BIOTECH

Application Note

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Selection and Design of Depth Filtration Process to Minimize Cell Lysis and Particle Counts

Contents

1 Abstract

Clarification using depth filtration is widely adopted in the manufacturing of monoclonal antibody (mAbs) as the first step to remove the cells and other colloids to prepare cell culture harvest for downstream chromatography and purification. This is a critical step with a strong impact on product recovery and subsequent downstream purifications steps, yet the impact of depth filtration on cell culture harvest has remained unclear, which creates confusion when selecting the right depth filters and clarification set-up for optimum clarification process. This work demonstrates the use of lactate dehydrogenase (LDH) assay and a particle counter to examine the efficiency of clarification and quality of Pall's Stax™ mAx platform depth filters. The results show that double-layer depth filters consisting of two layers of media in different size regime achieve lower LDH activity, less particle counts, and longer filter life than single-layer depth filters in a similar size regime. The results also demonstrate that clarification using both primary and secondary depth filtration further enhances the cleanliness of cell culture harvest, production yield, and filter life. We have generated an illustrative model to help explain the interaction between the cells the depth filter media, providing guidance for future design of clarification steps and selection of depth filters.

2 Introduction

The production of both mAbs and viral vectors require a clarification step to prepare harvest for downstream chromatography and purification steps. Clarification using depth filtration is widely adopted in the industry since depth filters are particularly well-suited for removing contaminants, such as cells, cellular debris, nucleic acids, host-cell-derived aggregates, and other colloidal compounds, that foul downstream processing steps and reduce product recoveries[1]. Titers and purification yields when manufacturing mAbs have improved significantly during the past decades, yet improper design of depth filtration processes and the selection of depth filters have big impacts on product recovery and subsequent purification steps. Furthermore, viral vector manufacturing still has a long way to go, borrowing experience from the manufacturing of mAbs. Researchers need to pay special attention to the design and selection of depth filtration for the more complicated viral vector production.

Turbidity and filter capacity/filter life have been the industry standard to evaluate the depth filtration process and depth filters[2, 3, 4, 5]. As technology advanced, researchers started to explore the interaction between cell culture harvest and depth filter media. Yu[6] *et al* examined the binding of high molecular weight species (HMWs) and low molecular weight species (LMWs) with depth filter media and have identified different degree of HMWs and LMWs binding on various depth filter media, providing guidance on the control of HMW and LMW in mAb manufacturing. Nejatishahidein[7] *et al* explored the host cell protein removal efficiency of Pall's depth filters, concluding the different binding degree of proteins with different electrostatics. However, the interaction between cell culture harvest and depth filter media remains unclear. This work aims at providing guidance to facilitate the decipher process and provide preliminary research results to demonstrate the selection of depth filters. Clarification using various Pall depth filters was performed to clarify cell culture harvest. Fractionate samples and pool samples were collected. LDH assay and a particle counter were used to evaluate samples collected. The results were analyzed and an illustrative model was generated.

3 Materials, Experiments, and Methods

3.1 Materials

3.1.1 Reagents and Solutions

Chinese Hamster Ovary (CHO) C38 for cell culture are from Pall Corporation. LDH assay is from BioVision♦. All other chemicals are from VWR♦.

3.1.2 Cell Culture and Antibody Production

Proprietary CHO cell lines were used to produce monoclonal antibody (mAb1). CD FortiCHO♦ media supplemented with 1X Gibco♦ HT supplement and 2X GlutaMAX♦ was used for cell culture. A bioreactor and flask were used to produce mAbs in two separate batches described below. Cell viability was measured using a Vi-CELL♦ XR cell viability analyzer; phosphate-buffered saline (PBS) was used as control.

For the first batch, the Allegro™ STR 50 L single-use bioreactors were run in fed-batch mode to produce mAbs. Cell culture and suspensions were harvested on Day 14 post inoculation in a production bioreactor with >90% cell viability and 22.87 x 106/mL cell density.

For the second batch, flask incubation was used to produce mAbs. Cell culture and suspensions were harvested on Day 13 post-inoculation. Cell culture and suspensions were further centrifuged at 800 G for 5 minutes. Clear suspension were disposed of, resulting in cell density of 21.24 x 106/mL and cell viability of >80%.

Harvest from both production batches were used as feed for two separate clarification processes.

3.1.3 Depth Filters

Stax mAx depth filters, shown in Figure 1, were used for harvest clarification. Specific removal ratings and filter properties were summarized in Table 1.

Table 1

Filter specification

Filter Media Part Number	Removal Rating (µm)	Construction
K100P	1.0 – 3.0	Single-layer
K700P	6.0 – 15.0	Single-layer
K900P	8.0 – 20.0	Single-layer
PDE2	0.2 – 3.0	Double-layer
PDH4	0.4 – 15.0	Double-layer
PDK5	1.0 – 20.0	Double-layer
PDP8	6.0 – 30.0	Double-layer
K100P	1.0 – 3.0	Single-layer

Figure 1

Stax mAx depth filter In Supracap™ 50 capsule format with luer lock connections

3.2 Experiments

Depth filtration was conducted by pumping cell culture and suspension to filter cartridges. Flow rate was set at 100 LMH and upper pressure limit was set at 1.7 bar (25 psi). Samples were taken approximately every 20 mL of filtrate generated. Throughput and pressure were recorded whenever a sample was taken.

