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Primary recovery of mammalian cell culture

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ABSTRACT

Mammalian cell culture process is widely being used in the biopharmaceutical industry to produce various therapeutics and diagnostics across the globe. The article sheds light onto the major unit operations employed in the industry for primary recovery of the protein. The clarification efficiency, protein recovery and the economics play the major role in deciding which unit operation to be used for clarifying the cell mass from the product. Centrifugation and depth filtration are the most popular ways of extracting the product initially, post-bioreactor stage. The trials and scale up calculations at the developmental stage based on the cell removal efficiency, processing time, contaminant reduction, and overall cost enables the drug manufacturer to select the optimal unit operation.

KEYWORDS

Mammalian cell culture; Therapeutics; Primary recovery; Centrifugation; Depth filtration

INTRODUCTION

Mammalian cell culture process also known as animal cell culture is widely used in the bioprocess industry for production of therapeutics and diagnostics. The therapeutic proteins such as monoclonal antibodies are commonly extracellular products. The first step in the product purification involves separating cell mass from the product. Initial separation of cell mass from the product poses a high degree of challenge as the cells are shear-sensitive. Therefore, it is critical to select an appropriate primary clarification step in order to maintain the final product quality and high protein recovery.

The desirable process for its primary extraction can be a centrifuge, a depth filtration or a combination of both which can ensure a very high product recovery and a considerable reduction in the contaminant profile under low cost.

CLARIFICATION OF MAMMALIAN CELL CULTURE PROCESS

The mammalian cell culture process generally follows a typical process flow with seed preparation, and a bioreactor stage in the upstream and a series of clarification, purification, polishing, and sterile filtration steps in the downstream as shown in Figure 1.

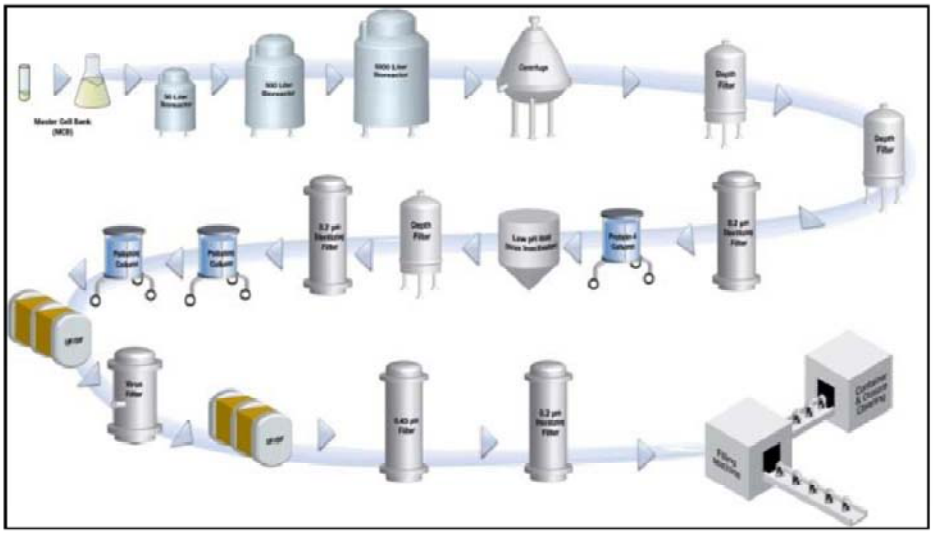


Figure 1: Typical Process flow for a mammalian cell culture process

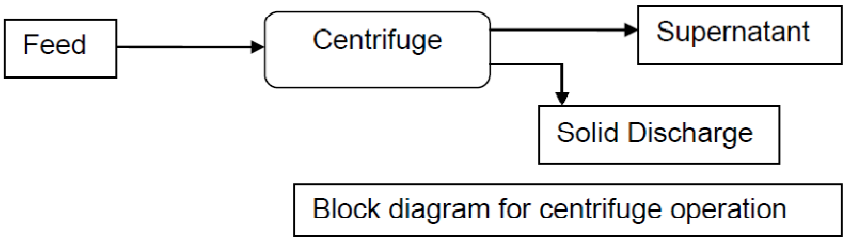
The common primary recovery operations in the bio-processing industry include centrifugation and/or depth filtration.

