Process Cost and Facility Considerations in the Selection of Primary Cell Culture Clarification Technology

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The bioreactor volume delineating the selection of primary clarification technology is not always easily defined. Development of a commercial scale process for the manufacture of therapeutic proteins requires scale-up from a few liters to thousands of liters. While the separation techniques used for protein purification are largely conserved across scales, the separation techniques for primary cell culture clarification vary with scale. Process models were developed to compare monoclonal antibody production costs using two cell culture clarification technologies. One process model was created for cell culture clarification by disc stack centrifugation with depth filtration. A second process model was created for clarification by multi-stage depth filtration. Analyses were performed to examine the influence of bioreactor volume, product titer, depth filter capacity, and facility utilization on overall operating costs. At bioreactor volumes <1,000 L, clarification using multi-stage depth filtration offers cost savings compared to clarification using centrifugation. For bioreactor volumes >5,000 L, clarification using centrifugation followed by depth filtration offers significant cost savings. For bioreactor volumes of ~2,000 L, clarification costs are similar between depth filtration and centrifugation. At this scale, factors including facility utilization, available capital, ease of process development, implementation timelines, and process performance characterization play an important role in clarification technology selection. In the case study presented, a multi-product facility selected multi-stage depth filtration for cell culture clarification at the 500 and 2,000 L scales of operation. Facility implementation timelines, process development activities, equipment commissioning and validation, scale-up effects, and process robustness are examined. © 2013 The Authors. American Institute of Chemical Engineers Biotechnol. Prog., 29:1239–1245, 2013

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Introduction

The first step in the purification of a secreted, therapeutic protein is clarification, in which intact cells, cell debris, and colloids are removed from the process fluid. The clarification technology used is often dependent on bioreactor volume, but the volume delineating the selection of primary clarification is not always easily defined. Commonly used technologies for pilot and commercial scale clarification are multi-stage depth filtration, tangential flow microfiltration (sometimes accompanied by depth filtration), and centrifugation followed by depth filtration.1,2 Because the clarification operation has traditionally been viewed as a straightforward separation of liquid from solid, the primary development drivers have been maximization of product yield and minimization of the unit operation cost, while maintaining a low risk of process deviation or failure. While investigations have been conducted to assess the efficacy of removing impurities such as host cell protein3,4 and DNA,5 particularly for depth filtration processes,6 this goal remains a secondary objective of the harvest process.

As product titers in fed-batch bioreactor systems have risen to 1–5 g L\(^{-1}\), with some companies reporting 10–13 g L\(^{-1}\),\(7,8\) cell densities have generally increased to greater than 10\(^6\) cells mL\(^{-1}\).\(9\) At the same time, cell viability at the time of clarification has generally decreased, often to <50%.10 This combination of high cell density and low cell viability has made tangential flow microfiltration a less viable option because increased cell density and higher cellular solids limit the extent to which the feed volume can be concentrated prior to diafiltration. Additionally, increased cell debris and colloid content due...
The decision as to which clarification technology to use is influenced by many considerations, including the cost of goods to manufacture (COGM), facility utilization rates, capital expenditure, scale-up experience, and process development and implementation requirements. For this study, process models were prepared to compare the COGM for bulk drug substance of a generic monoclonal antibody (mAb) process. Sensitivity analyses examined a range of bioreactor volumes, product titers, facility utilization levels, and depth filter capacities using either multi-stage depth filtration or centrifugation for primary clarification. A case study is presented in which a multi-product clinical manufacturing facility initially selected multi-stage depth filtration for cell culture clarification at the 500 and 2,000 L bioreactor scales. Prior to this investigation, this type of analysis had not yet been performed and centrifugation was widely accepted in the biopharmaceutical industry as the only viable primary clarification step above the 500 L bioreactor scale. The economic analysis and case study presented here establish that depth filtration for primary clarification is a feasible choice for the primary clarification of bioreactors up to 5,000 L.