Two sets of depth filtration experiments were performed. The first set of depth filtration was conducted using only primary depth filters and CHO harvest from bioreactors in fed-batch mode. Supracap 50 depth filter cartridges with surface area of 22 cm^2 were used to clarify CHO harvest from bioreactor.

The second set of depth filtration was conducted using both primary depth filters and secondary depth filters. CHO harvest from flask incubation was used as feed. Depth filter sheets were assembled in stainless steel Velapad™ housing for clarification. For K700P+EKMP two stage filtration set-up, Velapad housing with K700P filter sheets was used before Velapad housing with EKMP filter sheets. For PDP8+PDE2 two stage filtration set-up, Velapad housing with P8 filter sheets was used before Velapad housing with E2 filter sheets.

3.3 Characterization Methods

3.3.1 LDH Assay

When cell lysis happens, cells may release enzymes that can reduce mAbs, lowering production yield. LDH assays were used to assess the degree of cell damage. Less cell lysis is preferred during clarification to achieve optimum mAbs production yield.

LDH is an enzyme that cells release only when broken and can be detected using LDH assay. Assay preparation protocol was followed in the sequence of standard curve preparation, sample preparation, and reaction mix addition, as described on the assay kit instruction manual. During sample preparation, samples collected from clarification were centrifuged at 10 G for 5 min.

3.3.2 Particle Size Analysis

Particle size distribution is a good indication of suspension cleanliness. In this work, a Multisizer♦ 4e Coulter Counter was used to characterize particle size distribution and total particle count in samples collected throughout the depth filtration process. Both 30 µm and 100 µm apertures were used to generate results on a wider size range.

4 Results

An efficient clarification step is critical to separate the cells and other colloids to prepare cell culture for downstream chromatography and purification. This work aimed at exploring the impact of clarification on cell culture harvest, providing guidance on process control and depth filter selection.

4.1 LDH Level and Particle Count Change During Primary Clarification Using Single-Layer Depth Filters

Supracap 50 capsules with single-layer depth filter sheets, K100P, K700P, and K900P, were used separately to clarify the cell culture harvest. Figure 2 shows the differential pressure (dP) and LDH level of filtrate fractions as a function of throughput for clarification using K100P, K700P, and K900P, respectively. For K100P, dP and LDH level increased with throughput until the filter reached breakthrough point. For K700P and K900P, dP increased with throughput until the breakthrough points were reached; but LDH level behaved non-monotonically, first increased and then decreased, for K700P and K900P.

We suspect that the LDH activity was impacted by the sheer force applied on cells. When the pore size was relatively small, cells couldn't squeeze through, thus LDH level was positively correlated with dP. When the pore size was slightly bigger than the cells, cells could squeeze through in the beginning and then started to form a caking layer on top of the filter media and around pores, resulting in bigger shear force on the cells when the cells squeezed through. Later, when the dP was high enough, some loose aggregates might have been pushed through the pores, resulting in a small increase in LDH activity. And eventually, when the cells couldn't squeeze through anymore, the shear force on the cells dropped again. Further experiment is required to provide evidence for validation.

As shown in Figure 3, LDH activity and particle count behaved non-monotonically for all three depth filters. K100P has pore sizes smaller than cells, thus it is hard for cells to squeeze through the pores, resulting in LDH activity positively correlated with dP. However, the particle count of the second fraction was bigger than others. This may be due to membrane breakthrough or sample contamination but needs further verification. For K700P and K900P, LDH activity increased with dP in the beginning when a caking layer was forming, and the cells were squeezing through the pores. Later when the cells couldn't squeeze through anymore, LDH activity dropped.

K900P has bigger pore sizes than K700P. We hypothesis that when the dP was high enough, some loose aggregates were pushed through the pores, resulting in a slight increase in LDH activity for K900P.

Figure 2

Pressure drop and LDH level as a function of throughput for K100P, K700P, and K900P single-layer depth filters. Test were taken on each fraction during clarification process.

