

## Bioprocess Tutorial

# Continuous Cell Culture Using the ATF System

### A New Way to Grow Suspension- or Anchorage-Dependent Cells

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Advances in the biomedical field have resulted in dramatic growth in the biotechnology and pharmaceutical industries. As new drugs and diagnostics evolve, so does the need for their characterization, processing and eventual production.

Many products are animal cell derived. Possibly the greatest gain in productivity is achieved by enhancing the cell expression system for the product. Selection of a cell line that is a high producer and that is adaptable to production requirements may be well worth the effort. The next greatest gain in productivity can be achieved from the production system and steps associated with the production process.

Animal cells grow substantially slower than most microorganisms, and lacking a protective cell wall, they are also more fragile. Therefore, one is limited to very gentle culture conditions and relatively low cell concentrations. One way to increase the cell concentration, yet maintain gentle culture conditions is the perfusion method.

A perfusion culture is one in which waste medium is continuously removed from the culture and the displaced medium is replenished with fresh medium. The constant addition of fresh medium and elimination of waste products provides the cells with the environment they require to achieve high cell concentrations and with that higher productivity.

#### Steady State

Unlike the constant changing conditions of a batch culture, the perfusion method offers the means to achieve and maintain a culture in steady state. In a batch culture cells are inoculated into fresh medium. As the cells grow, log phase, they consume the nutrients in the medium.



**Figure 1. The ATF-4 System Shown with the Silicone Diaphragm, Screen Module and Hollow Fiber Module**

The nutrients are exhausted, waste products accumulate and the culture decays. Various methods have been developed to optimize the batch process, including the use of fed-batch techniques. In each case, nevertheless, the process undergoes a rapid growth phase, then a short stationary phase, which is followed by a decay cycle.

In perfusion, it is possible to achieve a state of equilibrium in which cell concentration and productivity are maintained. Typically, about one to two culture volumes are exchanged per day; the cell concentration achieved in perfusion is typically two- to more than 10-fold of those achieved at the peak of a batch culture.

Daily output by a continuous culture can be equal or significantly greater than that achieved in an entire batch run, in an equivalent size bioreactor.

In spite of the potential benefits of the perfusion production process, it has gained only modest acceptance. As a carry-over technology from bacterial fermentation, batch culture is well characterized and is preferred by many of its past users. It is also a more natural carryover from the lab process of flasks and roller bottles. Another reason is the low reliability of available perfusion devices.

Some perfusion systems frequently damage the cells they're designed to separate. At the high cell densities achieved in perfusion, growth-limiting factors, such as the rapid depletion of certain nutrients in the medium, changes in physiological conditions, and exposure to

growth suppressing elements released by the cells may stress the culture.

Under these conditions, the cells become more fragile and subject to greater damage and death from shear conditions. In addition, cells die naturally or by apoptosis.

In sum, the end product of cell death and lysis is the accumulation of cell debris and aggregates. Such cell matter in combination with the viable portion of the culture may build up resulting in eventual clogging and failure of the perfusion devices.

One type of perfusion system is the Spin Basket. A basket-like device is attached to drive shaft and is located inside or outside the bioreactor. The perimeter of the basket is covered by a screen with about a 20 micron opening.

The rotation of the basket inhibits the attachment of cells to the screen or their penetration into the basket. The "cell free" waste medium is removed from inside the basket. Medium removed is replaced with fresh medium.

The system, which has been used by a number of companies to produce product, is prone to failure. Cells and cell debris, while inhibited from attaching to, gradually accumulate on the screen and also go through it. The increasingly obstructed screen loses its capacity to keep up with the demand of the culture, eventually causing it to collapse.

Another limitation of the Spin Basket is its limited scale-up potential. As the volume of the culture increases by the cube power, the surface of the basket can only be increased by the square power. Therefore, as the size of the bioreactor increases, the Spin Basket will occupy proportionally a larger volume of the vessel.

#### External Filter Perfusion

In external filter perfusion systems, a culture is circulated from the vessel, through a hollow fiber filter module (HFM), and back to the vessel. A pump attached to tubing in this loop provides the energy to circulate the culture in a continuous loop.

A second pump on the filtrate side of the HFM controls the rate of filtrate removal or perfusion rate. Volume re-

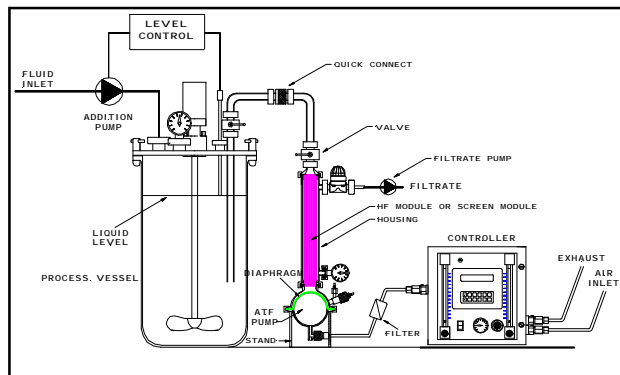


Figure 2. The ATF System consists of the controller, pump, housing, filtration device, and joint assembly (connects the housing to the vessel). After entering the process parameters and pressing the RUN button, the controller takes over the steady state control of the ATF System. Pressurization and depressurization cycles alternate according to the time sequences entered. A pump can be used to control the filtrate flow rate through the filtration device.

moved is replaced with fresh medium. Scale-up is achieved by a proportional increase in the number of hollow fibers.

