

Fouling Mechanisms of Filters During the Harvest Development of Monoclonal Antibody Therapeutics with Intensified Upstream Processes—Part 1

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ABSTRACT

The harvest step in a monoclonal antibody downstream purification process removes biomass, particulates, and other material from the cell culture to generate a product stream suitable for purification by liquid chromatography. In a widely employed, two-step harvest process, coarse solids and intact cells are first removed using a centrifuge, and the resulting centrate is further clarified by depth and membrane filtration in series. Conversely, direct depth filtration has the potential to greatly simplify the overall harvest process by directly loading the cell culture broth on high-capacity depth filters and consequently removing the need for centrifugation. The authors evaluated this potential of direct filtration for multiple biopharmaceutical candidates to understand the underlying mechanisms that define fouling and the resulting capacity limitations in these filters. Here, the authors present a methodology to assess the contribution of individual filters to overall capacity of the direct filtration train, and elucidate how capacity is affected by characteristics of the cell culture broth and process parameters, including for enhanced upstream processes such as those incorporating high cell density perfusion. The findings highlight the limitations of direct filtration when implemented in large-scale facilities for high cell density processes. Ultimately, increased understanding of the fundamental principles that govern depth and membrane filtration can aid in moving from a trial-and-error to a more predictive and methodology-based approach for harvest development. This article is Part 1 of the study.

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The harvest or clarification step in the manufacture of biopharmaceuticals, such as therapeutic monoclonal antibodies (mAbs), is the bridging process between the upstream production and downstream purification. It generally involves the removal of cells, cell debris, and other particulates from the cell culture broth after the bioreactor stage to prepare the feed stream for the more sensitive

downstream techniques such as affinity chromatography. The removal of particulates is typically accomplished with depth filtration, which employs porous filters that retain particulates through mechanisms such as volumetric partitioning (1). For manufacturing simplicity, completing the harvest process using solely depth filtration, here termed direct depth filtration (DDF), is often preferred (2). However, other additional techniques

such as flocculation or centrifugation are sometimes necessary to increase filter capacity or reach the required product quality, particularly if cell densities in the cell culture broth are relatively high, but these additional techniques introduce increased complexity and cost to the manufacturing process (3–5).

Depth filters are generally composed of three main components: a fibrous base structure that provides filtration capacity for particles, a filter aid for increased surface area and impurity capture (1,6), and a polymeric binder, which can carry a charge (2). Due to the different functionalities of these components, depth filters can not only filter out particulates but also adsorb common soluble impurities (7,8), including host cell proteins and DNA (9,10). Recent accomplishments in depth filter technology, such as high-capacity synthetic depth filters, have allowed the use of direct depth filtration with increasingly high cell densities (2,11,12). Synthetic filters have been shown to have improved capacity for such impurities (12).

Still, the push for higher titers and intensified upstream processes continuously challenges the capacity of these depth filters. Unfortunately, the fouling mechanisms involved in determining the capacity of depth filters for harvest clarification can be quite complex and are currently not well understood. The load capacity of depth filters for a particular feed stream of cell culture broth is generally determined experimentally. A better understanding of the fouling mechanisms can consequently aid in moving from an experimental to a more predictive approach to harvest development, which would be particularly useful in the light of ever-evolving upstream processes and accelerated timelines for development.

The following outlines the methodology during the direct depth

filtration of a wide range of mAb processes, produced in different upstream conditions leading to varying properties of the cell culture broth such as cell densities. All batches were clarified through a typical filter train setup containing a coarse depth filter, fine depth filter, and membrane filter in series, using synthetic depth filters (MilliporeSigma). These depth filters contain polyacrylic fibers and silica filter aids. For each filter, mechanisms that can lead to fouling are identified, as well as those properties of the cell culture broth that can best predict overall filter train capacity. While the results are limited to a certain filter type and range of mAb candidates, the conclusions illustrate the limitations of direct depth filtration as the industry moves toward intensified upstream processes and the outlined methodologies can be applied to a broad range of biopharmaceutical filtration processes.

