The clarification of biological products such as recombinant therapeutic proteins, monoclonal antibodies, and vaccines requires choosing a sequence of operations that ensures high yield, product consistency, and reproducibility. Depth, or pad, filtration is gaining acceptance as one technique for the removal of cells, cell debris, colloids, insoluble precipitants, aggregates, and other materials found in mammalian-cell and bacterial fermentation broths. One or more depth filters can be used in a clarification sequence to optimize the filtration with each successive filter, thereby removing progressively smaller particles by means of mechanical sieving and adsorption. Because optimized depth filtration can play such an important role in enhancing the efficiency of the entire clarification process, this article primarily addresses depth filtration technologies and applications. The authors wish to emphasize secondary clarification of effluent from various bioreactor types and methods of primary clarification and the effects of the various bioreactor types on the sizing of downstream sterile filters. The authors also describe considerations that should govern the design of the filtration stream as a whole.

The choice of technology for any given process must depend on the fluid properties of the stream as well as requirements for integration with downstream processes such as chromatography and ultrafiltration. The goals of clarification include high yield, product consistency, and reproducibility.

The clarification process can be separated into primary recovery, secondary clarification, prefiltration, and sterile filtration. The process as a whole is designed to handle high particulate loading and the wide particle-size distribution found in biological streams (see Figure 1). The efficiency and capacity of the total process is improved by distributing the particle loading across several steps.

Primary recovery, the first phase of the process, removes the bulk of large particles, whole cells, and cell debris. This recovery step is usually performed by tangential-flow filtration–microfiltration (TFF–MF), centrifugation, or, in some cases, depth filtration. Secondary clarification treats the product of primary recovery and is used for the removal of colloids, lipids, DNA–RNA, residual cells, and other particles not removed in the primary recovery process. Secondary filtration typically includes one to several sequences of depth filtration designed to...
Primary recovery and secondary clarification scenarios for removal of cells, cell debris, and contaminants from a bioreactor process stream.

<table>
<thead>
<tr>
<th>Type of Filter</th>
<th>Filter Media</th>
<th>Process Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenticular depth filter</td>
<td>Single-layer cellulose with filter aid</td>
<td>General clarification; usually requires multiple filters.</td>
</tr>
<tr>
<td>(positive charge)</td>
<td>Double-layer cellulose with filter aid</td>
<td>Improved capacity and retention.</td>
</tr>
<tr>
<td></td>
<td>Double-layer cellulose with filter aid and membrane filter</td>
<td>Highest retention of particles and colloids. Reduces the total number of required filters.</td>
</tr>
<tr>
<td>Pleated prefilter</td>
<td>Membrane, glass microfiber, polypropylene</td>
<td>High retention efficiency for membrane protection; low capacity in high colloid streams.</td>
</tr>
<tr>
<td>Wrapped depth filter</td>
<td>Polypropylene</td>
<td>Moderate contaminant capacity. Poor retention of colloids. Use for small batches.</td>
</tr>
</tbody>
</table>

Figure 1: Typical filtration of a biological product.

Figure 2: Primary recovery and secondary clarification scenarios for removal of cells, cell debris, and contaminants from a bioreactor process stream.

filtration step before sterile filtration is designed to remove trace bioburden and colloids, thereby protecting the sterile filter. After sterilization, the sterile product is then captured and purified using chromatography, ultrafiltration, and other purification processes.

The keys to an optimal secondary filtration are the quality of effluent from the primary separation and the effect that the product of secondary filtration has on downstream sterile filters. Although multiple linked stages are common within secondary filtration to achieve product clarity, each unit process has the potential for yield loss. One of the process developer’s goals is to achieve the highest level of product recovery and contaminant removal with the fewest number of unit processes.

Various technologies such as TFF–MF and normal-flow depth filtration (NFF) may be used for primary recovery before secondary filtration. The choice of technology will have an effect on the selection and sizing of depth filtration. Figure 2 shows the possible combinations of unit processes for the effective recovery of a biological drug product.

For large-volume processes (typically >2000-L fermenters), either TFF–MF or centrifugation may be used as the primary recovery step. For small-volume streams (<2000-L fermenters), depth filtration may be used as a primary recovery device, but its sole use may be limited by the large amount of solids accumulating in the filter media. For streams with high solids, the amount of material retained by the depth filter may be too large to handle practically. In some cases, a pretreatment device such as a settling tank or coarse screen may be used immediately after the bioreactor, and in other cases a complete bypassing of the secondary filtration may be possible with the use of certain tight TFF–MF membranes in the ≤0.1-μm range.

