Impact of Cell Density and Viability on Primary Clarification of Mammalian Cell Broth

An Analysis Using Disc-Stack Centrifugation and Charged Depth Filtration

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Production of monoclonal antibodies from mammalian cell culture has become ubiquitous in the biotechnology industry as companies continue to identify opportunities to treat diseases with such therapeutic proteins. The first step in recovery of secreted antibodies is to remove most insoluble components of the cell culture from the product stream. These components consist of whole cells, cell debris, colloids, and other such impurities. One industry-preferred method for accomplishing this initial separation is to use continuous disc-stack centrifugation coupled with depth filtration. These primary recovery steps are intended to remove most particulates from cell broth to ease the burden on the subsequent purification steps.

PRODUCT FOCUS: MONOCLONAL ANTIBODIES

PROCESS FOCUS: DOWNSTREAM PROCESSING

WHO SHOULD READ: PROCESS DEVELOPMENT AND MANUFACTURING

KEYWORDS: DEPTH FILTRATION, DISC-STACK CENTRIFUGATION, CLARIFICATION, TURBIDITY, PRIMARY RECOVERY

LEVEL: INTERMEDIATE

A disc-stack centrifuge can remove whole cells and larger cell debris from a cell culture using stacked, inclined conical discs to separate the solids (**1–3**). General centrifuge separation theory with computational fluid dynamics (CFD) has been described elsewhere (**4, 5**). For disc-stack centrifugation, cell culture broth is fed into a rotating bowl, and centrifugal force causes solids to separate in a narrow channel between the discs. Those separated solids slide down the underside of the discs into a solids-holding space, from which they can be discharged regularly. Clarified liquid containing the protein of interest continues up the disc stack and out of the bowl. This technique works extremely well for removing whole mammalian cells, provided that centrifuge conditions cause no shear-induced damage to those cells (**4**). Cell shearing will increase the amount of submicron particles that cannot be removed by the centrifuge.

The minimum particle size that a continuous disc-stack centrifuge can remove is a function of cell culture properties, centrifuge feed rate, and bowl geometry and rotational speed. The rotational speed and geometry are taken into account by the Sigma factor (Σ) for the centrifuge. This factor denotes the area of a gravity-settling tank needed to achieve the same amount of clarification as the centrifuge. For a disc-stack centrifuge, Equation 1 gives this equivalent clarification area (**2**).

Disc-stack centrifuge used in biotech and pharmaceutical applications.
WESTFALIA SEPARATOR AG (WWW.WESTFALIA-SEPARATOR.COM)

The ratio of the feed rate to the centrifuge (Q) and the equivalent clarification area (Σ) gives the equivalent settling velocity that can be achieved for a given set of operating conditions. Combining this Q/Σ ratio with Stoke's Law for a gravity-settled spherical particle (assuming a Newtonian fluid and low particle density) allows the minimum particle

size that can be separated in a disc-stack centrifuge at the given conditions to be calculated using Equation 2 (**1, 3**).

As the value of Q/Σ decreases, so does the size of particles that can be removed by the centrifuge for better clarification. Typically, it is difficult to effectively remove cell debris much smaller than 1 µm using a disc-stack centrifuge at practical operating conditions (**1**). Once such conditions are optimized at small scale, the Q/Σ ratio is typically kept constant upon scale-up between different disc-stack centrifuges to maintain a minimum particle size removal and provide similar product clarity at each scale (**3**).

Because there is a limit to the particle size that can be removed by a disc-stack centrifuge, further clarification by depth filtration is typically used to remove smaller solid particulates that still remain in the centrifuge product(**6–8**). Commonly used depth filters consist of a thick, porous matrix of cellulose fibers with inorganic filter aids bound to them by a positively charged resin. The thick matrix provides a tortuous path to retain a range of particle sizes, and the positive charge imparts adsorptive properties to the filter. The minimum particle size that can be effectively removed solely by the sieving mechanism of a depth filter is about 0.1 µm (**8**). The adsorptive mechanism, however, can remove much smaller negatively charged impurities such as DNA and host-cell proteins to further improve product quality (**6**). The key operating parameters for a depth filtration step are filter flux (L/m^2/hr), filter loading (L/m^2), and pressure drop across the filter (ΔP). Flux and loading are typically kept constant during scale-up, with the intention of providing the same product clarity and pressure drop at each scale. For most filters, lower filter flux and loading lead to better clarification.