MATERIALS AND METHODS

Materials

Cell culture material for five mAb candidates, labeled here as IgG1 A, IgG1 C, IgG1 D, IgG1 E, and IgG2, was produced in multiple batches. Coarse depth filters of type D0SP (nominal size rating 9–0.6 μm) in the μPOD format (23 cm^2), finer depth filters of type X0SP (nominal size rating 0.09–0.05 μm) in the μPOD format (23 cm^2), and sterilizing-grade high capacity (SHC) membrane filters with a polyethersulfone (PES) membrane (size rating 0.2 μm) were used (MilliporeSigma, MA).

Filtration studies

Filtration studies were performed using a filter screening system (PendoTech), linked to a feed pump (MasterFlex L/S) and scales (Mettler Toledo), and using cell culture broth freshly collected from the bioreactor at the end of the production phase

without preconcentration. Cell culture broth was kept mixed throughout the experiments and held at room temperature. Pressure traces were collected with reusable pressure sensors (PendoTech) placed in line before each of the filter stages. All equipment is maintained by facility engineers and periodically calibrated by scientists to ensure accuracy of measurements.

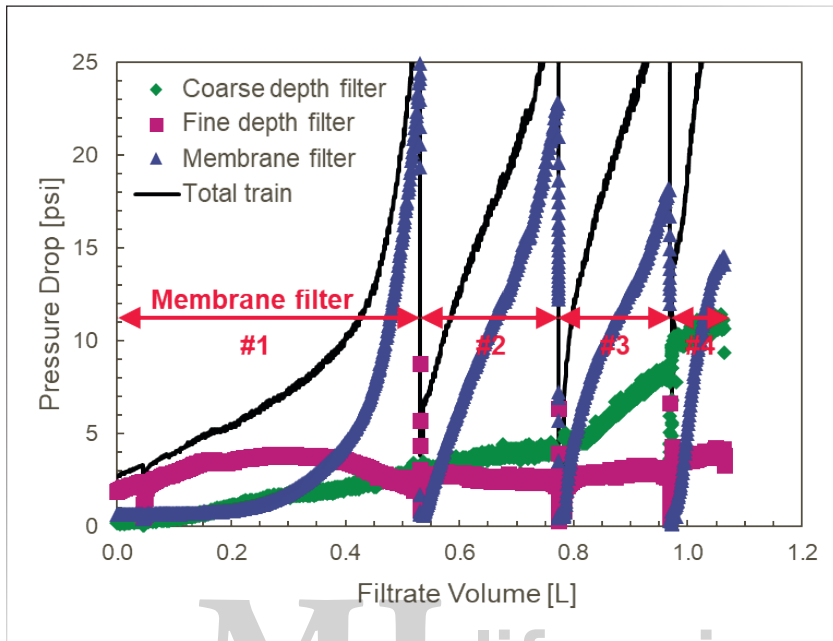
Generally, cell culture broth is filtered in each study through a series of three filters: a coarse depth filter, a finer depth filter, and a membrane filter. The depth filters were flushed with deionized (DI) water and vented according to manufacturer's recommendations prior to filtration of the cell culture feed material ($\geq 50 \text{ L/m}^2$ at 300 $\text{L/m}^2/\text{h}$ based on first-stage area). The approximate hold-up volume of the filters, as specified by the manufacturer (16.8 and 8.6 L/m^2 for D0SP and X0SP, respectively), was collected and discarded prior to collection of the filtrate. The membrane filter was connected after flushing of the depth filter and vented during collection of the hold-up volume. Filter trains were run continuously at constant flow up until the predefined endpoint when the pressure drop over the whole filter train reached 30 psi.