Centrifugation

Centrifuges are the conventional means of separating biomass and contaminants from the product stream. Centrifuges such as the disc stack centrifuge play a vital role in the continuous separation of biological products from fermentation broths. There are currently many varieties to such continuous centrifuges available in the market and their modes of operation are varied depending on the product and industry. This block diagram below depicts the operation of a continuous centrifuge.

A disc stack centrifuge contains a stack of truncated conical discs separated by spacer ribs of around 0.5 – 1 mm thickness. The suspension under consideration is fed through a feed channel located in the centre of the centrifuge which leads the feed at the bottom of the rotating bowl. Under high centrifugal force that the bowl generates the solids are thrown radially outward, and those are still present in the suspension during its upward motion on the discs slide down continuously joining their

earlier separated counterparts deposited at the sediment holding space (1). The supernatant on the other hand is continuously discharged at the top of the rotating bowl.



Block diagram for centrifuge operation

The rate of sedimentation is a function of many forces acting on the particle, namely, gravity, the drag force and buoyancy. The relation is described by Stokes' law which advocates the presence of a terminal velocity (u_c) at which all forces balance each other and net forces approaches zero.

$$U_C = \left(\frac{\Delta\rho}{18\mu}\right)(d_s)^2\,\omega^2\,r$$

$\Delta\rho\rho$- Density difference between the particle and the fluid
μ- Fluid viscosity
d_s- Diameter of the particle
ω- Angular velocity
r – Distance of particle from axis of rotation

Continuous-flow centrifugation can be successfully scaled from laboratory to pilot scale as well as be compared to other centrifuges using the concept of equivalent settling area, Sigma (∑). The settling area, ∑ is an index of the size of centrifuges and relates the centrifuge to the area of a gravity settling tank that would give equivalent clarification performance. Thus the performance of two centrifuges of different sizes can be compared as follows;

$$\frac{Q_1}{\Sigma_1} = \frac{Q_2}{\Sigma_2}$$

Where Q represents flow rate, ∑ represents the equivalent settling area.

The clarification efficiency of a continuous centrifuge is evaluated as a percentage using the following relation.

$$\%\ \text{clarification} = \frac{OD_f - OD_s}{OD_f - OD_w} \times 100$$

Where,
OD_f - OD of feed
OD_s - OD of a sample at steady state
OD_w- OD of a well-spun sample

The sigma factor for a disc stack centrifuge is calculated using the following equation;

$$\Sigma = \left(\frac{2\pi F}{3}\right)\left(\frac{z}{g}\right)\omega^2 cot\theta\left({R_0}^3 - {R_1}^3\right)C_{ds}$$

Where,

F = correction factor for area occupied by caulks
Z = number of discs in the stack
g= acceleration due to gravity
ω = angular velocity
θ = Half disc angle
R_0 = Outer disc radius
R_1 = Inner disc radius
C_{ds}= Calibration factor for non ideal flow

The performance of a continuous centrifuge is affected by the residence time of the liquid in the centrifuge and it is determined by the flow rate. Therefore, a comparison needs to be done between throughput and clarification before selecting the optimum flow rate for a process.

Furthermore, the scale-up of centrifuges for higher volumes is the biggest challenge which the industry faces in the practical scenario.

Filtration

Mechanical filtration is a physical operation which is used for the separation of solids from fluids by interposing a medium through which only the fluid can pass. Oversized solids in the fluid are retained on the feed side while the other particles that are small enough will pass through the partition. There are many types of filtration techniques based on the size or type of particles to be filtered. These operations are sometimes, more often than not subjected to a pressure difference which as a pressure drop, in order to ease the process of separation. Nevertheless, as the process continues over a period of time, a layer of deposit called the cake is formed which leads to further increase in the pressure drop thereby hindering the process.