Cell culture variability

In today’s bioreactor processes, higher concentrations of cells and longer fermentation times result in higher drug titers and greater product yields. These bioreactor conditions reduce cell viability, increase cell debris, and raise concentrations of organic constituents in the cell broths. The amorphous, colloidal nature of these components tends to complicate the separation process. The choice of a clarification technology also should take into account any requirements for integration with downstream processes such as chromatography and ultrafiltration.

The characteristics of cell fermentation broths vary according to the type of cell expression system and the type of bioreactor used. Figure 3 shows the relative difficulty that various bioreactor types contribute to clarification. Fed-batch and perfusion-style reactors show lower cell viability, higher cell densities, higher turbidity, and greater concentrations of colloids.
and polynucleic acids than traditional batch reactors. These bioreactor types present new challenges for clarification and secondary filtration that can be addressed with filtration and mechanical separation technologies.

**Primary recovery**

**TFF–MF.** TFF–MF is a membrane filtration process that separates particles using size exclusion. Membrane TFF–MF generally produces the highest product clarity because the membrane provides a positive size exclusion barrier to particles larger than the pore-size rating of the membrane. The filtration process uses a microfiltration membrane that has a membrane pore size that can range from 0.1 to 0.65 μm. The process is highly efficient—it removes as much as 99.5% of all whole cells, cell debris, and other particulates. The selection of tight membranes in the 0.1–0.2-μm range can facilitate the removal of lipids and other colloids, thereby minimizing or eliminating the need for further filtration devices downstream.

The process of bulk cell removal in primary clarification determines to a large extent the particle load on the subsequent secondary filtration step. TFF–MF provides the most consistent separation in terms of particle size by limiting the upper end of the range to the nominal pore size of the membrane used. The TFF–MF system must be designed to prevent rupture and fragmentation of cells in the recirculation loop. For this reason, secondary clarification or prefiltration may be required after TFF–MF to reduce the small-particle load before sterile filtration.

The TFF–MF membrane barrier ensures high removal efficiency throughout the process run and is resistant to variability in the process feed. This efficiency and uniformity allows for a more consistent feed to the secondary depth filters and ensures lot-to-lot consistency in the clarified product. This consistency, in turn, helps avoid costly product loss and reduced efficiency in downstream purification or sterilization equipment. TFF–MF devices offer advantages in scale-up because of the modularity of the device. Small-scale devices are available for some types of TFF–MF devices that can be used to initiate process development work with laboratory-scale volumes.

**Centrifugation.** Centrifuges separate particles by variances in density by subjecting the stream to a centrifugal force. Centrifugation typically is capable of handling high concentrations of insoluble material in the feed but may be limited in its ability to produce a clear product, and as a result will require more secondary filters than TFF–MF. Some centrifuges can concentrate the cell mass to ≥40% by volume and have advantages in their ability to process large volumes with high cell-mass concentrations. For some newer centrifuge types, the levels and durations of mechanical stress on cells may be low, thus minimizing cell lysis and the release of cell fragments and other intracellular debris.

Depending on the centrifuge design, some disadvantages can be found in the centrate quality, the ability to scale up predictably, high maintenance costs, and the high initial expense of the equipment. Centrifugation produces a measurably different effluent than TFF–MF does. Conventional large-scale disk-stack centrifuges with a 10,000–12,000-g operating capacity are not effective in the removal of particles <1 μm, and therefore yield a product stream more heavily solid-laden than that produced by microfiltration. As particles decrease in size, removing them efficiently decreases exponentially. A study by Kempken and Preissmann showed that high numbers of small particles (ranging from 1 to 6 μm) were not effectively removed by the centrifugation process (1). Because this small-particle loading may have serious effects on downstream chromatography or other purification, the study suggests that at least one secondary clarification step be used after the centrifuge. Centrifuges can be applied to the removal of very high cell densities, but their use as the sole clarification step is often limited.

**Secondary clarification**

**Types of NFF depth filters.** Secondary clarifying depth filters are most commonly cellulosic filters in a lenticular stacked-disk cartridge design. Depth filters function by retaining particles within the porous matrix of the media. The available filters range from 0.1 to >10 μm, the choice of which should be based on the process steps that immediately precede and follow the depth filtration. These high-area depth filters provide the needed contaminant capacity to cost-effectively handle the high levels of colloidal particles present in the fluid stream.

As cell culture fluids have high colloidal content, depth filters are commonly used in stages by using a more open filter ahead of the grade that will provide the needed protection to the downstream step. Some depth filters combine sequential grades of media into one filter along with a membrane prefilter layer. These depth filters provide a high level of protection to trailing downstream membranes or chromatography columns. The multimedia, multilayer design minimizes the number of
unit operations while making more efficient use of the filter media through improved flow distribution.