A typical pressure trace for a filter train with an undersized, smaller membrane filter is shown in **Figure 1**. In this particular example, the small membrane filter was replaced with a new one each time the total pressure drop reached 30 psi. **Figure 1** illustrates how the lifetime of each membrane filter decreases as the particle composition in the depth filter filtrates changes.

Turbidity and particle size

Turbidity measurements were performed using a turbidity meter (Hach). Particle size measurements were performed using both laser diffraction and dynamic light scattering

Figure 1. Pressure drop profiles over each of the filters and the total filter train as a function of filtrate volume for the case the membrane filter is the first to fail. Replacing each membrane filter after failure progressively reduces the capacity due to increasing impurities in the depth filter filtrate.



(DLS). For the laser diffraction particle size analyzer (Malvern Panalytical Mastersizer 3000 with Hydro MV, Malvern, United Kingdom), samples were added until minimum obscuration was reached and measured in triplicate for 10 s each assuming spherical particles. Results were analyzed using the General Purpose method in the accompanying software.

DLS measurements were obtained from a DLS probe (Nanotracs Flex, Microtracs MRB, PA). Sample volumes of about 2 mL were transferred into a 15 mL beaker and measured undiluted. The probe was lowered to just about 3 mm below the top level of the fluid in the beaker and measurement time was 90 s.

Wet cell mass

The wet cell mass (WCM) was defined as the mass fraction of the solid particulates present in the cell culture broth. The mass fraction of solids was measured in duplicate by

batch centrifuging 50 mL of cell culture broth in a conical tube for 20 min at 5000 rpm (3444 g) in a centrifuge (Avanti Centrifuge J-30I with JA-17 rotor, Beckman-Coulter, CA), decanting the supernatant, and taking the mass fraction of centrate to initial total mass without further drying.

Host cell protein concentration

The residual Chinese hamster ovary (CHO) host cell protein (HCP) enzyme linked immunosorbent assay (ELISA) employed is a microplate-based sandwich ELISA format utilizing a proprietary custom affinity-purified polyclonal goat anti-CHO antibody (Cygnus Technologies, NC). This antibody is specific to the CHO cell line utilized within the process and therefore has strong coverage of the host proteome as evaluated by 2D differential in-gel electrophoresis (DIGE). The custom antibody serves as both the capture and the biotinylated detection antibody.

The microplate is coated with the custom antibody to capture the analyte to the plate. The samples containing the analyte (i.e., HCPs) are added to the microplate and incubated to facilitate binding to the capture antibody. The biotinylated detection antibody is added, and through conjugation of the antibody to biotin, the detection antibody is then able to bind streptavidin horseradish peroxidase (SA-HRP) due to the high affinity of biotin for avidin/streptavidin. Once the SA-HRP is bound to the biotinylated detection antibody, the enzymatic activity of the horseradish peroxidase can act on the hydrogen peroxide contained within the colorimetric substrate added to the plate to produce a color change directly correlated to the amount of analyte present within the antibody “sandwich”. This reaction is stopped at a specified timepoint to ensure an accurate and precise reading and the color intensity is read on a spectrophotometric plate reader.

Each ELISA plate assay is performed with a standard curve of known protein concentration that is used to interpolate the concentration of residual HCP contained within the unknown process samples. Additionally, controls are contained within each plate to verify the assay is performing as expected and the data is accurate. All standards, controls, and test samples are plated in duplicate wells to demonstrate the data obtained is precise.

DNA concentration

The residual CHO host DNA assay is a real-time quantitative polymerase chain reaction (qPCR) which is a kinetic chain reaction that uses a specialized Peltier thermocycling and detection system in conjunction with fluorescence to quantitate the amount of PCR product being produced each cycle, thus providing real-time quantitation of DNA amplification performed by the DNA polymerase.