In addition to the key operating parameters for the disc-stack centrifuge and depth filtration steps, another major factor determining the quality of a primary recovery product is the amount of solid particles present in the initial cell culture at harvest. This level is mostly dictated by the cell density and cell viability of a culture. Higher cell densities and lower viabilities lead to larger amounts of whole cells, cell debris, colloids, and other solid impurities in the broth. This high cell density, low viability condition for a cell culture also tends to provide the highest product titers (**8**), which necessitates relatively robust primary recovery steps to provide the desired product clarity.

Over the course of process development for a new monoclonal antibody (MAb) product made by mammalian cell culture, we harvested a number of cell culture batches at varying cell densities and viabilities to investigate the effect of the different harvest conditions on the quality of our primary recovery product. We could then draw correlations between those cell culture properties and the performance of both the disc-stack centrifugation and depth filtration steps.

Equation 1

$$\Sigma = \frac{2\pi}{3g} \times \omega^2 \times N \times \cot\alpha \times (r_o^3 - r_i^3)$$

where

Σ = equivalent clarification area of centrifuge (m^2)
g = acceleration due to gravity (m/s^2)
ω = angular bowl velocity (rad/s)
N = number of discs in stack
α = disc half-conical angle (°)
r_o = outer radius of disc (m)
r_i = inner radius of disc (m)

Equation 2

$$d_{min} = \sqrt{\frac{Q}{\Sigma}} \times \sqrt{\frac{18\eta}{\Delta\rho g}}$$

where

d_{min} = diameter of minimum particle size that can be separated (m)
Q = centrifuge feed rate (m^3/s)
Σ = equivalent separation area of centrifuge (m^2)
η = dynamic viscosity of culture medium (kg/m·s)
$\Delta\rho$ = density difference between cells and medium (kg/m^3)
γ = acceleration due to gravity (m/s^2)

Materials and Methods

The disc-stack centrifuge we used for processing was from Westfalia Separator AG of Oelde, Germany (www.westfalia-separator.com). The model CSA-1 centrifuge contains a stack of 46 conical discs for separation, with a total bowl volume of 600 mL and a 250-mL sediment holding space. Solids are discharged periodically using a hydraulic discharge mechanism, and the feed zone contains a hydrothermetic inlet to minimize shear. The maximum bowl speed is 9,470 rpm (5,200*g* at the outer disc diameter), which provides a maximum equivalent clarification area (Σ) of over 1,400 m^2.

The depth filters we used for further clarification were provided by 3M CUNO of Meriden, CT (www.cuno.com) and Millipore Corporation of Billerica, MA (www.millipore.com). We performed a two-stage depth filtration using either Zeta Plus Maximizer EXT filters from CUNO or Millistak+ HC filters from Millipore. For the CUNO filters, the first-stage filtration involved a 60ZA05A filter (nominal retention rating >0.3 µm) and was followed by a second stage using the 90ZA08A filter (nominal retention rating >0.2 µm). For the Millipore filters, the first-stage filtration involved a C0HC filter (nominal retention rating >0.4 µm) and was followed by a second stage using the A1HC filter (nominal retention rating >0.1 µm). All these depth filters consist of dual-layer cellulose-based depth media containing inorganic filter aids bound to cellulose fibers by a positively charged resin binder. The Millipore A1HC filter is backed by an additional mixed cellulose ester membrane layer following the two layers of depth media.

Batches of mammalian cell culture were provided by the fermentation and cell culture group in bioprocess R&D at Merck & Co., Inc. (Rahway, NJ). The cell broth contained a MAb product secreted by a murine myeloma cell line. Cell density and viability of each batch at harvest were determined using the Cedex system from Innovatis AG (Bielefeld, Germany).