The hollow fiber system is also prone to clogging by accumulation of particulates and gelatin on the membrane surface. Recirculation in one direction through the HFM can also clog the inlets to the hollow fiber lumens with aggregates.

These aggregates grow in size and as more hollow fibers are blocked, filtration capacity declines. The damage to the cells from the recirculation pump head is another consideration.

Other perfusion devices based on cell settling have, to date, been used with mixed success. With the exception of the hollow fiber system described, the other devices produce an unfiltered stream that must be filtered prior to further processing.

### ATF System

The ATF System™, used in the ATF Process (patent pending) from **Refine Technology** (Edison, NJ), is designed to address some of the shortcomings of existing perfusion devices. The system consists of a unique diaphragm pump mounted to one end of a hollow fiber housing (Figure 1).

The other end of the housing is attached to a joint assembly, which, in turn, is connected to a bioreactor through an available port. The ATF System can be used in different orientations including connected through a top vessel penetration or a side penetration (Figure 2).

The diaphragm pump and control system serve to generate Alternating Tangential Flow (ATF) through the

hollow fibers. ATF is a pulsating, reversible flow of liquid moving back and forth, between bioreactor and pump.

The pump is partitioned into two chambers by a flexible, medical-grade silicone diaphragm. The controller cycles filtered air to and from one of the pump chambers and a positive or negative pressure gradient is produced relative to the bioreactor. The stainless-steel sanitary design enables the ATF system to be sterilized-in-place with the process vessel or autoclaved and then attached to the process vessel.

During an extended run, a filter may be changed and the system resterilized without terminating the run. The system configuration and pump action have a number of benefits:

- Low shear- In the ATF process, energy is added to the surface of the liquid, generating a low shear laminar flow.
- High flow / scalability- Since air flow is used to drive the culture through the HFM, one can generate very rapid, low shear tangential flow rates enabling the technology to be used from R&D to production.
- Longer operating life- The dynamics of the ATF system can extend the operating life of a perfusion run in two ways. First, the ATF action inhibits the attachment of aggregates to the hollow fiber inlets by the repeated and rapid reversal of flow between the pump and the bioreactor. Second is from the changing pressure differential across the filter membrane during the cycle of the pump. That pressure differential has a back flush

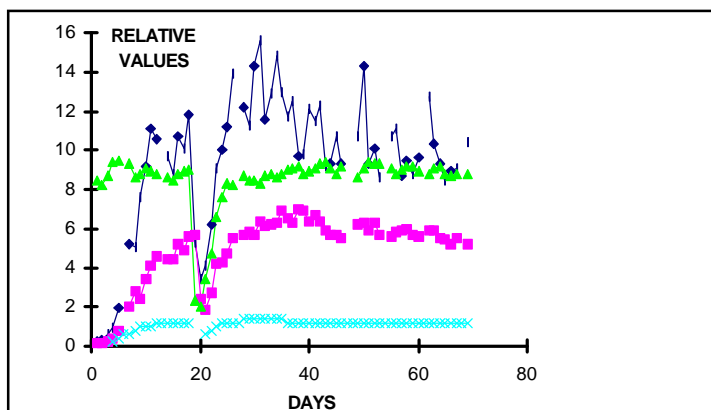


Figure 3. Production of a Humanized MAb in a 14 Liter Stirred Tank Bioreactor. Live cell concentration x10<sup>6</sup> cells/ml (♦), culture viability/10 (%) (▲), relative MAb concentration (■) and perfusion rate, vessel volumes/day (x). The cells were grown in serum free medium without antibiotics. Temporary failure of DO control on day 18. The perfusion hollow fiber module, filter surface area of 2.0 ft<sup>2</sup>, was changed on day 31 and 50. The 0.2micron filtered harvest stream was directly applied to protein-A for MAb purification, without further conditioning.

component which inhibits gelatin formation on the HF inner wall and extends filter life.

- Adaptable- The ATF system can be used with most bioreactors, from small glass vessels to large systems.

- Filtered product stream- The ATF process produces a filtered stream ready for processing.

### Applications

The system can be used in a number of applications, including suspension cells (Figure 3). The system delivered a 0.2 micron filtered product stream and demonstrated the capability to sustain cultures at high cell concentration with high viability for an extended period.

In anchorage dependent, microcarrier-based cultures, the modular nature of the ATF System allows replacement of the HFM with a screen module (SM). This converts the ATF System to a highly effective perfusion device for microcarrier based cultures.

The SM retains the microcarriers in the system as microcarrier free medium is removed. The active nature of the ATF process dislodges and inhibits the attachment of microcarriers or aggregates to the screen. Also, rapid and sustained media exchange rates have been achieved with this method.

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