Other types of filters used for primary recovery are wrapped polypropylene depth filters. These have been successfully applied in some low-volume primary clarification applications and should be limited to processes in which cell density is low and viability is high. Low effective filtration areas and inadequate retention of colloids preclude the successful application of these filters in most large-volume fermentation broth clarifications. Table I describes several depth filtration products and their process characteristics.

**Performance of normal-flow filtration.** Normal-flow filter performance is typically judged according to two performance criteria: capacity and retention. Capacity is simply the volume of fluid that can be processed at a maximum differential pressure. Capacity measurements can be acquired by using either constant flow (a maximum pressure limit) or constant pressure (a minimum flow limit) test conditions. Retention, on the other hand, has traditionally been determined according to the prefilter’s dominant role in bioprocessing, which is the protection of downstream sterilizing-grade filters (typically 0.22 μm). The retention of the depth filter is generally expressed as the process capacity of the trailing sterile filter or filters (i.e., the process volume for a maximum ΔP at a prescribed flux rate). Therefore, scalability in secondary clarification implies that accurate sizing of both the depth filters and the companion sterile filter at full process scale is required.

After secondary clarification, cartridge prefilters may be required to remove trace components that foul the relatively expensive sterile filters. The ideal situation is the selection of primary or secondary clarification devices that significantly reduce or completely eliminate the need for such prefilters.

**Scaling secondary filtration.** The scale-up of a secondary filter to perform a given service is expected to follow in proportion to the available frontal area of the filter media—doubling the filter area should double the capacity or volume of fluid that can be processed within an acceptable range of variation. Unfortunately, this ideal case is rarely, if ever, encountered in actual production, and one must be mindful of a number of variables that influence the design of full-scale prefiltration systems.

The accuracy to which full-scale depth filter performance can be predicted from small-scale tests—no matter how well controlled the process—can never be greater than the variability of the filter itself. The underlying presumption of the process of filter scale-up is that if all operating parameters are held constant, the filter media at any scale (or area) will behave identically, if not predictably. If media use is somehow affected by the device’s geometry or size, these variations can be comfortably addressed with a correction factor that is largely insensitive to process conditions.

The consistency of depth filter media also influences scale-up. The cellulosic depth filter media is composed of three main materials: refined cellulose fibers, an inorganic filter aid (such as diatomaceous earth), and a charged (cationic) polymeric binding agent. The manufacture of cellulosic filter media (a wet-laid process) does not lend itself to the creation of a perfectly uniform porous matrix—variations in sheet thickness, density (both lateral and longitudinal), and composition are inherent in the process when measured using the square-centimeter (rather than square-meter) scale.

Laboratory-scale tests have shown that—even when drawing from the same feed lot and operating under identical conditions—capacities for small-scale depth filter devices can vary as much as ±30% within a single test set. The scale-up comparison between laboratory- and process-scale devices (lenticular stacked disks) indicates that deviations in capacity as great as ±50% are possible even under tightly controlled test conditions. When scaling up to different device geometries (from flat disk to lenticular disk stacks), the variations in capacities are no greater than those witnessed for repeat runs on the same
laboratory-scale device. Thus, the change in flow patterns that occurs when scaling up to different device designs does not appear to influence performance as much as the variability of the filter media itself.

Given this variability, multiple repeat tests must be run to obtain a statistically meaningful result, which especially applies to laboratory-scale operations in which the prefILTER media sample is small and the probability is relatively high that any single sample will misrepresent one or more average properties of the media as a whole. Multiple laboratory-scale tests (≥3) run with a controlled feed, and tightly prescribed operating conditions will provide the most accurate measure of performance as well as an estimate of the precision to which scale-up performance can be predicted.

Sizing methods for secondary filtration. Depth filtration generally invokes two separation mechanisms: mechanical sieving and adsorption. Adsorptive capture often is effective in the removal of small particles from cell culture (<1 μm) by making use of the native net-negative electrical charge that most biological solids carry at neutral pH conditions. Depth filter media such as the cellulosic composite can be modified to carry a positively charged (cationic) surface that will bind suspended particles by simple electrostatic attraction.

Adsorptive capture, unlike mechanical sieving, typically produces no measurable change in the operating pressure of the filter. The capacity or service life of the filter is instead determined according to the number of binding sites available. Any sizing or test method that ignores the quality of the filter effluent by focusing exclusively on pressure build-up can yield misleading results.

