Exploration of synthetic depth filtration applied to mammalian cell harvest

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Abstract

Depth filtration is typically applied post-bioreactor for clarification in mammalian cells processes. The objective of this study was to explore the performance of newly developed synthetic depth filtration media compared to traditional cellulose and filteraid based media and/or pretreatement. These filters were tested on three different monoclonal antibody cell cultures. The trials show promising results using synthetic media over traditional depth filter by exhibiting a capacity 2 to 4 times superior along with lower flushing volumes and reduced footprint.

Introduction

When implementing a clarification step for monoclonal antibody (mAb) production, manufacturers are looking for robustness and cleaner processes, moving away from naturally-derived raw materials.

We investigate the comparative performance of a novel synthetic depth filtration media (Millistak+® HC Pro depth filter) that demonstrates a greatly increased filtration capacity for the clarification of mAb from CHO harvests. When compared to commercially available depth filtration media derived from natural components, this synthetic depth filter media has shown to have significantly decreased organic extractables and reduced WFI flushing requirements by TOC analysis. Three monoclonal antibodies (mAb) feeds from CHO harvest were examined.

Novel Synthetic Media Millistak+® HC Pro

The synthetic media grade are comprised of:

- proprietary mixtures of a polyacrylic fiber
- a silica filteraid
- a wet-strength binder resin.

Materials

Millistak+® HC Depth Filters	Novel Synthetic Media Millistak+® HC Pro Components	
CE25 DE40 *D0HC*	Layer 1 Layer 2 Layer 3 Layer 4	**D0SP** • *primary clarification (direct harvest)* • *density gradient improves capacity + retention*
IM75 IM83p	Layer 1 Layer 2	**X0SP** • *secondary clarification (direct harvest, centrate clarification)* • *largest contributor to HCP clearance*
DE30 DE60 *C0HC*	Layer 1 Layer 2 Layer 3 Layer 4	**C0SP** • *primary+secondary clarification (direct harvest, centrate clarification)*

Study background

Customer: Biosimilar producer

Scope of the study: Investigate different options for clarifying three mAbs from CHO harvest.

A templated approach is preferred.
The typical final product volume is 1000 L bioreactor.
Three general approaches were proposed and tested:

1. Untreated feed on traditional filteraid and cellulose based clarification depth filters (Millistak+® HC)
2. Untreated feed on novel synthetic depth filters (Millistak+® HC Pro)
3. Pretreated feed, using pDADMAC (Polydiallyldimethylammonium chloride) cationic polymer in the case of low viability cell culture. The flocculant aggregates cell debris and contaminants, creating larger size particles, which can be removed using specifically designed depth filters (Clarisolve®)

All of these options were not tested on each feed depending on users requirements; untreated feed options were always compared.

Key advantages of synthetic media

Key features:

- All synthetic materials: Using synthetic media eliminates β-glucans interferences with LAL testing
- Increased process capacity reducing installed surface leading to lower water consumption.
- Delivered in a disposable Millistak+® Pod device format.
- Lower flushing requirements: reducing flush volume by 50% at least (illustrated in figure 4 and 5).

Figure 4. Flushing volume and corresponding residual TOC level

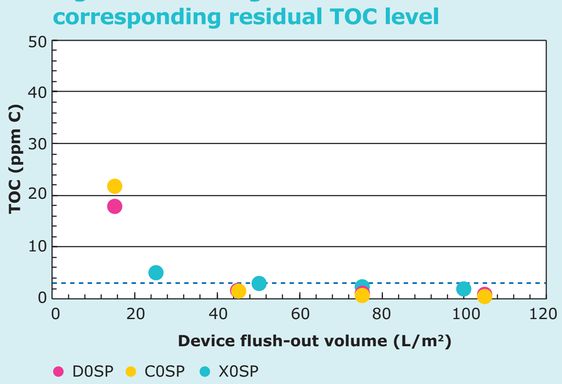
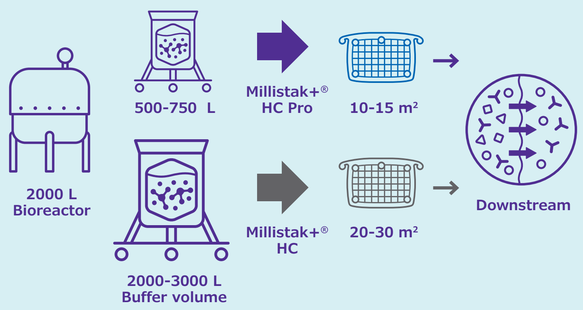


Figure 5. Example of comparison of water flush volumes necessary for a traditional media and an all synthetic clarification train.



