Factors for successful clarification and cell harvesting

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Factors for successful clarification and cell harvesting

This technical brief describes process variables you must consider when selecting a hollow fiber cartridge for clarification and cell harvesting processes.

Four key variables include:

- Membrane pore size
- Hollow fiber path length
- Fiber lumen diameter
- Process conditions

Membrane pore size

Membrane pore size can influence process efficiency in unexpected ways, and specifying a membrane with an appropriate pore size is a key step in developing an effective and efficient clarification or cell harvesting process. For example, when harvesting cells, smaller pore size membranes often provide the highest permeate flux once the system is in a steady state (Fig 1). Hence, experts often select our large pore size ultrafiltration membrane for harvesting *E. coli*, even though it has a relatively small pore size compared to the size of the cells.

Protein passage is paramount in clarification processes, and experienced users typically select open pore size microfiltration membranes (0.2 to 0.65 µm pore size), especially for larger recombinant proteins and monoclonal antibodies. In general, choose a membrane pore size that is at least ten times larger than the target material that you want to pass through the membrane.

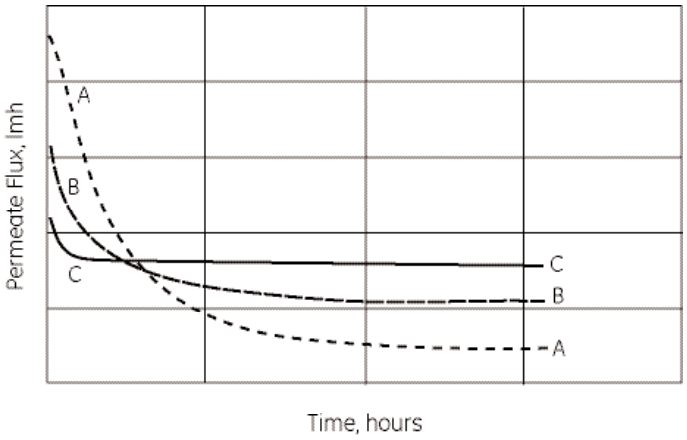

Figure 1. Flux of three membranes with all parameters held constant except pore size. Membrane A has a larger pore size than membrane B, which has a larger pore size than membrane C.

In general, cell harvesting is best performed with tight pore size microfiltration membranes or open pore size ultrafiltration membranes (Table 1). For clarification applications, choose a membrane pore size that is at least ten times larger than the target material that you want to pass through the membrane (Table 2).

Hollow fiber path length

The concentration of particulates (cells and cell debris) in the feed material is a primary factor in selecting the length of the cartridge. If the particulate level is high, as is often the case in cell processing applications, you should use short path length cartridges (nominally 30 to 60 cm). Longer path length cartridges (nominally 110 cm) exhibit higher inlet pressure due to increased dynamic friction at a given flow rate.

Fiber lumen diameter

When using hollow fiber cartridges, the inside diameter of the fibers influences other process variables. Feed streams with particulates such as cells and cell debris, flow readily through fibers with larger lumens (inside diameters). For example, hollow fibers with inside diameters of 0.75 to 1.0 mm perform well with particulated feed streams. Likewise, large lumen diameters perform well when processing viscous starting broths and the fouling components often found in upstream processes (for example, lipopolysaccharides in the case of *E. coli*).

Process conditions—cell harvesting

Cell harvesting is typically treated as a dewatering process. During dewatering, broth passes through the hollow fiber membrane and out of the permeate port of the filter. Permeate flows can be high (80-120 lmh) when the feed has a low cell density or lower when the feed has higher concentrations of cells, as with *E. coli*, yeast, or lysate concentration.

Process conditions—clarification

Clarification processes are generally influenced by three key process conditions including recirculation flow rate, permeate flow control, and the timing of diafiltration.

Recommended recirculation flow rate

Experience shows that the following recirculation flow rates work well:

2,000 to 4,000 sec^{-1} for fragile mammalian cells and viruses
6,000 to 8,000 sec^{-1} for yeast due to high viscosity
8,000 to 16,000 sec^{-1} for bacterial cells, lysates, and most proteins

Permeate flow control

Operators must exert deliberate control over transmembrane pressure and careful timing of concentration and cell washing to promote protein passage. During clarification, excessive transmembrane pressure can foul the membrane. Even when retentate pressure is zero, you can reduce transmembrane pressure further by controlling (limiting) the permeate flow from the cartridge. Many users thus add a pump to the permeate line to limit the flow of permeate from the cartridge and thereby exerting some backpressure on the membrane (Fig 2).