### Methods

A process model for a generic monoclonal antibody purification process was generated using a commercially available modeling software, BioSolve software (version 1.3.0)\(^1\)\(^6,17\) by Biopharm Services. Versions of the model were generated using either multi-stage depth filtration or centrifugation with secondary depth filtration for bioreactor clarification. In both cases, the use of encapsulated, disposable depth filters were modeled, which do not require stainless steel housings. Table 1 lists the key process parameters selected for each of the process unit operations. The base-case process was created for a 5,000 L CHO cell bioreactor with the culture conditions listed in Table 1. Both depth filtration and centrifugation were modeled for the clarification step. Protein A chromatography was used as the model capture step at a load ratio of 20 g mAb per liter of resin. The remaining steps were low pH virus inactivation, two ion exchange chromatography steps, virus filtration, and ultrafiltration.

The model assumed that a new facility was built solely for this manufacturing process. As summarized in Table 2, at a facility utilization of 80%, the facility was calculated to produce 27 batches year\(^{−1}\) at the base-case, though the model was used to assess varying facility utilization rates. Changeover time between products was not specifically addressed in the model. The facility model was based on a single bioreactor feeding into one downstream production train. In the model, the bioreactor was the bottleneck for the process run rate. Based on historic process information, product recovery for the two clarification methods was set at 90%. The overall product recovery was 66% for both methods. For analyses in which the product titer was varied, it was assumed, based on experience with multiple products, that increases in titer were due largely to increases in specific cell productivity and not due to the final cell density and viability in the bioreactor (data not shown). Thus, an increase in product titer has no direct impact on depth filter capacity. For this model, capital assets included the manufacturing facility and all facility and process equipment. For the clarification steps, capital equipment included the centrifuge, depth filter holders, and peripheral equipment such as pumps and control systems. Capital assets were depreciated over 10 years with a final capital value of 0%. Installation, validation, and qualification were taken as a one-time cost. The cost of capital, assumed to be the minimum return that investors expect for providing capital to the company, was set at 10%.

Once the process models were generated utilizing the two clarification technologies, a comparison of the overall cost and a breakdown of cost by category was calculated. Sensitivity analyses were performed to understand the influence of several key factors on the cost of the processes (see Table 3). Other model parameters were investigated, but were found to have no substantial influence on the cost difference between the processes. These parameters included use of disposable bags and containers, labor and quality control costs, and batch failure rates. For this study, a result was considered significant if the cost difference between the two

### Table 1. Process Model Assumptions for a Generic mAb Process

| Unit Operation | Key Process Parameter |
|----------------|-----------------------|
| Bioreactor     | A single, production scale bioreactor 5,000 L working volume (base case) |
|                | 3 g L\(^{−1}\) mAb titer, 5 × 10\(^6\) cells mL\(^{−1}\) |
|                | CHO cell density, 4–6% packed cell volume, 65% viability at harvest |
| Clarification  | Centrifuge clarification (two options) |
|                | Primary clarification: Continuous disc stack centrifuge, 25 L min\(^{−1}\) maximum capacity; Secondary clarification: Millistak+ XOH Pod filter, 290 L m\(^{−2}\) loading; 5 h process time, 10 L m\(^{−2}\) recovery buffer flush |
|                | Depth filter clarification: Primary clarification; Millistak+ D0HC Pod filter, 69 L m\(^{−2}\) loading; Secondary clarification: Millistak+ XOH Pod filter, 320 L m\(^{−2}\) loading |
|                | 4 h process time, 10 L m\(^{−2}\) recovery buffer flush |
| Protein A Capture | 20 g product L\(^{−1}\) of resin load |
| Virus inactivation | Low pH hold |
| IEX bind/elute | 50 g product L\(^{−1}\) of resin load |
|                | 150 cm h\(^{−1}\) linear velocity for all steps |
| IEX flowthrough | 50 g product L\(^{−1}\) of resin load |
|                | 150 cm h\(^{−1}\) linear velocity for all steps |
| Virus filtration | 880 g product m\(^{−2}\) filter area load |
| Ultrafiltration/ 
  diafiltration | 500 g product m\(^{−2}\) filter area load |
|                | 4 h process time |
|                | 10 diavolumes |
|                | 20 cycle membrane lifetime |

