Mitigation of Protein Retention in High Density Perfusion Process

Shashi Kudugunti, Roy Lin* and James Rusche. Repligen Corporation, Waltham, MA 02453, USA

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Abstract

Protein retention, as defined below, is one potential complication encountered in high density (HD) perfusion processes using filter-based cell retention devices. I n contrast to TFF-based perfusion system, it has been reported that XCell™ Alternating Tangential Flow (ATF) demonstrates less protein retention due to its uninterrupted pressure and exhaust cycles creating a continuous self-cleaning process (backflush) through filter pores (Karst, *et al.* 2016[1]; Clincke, *et al.* 2013, Part I & Part II[2]). The continuous backflush in ATF is assumed to decrease rate of filter fouling and reduce the potential for protein retention.

$$\%\ Protein\ Retention = \left[1 - \left(\frac{Harvest\ Protein\ Conc.}{BR\ Protein\ Conc.}\right) \times 100\right]$$

In an HD perfusion culture, a number of factors, either alone or in combination could lead to protein retention including presence of antifoam, accumulation of cell debris, and protein specific issues. Even though some level of protein retention is observed in most perfusion processes, the potential root cause may be different from one process to another. The objective of this work is to identify the root cause of protein retention and provide troubleshooting guidance.

A number of XCell™ ATF perfusion and shake flasks experiments were conducted to determine the impacts of antifoam, cell lysis and the presence of cell debris on protein retention. In addition, particle size analysis was also conducted on HD perfusion cell culture samples to determine size distribution and concentration of cell debris. In summary, results suggest that cell debris is a potential root cause for protein retention, while Antifoam C had no major impact. Furthermore, reducing cell debris in XCell™ ATF perfusion culture also decreased protein retention.

Material & Methods

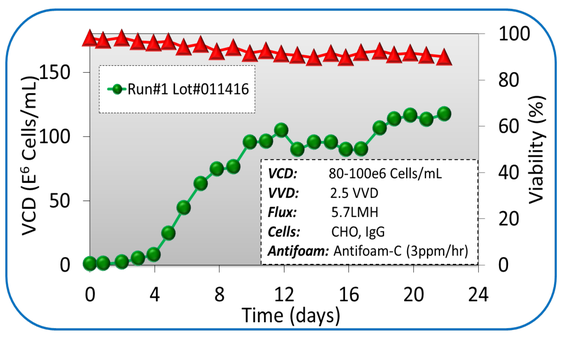
- An industrially relevant mammalian CHO DP12 cell line (ATCC# CRL-12445™) was selected to evaluate and troubleshoot protein retention problems. These cells were adapted to grow as suspension culture in CD OptiCHO medium supplemented with 100 ng/mL LONG®R³ IGF-I, and 4 mM Glutamax. This cell line is 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 0.2 µm PES hollow fiber (Repligen). The ATF 2 system was operated by a C24 controller (version 2.5), with a perfusion rate of 2.5 vvd, an ATF rate of 0.9 PLM 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/m²/H), LPM (Liter/Minute), VCD (Viable Cell Density), VVD (Vessel Volume per day)

