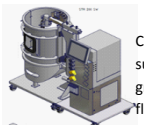
Comparison of different methods of clarification of CHO cells

Dustin Allen, Samantha Ricketts, Jenica Lee, Christa Short, Frank Agbogbo

Abstract

The development of clarification process for commercial biotherapeutic manufacturing requires a simple and scalable process. This begins with the selection of scaled-down models of large-scale production process for clarification. Separation of CHO cellular material from the product containing media can be tested by different methods. In this study, alluvial filtration, depth filtration and flocculant filtration have been evaluated. Alluvial filtration involves the addition of various grades diatomaceous earth (DE) into a vessel containing CHO cell culture and the resultant cake formed upon a filter disc during separation provides the filtration. Depth filtration is done with filters designed to have a tortuous path providing an avenue for the media to separate from the cell culture without allowing the filter to become quickly clogged by the cells. The flocculant design of filtration uses an addition of a stimulus responsive Smart Polymer (mPAA) which is designed to aggregate the cells from the media and allow the media to pass through the cell aggregate. This aggregate is then filtered against a depth filter. Each of these methods is scalable from 500 mL to 5000 L or beyond. Data generated with these three methods at laboratory scale is discussed below.

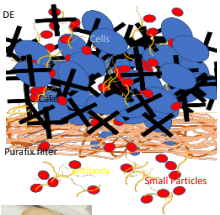
Materials and Methods



Cytovance harvested a 200L SUB of Freedom CHO-S line. Turbidity of the cell culture was 2220 NTU. The turbidity of the centrifuged supernatant was ~175 NTU. Cell culture suspension was 12.7 × 10^6 cells /mL @ 80 % viability. Material generated was tested across three filtration techniques. Filtrox's Alluvial filtration was optimized using different grades of Celpure Diatomaceous Earth (DE), Purafix filters, and Filtrodiscs. The depth filtration was done with Millipore's D0HC, DOSP, X0HC, and X0SP pods. Millipore's flocculant, the Smart Polymer (mPAA) was optimized with a dosing study and then the optimal percent was chosen and delivered across a CS60HX pod.

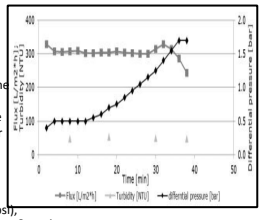
Results

DE CelPure C65, C100, C300 and C1000 was used as the filter aid. Some trials introduced a precoat of 3 g/L of filter aid with 100 mL 1XDPBS solution onto the Purafix® filter sheet. The DE/cell culture concentration was generally thirty (30) grams of filter aid to one (1) liter cell culture and kept in suspension with a magnetic stirrer. This suspension was pumped through the tubing, to a pressure gauge, and to the pre-wetted (and pre-coated) Filtrox Filtrodisc Bio SD 2" capsule (20 cm²) which was developed for alluvial filtration. The flowrate for the precoat was 15 mL /min and the clarification flowrate was 12 mL/min (Flux of 300-350 LMH). Turbidity was measured for each 100 mL of permeate and at the end of filtration. Total volume was measured at the end. Titer of recovered product was measured by the Cedex BIO Fast IgG. When the pressure reached 2 bar, the flow of suspension was followed by air for displacement



The DE pre-coat addition to the alluvial filtration provided a stacking mechanism which formed a cake on top of the Purafix filter within the Filtrodisc. This allowed the cell material to stack above the filter and the combination of the DE with cell material became a filter as more DE and cell material continued to stack within the Filtrodisc. Smaller particles than the cells and the antibody passed through this matrix and the filter .

As the optimum DE/cell culture slurry was being pumped across the filter, the pressure rose, and the turbidity decreased but the mAb retained a very similar titer. Recovery was high (> 94%).



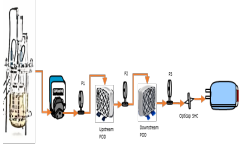
When the pressure was close to 2.0 bar (~28 psi), the flux decreased, and the filtration ended. The final height of the cake was 32mm. The average measured turbidity of the filtrate was 50 NTU. A second filtration was done to the filtrate with a 30 g/L of Celpure 65 DE concentration. A similar clarification process proceeded resulting in a turbidity of 26 NTU. The resultant permeate was able to easily go through a 3.5 cm² SHC filter.

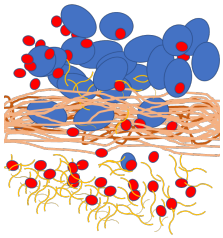
Capacity
- 177 L/m²-- 30g/L Celpure 300 Cell mixture + 3 g/L Celpure 300 precoat 50 NTU >94% Recovery
- Permeate ~ 191 L/m²--30g/L Celpure 65 Cell mixture + 3g/L Celpure 65 precoat 26 NTU >94% Recovery

Depth Filtration

The cell culture was kept in suspension in a beaker on the stir plate and pumped through tubing at a rate of ~4 mL /min (100 LMH for 23 cm² device). Two types of depth filter pod medias were tested, the High Capacity Synthetic Media (silica)(D0SP & X0SP) and the Diatomaceous earth (DE) and cellulose (CE) based filter media(D0HC & X0HC). Wetted and equilibrated were two of the "upstream D" pods in parallel and connected in series to one "downstream X" pod which in turn was connected in series to a Polyethersulfone (PES), Express SHC (0.5/0.2 µm) final filter.

