



## CHO cell separation by microfiltration using crossflow systems



Application  
Note

Purification by  
Crossflow Filtration  
Hydrosart® 0.2 µm

#8

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**Abstract**

Chinese Hamster Ovary (CHO) cells are used for fermentation of high value proteins in the pharmaceutical- and biotechnology industry. During the fermentation process the cells are grown under specific conditions in a defined media. The CHO cells produce extra cellular proteins. Separation and purification of these proteins result in the final product of high value.

The production steps utilized in any biopharmaceutical process must deliver the target protein with high purity and yield.

The first step in the production process is the separation of cells from the rest of the fermentation broth. Currently, stacked depth filters are used in this separation. The disadvantages with this method are: -1) low product yield (high hold-up|wetting volume) and 2) the messy "clean-up" that is inherent with stacked filters.

One of the main process requirements is that the CHO cell removal be accomplished with low cell mortality. This eases the subsequent process steps to purify the target protein with high yields free of DNA and other intracellular proteins. Additionally, the CHO cells can be reused.

The optimal cell separation technique that meet these requirements is Crossflow microfiltration.

**Principles of crossflow filtration**

In crossflow filtration, the influent stream (feed) is divided into two effluent streams, defined as the retentate and the permeate. The concentrate or nonfiltered portion "crossflows" over the membrane at high linear velocities. This flow creates a continuous self-cleaning sweeping action. In an optimization procedure the crossflow efficiency is maximized. Thus allowing the use of higher transmembrane pressures which result in increased flux, translating to higher permeate rates. The goal is to reach an optimal flux with a minimal decline during filtration. Variables that can be manipulated include inlet pressure (Pi), retentate back pressure (Po), and permeate back pressure (Pf).

Fluid flow through a membrane is best described by the Hagen-Poiseuille equation for streamline flow through channels:

$$J = [e \cdot r \cdot (TMP)] / [8 \cdot u \cdot x]$$

where

J = flux  
e = membrane porosity  
r = mean pore radius  
TMP = transmembrane pressure  
u = viscosity of fluid  
x = thickness of the membrane

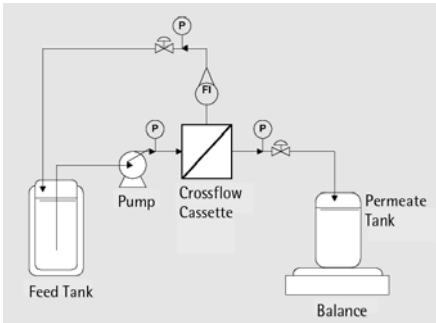


Figure 1: Typical Crossflow System configuration

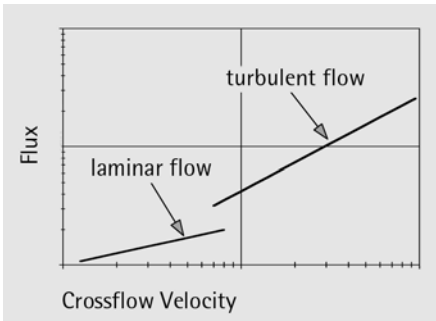


Figure 3: Effect of Crossflow Velocity on Flux when a Gel Layer has Formed

Based on this mathematical model, the ability to influence the flux for a given membrane is limited to using the maximum possible transmembrane pressure.

During filtration the solution will build up on the membrane surface a gel layer, despite the cross-flow sweeping action, by a phenomenon known as concentration polarization.

Once the gel forms, the flux declines and no longer depends on transmembrane pressure as described by the Hagen-Poiseuille equation independent from TMP.

### Transmembrane independent relationship

This phenomenon is expressed best by the following relationship:

$$J = k \ln [C_g / C_b]$$

Where

$J$  = flux

$k$  = mass-transfer coefficient

$C_g$  = concentration of retained species forming gel layer

$C_b$  = concentration of retained species of the bulk fluid

An optimization process is intended to balance cross-flow dynamics with filtration flux to generate the highest sustained flux. This goal is realized when the gel layer is minimized by disrupting the laminar-flow fluid boundary at the membrane surface.

In practical terms, this is described as  $\Delta P$  (Different pressure) versus TMP (Transmembrane pressure) optimization.

The first stage of an optimization procedure requires **optimization of cross-flow rates** by controlling differential pressure,  $\Delta P$ . In order to limit the influence of the other variables, transmembrane pressure and the feed concentration are constant while the cross-flow rates are varied. The feed concentration is held constant by recycling both the permeate and retentate back to the feed vessel.

To establish the proper profiles, the following formulas are used to describe the relationship of  $\Delta P$  and TMP.

$$\Delta P = P_i - P_o$$

and

$$TMP = [(P_i + P_o) / 2] - P_p$$

Where

$P_i$  = Inlet pressure (Feed pressure)

$P_o$  = Outlet pressure (Retentate pressure)

$P_p$  = Permeate pressure (Filtrate pressure)

By plotting the resulting values of flux versus the corresponding cross-flow rates, a profile can be established in order to determine the optimum cross-flow. The optimum rate should correspond with the point which gives the maximum flux or on the upper end of the plots plateau.

The next stage entails **optimization the transmembrane pressure**. In this stage, the transmembrane pressure (TMP) is manipulated while holding the cross-flow rate and feed concentration constant. Another profile is established this time by plotting the resulting flux values versus the corresponding transmembrane pressures. In this case the optimum rate is chosen as the upper-most point on the linear portion of the plot.

