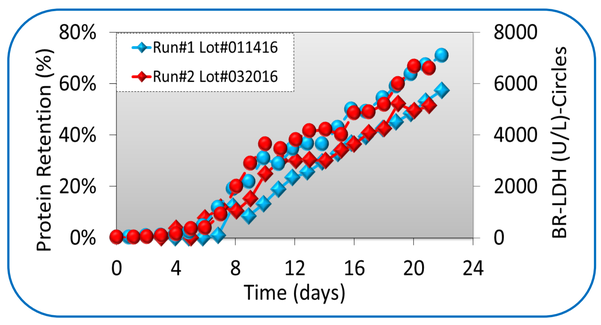
Data from above experiment (spiking antifoam-C and IgG to a cell free perfusion system) indicated that Antifoam-C has no impact on protein retention.

Results (continued)

Effects of Cell Lysis on Protein Retention

LDH measurements were used as a surrogate marker for quantification of cell lysis

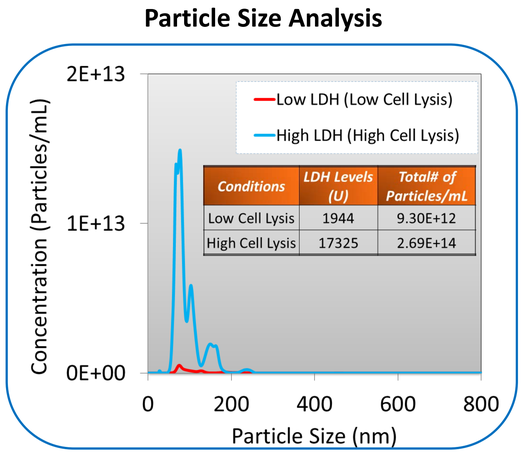
% Protein Retention and LDH



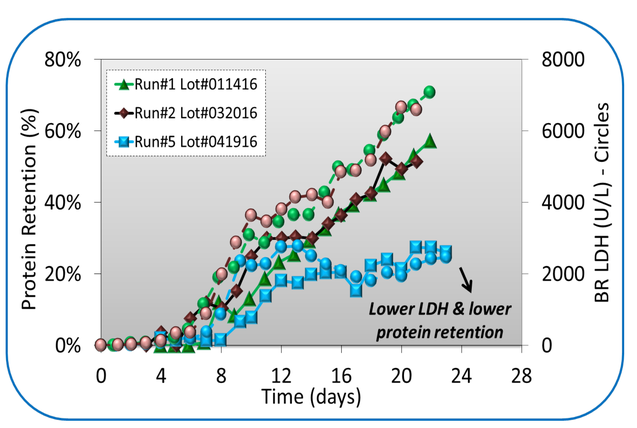
Protein retention and LDH profiles correlated well in both test runs, indicating that cell lysis (cell debris) plays a crucial role in protein retention

Particle Size Analysis

Particle Size Analysis



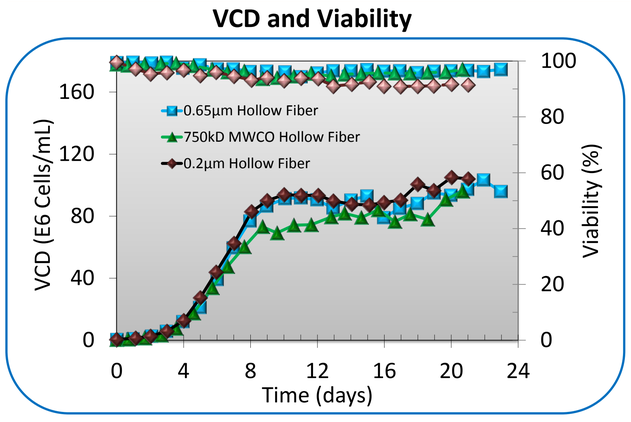
Low LDH → Decreased Protein Retention



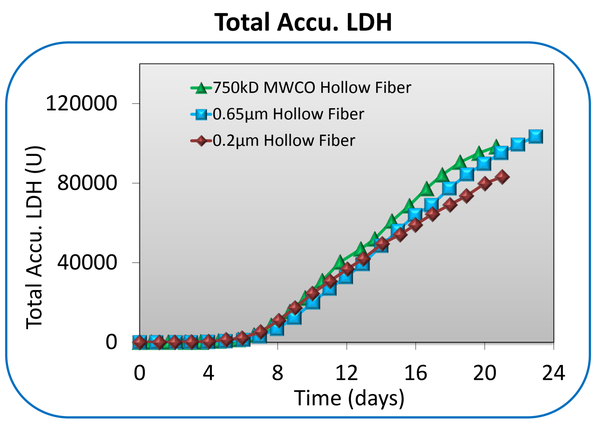
- Results from the particle size analysis suggested that cell lysis leads to cell debris with resultant particle sizes around 70 – 200 nm, similar to filter pore size
- A higher number of particles was observed in the sample containing high LDH levels indicating a correlation
- Minimizing the cell lysis by reducing the usage of micro-sparger (low LDH levels) mitigated the protein retention

Pore size Vs Protein Retention

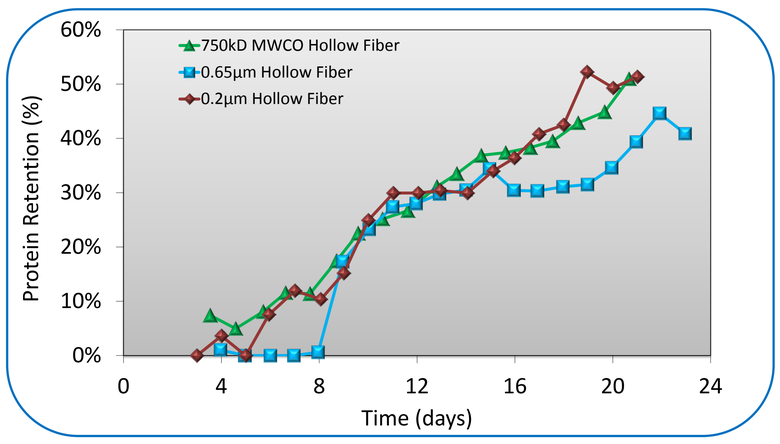
VCD and Viability



Total Accu. LDH



Protein Retention



- Similar VCD and total accumulated LDH levels indicate that that bioreactor culture characteristics were maintained similar across all perfusion cultures.
- The hollow fiber with 0.65µm pore size demonstrated slightly improved protein retention at end of perfusion culture compared to 0.2µm and 750kD MWCO hollow fibers.

Conclusions

- At flux of 5.7 LMH, antifoam C alone in cell free medium, has no impact on protein retention in a 30-day cell free perfusion run.
- Correlation between cell lysis (LDH levels) and protein retention profiles indicates cell lysis as a potential root cause.
- Pore size 0.2µm and 750kD hollow fibers performed similarly in terms of protein retention whereas, 0.65µm demonstrated slightly better protein retention.

References:

1) Daniel J. Karst *et al,* Characterization and comparison of ATF and TFF in stirred bioreactors for continuous mammalian cell culture processes. *Biochemical Engg. Journal, Volume 110, 15 June 2016, Pages 17–26.*

2) Clincke MF *et al*, Very high density of CHO cells in perfusion by ATF or TFF in WAVE bioreactor™. Part I: Effect of the cell density on the process. Part II: Applications for antibody production and cryopreservation. *Biotechnol Prog. 2013 May-Jun;29(3):754-67.*