For many filter types, a bench-scale, short-duration, constant-pressure test (commonly referred to as the Vmax method) will yield a reasonable estimate of the filter area required for scale-up (2). The conventional pore-plugging model that underlies the Vmax method allows one to linearly extrapolate the maximum volume of fluid that the filter can process from the initial flow decay rate. Although sufficiently accurate and convenient for the evaluation of most surface or membrane filters, the Vmax method has been shown to be inappropriate for depth filter media, which do not generally comply with this operating model.

In most bioprocess applications in which depth filters are used, the flow rate per unit cross-sectional area (or flux rate) will have a significant effect on the capacity of the filter—the higher the flow rate, the lower the capacity. Capacity is defined as the volume of fluid that can be processed at a maximum allowable pressure differential and is generally expressed in liters per square meter (L/m²) of filter frontal area. For this reason, a test method that allows for a variable flow rate over time, as Vmax does, will generally result in an underestimation of filter capacity—the initial surge in feed flow that occurs in a constant pressure test can load the filter inefficiently and thereby create premature clogging, as documented by Yavorsky and McGee (3). Moreover, the Vmax method does not conveniently permit filtrate quality monitoring, which is critical to properly assess depth filter performance in which adsorption is the dominant mechanism of particle capture.

To get an accurate profile of performance for a given depth filter medium, a constant-flow test method (referred to as the Pmax method) should be applied. In this method, the feed flow rate to the filter is held steady, and the differential pressure is measured against throughput until the maximum allowable operating pressure is reached. In addition, the Pmax method allows for the effluent quality to be monitored either according to filtrate turbidity or, more precisely, according to the pressure response of a trailing sterile filter. By using flow control and filtrate monitoring, the Pmax method provides a truer measure of depth filter capacity and retention than is achievable by the Vmax method. Pmax testing should be conducted at several selected flow rates throughout a range that includes the lowest practical flow rate for full-scale processing. In this manner, a production system sizing that is based on a specific batch volume and processing time can be accurately calculated at the actual flow rate required.

Considerations for hardware design. Full-scale process systems are constructed with complex piping networks between large filter housings. As the size and number of filters in a housing increase, the flow through the filter elements changes and is affected by hydraulic head and dynamically changing pressure profiles as the filtration continues. Flushing, steaming, cleaning, and filtration are all affected by the size and complexity of the system. Cleaning and preparation steps can require a significant amount of time and labor, especially if steaming, cooling, assembly, and disassembly are required. The clarification step can easily become an obstacle or limiting step in the harvesting of a fermenter.

Large clarification systems may require significant areas in a fermentation suite as well as enough headroom to disassemble housings. In addition, if disposable elements are used for clarification in a large-scale operation, room must be available for handling the new and used elements. Significant crowding of equipment may occur, which can impede operations and safety. The handling of solid waste must also be considered in the environmental effect and the operational cost computation.

Safety. The final, but very important, factors in the operation of the clarification system are safety and containment. The safety aspects range from the proper design and fabrication of the equipment for steaming, to the physical aspects of removing the spent filters from the housing, to the containment of the live genetically engineered organisms. The design of the housings, piping, and connections for operation at elevated temperatures and pressures becomes more critical with increasing housing size. As housing diameter and height increase, a special design effort must be made to ensure system safety. The physical operations required to remove the spent filters, which can weigh ~20 kg, can cause severe back injury in operational personnel if they are required to manually lift the cartridges from the housing. Not only are the cartridges heavy, but they also may be hot after steaming to decontaminate the solids collected.

When designing a system, one must also take containment into account. For example, the system design should not limit the capability to decontaminate new filters with heat or spent filters with steam. Any failure to address this problem becomes evident after construction is complete and start-up is in progress.
The temptation to shorten the steaming cycle or reduce the temperature may compromise decontamination.

**Secondary clarification: case studies**

Commonly available stacked-disk cellulosic-pad prefilters have proven to be a versatile and effective tool for a variety of clarification duties in the downstream processing of mammalian-cell cultures. The following case studies describe three applications in which cellulosic-pad prefilters have been applied to cell-culture secondary clarification.

**Perfusion bioreactor harvest.** In this example (see Figure 4), a mammalian-cell culture is sustained during an extended 30–60-day period, and the soluble protein product is harvested continuously from two effluent streams. The primary product harvest takes place through an integrated spinning basket filter that uses a fine screen to separate the broth from the cells. The cells are returned directly to the bioreactor. The coarse structure of the spin filters yields a harvest fluid that contains substantial amounts of cell debris and other suspended solids as reflected by its relatively high turbidity. To maintain a stable and productive cell population, the bioreactor is also periodically purged (the purge volume represents roughly one-third of the fluid harvested through the spin filter). These two effluents are subsequently batch-clarified and sterile-filtered each in turn through a depth filter–sterile filter train. Figure 4 shows the major process steps for product harvest.