### Table 2. Key Model Assumptions for COGM Analyses for 5,000 L Bioreactor Base-Case

| Model Parameter | Base-Case Value |
|-----------------|-----------------|
| Facility utilization | 80% |
| Production rate | 27 batches yr\(^{−1}\) |
| Product yield | 9.9 kg batch\(^{−1}\) |
| Total process recovery | 66% |
| Cost of capital | 10% |
| Capital depreciation schedule | 10 years |
| Final capital value | 0% |

The base-case process was generated using a commercially available
processes for the final bulk drug substance was >1%. All process costs are reported in cost per gram of bulk drug substance, unless otherwise specified.

Results

Process comparison at the 5,000 L bioreactor scale

The process models utilizing either centrifugation or depth filtration as the primary clarification technology were run at a bioreactor volume of 5,000 L for initial comparison of bulk product cost. This comparison was made at the base-case assumptions of 3 g L\(^{-1}\) product titer in the bioreactor, 80% facility utilization, and 27 batches year\(^{-1}\). Both processes were calculated to produce 268 kg year\(^{-1}\) of bulk drug substance. For the centrifuge clarification process, the cost of bulk drug substance was $78.54 g\(^{-1}\) and for the depth filtration clarification process, the cost was $82.00 g\(^{-1}\), a 4.4% difference. On an annual basis at the 5,000 L scale, the overall process costs using multi-stage depth filtration were approximately $0.9 million more than the overall process costs using centrifugation and depth filtration (Table 4).

The greatest cost increase for the depth filtration clarification process, relative to the centrifugation clarification process, was the increase in consumables associated with the use of disposable depth filters. At the 5,000 L scale, the recurring cost of the depth filters in this process was significantly higher than the capital cost associated with the purchase of a centrifuge. Although not a significant cost lever, there was also an increase in water usage for the depth filter clarification process due to the 100 L m\(^{-2}\) pre-use water flush for the additional depth filters.

The capital cost comprised the largest fraction of the overall cost for both processes (Table 4). The centrifuge clarification step cost was largely capital driven, while the depth filtration clarification step cost was largely consumables driven.

Influence of process scale on relative process cost

The costs of the centrifugation and depth filtration clarification processes were compared at bioreactor volumes ranging from 500 to 12,000 L. As seen in Figure 2, the centrifugation process held a substantial cost advantage in bioreactor volumes of 5,000 L and above, while the depth filtration process held the cost advantage at bioreactor volumes of 1,000 L and below. Between the 1,000 and 2,000 L scale, the difference in cost per gram of product was <1%. A cost premium value below 0% indicated a bioreactor volume at which the depth filter clarification process provided cost savings.

Table 3. Model Parameter Ranges Examined During Sensitivity Analyses

| Model Parameter                  | Base-Case Value and Analyzed Range |
|----------------------------------|-----------------------------------|
| Bioreactor volume                | 5,000 L (500–12,000 L)            |
| Facility utilization rate        | 80% (20–100%)                     |
| Product titer (at time of clarification) | 3.0 g L\(^{-1}\) (0.5–10.0 g L\(^{-1}\)) |
| Primary depth filter capacity    | 69 L m\(^{-2}\) (35–500 L m\(^{-2}\)) |

Table 4. Comparison of Overall Process Costs Per Year by Expense Category for 5,000 L Bioreactor Base Case

| Process Comparison  | Centrifuge Clarification | Depth Filter Clarification | % Change |
|---------------------|--------------------------|----------------------------|----------|
| Annual COGM* (M$)   | 21.1                     | 22.0                       | 4        |
| Capital             | 7.8                      | 7.7                        | 0        |
| Materials           | 2.6                      | 2.6                        | 0        |
| Consumables         | 4.7                      | 5.7                        | 0        |
| Labor               | 3.5                      | 3.5                        | 0        |
| Other               | 2.5                      | 2.5                        | 0        |
| Water usage (m\(^3\) batch\(^{-1}\)) | 127                      | 132                        | 4%       |
| Batches per year    | 27                       | 27                         | 0%       |

*Capital costs include the manufacturing facility, process and facility equipment, installation and validation, and other capital expenditures. Material costs are media, buffer, other raw materials, CIP solutions, and QC tests. Consumables costs are chromatography resins and process filters. Labor costs are process, quality and indirect labor. Other costs are insurance, waste management, maintenance, and utilities.