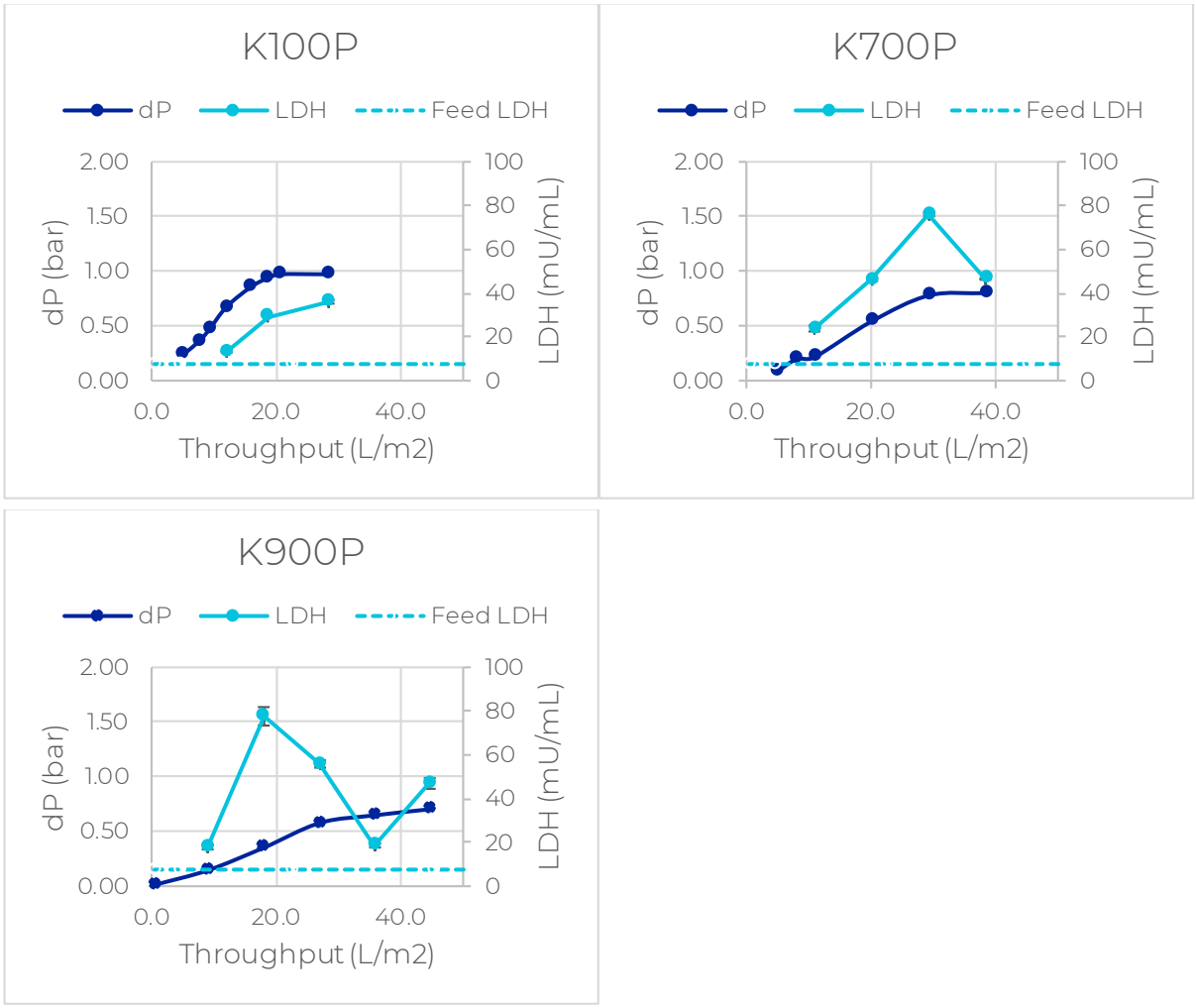