Analytical Techniques: Turbidity of the cell culture, centrifuge product, and depth filter product was measured

Equations 3 (top) and 4 (bottom)

$$\text{Centrifuge Clarification Efficiency} = \left(1 - \frac{\text{Centrate Turbidity}}{\text{Cell Culture Turbidity}}\right) \times 100$$

$$\text{Depth Filtration Clarification Efficiency} = \left(1 - \frac{\text{Filtrate Turbidity}}{\text{Centrate Turbidity}}\right) \times 100$$

using a Micro 100 Laboratory Turbidimeter from HF Scientific, Inc. (Fort Myers, FL). Host-cell protein (hcp) levels in the process streams were analyzed with an in-house protein ELISA assay specific to the murine myeloma cell line. DNA levels were determined using the fluorescence-based PicoGreen assay from Invitrogen (Carlsbad, CA).

Centrifugation Methods: Throughout the course of process development for the MAb product, several cell culture batches were harvested at various cell densities and viabilities. The densities ranged from 5.6×10^6 to 13.6×10^6 cells/mL, and cell viabilities ranged from 20% to 82%. The batches came from fed-batch fermentations at 30-L or 300-L scale. All batches were initially clarified through the CSA-1 disc-stack centrifuge at the maximum bowl speed using various feed rates so we could explore a range of Q/Σ values from 3.4×10^{-9} to 13.5×10^{-9} m/s. We controlled the feed rate by changing the pump speed of the peristaltic pump used to introduce the cell culture into our centrifuge. Pressure fluctuations caused by the pulsatile nature of that pump were minimal for all feed rates tested, so we did not expect them to affect our centrifugation results. For each Q/Σ value, we monitored the turbidity of the centrate stream and used the steady-state value to determine the clarification efficiency of the centrifuge. This was calculated using Equation 3.

To examine the relationship between cell density and viability and the resulting centrifuge product, we then correlated clarification efficiency and centrate turbidity to the cell culture properties. In addition, for one batch we analyzed centrifuge products from a wide range of Q/Σ values for hcp and DNA levels to determine what cell shearing may have occurred during processing.

Depth Filtration Methods: We further clarified the centrate streams using the 60ZA05A–90ZA08A and C0HC–A1HC two-stage depth filtrations initially with 2-in. diameter filter discs and eventually scaling-up to 16-in. diameter filters. All filtrations used a constant feed rate method of operation controlled with a peristaltic pump. Flux and loading targets for the filtrations were dictated by constraints at the proposed manufacturing site for the product. The required loading was 500 L/m^2, and the target flux was 180 LMH, with a maximum pressure drop of 25 psig across the filters. We monitored filter inlet pressure and filtrate turbidity throughout the filtrations to examine the profile of both parameters as the loading increased. Performance of the two filter trains was compared, and we used the leading candidate for scale-up to evaluate the effect of cell density and viability on the depth filtration step. We used the turbidity of the pooled filtrate at the target loading to calculate the clarification efficiency of the depth filtration with Equation 4.

Then we correlated depth filtration clarification efficiency and filtrate turbidity to the cell density and viability of the cell culture at harvest (before centrifugation) to determine the effect of those cell culture properties on the depth filtration product.

Results

Disc-Stack Centrifugation: Upon harvest of a cell culture batch, a company's cell culture group typically reports a viable cell density (VCD) and percent viability for the batch. These numbers can be used to calculate the total cell density (TCD) of the batch using Equation 5, in which the units of TCD and VCD are cells/mL and the percent viability is greater than zero. TCD can be used to gauge the amount of large cell solids that are present in a batch. If that number is significantly higher than expected, then centrifugation operating parameters may need to be adjusted to achieve desired centrate clarity.