The residual host DNA assay uses a Taq DNA polymerase-based master mix (Master Mix, Applied Biosystems, MA) and proprietary primers and probe targeting the type-2 Alu-equivalent sequence of the CHO genome. The assay achieves fluorescent signaling via the probe labeled with both a 5-inch reporter dye and a 3-inch quencher dye or non-fluorescent minor groove binder (MGB). This is commonly referred to as a TaqMan probe. The probe itself is an oligo comprised a nucleotide sequence that will bind a portion of the target sequence downstream of the forward or reverse primer. When both fluorochromes are within close proximity on the probe, the quencher molecule stops the detectable fluorescence of the reporter due to fluorescence resonance energy transfer (FRET). As the Taq DNA polymerase extends the primer, the intrinsic 5-inch to 3-inch nuclease activity of the polymerase degrades the probe, releasing the reporter fluorochrome; the fluorescence can then be read by the 7500 real-time PCR system (Applied Biosystems). The amount of fluorescence released during the amplification cycle is proportional to the amount of product generated in each cycle.

Prior to quantitation via qPCR, biopharmaceutical in-process and bulk drug substance samples require dilution and extraction to allow for accurate quantitation of the residual CHO genomic DNA, because protein concentration, pH, and buffer components within the samples may interfere with the qPCR assay. A sodium iodide based manual extraction using extractor kits (Wako Extractor, FujiFilm Wako Chemicals, VA) or an automated proprietary magnetic silica-based extraction using a purification system (ThermoFisher KingFisher, Thermo Fisher Scientific, MA) may be used

to purify residual CHO genomic DNA from samples. Samples undergoing extraction are also performed with a spike of known DNA concentration to demonstrate good recovery of purified DNA within the extraction method. The qPCR assay is then performed using these purified samples and a standard curve of known concentrations of the host genomic DNA, the logarithmic amplification of which is converted to a linear scale using the cycle time (Ct) at which the fluorescence crosses the set threshold. The standard curve can then be used to calculate the host DNA concentration within the samples using the Ct value of the sample reaction wells.

RESULTS AND DISCUSSION

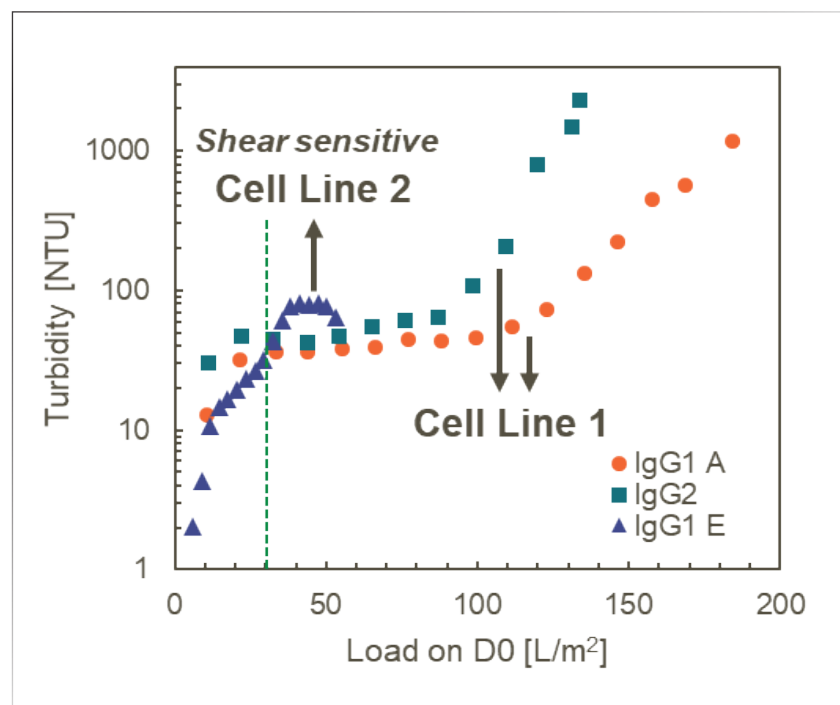
Fouling mechanisms of coarse depth filters

In direct depth filtration processes, the primary or coarse depth filter is

designed to remove larger particulates such as whole cells and cell debris from the upstream feed stream. As opposed to the membrane filters typically used for sterile or tangential flow filtration, the pore sizes of depth filters are not as strictly defined, and retention is achieved by particle capture in the porous bulk of the filter. Consequently, coarse depth filters are prone to breakthrough of particulates at the onset of fouling. While the uptake of particulates in the filters can increase the pressure drop, breakthrough of particulates through the coarse depth filter generally leads to a significantly higher pressure increase on the consecutive finer filters.