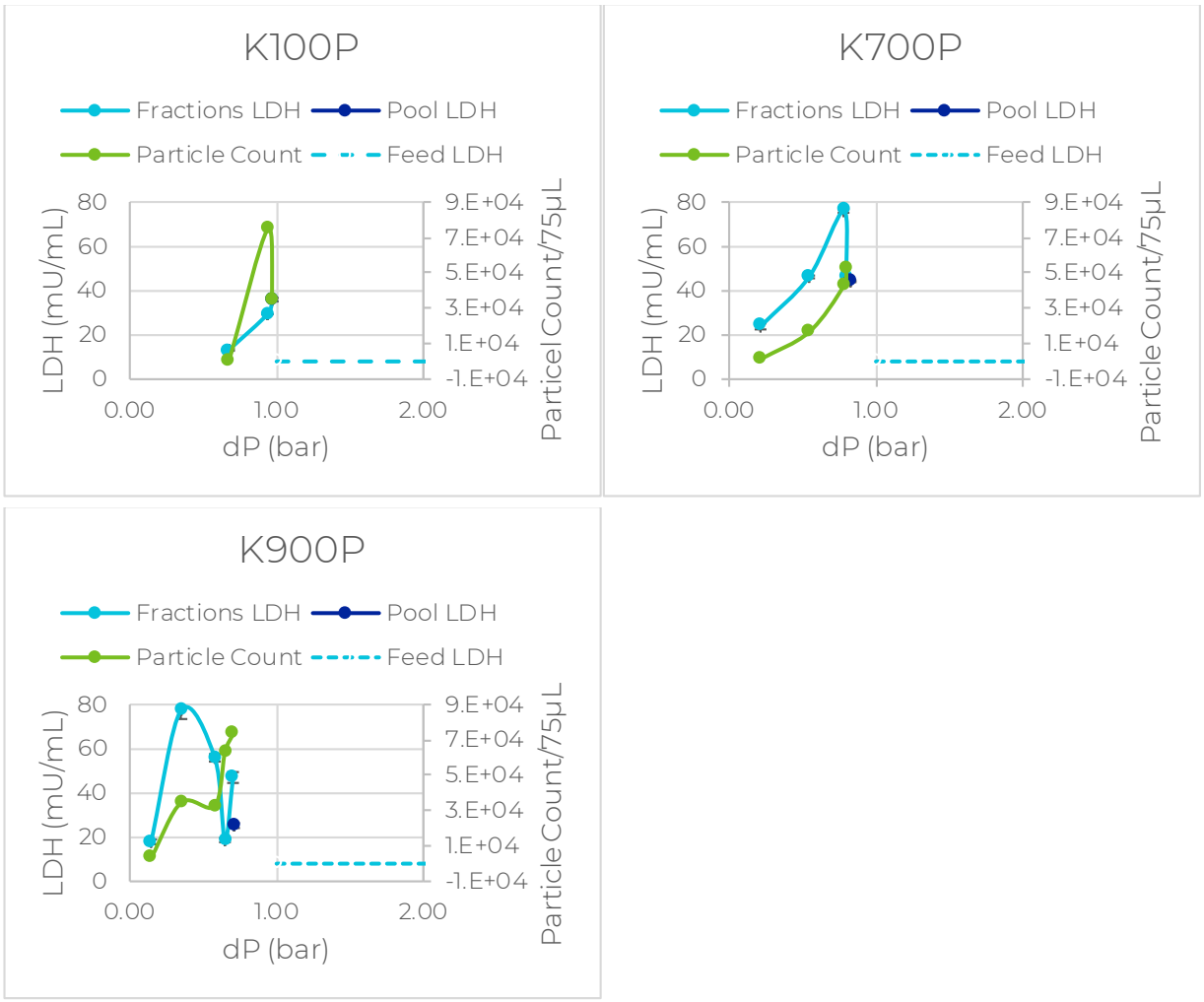