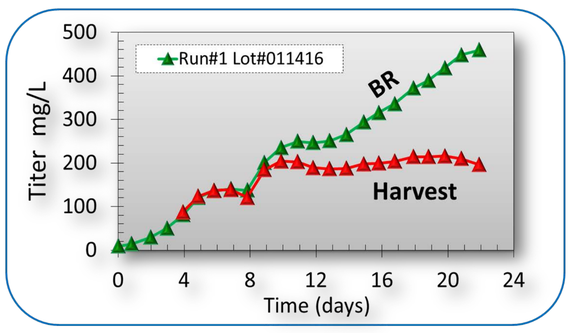
Results

Protein Retention vs Protein Recovery

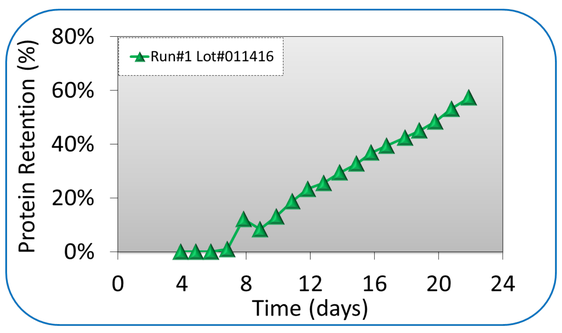
VCD and Viability



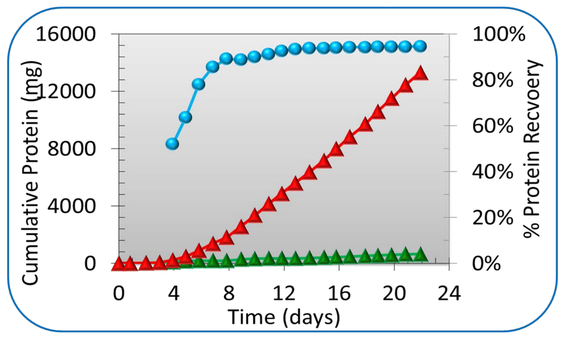
Protein Concentration



% Protein Retention



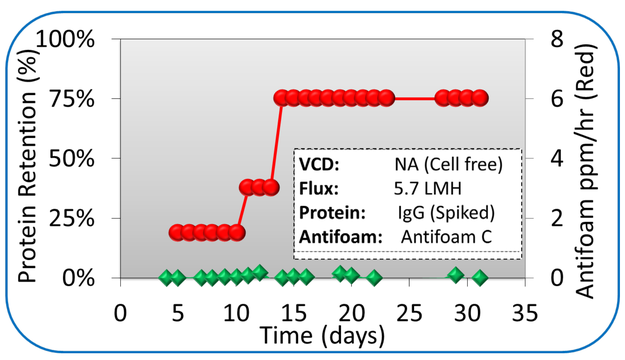
% Protein Recovery



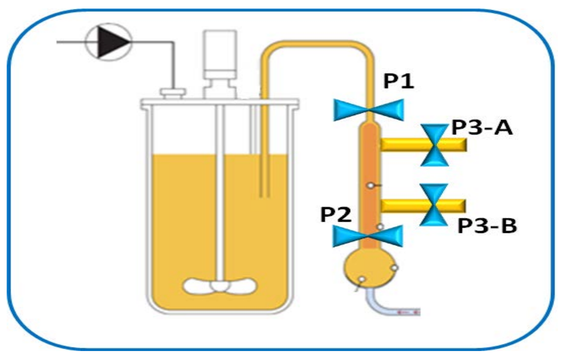
Even at 60% protein retention, the total protein recovered in the harvest is > 95% (blue circles) and the total protein retained in BR is negligible

Effects of Antifoam C on Protein Retention

% Protein Retention & Antifoam C

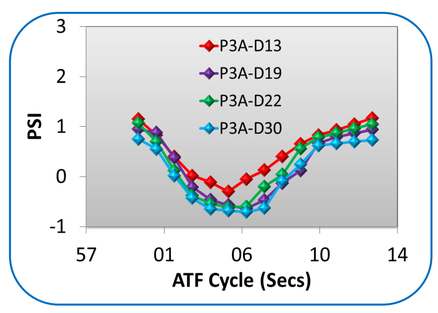


Location of Pressure Sensors

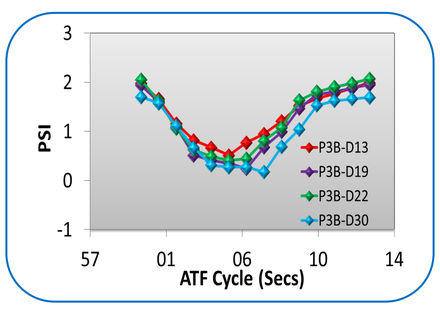


Results (continued)