Figures 1 and 2 show centrifugation results for batches with similar TCDs. Centrifugation clarification efficiency is plotted against the percent viability of the batch for each centrifugation condition evaluated. We found that for batches with similar cell densities this relationship was linear for each Q/Σ value tested. As the percent viability drops, so does the clarification efficiency. We also observed that for high viabilities, the clarification efficiency seemed to converge for all Q/Σ values tested, indicating less sensitivity to feed rate at higher viabilities.

A similar correlation was attempted for the centrate turbidity to determine whether cell density and viability of a cell culture could be used to estimate the quality of the centrifugation product. As expected, we saw no correlation between centrate turbidity and TCD for a given Q/Σ value (data

Figure 1: Effect of cell viability on disc-stack centrifugation clarification efficiency for low–cell-density cultures. Batches were harvested with an average total cell density of 7.2×10^6 cells/mL.

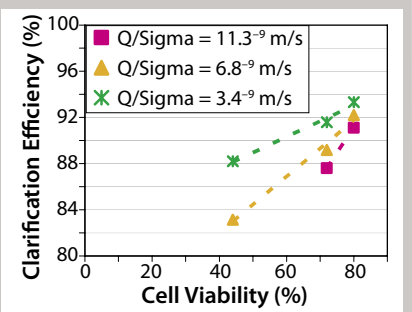


Figure 2: Effect of cell viability on disc-stack centrifugation clarification efficiency for high–cell-density cultures. Batches were harvested with an average total cell density of 11.1×10^6 cells/mL.

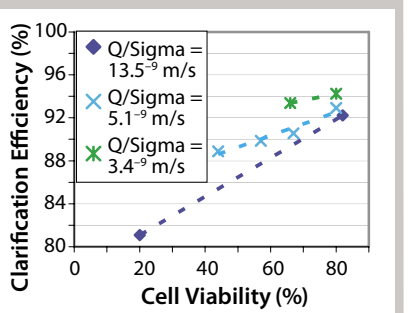


Figure 3: Impact of nonviable cell density (NVCD) at harvest on centrate quality for different disc-stack centrifugation conditions.

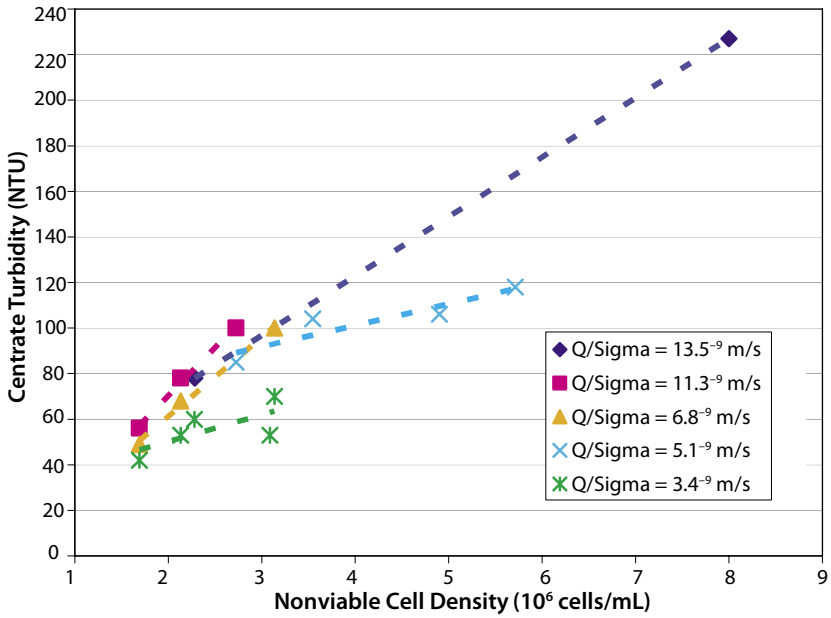
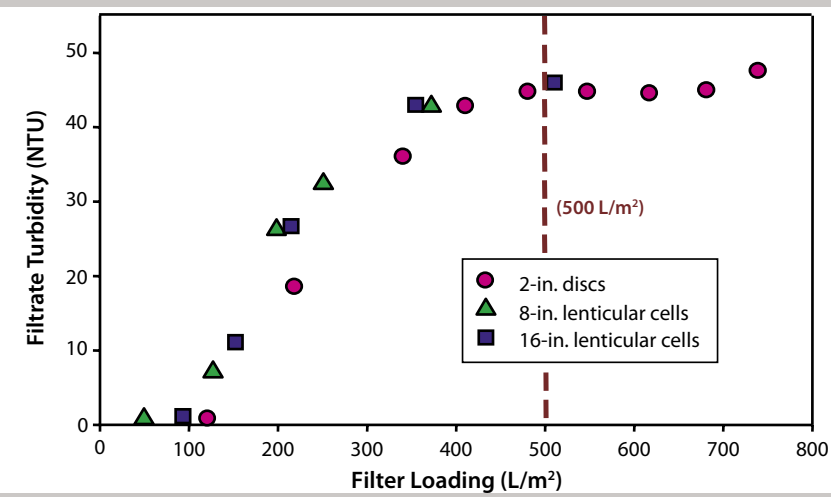


