Troubleshooting Tangential Flow Filtration

Follow this expert's advice to maintain good filtrate and extracellular product recovery rates.

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TANGENTIAL FLOW FILTRATION (TFF) IS commonly used to concentrate and dialyze solutes measuring from hundreds to thousands of Daltons in size (such as in reverse osmosis, one of several types of TFF) to macromolecules measuring from tens to hundreds of kiloDaltons in size (such as in ultrafiltration) and cellular biomass measuring microns in size (such as in microfiltration). TFF generally involves the circulation of the feed solution over a filter surface — *i.e.*, solute is swept over the surface of the filter as filtrate is pushed through the filter by the pressure of the feed stream (Figure 1). The sweeping action of the circulating feed solution prevents solute from entering the filter matrix and obstructing or fouling the flow paths within the filter matrix.

This article presents a brief overview of the principles governing TFF, and offers an empirical approach to optimizing operating conditions for mammalian cell-culture clarification applications. In the case of mammalian cell culture clarification, this optimization is complicated by the fact that the cells are damaged by high feed flowrates.

Filters designed for TFF applications come in a variety of configurations offering different flow path geometries (depending on the shape of the feed channel's cross section), surface areas and filter types. The filter surface presented to the feed must be uniform and relatively smooth. Therefore, membrane-type filter matrices are used in TFF filter modules. The nature of the feed material and the configuration of the TFF filter are major determinants of the performance potential of the separation process. With a given feed material and TFF system, the maximum efficiency of a TFF separation generally depends upon the optimum combination of feed velocity and the pressure across the filter surface, also called the transmembrane pressure (*TMP*). *TMP* is generally calculated by adding the measured line pressures of the feed flowing into the TFF system and out of the TFF system, dividing the sum by two, and subtracting any line pressure of the filtrate flowing out of the TFF system:

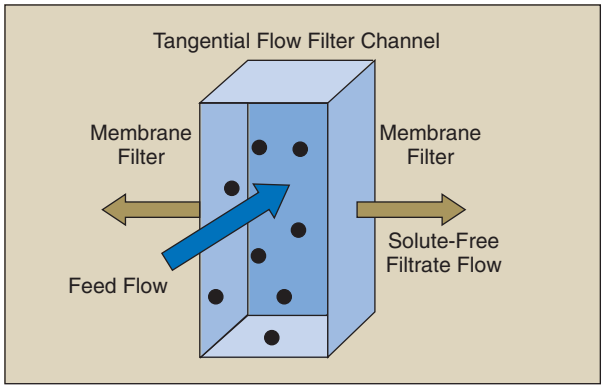

Figure 1. In a tangential flow filtration (TFF), solute is swept over the surface of the filter as filtrate is pushed through the filter by the pressure of the feed stream. The sweeping action of the circulating feed solution prevents solute from entering the filter matrix and obstructing or fouling the flow paths within the filter matrix.

$$TMP = (P_{fi} + P_{fo})/2 - P_{po} \quad (1)$$

where P_{fi} and P_{fo} are the feed inlet and outlet pressures, respectively, and P_{po} is the permeate (or filtrate) outlet pressure, all in psig.

Feed flow velocity is not uniform within the flow path or feed channel of the TFF filter module. The feed flowing along the filter surface and walls of the feed channel experiences drag and loses velocity relative to the feed flowing in the center of the channel. As filtrate passes through the filter, the concentration of the solute increases at the surface of the filter. Therefore, during a TFF process, solutes may begin to concentrate, flow along the filter's surface, and potentially interfere with the flow of filtrate across the membrane. This area of lower feed velocity and higher solute concentration is often referred to as the boundary layer.

In TFF separations involving solutes smaller than mammalian cells, the concentration of solute at the filter surface can be addressed by increasing the feed flowrate, Q_{feed}, to the point where the stable, laminar flow-velocity profile within the channel becomes unstable and transitional. Transient flow conditions create a non-uniform pattern of velocities near the edges of the feed channel. Eddies form and extend from the center of the feed flow into the boundary layer, where they break up the boundary layer and improve filtration conditions. The solute in the feed channel experiences more shear force due to the increasingly random velocity pattern within the flow channel.