In filtration, the resistance to flow increases with time as the filter medium gets clogged or as cake formation occurs. The quantities that are closely measured are the flow rate through the filter which reduces over time and the pressure drop across the filter which increases over time. Thus filtration processes are either operated at constant pressure or constant flow rate. As with every rate processes, the rate of filtration as per Darcy's Law can be calculated as a product of the driving force, in this case the pressure drop (ΔP) times area (A), the inverse of the resistance which is the viscosity (μ) and thickness of bed (L) and a proportionality constant (k).

$$Q = \frac{kA\Delta P}{\mu L}$$

This equation can be further simplified into the following equation thereby allowing us to differentiate between the two resistances.

$$Q = \frac{\Delta P}{R} \; where \; R = R_m + R_c$$

Where R_m is the resistance offered by the membrane/ filter and R_c is the resistance offered by the cake. In conventional filtration, R_c is always variable and defined as;

$$R_c = \mu\alpha'\Delta P^s(\frac{wV}{A})$$

Where,
μ is viscosity of the filtrate (Pa.s)
α' is a specific cake resistance (m kg^{-1})
ΔP is pressure drop across the filter medium and cake (Pa)
s is the cake compressibility

w is the mass of dry cake solids per volume of filtrate (kg m^{-3})
V is the total volume of filtrate (m^3)
A is the membrane area (m^2)

There are over six well established filtration technologies used in industry. Their use varies with industry, process and even on what part of an industrial they are employed. The two major types of filtration namely Microfiltration (MF) and Ultrafiltration (UF) are being employed for Mammalian cell culture clarification presently. Microfiltration is among the oldest membrane application second only to dialysis. However, Ultrafiltration evolved from Reverse Osmosis which in itself did not evolve from MF. These development differences still divides MF and UF as two fields today with firms outstanding in one field having little presence in the other.

Moreover, these two filtration technologies can be used as either dead end filtration or Cross flow filtration. In *Dead end filtration*, the fluid flows directly towards the filter under the influence of pressure. While using the dead-end filtration method, all the smaller particles passes through the membrane and all particles those are larger than the pore size of the filter accumulates at its surface. The size of those particles prevents them from passing through the filter medium. Cross flow or Tangential flow filtration (TFF) involves fluid flow parallel to the surface of the filter under pressure. The particles present in fluid greater in size than that of the pore are swept away by the current and hence prevent accumulation to a certain degree. Initial clarification/ coarse clarification efficiency is better with dead end filtration/ depth filtration in comparison with cross flow, whereas finer clarification is better with cross flow filtration.

The mechanical sieving along with the electro-kinetic properties of depth filters such as Zeta Plus™ (2) ensures high product purity and recovery with minimal shear forces to the cell mass. The choice of using a centrifuge/ depth filtration for primary clarification is usually dependent on the clarification efficiency in practical conditions as shown below:

$$Centrifuge\ Clarfication\ Efficiency\ = \left(1 - \frac{Centrate\ Turbidity}{Feed\ Turbidity}\right) \times 100$$

$$Filter\ Clarfication\ Efficiency\ = \left(1 - \frac{Filtrate\ Turbidity}{Feed\ Turbidity}\right) \times 100$$

The higher clarification efficiency leads to higher product quality and less downstream processing which in turn is beneficial in terms of time and cost.

Depth filtration is generally preferred over centrifugation for mammalian cell culture clarification due to various reasons such as shear sensitivity of the cells, scalability of the equipment, and so. The chart (Figure 2) depicts the advantages of depth filtration over centrifuge.

Parameter	Depth Filtration	Centrifuge
Scalability	Linear	Difficult
Effluent Quality	Excellent	Require additional filtration
Recovery	Excellent (> 95%)	Dependent on the Solid dryness achievable
Shear Forces	Low	High
CIP[a] Validation	Simple-Single use filters	Complex: May require equipment disassembly
SIP[b] Capability	Yes	Yes
Cross batch contamination	No: Single Use filters	May occur depending on the CIP & SIP efficiency
Capital Cost	Low	High
Running Cost	Low/ Moderate- Depends on consumable cost	Low- Depends on power consumption

[a:] Cleaning In Place, [b:] Sterilization In Place

Figure 2: Centrifuge versus depth filtration (2)

CONCLUSION

The choice of the unit operation employed in a mammalian cell culture clarification/ primary recovery depends on various factors such as the clarification efficiency, cost-effectiveness, protein recovery, and so on. The selection is basically a tradeoff between the cost incurred throughout the drug life cycle and the quality at which the drug to be produced. Most of the manufacturing processes deploy either centrifugation or depth filtration or both depending on the quality at which the downstream processing and the polishing steps are meant to be operated.

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