Advances in Single-Use Technologies for Cell Clarification

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One of the major challenges in batch mammalian cell clarification is cost-effective improvement in filtration in the face of high cell densities. Single-use technology has serious benefits for flexibility in production, pilot or clinical batches, but with existing filtration equipment, the economic benefits have not been fully realized at volumes ≤ 2,000 liters. Developers and CMOs alike require cell clarification technology that offers higher yields, fewer failures, and the ease of use of single-use, with reduced consumables costs for cost-effective drug development and production.
Limitations of Existing Technologies

**Centrifugation**

Though centrifuges have been an established filtration solution for decades, the hardware, maintenance and implementation continually require significant investments in both capital and time for small-scale production. In addition to the capital costs, they require considerable upfront work with cleaning validation and are difficult to customize or scale-down. Once validation has been completed, facilities endure relatively long cycle times due to cleaning, assembly and disassembly during processing.

**Depth Filtration**

Easy to implement and available as a single-use solution, depth filtration is currently the most commonly used option for small-scale batch mammalian cell filtration. The technology allows for rapid product changeover and customization, but depth filters do have drawbacks: they can be costly, they require time and resources for pre-flushing, are more suitable for low flow rates, and can clog with higher cell densities. Current depth filtration processes may often take up to 10 hours for set-up, pre-flushing, filtration and harvest, and a recent industry survey reported that single-use facilities spent an average of $204,000 in 2013 on depth filters alone.¹

Improving Single-Use Clarification

Fortunately, products have been developed to address the demand for improved high cell density clarification in the < 2,000 L scale. New dynamic bodyfeed filtration (DBF) combines the ease of single-use with superior filtration capabilities using ultrapure diatomaceous earth as a filter aid suspension, which precludes the need for pre-flushing. Outfitted with medical grade polyethylene membranes, DBF filters allow for high flow rates and a single-step clarification, effectively converting the cell harvesting step from a two-stage process into a single-stage operation, saving both time and money.²
DE Suspension in Cell Filtration

In other industries with similar applications, such as the plasma fractionation industry, bodyfeed filtration is used for the clarification of high solid content solutions. The technology uses diatomaceous earth (DE) as a suspended filter aid to form a denser and higher flowing filter cake. DE is a very porous material and acts as a spacer between the solid particles (Figure 1). Composed of the shells or exoskeletons of fossilized diatoms (microscopic, single-cell algae), DE has a long history of use in the biotechnology industry in depth filters during the harvesting stage. While the particles are entrapped in the filter cake, the fluid, including the dissolved products, pass through the filter cake by flowing through the porous structure of the DE particles. The efficiency of the separation is dependent on the proportion of the DE and the biomass and influenced by the particle size of the solid content. With the use of a disposable filtration module, and a DE containment strategy, an introduction of a single-use platform in the upstream process has significant potential to improve this difficult processing step.

Typically, ultrapure grades of DE are available for different applications. Sartorius Stedim Biotech has invested significant time and effort in qualifying Celpure C300 as the best filtration aid for mammalian cell culture clarification.

Figure 1: Dynamic bodyfeed filtration (DBF) with diatomaceous earth (left) and conventional filtration (right).

![Figure 1: Dynamic bodyfeed filtration (DBF) with diatomaceous earth (left) and conventional filtration (right).](image)

Figure 2. Indicative DE requirements at a lower pH of 5.0

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Enhancing Filtration Productivity with Single-Use

Single-use technologies have become extremely important as manufacturers look to the future, where bioprocessing flexibility will be instrumental in achieving:

- Smaller bioreactors and multi-product sites
- Meeting small-scale clinical needs and production rates
- Continuous processing
- Shorter process development times

By using DE suspension in single-use dynamic filtration, setup, harvest and cleaning are far more efficient than with traditional centrifuges or depth filters. The technology can handle high volume and high solids capacity without fouling, and does not require pre-flushing, meaning that both WFI costs and overall filtration time can be reduced compared to depth filtration. Optional processes such as pH adjustment and the addition of flocculants will create aggregates that are too big to migrate into the filter cake, so that the cake stays permeable. This decreases the amount of DE required and can improve filterability and lead to clearer filtrates.

Additionally, single-use systems are easy to implement, have a short time to proficiency, and can integrate into new or existing operations quickly.
Case Study:
Single-Use Becomes Economical with DBF

A pilot-scale run at Rentschler Biotechnology presented in 2014 sought to compare various filtration methods, including depth filtration and DBF at neutral and reduced (5.0) pH. Results showed that DBF allowed for higher capacities, while processing time could be reduced by 2-3 hours for a 2,000 liter batch. The study concluded that with DBF, “Harvest of high volumes using single-use technology becomes economical.” Additionally, they reported that DBF at low pH can better utilize the diatoms and lower turbidity, resulting in increased filter capacity.

The DBF runs were performed using Sartoclear® Dynamics, a single-use filtration module with suspended DE (highly purified Celpure C300) and fully contained powder transfer. The study determined that DBF is a robust technique for clarifying high-cell density processes in a single-use setup without centrifugation for volumes found in single-use applications (≤2,000 L) with the advantages of:

• High solids capacity
• Ease of scale-up
• One size fits all configuration, though the technology is customizable

DBF technology offers another advantage over depth filtration, in that the main control parameter, total wet cell weight, is a relatively easy value to determine, where depth filtration performance relies on more unstable parameters like cell viability and cell size. This feature can save time during process development by making the harvest step more predictable.
Upstream filtration technologies must be able to accommodate high titers while delivering increased product yields, particularly in the ≤ 2,000 liter scale. As the industry moves toward developing drugs for smaller indications and more effective drugs (allowing for lower dosages), the need for more flexible small-scale manufacturing facilities continues to grow. It is expected that a majority of the future biomolecules will be produced in single-use vessels of 2,000 liters or smaller.

With faster processing and reduced utility costs, novel single-use DBF systems save considerable time and money in cell clarification operations. Additionally, they offer unprecedented flexibility for customization and product changeover, which is becoming more critical as facilities shift to multi-product and personalized batches. Coupled with the short time to proficiency and reduced capital expenses required, solutions like Sartoclear® Dynamics from Sartorius Stedim Biotech are an appealing investment for pharmaceutical manufacturers and CMOs looking to cost-effectively produce pre-clinical and clinical batches in right-sized equipment.

Closing Thoughts

A profile of Sartorius Stedim Biotech

Sartorius Stedim Biotech is a leading provider of cutting-edge equipment and services for the development, quality assurance and production processes of the biopharmaceutical industry. Its integrated solutions covering fermentation, cell cultivation, filtration, purification, fluid management and lab technologies are supporting the biopharmaceutical industry around the world to develop and produce drugs safely, economically and in a timely manner. Sartorius Stedim Biotech focuses on single-use technologies and value-added services to meet the rapidly changing technology requirements of the industry it serves. Strongly rooted in the scientific community and closely allied with customers and technology partners, the company is dedicated to its philosophy of “turning science into solutions.”

ambr® systems are designed and manufactured by TAP Biosystems (now part of the Sartorius Stedim Biotech Group), a leading global provider of automated cell culture and fermentation systems for life science research, development and production. ambr systems are widely used for cell line development and process optimization at pharmaceutical, biotechnology and academic laboratories. They are proven to provide a reliable model and consistent scalability to a range of upstream processes.