Hypothesizing that turbidity is a good indicator of filter performance for these larger particulates, the filtrate turbidity of a D0SP coarse depth filter was monitored as a function of the filter loading for three

Figure 2. Filtrate turbidity as a function of load on the coarse (D0SP) depth filter for three different IgG projects. IgG1 E uses a cell line that is more shear sensitive than IgG1 A and IgG2. Inflection in the turbidity trend for IgG1 E, suggesting breakthrough, is indicated by the green dashed line.

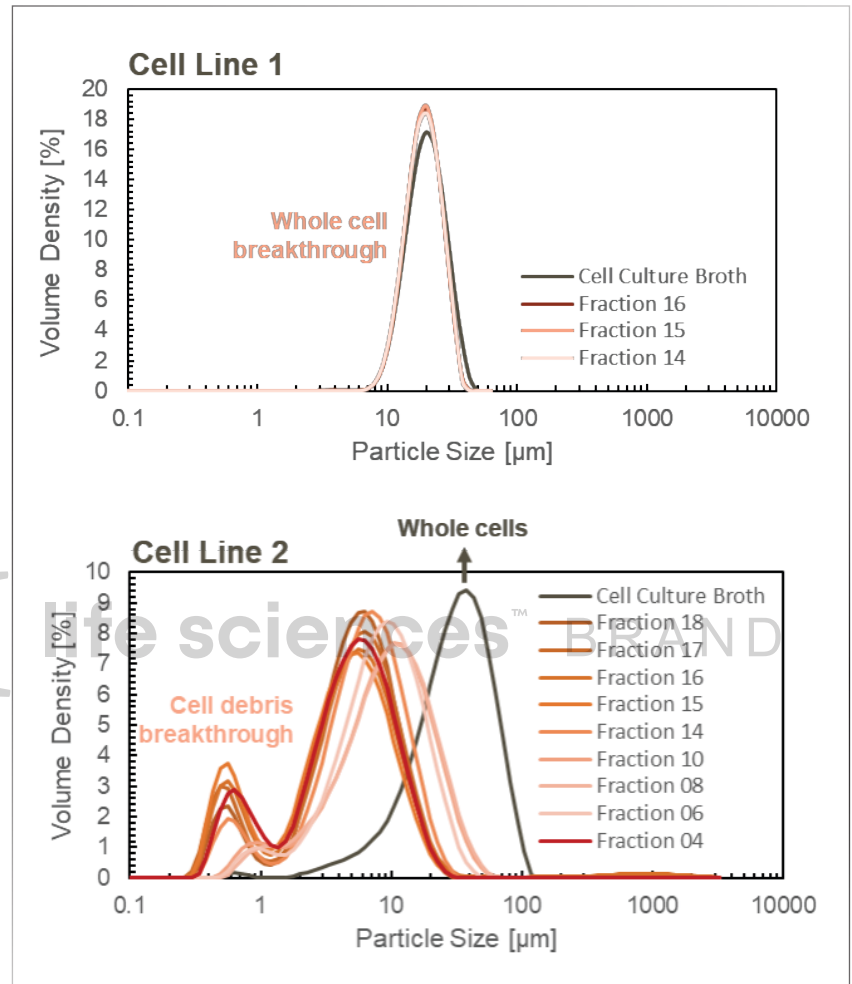


different mAb feed streams. The results in **Figure 2** show that in a semi-logarithmic plot, the filtrate turbidity initially plateaus and only increases gradually during the lifetime of the filter. However, after reaching a certain loading, turbidity increases rapidly as larger particulates start breaking through the filter. The pressure drop over the filter was generally around 10–15 psi at this breakthrough loading. The turbidity breakthrough occurred around 100 L/m² for IgG1 A and IgG2, which originated from a similar cell line. However, IgG1 E, which originates from a different cell line, showed much faster turbidity breakthrough.