Cell culture

The optimum value of Q_{feed} for most TFF applications is driven by the maximum operational inlet pressure of the TFF filter or system; conversely, the optimum value of Q_{feed} for cell culture is usually driven by the shear sensitivity of the mammalian cells. When subject to moderate levels of shear (*e.g.*, 3,000 s^{-1}) due to high feed flowrates, mammalian cells can rupture and spill their contents into the feed stream. Damage to the cells can increase the fouling of the filtrate step (by adding smaller solutes to the feed stream), resulting in less-efficient filtration and/or product quality problems. Therefore, it is usually necessary to operate TFF processing of mammalian cell culture under laminar feed flow conditions where shear rates are lower.

The laminar flow conditions also provide a benefit in terms of the efficiency of the filtration process. Under these conditions, the velocity gradient within the cross section of the flow channel is parabolic. The center of the feed channel has the highest velocity, and the velocities decrease asymptotically as one moves from the center of the channel to the walls of the channel. Mammalian cells are large enough (*i.e.*, tens of µm in dia.) that this velocity gradient can exert lift forces upon the cells. Lift forces are created when gas or liquid flows at different velocities across opposite surfaces of an object (*e.g.*, the cell). The stream with the higher velocity creates a lower pressure against the object, which experiences a force that draws it into the higher velocity stream. The feed flow velocity is higher the closer it is to the center of the feed channel, therefore mammalian cells are drawn towards the center of the feed channel by lift forces.

Table. Data for Figure 2.

$Q_{filtrate}$, L/min	Q_{feed}, L/min	*TMP*, psi
2	12	2
3	12	5
3	12	7
3	16	3
4	16	6
4	16	9
4	20	5
5	20	8
4	20	12
4	25	7
5	25	10
3	25	15

Nomenclature

- P_{fi} = feed pressure on inlet side of membrane, psig
- P_{fo} = feed pressure on outlet side of membrane, psig
- P_{po} = permeate (or filtrate) pressure on outlet side of membrane, psig
- Q_{feed} = feed volumetric flowrate, L/min
- $Q_{filtrate}$ = filtrate volumetric flowrate, L/min
- *TMP* = transmembrane pressure, psi (Eq.1)

Mapping out a better separation

Optimization of TFF processing of mammalian cell culture involves balancing the force of lift provided by feed flow, with the force of *TMP*, which produces filtrate flow. Due to the complexity of the separation process produced by the nature of the feed solution, (*i.e.*, the viscosity and density of cells) and the fluid dynamics of the TFF system (*i.e.*, the geometry of the feed channel can be circular or rectangular, less than 1 mm to 2–3 mm wide, and the feed channel can be in. to feet long), the optimal processing conditions must be determined empirically. One approach to determining the optimal processing conditions is to construct a process map.

The construction of a such a map involves operating the TFF separation process under different conditions with the same or similar feed solutions: Q_{feed} and *TMP* are independently varied and the flowrates of the retentate and permeate, or fluid containing extracellular product, are measured after conditions have stabilized at each combination of flow and *TMP*. Note that *TMP* can be controlled in two ways — first by partially closing a valve on the feed flowing out of the system, and secondly by restricting the flow of the filtrate out of the system, thereby creating filtrate line pressure.

The process mapping data in the table (above) is plotted on a two-axis graph, with Q_{feed} and *TMP* occupying the *x* and *y* axes (Figure 2). Each *x, y* coordinate is labeled with the $Q_{filtrate}$ generated with the corresponding Q_{feed} and *TMP* conditions. Lines are drawn between the *x, y* coordinates that have the same corresponding $Q_{filtrate}$ values to form the map. The lines connecting the *x, y* coordinates with the equal $Q_{filtrate}$ should form a circle surrounded by concentric