Figure 4: Scale-up performance of the 60ZA05A and 90ZA08A depth filters at an average flux of 185 LMH for the same pool of centrifuged product. In-line turbidity of the depth filtrate was monitored as a function of filter loading at each scale.



not shown) because the amount of cell debris at harvest from different cell viabilities is not directly taken into account with this method. As shown in Figures 1 and 2, lower viabilities result in reduced clarification. However, we also found no linear correlation by plotting centrate turbidity against either viable cell density or viability for all harvested batches (data not shown) because neither method completely takes into account the effect of increasing TCD on the observed clarification.

To try capturing the effects of both TCD and viability at harvest on centrate turbidity, we decided to examine the nonviable cell density (NVCD) of the cell culture. This can be calculated using Equation 6, in which the units of NVCD, TCD, and VCD are all cells/mL. NVCD is essentially a measure of the number of dying cells in a batch, which reflects both TCD and viability. The number of dying cells present is an indicator of both the number of whole cells present and the amount of cell debris present from cells that have already lysed. The higher the NVCD, the more total cells and cell debris must be removed across primary recovery. Figure 3 shows the relationship between centrate turbidity and NVCD for the different Q/Σ values we evaluated. The relationship appears linear for the values tested, and (as expected) lower turbidity values correspond to lower NVCD values for each centrifugation condition.

The reason these linear correlations could be made for the disc-stack centrifuge was that there were minimal differences in shear damage between its different operating conditions. Table 1 shows the relative levels of hcp and DNA present in the centrifuge product for a wide range of centrifuge conditions. Data indicate no discernible pattern in impurity values with changes in bowl speed and feed rate. In fact, when assay variability (10–20%) is taken into account, the impurity values are essentially the same for all centrifuge conditions, indicating little change in cell shearing with operating conditions in the CSA-1. Because no condition increased the generation of more particles due to shear, we could draw the linear turbidity correlations.

Depth Filtration: We also made correlations similar to those for centrifugation for the subsequent depth filtration step. Centrifuge product for all filtrations was filtered at a constant flux of ~185 LMH with a target loading of at least 500 L/m^2 and a maximum pressure drop of 25 psig to match conditions at the proposed manufacturing facility for the product. Table 2 shows a relative performance comparison for the two different filter types initially evaluated. This development work suggested the CUNO 60ZA05A–90ZA08A filters were the leading contenders to meet the required constraints based on capacity and scale-up performance.

Figure 4 illustrates the consistent performance of those filters upon scale-up at the desired operating conditions. This figure also illustrates the three operating regimes for a typical depth filter. At low loadings, very low filtrate turbidity is achieved because of the

Equations 5 (top) and 6 (bottom)

$$TCD = \frac{VCD}{\%\ Viability} \times 100$$

$$NVCD = TCD - VCD$$

Figure 5: Effect of cell viability at harvest on depth filtration clarification efficiency. Centrifuge product harvested at an average cell density of 11.7 × 10^6 cells/mL was depth filtered at an average flux of 185 LMH and average loading of 560 L/m^2.