Figure 3

LDH level change for single stage depth filters and double stage depth filters

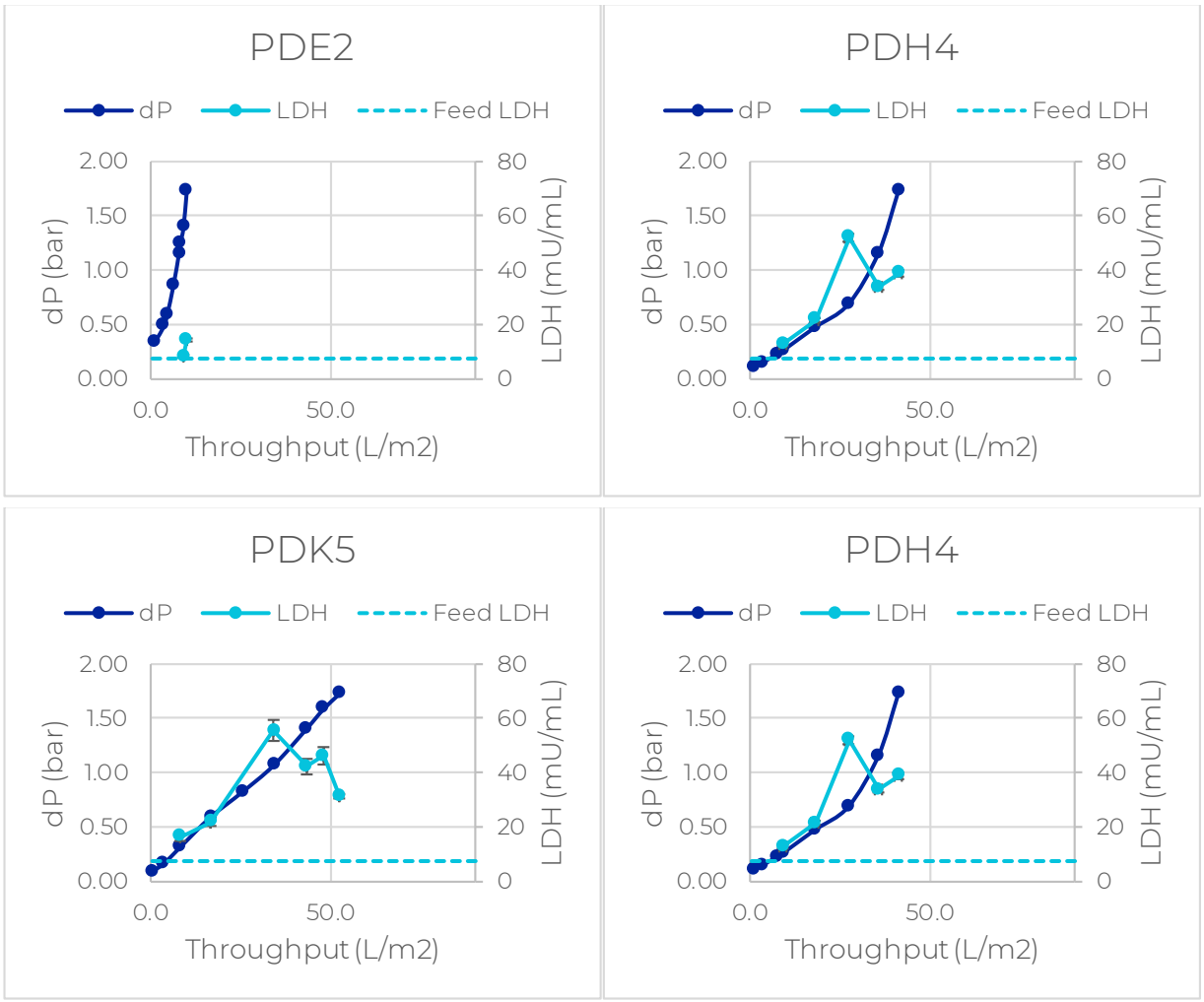


4.2 LDH Level and Particle Count Change During Primary Clarification Using Double-Layer Depth Filters

Supracap 50 capsules with dual-layer depth filter sheets, PDE2, PDH4, PDK5, and PDP8, were used respectively to clarify the cell culture harvest. As shown in Figure 4, the dP was positively correlated with throughput for all depth filters. PDE2 has very tight pores and plugged quickly. However, LDH level behaved non-monotonically for PDH4, PDK5, and PDP8 depth filters. The LDH levels increased in the beginning of clarification, then decreased slightly for PDH4, PDK5, and PDP8. LDH level increased in the beginning possibly because of the cells squeezing through the pores and forming a caking layer. When less cells squeezed through, LDH activity dropped slightly. For PDK5 and PDP8, a second increase in LDH activity was seen, followed by further decrease in LDH activity. The sudden increase may a result of some loose aggregates squeezing through pores when the dP was high enough.

Figure 4

dP and fractionate LDH level as a function of throughput for clarification using PDE2, PDH4, PDK5, and PDP8 filters, respectively.