Figure 1. Clarification unit operation cost breakdown by category.
The difference in total installed capital remained nearly constant across all scales, at approximately $1,000,000 more for the centrifugation process. At scales at or below 1,000 L, this additional capital accounted for a higher overall process cost relative to the depth filtration process. As the process scale increased from 1,000 to 12,000 L, increasing consumables and labor costs of the depth filtration process overcame the difference in capital cost.

**Facility utilization and production rate**

Facility utilization rate had a significant impact on the relative cost of the depth filtration and centrifugation clarification processes. At lower facility utilization rates, the relative cost of the centrifugation clarification process increased, as the higher capital cost process was spread across fewer batches. Figure 3 illustrates the effect of facility utilization rate on relative process cost at bioreactor scales of 500–5,000 L. At lower facility utilisations, particularly at 40% or lower, the centrifugation clarification process cost exceeded the cost of the depth filtration clarification process for bioreactor volumes of 2,000 L or less. At the 5,000 L scale, the centrifugation process held a cost advantage at all facility utilization rates examined.

**Product titer**

As discussed above, at process volumes at or above 5,000 L, the centrifugation process held a cost advantage. Figure 4 shows that this cost advantage decreased as product titer increased. As the titer increased, the depth filter cost per gram decreased because the filter surface area was held constant, allowing the consumables cost to be distributed over more mass of product. For a 5,000 L bioreactor, the cost advantage for centrifugation decreased from 6.2% to 2.4% as titer increased from 0.5 to 10 g L\(^{-1}\). At bioreactor volumes of 2,000 L or less, the increase in product titer had a minimal influence on the relative cost.

**Primary depth filter capacity**

The sensitivity of costs to primary depth filter capacity was examined over a range of 35 to 500 L m\(^{-2}\). Figure 5 shows that the base-case filter capacity of 69 L m\(^{-2}\) leads to a cost premium of 4.4% for the depth filtration process at the 5,000 L scale. As the capacity of the primary depth filter increased, the relative cost of this clarification method decreased. Parity was reached for the two processes at a primary depth filter capacity of 150 L m\(^{-2}\), and at primary depth filter capacities above 150 L m\(^{-2}\), the depth filtration clarification process became cost advantageous. The benefit of increasing primary depth filter capacity paid large dividends at capacities up to 200 L m\(^{-2}\), but resulted in diminishing returns beyond this range. A similar analysis revealed that secondary depth filter capacity was not a significant cost lever in the comparison of the two processes.

**Discussion**

The analysis of COGM for a monoclonal antibody process in a 5,000 L bioreactor scale facility utilizing either depth filtration or centrifugation for clarification clearly quantified the difference in total installed capital remained nearly constant across all scales, at approximately $1,000,000 more for the centrifugation process. At scales at or below 1,000 L, this additional capital accounted for a higher overall process cost relative to the depth filtration process. As the process scale increased from 1,000 to 12,000 L, increasing consumables and labor costs of the depth filtration process overcame the difference in capital cost.
the difference between the consumables-driven depth filtration process and the capital-driven centrifugation process. For the base-case conditions, the centrifugation clarification process demonstrated a clear cost advantage at bioreactor volumes of 5,000 L or greater. This base-case closely modeled a commercial scale process and facility focused on large volume production of a single product. This situation allowed for high facility and capital utilization while maximizing production. Even at lower facility utilization and production rates, at a 5,000 L scale or greater, the use of centrifugation for primary clarification minimized overall product costs relative to depth filtration clarification.

With the lower process volumes and facility utilization commonly seen in multi-product and clinical production facilities, the use of depth filtration for clarification became cost competitive or advantageous. For bioreactor volumes of 1,000 L or less, the process costs favored the use of depth filtration for clarification over centrifugation and depth filtration. For 2,000 L bioreactors, at facility utilizations of ≤40%, the depth filtration clarification process cost was effectively equal to the centrifugation clarification process cost.