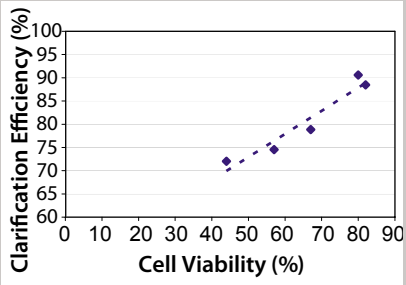


Figure 6: Effect of cell viability at harvest on the quality of depth filtration product with loading. Two batches of centrifuge product harvested at similar cell densities were depth filtered at 185 LMH while in-line turbidity of the filtrate was monitored.

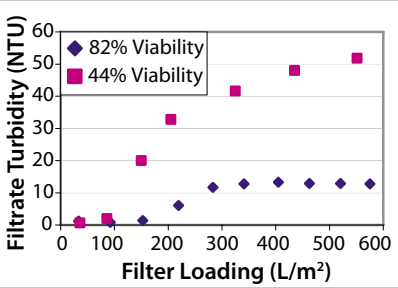
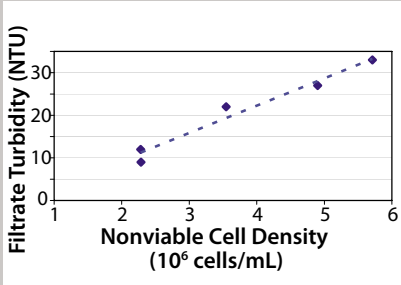


Figure 7: Impact of nonviable cell density (NVCD) at harvest on the quality of the depth filtration product. Centrifuge product initially harvested at an average cell density of 11.7 × 10^6 cells/mL was depth filtered at an average flux of 185 LMH and average loading of 560 L/m^2.



combined effect of physical particle retention along with physical adsorption. As loading increases, however, the number of adsorptive sites is reduced, so filtrate turbidity begins to increase as some of the negatively charged particles (hcp and DNA) begin to break through. Once the filter's adsorptive capacity is completely exhausted at high loadings, filtration functions solely by means of particle retention, and the maximum filtrate turbidity is reached. The 60ZA05A–90ZA08A filters met the 500-L/m^2 loading requirement with the required pressure drop of <25 psig across the filter bank while providing acceptable filtrate quality and consistent scale-up performance.

Once we identified a leading filter candidate, we could closely examine the impact of cell density and viability on the depth filtration step. Figure 5 plots clarification efficiency for the different batches of centrate material processed using the 60ZA05A–90ZA08A filters against viability of the cell culture batch at harvest. The plot demonstrates a linear trend, with the clarification efficiency decreasing with a drop in viability. That decrease directly results from the number of small solid particles carried over from the centrifugation step.

We examined that phenomenon more closely by tracking the turbidity of the depth filtrate with loading for two batches of similar TCDs harvested at different viabilities. The resulting data are plotted in Figure 6. For the batch harvested at 44% viability, turbidity at the end of loading is four times larger than that of the 82% viability batch. Initial break-through of impurities occurred later for the high-viability batch at a loading of ~180 L/m^2 compared with ~120 L/m^2 for the low-viability batch. It should also be noted that we centrifuged the high-viability batch at a feed rate and Q/Σ value 2.5 times higher than the low-viability batch, which still resulted in a lower centrate turbidity. These data clearly indicate the negative impact of a low-viability batch on the depth-filtration step.

We further correlated our 60ZA05Z–90ZA08A depth filtration results with the NVCD. Figure 7 shows turbidity of the depth filtration product pools plotted against NVCD of the initial cell culture. As expected, there was a linear trend, with a lower NVCD corresponding to a lower depth filtrate turbidity.