A series of laboratory-scale trials were conducted to identify the best prefilter in combination with a select sterile filter that would yield the highest overall system capacity (liters processed per unit prefilter area). The prefilters chosen represented an assortment of bilayer cellulosic-pad filters and one high-charge (cationic) single-layer pad. The total system pressure was tracked in relation to the volume of fluid processed and limited to a maximum of 20 psid. Figure 5 shows the results.

The single-layer high-charge capacity pad showed little capacity for the heavier biomass material because of its tight pore structure, which easily blocked the front surface. Several of the bilayer pad combinations also reached the 20 psid system limit after processing only moderate volumes of either the harvest fluid and/or biomass material. However, one particular dual-layer pad filter optimized for this application outperformed all contenders.

**Microfiltration permeate polishing.** The use of TFF–MF in cell-culture clarification produces a high-quality product stream. In cases where a 0.45–0.65-μm membrane is used, further polishing may be required because some suspended solids are still present in the permeate that will significantly diminish the process capacity of a typical 0.2-μm sterile filter (see Figure 6).

In this example, a manufacturing process was being developed to produce a monoclonal antibody starting with a fed-batch Chinese hamster ovary cell culture. When the culture is terminated, the fluid is first clarified using a cassette-type TFF system with a nominal 0.65-μm membrane. Samples of this permeate were then processed using several laboratory-scale cellulosic-pad prefilters, each paired with a trailing 0.22-μm sterilizing filter.
Because of the small sizes and low concentrations of particles contained in the MF permeate, none of the cellulosic-pad prefilters exhibited any significant pressure build-up through the performance tests—the capacity of the secondary clarification step was determined instead by the capacity of the sterile filter. The pad prefilters behave much like an adsorbent bed, wherein the effluent (or filtrate) quality initially is high until the media is saturated with adsorbed material. At this point, there is a breakthrough or abrupt rise in filtrate particles. Figure 7 shows the measured capacities of the trailing sterile filter for each of the pad prefilters tested at a pressure differential of 20 psid. These tests were conducted at a constant flow rate and a prefilter–sterile filter area ratio of approximately 5:1. Again, the dual-layer graded-density pad filter with membrane demonstrated greater performance.

**Centrate clarification.** A scale-up study conducted on a typical recombinant protein production process demonstrates the accuracy with which the results of laboratory- or pilot-scale trials can be used to predict performance at full-scale production. In this study, the cell culture was preclarified by centrifugation, as shown in Figure 8. The centrate was further clarified using a prefilter, and the filtrate then delivered to a 0.22-µm cartridge filter for sterilization.

![Prefilter and sterile filter scale-up](image-url)
Constant-flow tests were conducted with the prefilter and sterile filter operating in tandem, and the combined system pressure was monitored in relation to throughput or capacity. Figure 9 records the results of multiple tests run at three prefilter device sizes.

As shown, filter capacities at the three scales of testing are in reasonably close agreement. Scale-up from laboratory to process size (a factor of 782:1 in prefilter frontal area) yielded filter capacities that deviate ±35%. The moderately higher capacities achieved with the pilot- and process-scale prefilters can be attributed to better flow distribution (and, consequently, better filter media use) in the multiple-stacked cell devices.

This example illustrates the utility and accuracy of the constant-flow (Pmax) method for evaluating clarification depth filter media. The constant-flow method not only mimics conventional process conditions but also allows for simultaneous testing of prefilter and sterile filter pairings. The disparity between laboratory- and full-scale device performance appears to mirror the variability of the prefilter media itself.

Conclusion
The development of a clarification process requires integrating several unit processes such as centrifugation, TFF–MF, depth filtration, and sterile filtration. Optimization of the total process requires an understanding of the effects of the unit processes on each other. The challenges are to select equipment that meets the increasing complexity of process fluids produced by today’s more efficient bioreactors. Increases in drug titer, cell density, cell debris, and cell lysis products add difficulty to the clarification process and confound the selection of separation and filtration devices.

As process-scale selections are made, consideration should be given to equipment design, ease-of-use, and cleanability. This will ensure efficient changeover and operator safety in handling spent filters.

To develop a clarification process, a robust integration of clarification steps is important to ensure the cost-efficient processing of cell-culture fluids. Successful product scale-up requires achieving uniform filtration consistency and compressing the number of unit processes. A range of filter devices is readily available to facilitate laboratory trials, pilot production, and full-scale processing. Through the implementation of a well-devised scale-up work plan that assesses several clarification options, one can confidently select and size clarification filters to protect downstream unit operations while reducing operating costs.

References