Methods

The three cell culture mAb feeds were tested on different filtration trains using the µPod® format (23cm²) to evaluate and define the best filter train. The testing utilized a constant flow methodology with the filter performance being assessed by monitoring back pressure (P_{max}) and filtrate turbidity breakthrough (T_{max}). The experimental set-up is illustrated in figure 1.

Figure 1. Experimental set-up
(Characteristics of the feeds are detailed in table 1)

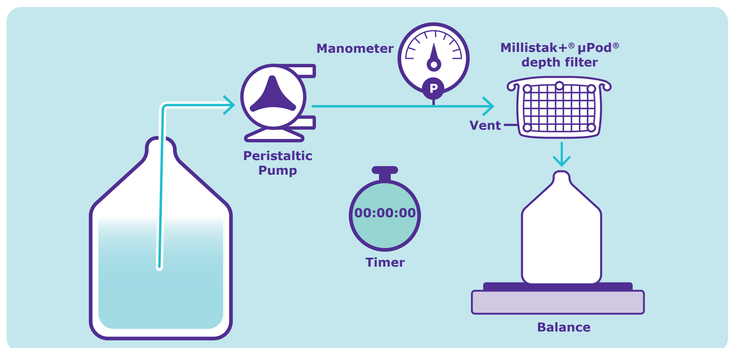
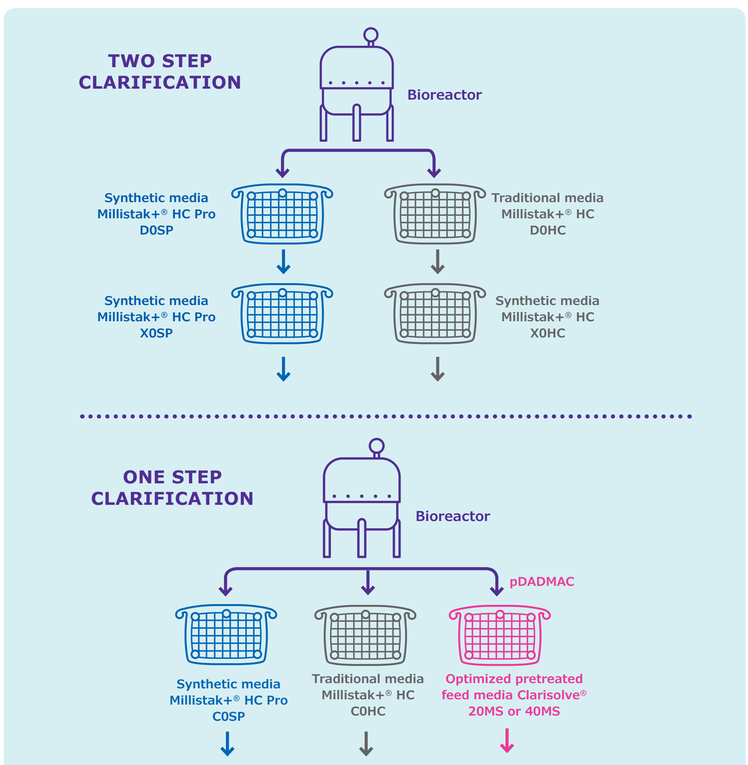


Table 1. Feedstream characteristics

Feed type	Product	Product Concentration (mg/mL)	Total cell density (million cells/mL)	Viability (%)	Turbidity (NTU)
CHO	mAb1	1	6	40	690
	mAb2	1,3	10	50	938
	mAb3	1,1	9.2	67	765

The different filtration train options are illustrated in figure 2. Pretreatment with pDADMAC takes place directly into the disposable bioreactor (gentle mixing for about 10-15 min).