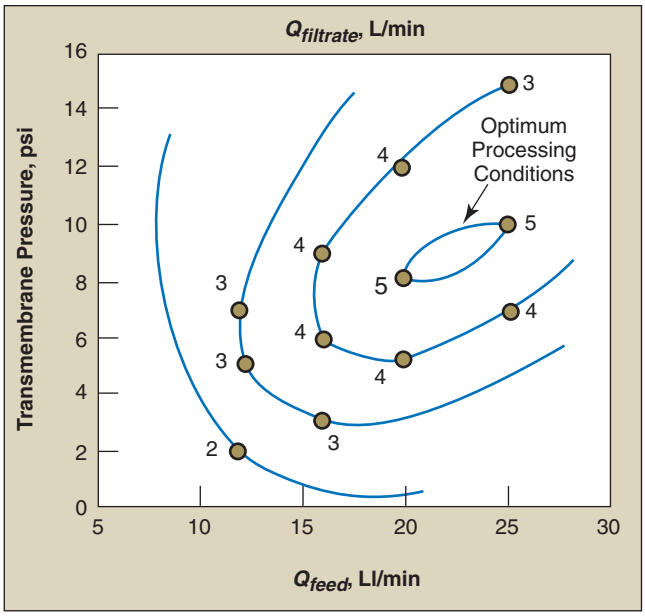

Figure 2. A process map can be effectively used to determine optimal processing conditions. Here, feed flowrate, Q_{feed}, is shown on the *x*-axis and transmembrane pressure, *TMP*, is shown on the y-axis. Each *x*, *y* coordinate is labeled with the filtrate flowrate, $Q_{filtrate}$, generated with the corresponding Q_{feed} and *TMP* conditions. Lines are drawn between the *x*, *y* coordinates that have the same corresponding $Q_{filtrate}$ to form the map.

rings or semi-concentric lines. The circle should contain the highest $Q_{filtrate}$ and product passage values. The Q_{feed} and *TMP* combinations within this circle represent the optimal processing conditions. This boundary of optimum processing conditions can be used to set preliminary process control limits, or further optimization within these boundaries can be conducted by generating more *x*, *y* coordinates within the optimum process boundaries.

It is important to note that the optimum processing boundaries are dependent upon the qualities of the feed solution, such as cell density and culture viability. If these qualities vary significantly from batch to batch, process mapping should be performed on multiple preparations of feed solution, or using a "worst case" feed solution if that is possible to define. Also, most TFF processing of cell culture will involve concentration of the culture; therefore, it is important to perform the process mapping under the conditions that are representative of both the start of the process and the end of the concentration process. It is possible that the optimum process format will involve changing Q_{feed} and/or *TMP* setpoints after the concentration portion of the process is completed.

It is always desirable to perform process optimization on a small scale. In the case of cell-culture TFF applications, "scaled down" versions of the major TFF microfiltration modules are available from the major TFF filter manufacturers. Using these scaled-down modules, the flow channel geometry and feed flow conditions can be replicated at a small scale, and optimum processing conditions can be determined, followed by a limited mapping exercise in the full-scale system.

It is also very important for the length of the feed channel in the scaled-down system to be representative of the length of the feed channel in the full-scale system. Filtration conditions are not uniform along the length of the feed channel because: drag decreases the velocity of the feed stream; feed fluid volume is lost to filtrate flow; and solute becomes more concentrated as filtrate is drawn from the feed fluid. This is why TFF modules with shorter path lengths will generally have higher optimum values of $Q_{filtrate}$. With applications involving the clarification of tens of thousands of liters of cell culture, TFF modules are often configured in series to reduce the feed flowrates and size of the feed pump. Due to the length of the feed channel in these large applications, it is generally not possible to develop a representative scaled-down model.

The optimum processing conditions for cell-culture TFF applications are fundamentally different from the optimum processing conditions for other TFF applications, such as ultrafiltration. By using the lift forces generated during laminar feed flow conditions, very efficient and reliable cell-culture TFF processes can be developed. Here's a step-by-step procedure for determining the optimum cell-culture TFF process conditions:

1. Using a representative cell culture feed fluid, perform TFF clarification under a variety of combinations Q_{feed} and *TMP*. Record $Q_{filtrate}$ at each combination of Q_{feed} and *TMP*.
2. Plot the *TMP* vs. Q_{feed} values on an *x*, *y* scatter plot.
3. Enter the correlating $Q_{filtrate}$ beside each point on the graph.
4. Draw a line that connects equal $Q_{filtrate}$ values; the lines should form a circle surrounded by concentric lines. The circle should contain the highest $Q_{filtrate}$ values — the *TMP* and flowrate conditions within this circle represent the optimum processing conditions.
5. Perform further process mapping by:

- using the worst-case feed fluid.
- working within the optimum processing-conditions boundary to establish final process conditions.
- conducting the process at full-scale flow conditions; perform limited mapping and optimization as necessary. CEP

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