Scale-Up: Following the extensive process development work for these primary recovery steps, the centrifugation and depth filtration were scaled up to the 5-kL scale. Table 3 shows the results. Both steps have performed consistently with desired performance across several batches.

Discussion

The results of our experiments demonstrate the impact of cell viability and nonviable cell density on the quality of primary recovery product from a mammalian cell culture. We used turbidity as a marker for product quality because it has been shown to correspond to impurity levels in the product (**2, 3**) and to affect loading on subsequent sterile filtration steps (**3**). For a given product concentration, the higher the turbidity of the primary recovery process stream, the higher the level of DNA, hcp, and other impurities in that stream.

Effect of Viability on Centrifuge Clarification: For a given centrifugation condition, clarification efficiency was linearly dependent on the viability of a cell culture at harvest (Figures 1 and 2). As viability decreased, so did clarification efficiency. The reason for this is that, at lower viabilities, more dead and dying cells are present in the culture, and thus more fine cell debris that is too small to be removed by the disc-stack centrifuge. Comparing data

Table 1: Relative host cell protein (hcp) and DNA levels in the centrifuge product across a range of Q/Σ values. Results indicate little difference in shear effects with operating conditions for the CSA-1 disc stack centrifuge.

Feed Rate (Q) (mL/min)	Bowl Speed (rpm)	Q/S (m/s)	Relative Amount of hcp in Centrate	Relative Amount of DNA in Centrate
200	9,470	2.3 × 10^{-9}	1.02	1.06
200	7,781	3.3 × 10^{-9}	1.01	0.89
200	6,000	5.6 × 10^{-9}	0.99	1.01
450	9,470	5.1 × 10^{-9}	0.90	1.18
450	7,781	7.5 × 10^{-9}	0.97	1.17
450	6,000	12.6 × 10^{-9}	1.06	0.99
1,000	9,470	11.3 × 10^{-9}	1.05	1.10
1,000	7,781	16.7 × 10^{-9}	1.00	1.00
1000	6,000	28.0 × 10^{-9}	0.99	1.15

from the high (11×10^6 cells/mL) and low (7×10^6 cells/mL) TCD batches, demonstrates that this effect of viability on clarification efficiency appears to be greater for the lower-TCD batches. The decline in clarification efficiency with decreasing viability is greater for the low-density batches because the rate of change in initial cell culture turbidity with viability is greater, most likely because of the greater influence of whole cells on the turbidity measurement. Feed turbidity for the high-density culture is much higher because of the number of whole cells present, and a drop in viability does not reduce turbidity as much as in the lower cell density batches.

Effect of Centrifuge Feed Rates on Clarification: The different centrifuge feed rates we tested for the high- and low-density batches performed as expected in regards to clarification efficiency, with higher feed rates giving lower clarification because of lower centrifuge residence time. The convergence of clarification values at high viabilities for both cell densities indicates less sensitivity to feed rate as the number of intact cells increases, which suggests that the disc-stack centrifuge can remove whole cells from a culture over a large range of Q/Σ (provided there is no increase in cell shearing). However, residence time in the centrifuge becomes a key parameter as the amount of cell debris increases for a given cell density.

Effect of NVCD on Centrate Quality: We also showed that the turbidity of the centrifuge supernatant depends linearly on the nonviable cell density of a cell culture at harvest. We used NVCD in an effort to take both cell viability and TCD into account for each cell culture batch, which allowed turbidity results for all harvest conditions to be plotted on the same graph. For each centrifugation condition we tested, as NVCD increased so did centrate turbidity (Figure 3). NVCD reflects both the number of total whole cells in a cell culture and the amount of fine cell debris that is too small to be removed by a disc-stack centrifuge. With the exception of the 5.4×10^{-9} m/s centrifugation condition, these lines also seemed to converge as the nonviable density decreased, indicating that similar centrate turbidities can be obtained over a large range of Q/Σ when the NVCD in a cell culture batch is low. However, residence time becomes a key parameter as the number of total cells and amount of cell debris increases.