Consequently, to better understand the nature of the turbidity breakthrough, the particle size distribution in the cell culture feed material and the filtrates at different filter capacities were evaluated using laser diffraction for two different cell lines (**Figure 3**). The particle size distribution of both cell culture feed materials indicates the presence of mainly whole cells around the expected size of 20 µm. While the IgG1 A filtrate also mainly contains whole cells after breakthrough, the IgG1 E filtrate contains smaller cell debris, particularly after breakthrough (fraction 14 onwards). This shows that Cell Line 2, which is known to be more shear sensitive, is vulnerable to cell rupture during the depth filtration. Note that the laser diffraction data only captures the relative particle size distribution, not the absolute number of particles. For example, while the breakthrough fractions for Cell Line 1 show a similar distribution of mainly whole cells breaking through, the number of whole cells in the filtrate is not expected to be the same as the feed material, based on the turbidity data.

Generally, the failure of the filter train manifests as an increase in the pressure drop over the fine depth

Figure 3. Particle size distribution of different filtrate fractions of the coarse depth filter (DOSP) as compared to that of the cell culture broth for IgG1 A (top, cell line 1) and IgG1 E (bottom, cell line 2). While the IgG1 A filtrate mainly contains whole cells after breakthrough, the IgG1 E filtrate contains smaller cell debris, particularly after breakthrough (fraction 14 onwards).



filter and/or the membrane filter (**Figure 1**). However, these observations show that while the pressure increases may appear in the finer filters, the coarse depth filter can be the underlying cause of the failure mechanism. After breakthrough, the rapid increase in large particles in the coarse depth filter filtrate clog the finer filters downstream, leading to the pressure drop increase. In this example, increasing the filter area of the fine depth or membrane filter, based on the fact they show the largest pressure drop, would do lit-

tle to alleviate the underlying problem: the coarse depth filter reaching capacity. This example highlights the importance of understanding the underlying failure mechanisms of the filter train to optimize the harvest process.

CONCLUSION FOR PART 1

In the direct depth filtration of high cell density feed streams for the harvest of biopharmaceutical products, the filter capacity is generally limited by the ability of the coarser depth filter to remove larger

particulates such as intact cells, which is described in this article (Part 1 of the study). In a subsequent article (Part 2 of the study), the authors will describe the study outcome regarding HCP removal by the finer depth filter and a correlation between wet cell mass and depth filter capacity in filtration systems and a more predictive approach to harvest development.

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The authors declare no financial or commercial conflict of interest.

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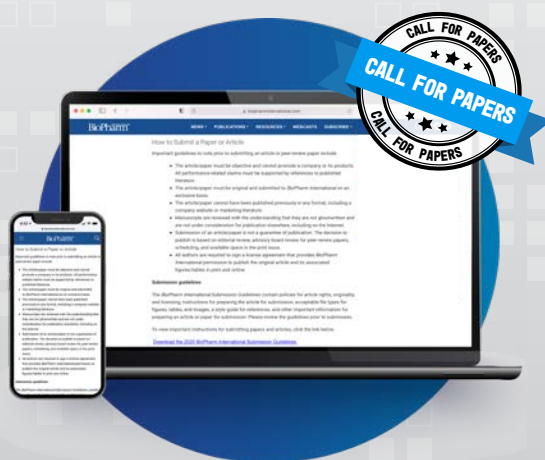
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