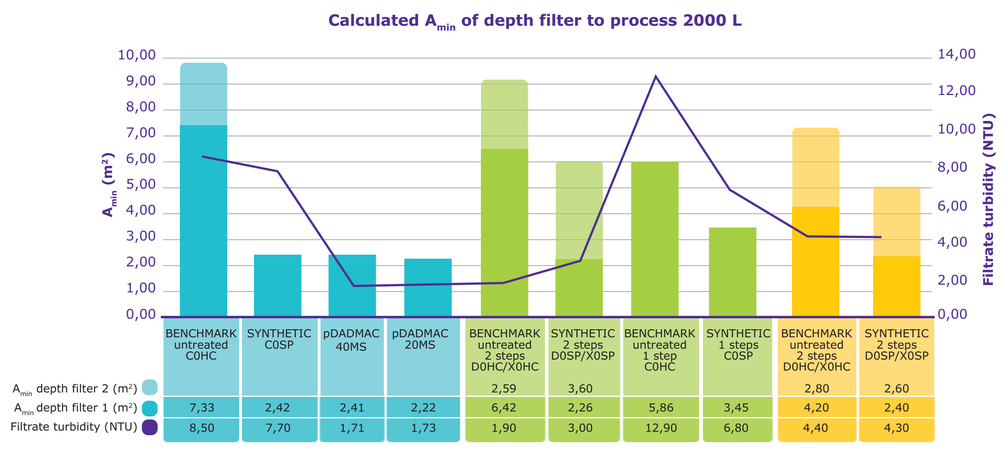
Figure 2. Filtration train options



Results

The resulting minimum area (A_{min}) is indicated for each mAb in figure 3:

Figure 3. Minimum filtration area



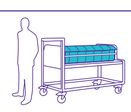
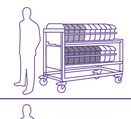
The most promising large scale suggested configurations are indicated in table 2, with an average safety factor of 50% filtration area.

For mAb1 and 3, the product yields on traditional depth filter and synthetic filter trains were measured and found to be comparable.

Table 2. Suggested large scale configurations

So as to evaluate further the different options, several factors could be considered:

- Filtrate quality (turbidity, contaminants)
- Process economics
- Ease of use and ergonomics (one step, pretreatment, versus two steps, number of devices to install)
- Use of all synthetic clarification train

Option	Filter	Suggested configuration	Holder/hardware No. of racks	
mAb 1				
Benchmark Millistak +® HC	C0HC	10 x 1,1 m²	1	
Millistak+® HC Pro	C0SP	5 x 0,77 m²	1	
pDADMAC & Clarisolve®	40MS	7 x 0,55 m²	1	
pDADMAC & Clarisolve®	20MS	7 x 0,55 m²	1	
mAb 2				
Benchmark Millistak+® HC primary clarification	D0HC	9 x 1,1 m²	1	
Benchmark Millistak+® HC secondary clarification	X0HC	4 x 1,1 m²	1	
Millistak+® HC Pro secondary clarification	D0SP	5 x 0,77 m²	1	
Millistak+® HC Pro	X0SP	5 x 1,1 m²	1	
Benchmark Millistak+® HC	C0HC	8 x 1,1 m²	1	
Millistak+® HC Pro	C0SP	7 x 0, 77 m²	1	
mAb 3				
Benchmark Millistak+® HC primary clarification	D0HC	7 x 1,1 m²	1	
Benchmark Millistak+® HC secondary clarification	X0HC	4 x 1,1 m²	1	
Millistak+® HC Pro primary clarification	D0SP	6 x 0,77 m²	1	
Millistak+® HC Pro secondary clarification	X0SP	4 x 1,1 m²	1	

Discussion

The use of alternative clarification options with either pretreatment or synthetic depth filters is promising as substitute to traditional depth filters.

In the scope of the three particular mAbs tested in this study, further elements could be evaluated additionally to define the final option adopted.

In particular, more tests are recommended to assess:

- Filtrate quality, through: following sterilizing grade capacity evaluation, contaminants removal (HCP, DNA), downstream performance (chromatography);
- Efficiency of pretreatment on the two other mAbs.

Other elements such as ease of use (flushing volumes for preparation step) and economics need to be considered as well.

The use of an all synthetic clarification train holds a great attraction as it has the advantage of increased robustness, lowers risk associated with natural sourced raw materials and running an overall cleaner process.

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