Introduction to DSP – Harvesting

Your Objectives:

At the end of the lesson, you should be able to sequence the steps of harvesting.

Harvesting

Cell harvesting is a critical step in connecting upstream monoclonal antibody production with downstream purification. Selecting the best cell harvesting technology based on the characteristics of the cell culture process can be challenging. This decision is made early on in process development; a sound understanding of the current process, as well as of the pros and cons of the various cell harvesting technologies available, is required.

Companies will take decisions as to which harvesting technique best suits their own process, and as to which variables would be preferential for them.

The selected harvesting method and equipment depends on (1) the type of cells, (2) product being harvested, and (3) properties of the process fluids. Traditional (standard) techniques include **membrane microfiltration, tangential-flow filtration** (TFF, or crossflow filtration), **centrifugation**, and **depth filtration**, as well as specialized solutions that are to be coupled with either microfiltration or centrifugation with TFF or then with depth filtration.

Centrifugation

Disc-stack centrifugation (e.g. from Alfa Laval Inc. or Westfalia Separator Group Ltd.) has been in use for cell harvesting for some time. A disc-stack centrifuge uses stacked, inclined conical discs to separate out whole cells and large cell debris.

Shearing, however, can damage cells and thereby increase the number of submicron particles, particles which cannot be removed. Instead, low-shear systems and techniques coupled with second-stage depth filtration is especially useful. The use of depth filtration can provide further clarification, removing smaller solid particulates.

Filtration

The bioprocess industry adopted membrane clarification (purification) methods from other technologies; but it has also developed modules specifically designed for biopharmaceutical purposes, including for primary clarification of fermentation and cell-culture systems. When a membrane technique is used, the size and fouling potential of the membrane is considered.

Microfiltration is highly sensitive to changes in feedstock quality such as cell culture viability, cell density, and medium components. High cell densities and low cell viabilities can result in high **transmembrane pressure** (TMP) for constant-flux membranes, the extent to which depends on the scale of the feed batch. TMP is relatively lower for typical cross-flow (TFF) microfiltration operations (MF–TFF). The flow of feed solution across the membrane reduces concentration polarization and creates a pressure drop, forcing a size-exclusion condition. Such filters are currently available with various pore sizes, typically between 0.1µm and 1µm for primary harvest (e.g. EMD Millipore's ProStak system and PallSep Biotech from Pall Corporation).

Depth filtration retains particles, both larger and smaller than their pore size, throughout their porous media. Particle retention involve both size exclusion and adsorption through hydrophobic and ionic (as well as other) interactions. Depth filtration provides some benefits, but is not without its limitations. Commercially available technologies include filter sheets (Seitz depth filter sheets by Pall Corporation); encapsulated modules (*Supracap* 200, by Pall Inc.); and disposable, scalable formats (e.g. *Millistak+* pod depth filter media by Emanuel Merck, Darmstadt Millipore).

Cell separation using depth filters

Benefits

- Low material and hardware costs
- Ease of use
- Fast process times
- High flexibility from scale-up options (e.g. for multiproduct facilities)
- Filtration with minimal shearing forces so as to offer cell and product protection
- FDA approved materials
- High-quality of filtrate
- Space-saving equipment

Disadvantages

- Necessity of monitoring differential pressure
- Need for conducting economic evaluation at all stages?
- Necessity of the removal and disposal of filters
- Scale-up limitations like budget limits or infrastructural limits

Tangential-flow filtration: Methods for harvesting small-scale bioreactors may not work for process scales of between 10,000 and 20,000 litres. As Ian Sellick observed, "Handling requirements for such large batches call for employing larger and more continuous types of processing systems, often some form of TFF". TFF is suitable for fine-sized-based separations since it retains particulates and other molecules too large to pass through its membrane pores, and are transported along the tangential flow.

TFF configurations include both cassettes (flat) as well as hollow-fibre formats. In his article, Sellick describes a compact TFF cassette device that uses polyethersulfone (PES) membranes and screens to form diagonal flow paths that prevent product channelling (which occurs when membrane surface area is not fully used and the feed channels to the retentate). He also describes enhanced hollow-fibre modules for cell perfusion that will have used a nonbinding polyvinylidene difluoride (PVDF). Disposable, pre-sanitized TFF cassettes provide several benefits over many traditional cassette formats, including cost-savings, lower risk of cross contamination, and greater manufacturing flexibility.

Source: https://bioprocessintl.com/downstream-processing/chromatography/a-decade-of-harvesting-methods-331186/

Notabene: "e.g." = one of other examples (zum Beispiel) / "i.e." = namely (sprich, nämlich, das heisst)

Optimisation

Efforts have been made to correlate the characteristics of a cell culture (e.g. viability, density) and the efficiency of a harvest process. In one study of a high cell density culture, the quality of the primary recovery depended on cell density and cell viability. High cell densities and low viabilities result in larger numbers of whole cells and solid impurities such as colloids and cell debris. High **titres*** typically generate from conditions of high cell densities and low viability. Using **turbidity** as a marker for product quality, researchers demonstrated a linear correlation between cell culture viability and clarification efficiency for a given centrifugation condition. Their results also showed that higher feed rates lowered clarification because of lower centrifuge residence times and that the correlations for the centrifuge were the same as those shown for the subsequent depth filtration step.

The increasing use of single-use technologies in upstream processes may require a shift in harvest strategies. With traditional **fed-batch culture** processes, harvest clarification is usually achieved by centrifugation followed by depth filtration. For processes based entirely on disposables, the disc-stack centrifuge needs to be replaced by filtration alone".

* **Titre**: (US: titer) = concentration of a solution determined by titration, the minimum volume of a solution needed to reach the end point in a titration; the concentration of an antibody, as determined by finding the highest dilution at which it is still able to cause agglutination of the antigen.

Scale Challenges

The current trend towards a **small-scale** batch process will necessitate primary recovery techniques that can provide quick turnarounds. A system of glass-fibre filters, coupled with a retentive membrane to provide "a rapid solution that can be implemented with very little preparation," might be used. Glass-fibre and polyethersulfone membranes are inherently low in extractables and have been validated for low levels of endotoxin and other contaminants. They are steam sterilizable and are compatible with gamma irradiation, enabling pressure sterilization with either method.

TFF and centrifuge–depth filtration are typical methods for harvesting **large-scale** batches. Process transfer, however, sometimes requires alternative, streamlined approaches. A *Genentech* study evaluated various dual-layer, single-stage depth filter media to find an alternative to its traditional two-stage depth filtration train to be used at a separate facility. The work involved testing at various turbidities, resulting in a system that demonstrated increased capacity at all levels without significant plugging of the internal pore structure of the depth filters. Researchers further suggest that "one key to increasing future depth-filter capacity may (…) be to control particulate clustering (…) or modulate flow rate to avoid or exploit critical shear thresholds within a depth filter."

High-density cell cultures

As titres increased during this past decade, so too did the importance of efficient product recovery methods. With monoclonal antibody titers reaching ~25 g/L and the expectation of even greater values in the future, clarification of high-density cell cultures will present unique challenges to biomanufacturers. Ever-increasing amounts of cell debris will need to be removed quickly to prevent bottlenecks in downstream processes.