Top Permeate Pressure



Bottom Permeate Pressure

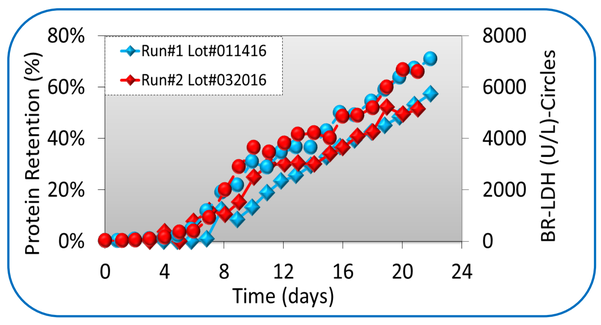


Based on the above experiment, antifoam C alone has no impact on protein retention (based on protein conc.) and filter fouling (based on permeate pressure)

Effects of Cell Lysis on Protein Retention

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

% Protein Retention and LDH



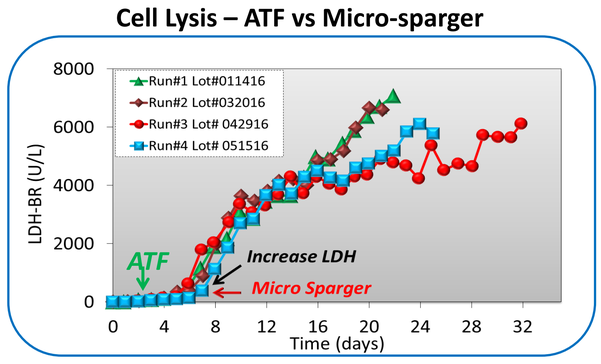
Protein retention and LDH profiles correlated well in both test runs, indicating that cell lysis (cell debris) plays a crucial role in protein retention

What Causes Cell Lysis?

Antifoam C Toxicity

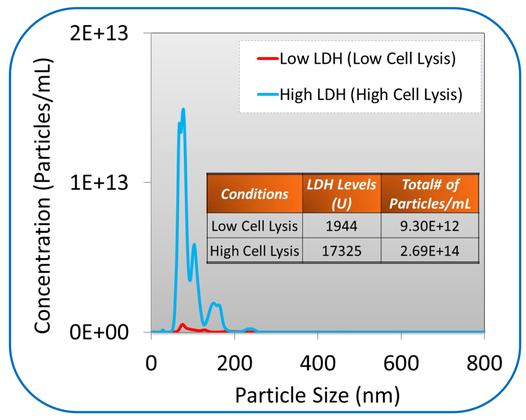
The antifoam C toxicity result suggested that there is no impact on cell growth/viability and cell lysis based on LDH levels (data not shown)

Cell Lysis – ATF vs Micro-sparger

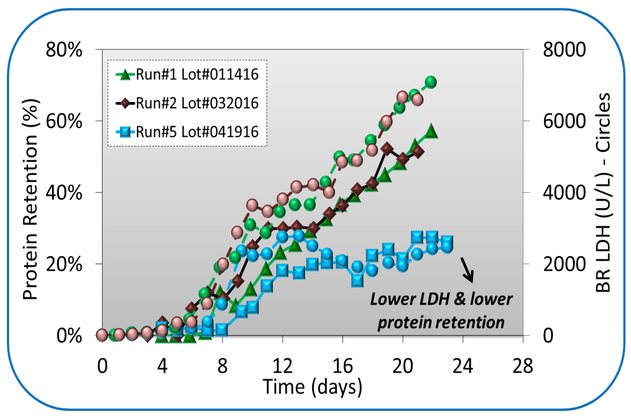


Increased LDH levels were observed only after turning on the micro-sparger (Day 06) and not from ATF (Day 03), indicating that high gas flow through the micro-sparger is a potential cause for cell lysis or high LDH

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
- Reducing flow rates through micro-sparger decreased cell lysis (low LDH levels) followed by decreased protein retention

Conclusions

- At flux rate of 5.7 LMH, antifoam C alone in cell free medium, ha no impact on protein retention and filter fouling in a 30-day cell free perfusion run.
- Correlation between cell lysis (LDH levels) and protein retention profiles indicates cell debris as a potential root cause.
- High LDH levels were observed only after turning on the micro-sparger, indicating that cell lysis followed by protein retention corresponds to high flow rates of aeration through the micro-sparger.

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.*