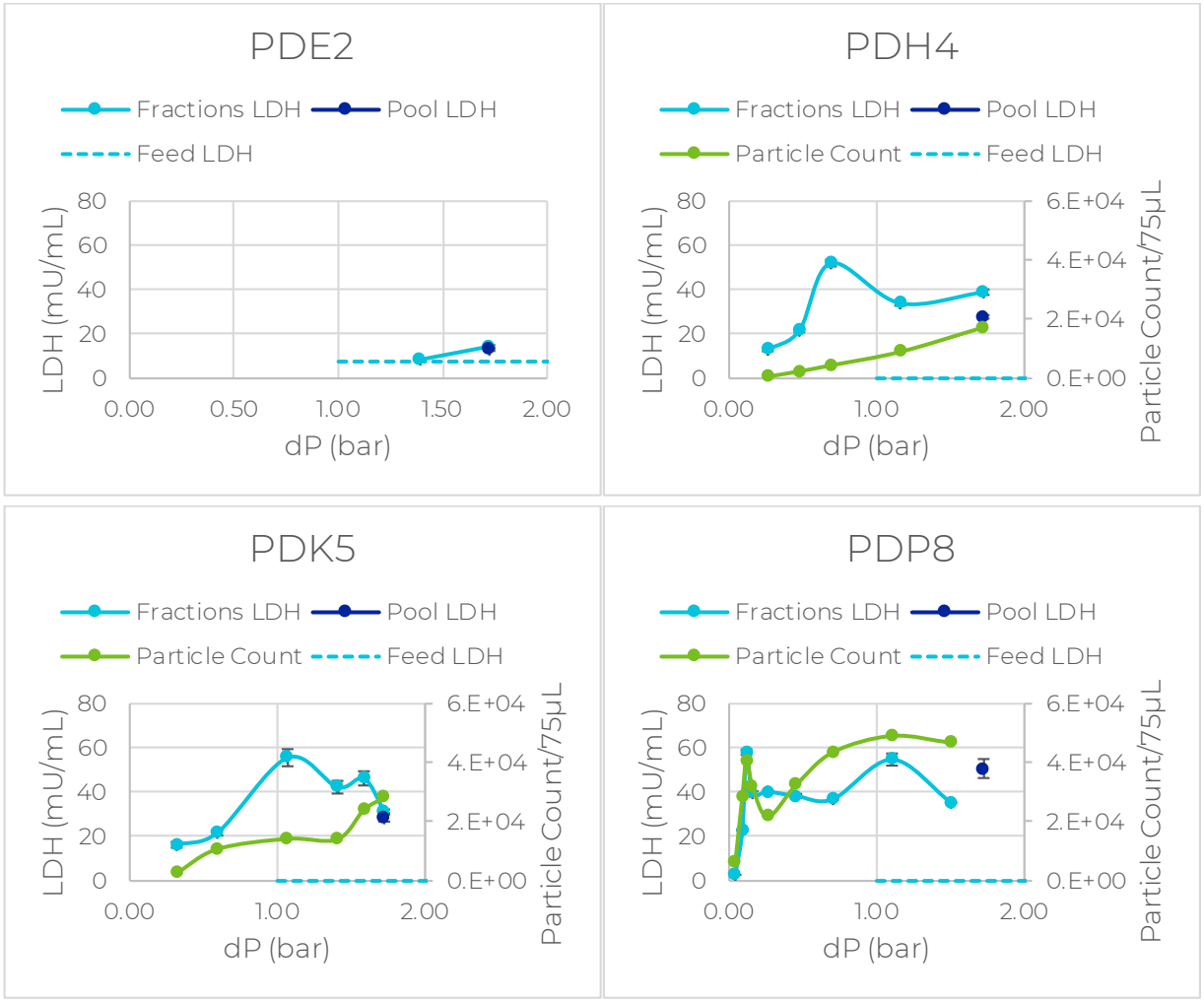


Shown in Figure 5, for PDH4, PDK5, and PDP8 depth filters, LDH level first increased with increasing dP, and then decreased slightly, which is followed by a second increase. For PDK5 and PDP8, a further decrease was noticed after the second increase in LDH activity. Similarly, this could be explained by our hypothesis mentioned above. During the filtration process, the cells squeezed through the pores and started to build a caking layer on top of the media and around pores, increasing the release of LDH. When some cells couldn't squeeze through anymore, LDH level decreased. As the dP continued to increase, some loose aggregates were pushed through the pores, resulting in a second increase in LDH activity. And if the dP didn't reach the limit after the second increase, LDH activity would drop again, given less shear on the cells.

For PDH4 and PDK5 depth filters, particle count were positively correlated with dP, while for PDP8, particle count behaved non-monotonically, reaching the first peak when LDH reached the first peak and then decreased slightly, followed by constant increase. PDP8 has the biggest pore sizes among all filters tested. We suspect that the initial jump in particle count for PDP8 was caused by large number of contaminants such as cell debris passing through the pores. Once a caking layer formed and narrowed the biggest pores, the passage of contaminants was greatly reduced, lowering particle count. Therefore, to achieve optimum clarification performance using only primary stage depth filters, avoid collection of filtrates in the beginning of the process would be advised. To avoid doing so, adding a secondary stage depth filter would solve the problem and creating cleaner filtrate.

Figure 5

LDH level and particle count as a function of dP for PDE2, PDK5, PDP8, PDH4 depth filters, respectively. Particle count was not tested for PDE2 due to limited fractionate sample volume collected. Feed LDH represents LDH level of cell culture harvest prior to clarification. Fraction LDH represents LDH level of fractionate samples taken during clarification. Pool LDH level represents LDH level of accumulated samples taken after clarification process ended.



4.3 LDH Level and Particle Count Change During Primary and Secondary Clarification Using Two Dual-Layer Depth Filters

Dual-layer depth filters provide higher efficiency and filtration life than single-layer depth filters in similar size range. Clarification with both a coarse primary stage depth filter and a fine secondary stage depth filter prepares cleaner cell culture harvest and better protection on downstream bioprocessing steps. Therefore, clarification using two stage dual-layer depth filters will maximize the filtration efficiency and provide the cleanest cell culture harvest. However, as shown above, clarification may have an impact on cell lysis, releasing enzymes that reduce mAbs chains and lowering production yield. Thus, the impact of clarification using two stage dual-layer depth filters were investigated in this work to provide guidance on this clarification process.

As shown in Figure 6, dP and LDH were positively correlated with throughput during clarification for both two-stage depth filtration set-ups. Filtrate produced by second stage filter EKMP and PDE2 had lower LDH activity than that of primary stage filter K700P and PDP8, resulting in much cleaner cell culture harvest. Moreover, for filtrate of PDP8+PDE2 double stage filter set-up, the LDH activity was lower than that of feed cell culture harvest stock until dP reached 0.34 bar (5 psi), indicating superior clarifying efficiency.

Figure 6

Particle sizer results on single-stage depth filters and double stage depth filters