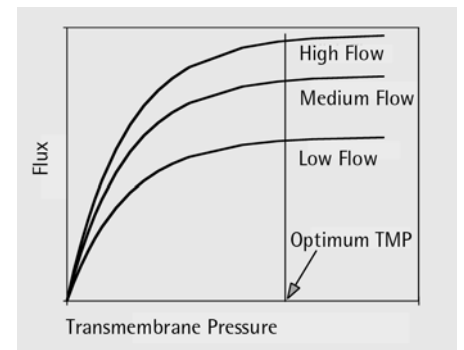


Figure 3: Effect of Trans-membrane Pressure (TMP) and crossflow velocity on Flux rates

If this process optimization steps are not done than it has a main influence in decreased flux and premature fouling of the membrane.

## Objectives

The objective of this Crossflow microfiltration study is to demonstrate the efficacy in using Sartorius Stedim Biotech crossflow technology in separating CHO cells from the then conditioned media followed by the concentration and diafiltration of target proteins in the media by crossflow ultrafiltration.

The microfiltration of CHO cells **must** be accomplished with low cell mortality.

The study focused on:

- membrane polymer
- process parameters
- cassette and equipment design...

each one of which is crucial in attaining the over-all objective.

## Vitality rate of CHO cells and membrane type

In the separation market different membrane material are available. Standard membrane materials (like PVDF-poly vinylidene difluorid- | PESU-poly ether sulfone- | CA-cellulose acetate- | nylon-etc.) are in use for different applications.

The relationship between membrane polymer and cell mortality is evident from the following data.

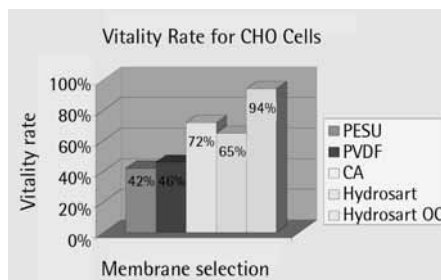


Figure 4: Relationship between membrane polymer and cell mortality

System size, type of recirculation pump and process parameters have also a main influence in product yield.

The optimal cell separation method that fulfills the main requirement i.e. low mortality is Crossflow microfiltration and the Sartorius Stedim Biotech Hydrosart® microfilter membrane. The Hydrosart® is a cellulose based membrane material which is naturally hydrophilic. It allows high product yields due to low protein binding characteristics and **excellent** cleanability due to its chemical resistance to caustic (1.0N | NaOH) solution. This gives the Hydrosart® membrane an economic advantage over other membrane polymers.

In the biotechnology industry, process steps using cellulose based membrane material (CA or Hydrosart® membrane) are the most commonly employed. In comparison to PVDF or PESU membranes, the yield commonly seen with CA or Hydrosart® membrane are significantly higher. (> 30%).

Trials under production condition have shown that the cassette design (geometry) has distinct advantages over other devices and geometries. With the cassette design it is possible to fulfill economical requirements. Cassettes stack in parallel.

Crossflow systems are modular. Very large membrane area can have a very low hold up volume in the system. This is necessary to achieve a high concentration rate. Easy and efficient cleaning reduce the production cost dramatically.

### Optimized Flow Characteristic

For this crossflow microfiltration application Sartorius Stedim Biotech developed a new and optimized cassette design. "Open channel" flow channels combined with a self sealing cassette are features in the design that influence increased yield.

With optimized flow channels it is possible to achieve very high cell concentrations.

The clarification of CHO cell laden media with high cell concentration ( $10^6$  to  $10^7$  cells/ml) can be performed with Sartorius Stedim Biotech Hydrosart® microfilter in Crossflow Cassettes (open channel version) very efficiently with insignificant cell lysis and high product yields.

This cell separation technique allows subsequent purification steps to be designed and performed much more efficiently. For the concentration and diafiltration of the conditioned media the Hydrosart® ultrafilter cassette is highly recommended. Low nonspecific adsorption and high pH resistance for efficient cleaning are hallmark characteristics of the Hydrosart® membrane.

Easy and high efficient cleaning during a short time are the main point for reduce production cost. High pH resistant membrane (Hydrosart® pH= 2-14) avoid cross contamination. Effective cleaning support the life time of the membrane.

### Flux graph versus transmembrane pressure

Typical Flux Graph (Figure: 5) during CHO cell separation with Hydrosart® 0,2 µm microfilter ("OpenChannel") under cross-flow conditions.

From this trial the conclusion can be drawn that the Sartococon Hydrosart® open channel cassette system can easily and efficiently (harvest) clarify CHO cell laden media to very high cell concentrations... ( $10^6$  to  $10^7$  cells/ml) with insignificant cell lysis.

### Prospective View

For separation of CHO cells Hydrosart® microfilter is the optimum alternative for common separation technique.

Economical results, scale ability and high yield are the main arguments for using crossflow microfilter to separate CHO cells from the fermentation broth.

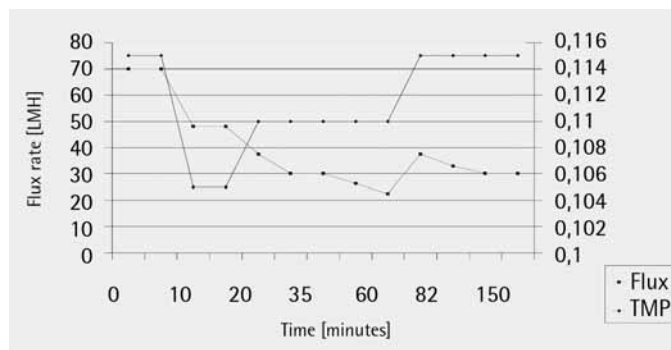


Figure: 5

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