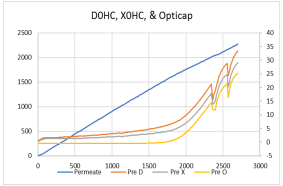
The turbidity was measured at the end of the process when the pressure reached 30 psi. Total volume was measured at the end. Titer of recovered product was read by the Cedex BIO Fast IgG.





The cell material, media, and antibody stacked upon the surface of the "D0" depth filter and within the porous torturous path of the depth filter. This provided pathways which lodged big material yet allowed smaller material to pass through. In a series of reducing porosity from the upstream POD to the downstream POD, the cell debris, is further separated from the mAb as it passes through the "X0" depth filter.

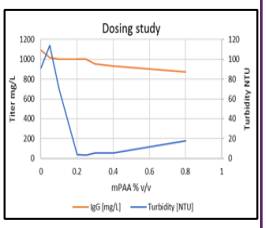
The pressures here were recorded with the Puretec System during clarification. The pressures between the pod series show that they have been sized to adequately clarify the amount of material. The Synthetic Pods were similar in clarification in this case.



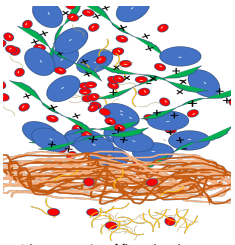
Harvest Descriptio	Filter Train	Filter Type	Capacity L/ m²	Turbidity	Recovery
No Pretreatment	2 xD0SP into X0SP Express SHC	Millistak D0SP POD	100		> 90%
		Millistak X0SP POD	200		
		Express SHC (0.5/0.2um)	192	9.0 NTU	
	2 xD0HC into X0HC Express SHC	Millistak D0HC POD	84		>90%
		Millistak X0HC POD	168		
		Express SHC (0.5/0.2um)	322	13.2 NTU	

mPAA Flocculation & Clarisolve Filtration :

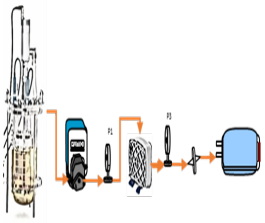
This clarification was similar to Depth filtration except that one Clarisolve filter media (Polypropylene (PP) Millistak Media) was attached to the final filter. The optimum concentration of Smart Polymer (stimulus responsive polymer, mPAA) to use was determined with a dosing study. The turbidity was used as the indicator of the percentage of polymer to add. The cell suspension had the additive of Smart polymer with the final concentration of 0.25% for the flocculation of the cell material.
There was a low impact on the titer with the greater addition of polymer .



The stimulus responsive polymer was added to the cell culture to stir at room temperature for 1 hour followed by 2M sodium phosphate solution. 15 minutes later a Tris Base solution was added to adjust the pH to 7. An aggregation of polymer chains and the multivalent anions (DNA, HCP, and cells) became insoluble and flocculated. The suspension is then passed through the Clarisolve filter CS60HX.



A large capacity of flocculated material was passed through the CS60HX pod at 100 LMH , and then passed through the SHC filter with ease. There was very little pressure measured < on the SHC throughout the clarification. This may have been due to the very low turbidity of 5 NTU



Harvest Descriptio	Filter Train	Filter Type	Capacity L/m²	Turbidity	Recovery
NO Pretreatment	Clarisolve 60HX into SHC	Clarisolve 60HX	314	5 NTU	>90%
		Express SHC (0.5/0.2u	2569		

- The throughput of the filtrate is high and the rate of 300 LMH is the highest of the filtrations.
- The turbidity of the permeate goes down as the cake is built.
- The recovery of the mAb is > 94%.

- Highest turbidity
- Extensive testing for optimal DE recipe which may be one or two steps before final sterile filter.
- Manufacturing would require additional equipment for both the mixing and filtering of the addition of DE.

- Standard depth filtration is a dependable manner of clarification.
- Easily sized and transferred to manufacturing.
- No additives to cell culture.
- >90% Recovery

- Scale- up including many PODS may require high flow rate to meet flux requirements so that product isn't lost in adsorption.
- Large number of pods needed for scale-up.

- One POD Type (60HX)
- Highest Capacity ≥ 314 L/m²
- Double the capacity and less # of pods
- Lowest Turbidity of 5 NTU
- > 90% Recovery

- Addition of Polymer to cell culture creates pH change which must be tested for product quality effects.
- Polymer removal and effects on recovery should be carefully characterized.
- Addition step could require additional mixing materials.

Conclusion

CHO cell culture clarification using alluvial filtration yielded > 94% Recovery, and the Smart Polymer and depth filtration had similarly high recovery of mAb of > 90% . Filtrox and Millipore's Smart polymer (mPAA) reduced the footprint by having higher capacities than the depth filtration processes. This reduced price and space for the clarification. The removal of cell debris and other colloidals was done best by the Smart Polymer/ Clarisolve process as indicated by the lowest resultant turbidity of 5 NTU. Addition of material to the cell culture was done in a separate vessel with the Filtrox and the mPAA as would be done in manufacturing. Yet the addition of chemicals to a process could also increase the testing of the product and removal of the chemicals. Using only depth filtration, the cell culture can be pumped directly from the bioreactor –already well established within manufacturing. All these techniques could be used interchangeably for the recovery of product but the determination of the one to use may be guided by these differences.