

Bioprocessing

Scale-up of a Cell Culture Perfusion Process

APPLICATION NOTE

A Low-Shear Filtration System that Inhibits Filter-Membrane Fouling

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Perfusion and batch / semi-batch are two typically used modes of operation for a mammalian-cell-culture-based production process.

In a batch culture, cells are inoculated into fresh media. As the cells grow, they consume the nutrients in the media and waste products accumulate. For a secreted product, when the culture has run its course, cells are separated from the product by a filtration or centrifugation step. For viral-vector production, cells are typically infected with a virus during the growth phase of the culture, allowing expression of the vector followed by harvest.

In perfusion culture, product and / or waste media is continuously removed and the volume removed is replaced with fresh media. The constant addition of fresh media, while eliminating waste products, provides the cells with the nutrients they require to achieve higher cell concentrations.

Unlike the constantly changing conditions of a batch culture, the perfusion method offers the means to achieve and maintain a culture in a state of equilibrium in which cell concentration and productivity may be maintained in a steady-state condition. For viral-vector production, the perfusion process provides a means to increase the cell concentration and, thereby the post-infection virus titer.

For a secreted product, perfusion offers the user the opportunity to increase the productivity from an existing production vessel or the ability to reduce the size and number of vessels, and, hence the capital costs to meet annual



Figure 1. Display of ATF-2 and ATF-4.

production goals.

A perfusion process requires a reliable cell-separation system that can operate from a range of 5 to >30 days. Ideally, a cell-separation system will be linearly scalable from the small-development scale to the production scale, so that time and resources are not wasted as a product moves to larger-size vessels as product requirements increase. One cell-separation system that meets these requirements is the *ATF System™*, used in the alternating tangential flow (ATF) process from Refine Technology, Co. (East Hanover, NJ).

ATF System

In this process, the diaphragm pump and control system serve to generate alternating tangential flow, through a hollow-fiber filter module. ATF is a constant-rate, reversible flow of liquid, back and forth, through the hollow-fiber lumens between bioreactor and pump. A small percentage of the cell-free spent media that moves through the fiber lumen is removed through the fiber's wall, typically with a 0.2-micron pore size.

Addition of energy in the form of air pressure over a large surface area of the diaphragm results in a system that is low shear and can generate rapid flow rates. The low-shear nature is important for fragile mammalian cells, and particularly for cells infected with a virus, as in the case of virus production.

The dynamics of the ATF system

inhibits fouling of the filter membrane as compared to a hollow-fiber module in a traditional tangential flow filtration (TFF) mode during a perfusion run, in two ways. First, the ATF action inhibits the attachment of aggregates to the hollow fibers' inlets with the rapid reversal of flow between the pump back to the bioreactor. Second, the changing pressure differential across the filter membrane during the cycle of the pump generates a back flush that inhibits gelatin formation on the hollow-fiber inner wall and extends filter life.

To minimize media consumption and cost, many companies choose to work in a small vessel, typically a glass bench top bioreactor, to test a new process tool or for initial process development work. At this scale, the volume of media required per day is manageable, particularly if supporting multiple bioreactors. The volumetric throughput to support the cell line and process objectives can be initially determined. The ATF-2, with a hollow fiber filter area of 0.042m² can be on these lab scale systems in the range of one to three liters. This unit is capable of sustaining a flux of 3 L/m²/hour (3 LMH) and total throughput of over 2000 L/m².

For scale-up of bench-scale operations, different size ATF units are available (Table). Such units have been used to scale a perfusion process successfully from a bench-scale to a pilot-scale vessel and can be scaled to a production vessel of 1,000 L (Figures 1, 2).

System Sizing Based on Filter Area

System	Approx. Filter Surface Area (m ²)
ATF-2	0.042 or 0.084
ATF-4	0.46
ATF-6	2.1
ATF-8	4.2
ATF-12	16.8

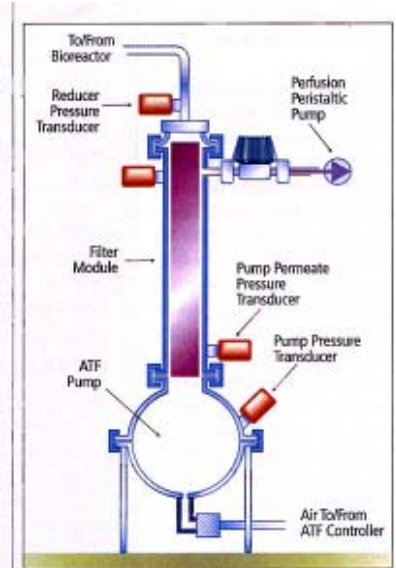


Figure 3. Representation of ATF-4 with transducers to measure flow characteristics.

Scale-Up of Bench-Scale Operations

When a company scales from the benchtop glass vessel to a stainless steel process vessel, one difference encountered is that a benchtop glass vessel is usually, for safety reasons, unpressurized (atmospheric pressure), while a stainless steel process vessel is usually maintained at a positive pressure of ~5 psi. As a process is moved from an atmospheric to a pressurized vessel, there is an interest to maintain the flow characteristics of the cell-separation system.

With an atmospheric vessel, the ATF

System controller requires a vacuum source to draw liquid through the hollow-fiber lumens to the ATF Pump. With a pressurized vessel, the vessel pressure is used to drive the liquid through the hollow-fiber lumens to the ATF Pump (exhaust cycle). In both cases, air at several psi greater than the vessel pressure is used to drive the liquid from the ATF Pump to the vessel (pressure cycle).

Using an ATF-4, some of these flow dynamics were characterized with water as the liquid medium (Figure 3).

The transmembrane pressure at the

pump ($P_{\text{pump}} - P_{\text{pump permeate}}$) and the pressure drop on the fiber lumen side ($P_{\text{pump}} - P_{\text{reducer}}$) was characterized for both a pressurized vessel and an atmospheric vessel at the same alternating flow rates and permeate flow rates. The data demonstrates that there is not much difference in the flow characteristics between the two vessel types (Figure 4). The data also demonstrates the back-flush through the filter membrane (negative transmembrane pressure).

A cell culture production run may require a high throughput through the perfusion system, such as for an extended run. While the ATF system has been shown to maintain a culture in continuous perfusion for extended periods of time, it also offers the ability for rapid exchange of filters without compromising a culture run. Because the ATF system requires only one vessel connection, it facilitates making or breaking the connection between vessel and ATF system, using steam sterilization of the joint between the two. In this manner, the process can be maintained producing a 0.2-micron product stream.

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