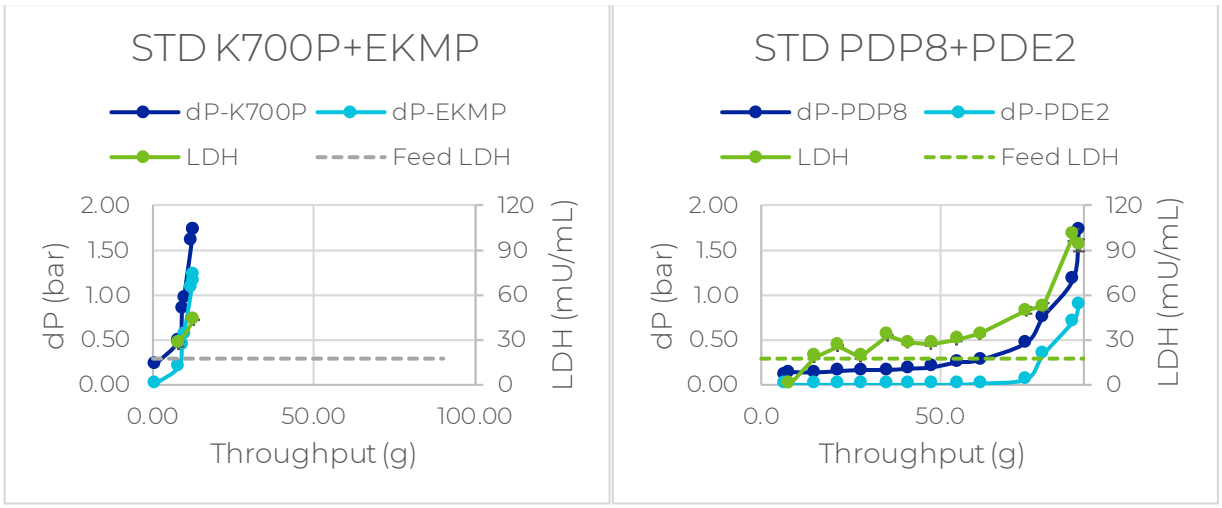
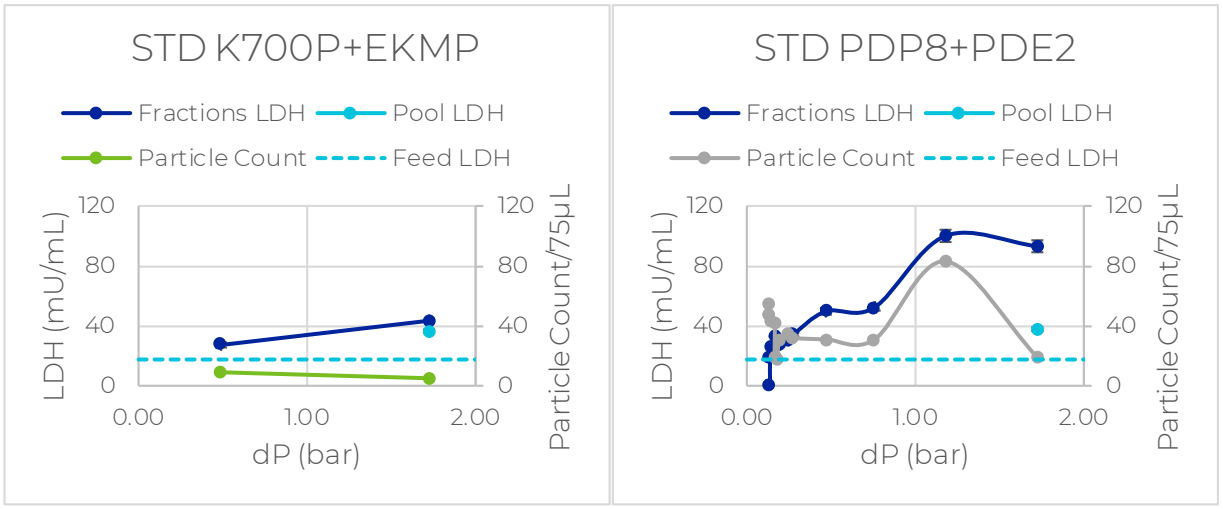


Figure 7 shows that LDH activities of fractionate filtrates were positively correlated with dP. While particle count for K700P+EKPM stayed low, the particle counts for Stax mAx PDP8+PDE2 decreased when the dP is in the range of 0-0.27 bar (0-4 psi) , stayed relatively stable when dP is in the range of 0.34-0.76 bar (5-11 psi) increased again at dP range of 0.83-1.03 bar (12-15 psi) and then decreased when dP approached the limit. Similarly, the sudden increase in particle count may be a result of some loose aggregates squeezing through the pores when the dP was high enough. However, further experiment is needed to verify the non-monotonical trend in data.

However, the overall LDH activity of filtrate pool from PDP8+PDE2 filtration set-up was similar to that of the feed cell culture harvest stock prior to clarification, indicating minimum impact on cell lysis.

Figure 7

LDH level and particle count as a function of dP for clarification using K700P+EKMP and Stax mAx PDP8+PDE2 two-stage depth filters.