The relative costs of the modeled processes were also influenced by the product titer in the bioreactor. At bioreactor volumes of 5,000 L and above, the cost advantage of the centrifugation process decreased with increasing product titer. At lower bioreactor volumes (<2,000 L), product titer had minimal influence on the relative process cost.

The process cost comparison between depth filtration and centrifugation clarification showed significant sensitivity to primary depth filter capacity between 35 and 200 L m$^{-2}$. Current filtration technology does not commonly allow for primary depth filter capacities greater than the model base-case of 69 L m$^{-2}$ for whole cell culture clarification. However, the use of cell culture pretreatment techniques such as acid precipitation and flocculation could significantly increase the depth filter capacity and impurity clearance during the clarification operation. This in turn may increase the bioreactor volume at which a financially viable depth filtration clarification process could be operated. Optimizing the primary depth filtration capacity beyond 150–200 L m$^{-2}$ did not lead to further significant cost savings.

Current trends in the biopharmaceutical industry point toward increasing product titers and smaller patient populations for new product indications. With product titers of 3–5 g L$^{-1}$ common for current clinical processes and titers of >10 g L$^{-1}$ on the horizon, a 2,000 L bioreactor could be expected to produce 4–14 kg of mAb protein. These expression levels, coupled with lower mass requirements for new products, may push the industry to use smaller bioreactors in multi-product facilities. This confluence of industry trends could lead to a situation in which depth filtration may become advantageous on an operational and cost basis, especially if next generation filters could achieve primary depth filtration capacities in excess of 150 L m$^{-2}$.

**Table 5. Equipment Commissioning and Installation Activities Required for Depth Filtration and Centrifugation Clarification Technology**

| Activity                  | Depth Filtration | Centrifugation |
|---------------------------|------------------|----------------|
| FAT/SAT                   | No               | Yes            |
| Cleaning validation       | No               | Yes            |
| Utilities other than electricity | No       | Yes            |
| WFI                       | Yes              | Yes            |
| Facility shutdown         | No               | Yes            |

The primary method for the clarification of 500 and 2,000 L bioreactors had to be selected for an existing multi-product biopharmaceutical manufacturing facility. Two options were considered: centrifugation followed by depth filtration and multi-stage depth filtration. In both cases, only encapsulated single-use depth filters were considered because all product contact surfaces would be disposable, eliminating the need for cleaning validation studies. Equipment commissioning and validation activities, implementation timelines, impact to process development, and the generation of centrifugation process experience and performance characterization were considered.

As shown in Table 5, the activities required to commission and validate each method were considerably different. Depth filtration required proximity to electricity and WFI with no other major commissioning and installation activities. Centrifugation required a factory acceptance test (FAT) to ensure the equipment was functional prior to installation into the facility. A site acceptance test (SAT) was also required to ensure that the equipment, once installed, was operating properly under actual conditions. In addition, the execution of a cleaning validation protocol, connection to advanced utilities such as clean in place (CIP) and steam in place (SIP) systems, and a partial facility shutdown were required to install the centrifuge.

To meet the demand for on-time delivery of clinical trial material, it was essential that implementation timelines be as condensed as possible. A centrifuge is a complex piece of equipment that typically requires customization. To ensure that the customized centrifuge matched process requirements, a lengthy capital procurement process was required. In contrast, depth filtration uses equipment available in the vendors’ inventory so procurement had little to no impact on project timelines and an abbreviated capital procurement process was used. Centrifugation installation required connections to CIP and SIP systems, in addition to custom electrical and temperature control loops that had a high impact to the timeline. Furthermore, these centrifugation installation activities required a partial facility shut down. Because of the straightforward operation of normal flow filtration, installing depth filtration into an existing facility was achieved while the facility was operational. An electrical source to drive the pump was required, as well as access to the process stream and WFI. This facility only considered single-use disposable flow path depth filters that did not require CIP and SIP operations, eliminating the need for cleaning validation. Centrifugation equipment required cleaning validation that would have further lengthened the project timeline. Process documentation timelines were considered roughly equal for both technologies. It was determined that procurement and installation of a centrifuge would require 8–12 months, whereas depth filtration could be installed into the facility in 2 months. The timeline advantages posed by depth filtration

**Practical considerations for clarification technology selection and implementation**

In situations where the difference in cost for the two clarification technologies is small, non-cost related considerations become essential for choosing the clarification method. Examples of some non-cost related considerations are discussed in the following case study.
steered the decision of clarification technology towards depth filtration for the facility start-up.

