Continuous perfusion: enabling maximum protein output

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The situation

Most suspension-adapted cell culture processes are operated in either batch or fed-batch mode for a period of several days. Cell densities increase, metabolites are consumed, and toxic waste products increase in concentration until the process is interrupted and the target protein is separated from the cell mass. As the need for the target protein increases during clinical trials, the process is scaled up in larger working vessels. At a process scale, these operations exceed the confines of the pilot plant, meaning that the process must be outsourced to a contact manufacturing operation or a dedicated bioprocess facility must be developed.

Continuous cell culture represents a means to maintain a consistently high cell density of healthy cells working in an equilibrium environment for a period of 30 to 60 days or more. A small bioreactor operating on a bench top or in a hood can produce large quantities of protein, but there is a critical need to introduce fresh media and to remove the protein containing cell-free media aseptically.

The solution: Autoclavable GE Healthcare hollow fiber microfiltration cartridges

Using GE Healthcare hollow fiber membranes, such processes can be operated using continuous perfusion to replenish media with filtrate flow rates from less than one liter per day up to 1,000 liters per day.

The solution is to create a subsystem for continuous perfusion, consisting of a pump that draws cells in their medium from the bioreactor, sends it through a GE Healthcare cross flow hollow fiber microfiltration cartridge, and returns the cells to the bioreactor (Fig 1).

In this system, the retentate consists of the cells, which flow past the membrane and are sent back to the bioreactor. The spent medium is the permeate that passes through the membrane.

A separate subsystem injects new media into the bioreactor, providing fresh nutrients for the culture. The entire system is sealed and aseptic. Perfusion is continued until the desired amount of product is obtained.

Specifying the right cartridge

As the popularity of continuous perfusion has grown, GE Healthcare microfiltration membranes are increasingly specified because of their consistency in performance, autoclavability, ease of incorporation in the system, and overall quality.

In this application, the cartridge size is based upon the total projected filtrate volume, not simply the size of the bioreactor. Therefore the duration of the process will heavily influence the size of the cartridge. Although a full scale process objective would usually be a 30- to 60-day operation, there may be reason to operate the same process for a shorter period of time during development.

The other variable for selecting an appropriate cartridge is the relative filterability of the media. Experience has shown most cell lines with reasonable cell viability will allow a throughput of 250 to 500 l/m^2 of membrane area.

For example, a researcher selected our CFP-2-E-9A cartridge (0.8 m^2) to perfuse 30 liters per day for a two-week period. If the process objective was extended to 60 days, a single CFP-2-E-65 cartridge (4.4 m^2) would be needed. Some scientists elect to operate a system with two cartridges in place. One cartridge is operational, while the second cartridge remains in "stand-by" if needed to reach the process time objective.

Although GE Healthcare offers four different microfiltration pore sizes, historically the 0.2 micron rating has been preferred. In order to insure that cells are not damaged due to shear stress forces, clients are encouraged to keep the recirculation flow near the 500 sec^{-1} shear rate for the cartridge. The peristaltic pump should be selected to operate at a speed less than 100 RPM and all of the components in the recirculation flow loop (including the bioreactor dip tubes) should be sized to maintain a consistent, low fluid velocity for improved productivity.

Easily adjusted for improved productivity

All of the critical parameters related to maintaining a conventional healthy cell culture environment remain intact. Adjustments in nutrient content or concentration can be made at any time during the perfusion process to control and/or improve productivity. Often, the media exchange rate, given in bioreactor volumes per day (BVD), has already been determined by the investigator.

The cells spend a very brief period of time outside of the bioreactor as they pass through the hollow fiber cartridge. The fluid returning to the bioreactor is dispersed back into the bulk material, maintaining a homogeneous environment for the cells.

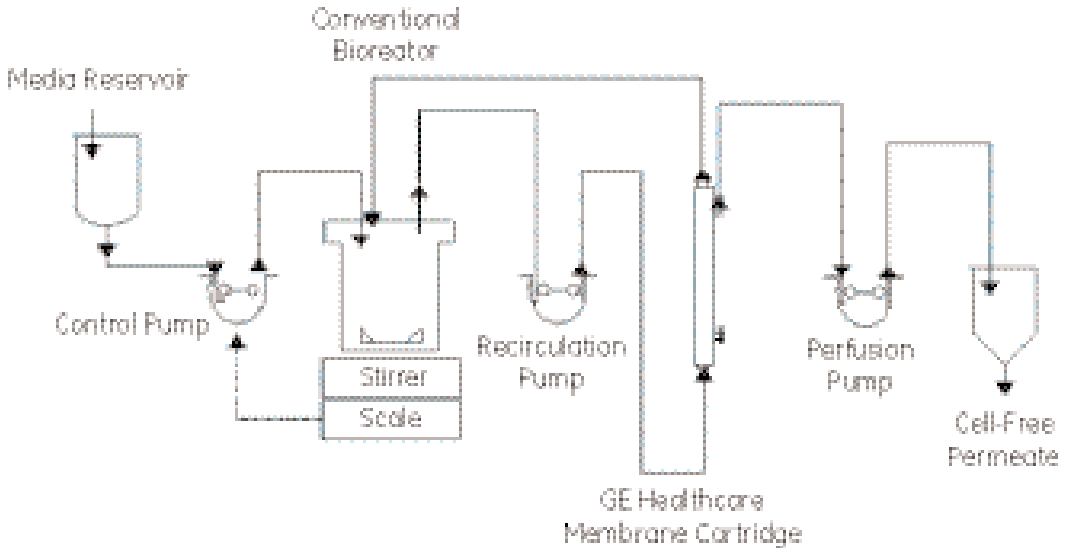

Figure 1. Continuous perfusion system for removing spent cell culture medium

Built-in controls for ease of operation

The controls for the operation of the hollow fiber membranes are quite simple. The pump that is used to recirculate the cells remains at a uniform rate. Pressure measurement on the cartridge inlet and/or permeate cavity is helpful for monitoring cartridge capacity as a function of time. In an effort to simplify the operation, many scientists operating at the lab scale do not include this feature in their studies.

The only critical parameter is the ability to maintain a constant fluid level in the bioreactor. The diagram shows a relatively simple operation using two peristaltic pumps to match the influent flow of replacement media to the outflow of spent media. In this scheme, the outflow is set at a prescribed rate (usually one bioreactor volume per day), while the pump controlling the influent flow is controlled by the signal from the load cell monitoring the weight of the entire bioreactor. Researchers who have hoped to use more manual systems with matching pumps for this operation have been disappointed with the flow variability, resulting in the need for chronic manual adjustments over such an extended process time.

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