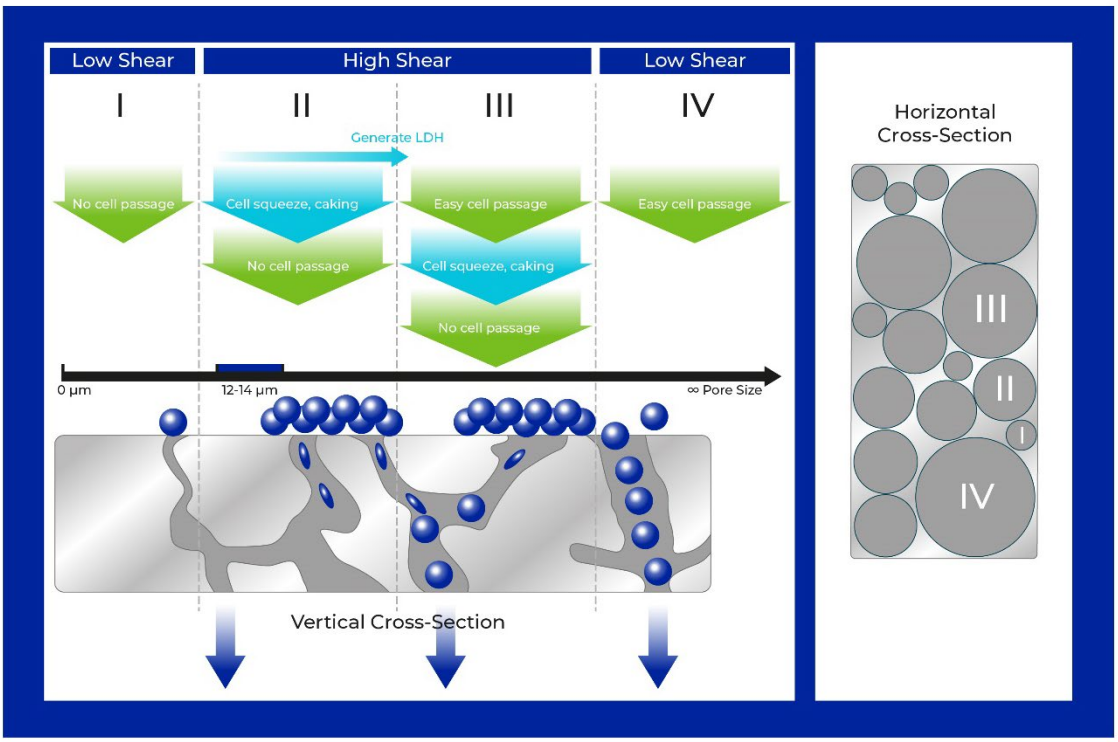
5 Discussions

As we hypothesized above, cell lysis is dependent on the shear force applied on the cells during clarification. We have constructed a simple illustration on the membrane structure of depth filters, helping to decipher the interaction between cells and depth filter media during clarification.

Depth filters have a relatively broad pore size range, capturing contaminants in various sizes while keeping the high flow. As shown in Figure 8, four different cell and pore interactions were illustrated, depending on the relative size of the cells to pores. Represented by type I, when the pores are much bigger than the cells, the shear on the cells is relatively low and it takes long before a caking layer can form on top of the membrane or around pores. For type II, when the pores are slightly bigger than the cells, cells can pass through easily in the beginning, but quickly start to form aggregates or caking when the shear on the cells starts to increase, until the cells can no longer squeeze through the pores. When the cells are slightly bigger than the pores, they start to squeeze through when clarification process starts, increasing shear on the cells until pores are blocked and no cell can be pushed through, as represented by type III. When the cells are much bigger than the pores, cells will try to squeeze and quickly form a caking layer when dP increases sharply and reach the limit, as represented by type IV.

Figure 8

Illustration of an interaction between cells and depth filters during different filtration stage



To obtain cleaner cell culture harvest post clarification, we need to consider particle count variation during the process. For type I, particle count in clarified cell culture harvest will stay high until when a caking layer can form to narrow the pores. For type II, particle count in the clarified cell culture harvest will be high in the beginning and then drop quickly after a caking layer forms. Therefore, it is preferred to dispose the filtrate collected in the very beginning of the process and start to collect later when particle count reduces. To achieve cleaner filtrate and increased production yield, we can consider using dual-layer depth filters and two-stage filtration process, as proven by the above-mentioned Stax mAx PDP8+PDE2 two stage dual-layer depth filter set-up.

6 Conclusions

This work employed LDH assay and a particle counter to examine the clarification efficiency and the quality of clarified cell culture harvest. We have demonstrated the use of LDH activity and particle count as effective indicator to select depth filters and design clarification process.

To conclude, Pall's dual-layer depth filter Stax mAx clarification platform contributes to cleaner clarified harvest, reduced cell lysis, and extended filter life, compared to single-layer depth filters. We have also proved that the use of two stage depth filtration with dual-layer depth filters, Stax mAx platform series, results in lower LDH activity and particle count. Thus, to achieve cleaner filtrates and higher production yield, we recommend the use of both primary and secondary depth filtration, applying dual-layer depth filters in each stage.

Lastly, we have generated an illustrative model to help decipher the interaction between the cells and depth filtration media during clarification process, providing guidance and a starting point for future work on clarification.

7 Acknowledgements

We would love to acknowledge the contributions from Pall Corporation, more specifically, Bioprocessing Research and Development for providing production of mAbs and analytical work on samples, and Central Media Research and Development for providing depth filtration media.

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