Whether depth filtration or centrifugation is selected for clarification, efficient robust processes must be developed and transferred into the facility. Each technology was attractive in terms of its ability to fit a process platform.

To facilitate bench scale process development, it is useful for the process development scientists to have a predictive scale down model for a unit operation. Scalable, predictive depth filtration bench scale devices are readily available. Most vendors offered several scale down filter sizes that require 300 mL or less of process feed. These predictive bench scale models are not readily available for centrifugation. Although scaled down centrifuges exist, they are generally pilot scale systems and require tens of liters of process material which can be difficult to procure during early development work. In addition to the scale down model limitations, the shear force applied to the cells in the larger scale devices can be difficult to accurately model. Additional work would be required to fully understand the implication of shear to product quality and process performance as the process was scaled up. Disk stack centrifuge operation can require significant optimization in order to minimize the shear force applied to the cells. High shear can lead to cell lysis, introducing additional cell debris and host cell related impurities to the downstream process. This optimization adds complexity to process scaling and may require additional development effort and time.

Despite the drawbacks involved in small scale process development, centrifugation at a representative pilot scale offers the opportunity to collect process data and gain experience that is valuable at larger scales where centrifugation was required. Although installing a centrifuge would have posed some time delays at the 500 and 2,000 L scales, the data and experience gained by using this clarification technology at this intermediate scale would have been valuable when scaling the process for commercial, centrifuge-based production. In addition, the work and expenses incurred to show process comparability when switching from depth filtration to centrifugation could have been avoided.

Because of the large timeline savings offered by depth filtration, in addition to the ease of implementation and process development, disposable depth filtration was selected as the cell culture clarification technology for the facility. A platform unit operation was developed and 59 batches using this technology were performed across 24 different molecules and two mammalian expression systems. The process has proven robust with no failures. Product recovery has remained ≥90%. Although the performance of the depth filtration process has been reliable and robust, installation of a centrifuge in the facility is now being pursued. The addition of this equipment will offer the facility the flexibility to use either clarification technology and the ability to support larger scale commercial production through the acquisition of representative process data and experience.

**Conclusions**

A cost analysis was prepared comparing a monoclonal antibody production process utilizing either multi-stage depth filtration or centrifugation followed by depth filtration for the bioreactor clarification step. The relative cost of the processes showed considerable dependence on process scale, facility utilization, and product titer. The total capital requirements for implementation of either clarification technology also influence the relative process cost.

Clarification using multi-stage depth filtration offered process flexibility, rapid product changeover, and capital savings at a cost that was less than that of the centrifugation clarification process for bioreactor volumes less than or equal to 1,000 L. The benefits of clarification by depth filtration were particularly clear in multi-product and clinical manufacturing facilities due to the inherent lower production rates where the preference is for flexibility over capacity utilization. For bioreactor volumes equal to or greater than 5,000 L, clarification using centrifugation and secondary depth filtration offered significant cost savings through the reduction in consumables. This cost benefit was maintained even at lower facility utilization rates.

For bioreactor volumes in the range of 1,000–2,000 L, the selection of clarification technology should depend on specific facility, project, and process requirements. In the case study presented, depth filtration clarification was selected for 500 L and 2,000 L bioreactor clarification due to shortened implementation timelines and decreased installation and commissioning requirements during plant startup. Depth filtration was also easier than centrifugation to develop in a scalable manner at the bench scale. Selection of depth filtration technology for clarification at this intermediate process scale, however, may limit the ability to gain experience and data using centrifugation for subsequent commercial-scale production.

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