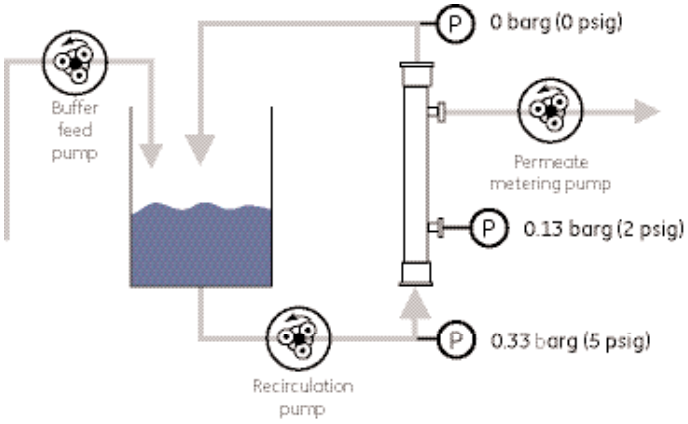

Figure 2. Typical cross flow filtration process showing a pump on the permeate line for permeate flow control

How much you reduce the permeate flow (compared to unregulated flow) depends on the nature of the starting feed stream. We recommend lower permeate flow rates: (1) for large target molecules, (2) if the particulates have a broad size distribution, or (3) if the particulates are sticky and fouling (Fig 3). A typical monoclonal antibody clarification from hybridoma cell culture with intact cells may be controlled at approximately 30 to 50 lmh, whereas enzyme clarification from bacterial lysate is almost always at approximately 10 lmh.

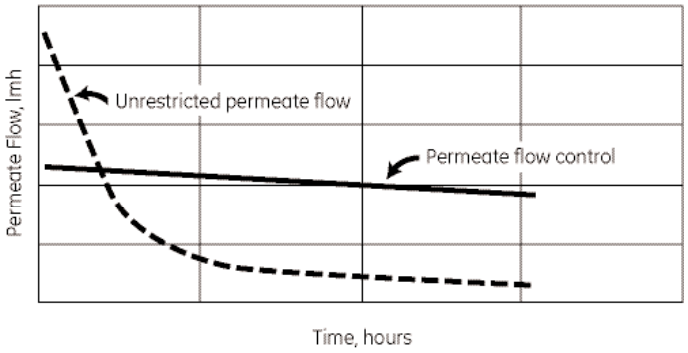

Figure 3. Typical permeate flow rates for controlled and uncontrolled process conditions. Permeate flow control results in a more stable flow, higher protein yields, and often shorter process times.

Diafiltration

To promote maximum protein passage during clarification, sound process design is required. For example, particulates can interfere with the passage of protein as the particulates become more concentrated in the feed stream. In addition, fine particles can coat the membrane surface, creating a secondary rejection layer that will retain the target protein. To overcome these problems, a partial concentration should be followed by diafiltration. Proper timing of the diafiltration step is essential. We recommend performing a brief diafiltration at a point where protein is still passing freely—that is, not being retained by the gel layer of concentrated particles that forms on the membrane surface.

Sizing up the process

During the early stages of development, it is important not to "overwhelm" a membrane with a large volume of starting material. You should try to anticipate the relative rate of productivity and introduce a proportionate amount of feed stock to result in a reasonable process time. Here is an example:

When working with a high solids load from yeast fermentation, how much starting material should be used for a one- to two-hour process?

The estimated flux rate is 30 lmh at room temperature. The membrane surface area is 50 cm^2 (cartridge model CFP-1-E-2U), and the process design includes a 2× concentration followed by a 3× wash. The calculated permeate flow rate is 150 ml/hr.

If you start with 150 ml and follow the process design, there would be 225 ml of clarified filtrate at the end. Using these estimates, the 150-ml starting volume would be appropriate for a two-hour run.

Summary

There are many factors influencing cross flow filtration clarification. Four key factors include membrane pore size, cartridge length, fiber lumen size, and process conditions.

You must understand these factors to develop an effective and efficient cross flow filtration clarification process.

For details, please contact your GE representative today.

Table 1. Hollow fiber selection guidelines for cell harvesting applications

Factor	Recommendation for cell harvesting
Membrane pore size	500,000 or 750,000 NMWC for ultrafiltration 0.1 or 0.2 µm for microfiltration
Hollow fiber path length	Short path length cartridges (nominally 30 to 60 cm)
Fiber lumen diameter	Large lumen diameter fibers (0.75 to 1.0 mm)
Process conditions	Recirculation flow rate—8,000 to 16,000 sec^{-1} shear rate for fouling feed streams, 2,000 to 4,000 sec^{-1} for shear-sensitive feed streams.
	Process sequence—Flux (and protein passage) is dependent on the concentration of particulates. To get the best protein passage, position a cell-washing step before flux declines significantly. Use low to moderate transmembrane pressure (<1 barg [15 psig]).
	Process temperature—Room temperature works best, but only if process components are stable at this temperature; otherwise 4°C-12°C (39°F-53.6°F) works well, but with lower flux.

Table 2. Hollow fiber selection guidelines for clarification applications

Factor	Recommendation for cell clarification
Membrane pore size	0.2 to 0.65 µm pore sizes for most mammalian cell culture clarifications
Hollow fiber path length	Short path length cartridges (nominally 30 to 60 cm)
Fiber lumen diameter	Large lumen diameter fibers (0.75 to 1.0 mm)
Process conditions	Recirculation flow rate—8,000 to 16,000 sec^{-1} shear rate for fouling feed streams, 2,000 to 4,000 sec^{-1} for shear-sensitive feed streams.
	Process sequence—Flux (and protein passage) is dependent on concentration of particulates. To get the best protein passage, position a cell-washing step after a partial concentration, but before flux declines. Use of permeate flow control is strongly recommended. Low transmembrane pressure (<0.7 barg [10 psig]) is usually very important for best protein passage to prevent blinding of membrane by particulates.
	Process temperature—Room temperature works best, but only if process components are stable at this temperature; otherwise 4°C-12°C (39°F-53.6°F) works well, but with lower flux.

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