Table 2: Relative performance comparison between 60ZA05A–90ZA08A and C0HC–A1HC two-stage depth filtrations. The C0HC–A1HC combination had the better filtrate quality, but the 60ZA05A–90ZA08A combination had the better capacity and scale-up path.

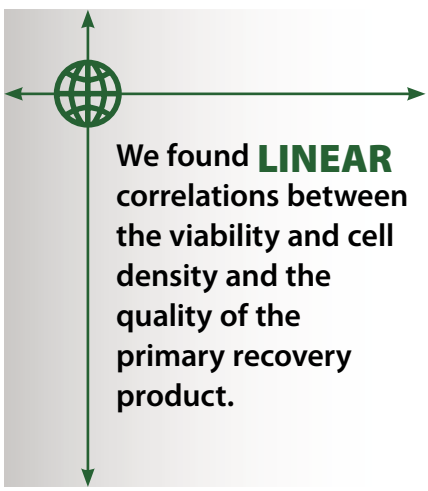
Filter Train	Filter Capacity (Pressure Drop)	Filtrate Turbidity (Particle Retention)	Consistent Scale-Up Path
60ZA05A/90ZA08A	++	++	++
C0HC/A1HC	+	+++	+/-

Table 3: Summary of primary recovery performance for three batches of MAb product at the 5-kL scale. Both the disc-stack centrifugation and depth filtration have performed consistently.

	Batch #1	Batch #2	Batch #3
Disc-stack centrifuge clarification efficiency	>90%	>90%	>90%
Pressure drop across depth filter bank	<15 psig	<15 psig	<15 psig
Loading on depth filters	498 L/m^2	496 L/m^2	499 L/m^2
Yield across primary recovery (disc-stack centrifuge + depth filters)	91%	89%	91%

Effects of Viability and NVCD on Depth Filtration: The correlations for the centrifuge were the same as those shown for the depth filtration of the centrifuge product. Because we did not finalize the exact types of depth filters until after cell culture conditions had been optimized, little variability occurred in cell density of the batches processed through the filters, so data are similar for both viability (Figure 5) and NVCD (Figure 7) plots. As for centrifugation, clarification efficiency across the filters increased with higher viabilities, and turbidity of the depth filtration product increased with NVCD for the required depth filter flux and loading.

Those results demonstrate the effect of fine cell debris in an initial cell culture propagating through both the centrifugation and depth filtration steps. Although the positive charge imparted on the depth filters does lead to clearance of some submicron particles, once all the adsorption sites are occupied, then break-through of such particles does occur (Figures 4 and 6). The effect can be illustrated further by examining the turbidity profile of the depth filtrate with loading for two batches harvested at different viabilities (Figure 6). At lower viabilities, the amount of submicron particles remaining in the centrate is greater, and break-through of the depth filter begins to occur earlier in the load, which results in a higher-turbidity depth filtrate pool. However, the break-through effect may be limited if sufficient depth filter area can be used to achieve more modest loadings (~100 L/m^2) (**6**). That would help dampen the effects of different cell culture viabilities and lead to a more consistent quality primary recovery product.



For Consistent Primary Recovery

Over the course of process development for a new monoclonal antibody product produced from a murine myeloma cell line, several batches of cell culture were harvested and processed through primary recovery. We found linear correlations between the viability and cell density of those batches and the clarification and quality of the primary recovery

product. High-viability and low-NVCD batches gave the best clarification efficiency and product quality for both disc-stack centrifugation and depth filtration. The reasons for this are limited removal of cell debris and fines from lysed cells using a disc-stack centrifuge and limited capacity of positively charged depth filters to remove the same fine particulates.

Our results led to setting a harvest specification of greater than 50% viability upon scale-up for the particular product clarified in our experiments because the depth filter area available at the manufacturing site was fixed. This specification limited the amount of cell debris and fines in our primary recovery product and reduced the burden on subsequent filtration and chromatography steps. The result has been a consistent primary recovery performance across several batches at the manufacturing scale (Table 3).

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