Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Ng, Paul K. and Obegi, I. Andrew(1990) 'Tangential Flow Cell Separation from Mammalian Cell Culture', Separation Science and Technology, 25: 6, 799 — 807
To link to this Article: DOI: 10.1080/01496399008050366
URL: http://dx.doi.org/10.1080/01496399008050366

SEPARATION SCIENCE AND TECHNOLOGY, 25(6), pp. 799-807, 1990

NOTE

Tangential Flow Cell Separation from Mammalian Cell Culture

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Abstract

Tangential flow cell separation from fermenter-derived mammalian cell culture has been studied. The process is governed by transmembrane pressure and interactions of carry-over cells with the membrane. Membrane regeneration and comparison with other cell separation systems were discussed. Higher throughput per unit area over conventional dead end filter was demonstrated.

INTRODUCTION

Industrial scale mammalian cell culture has produced large quantities of recombinant DNA products. Consideration must be given to the removal of cellular DNA. Clarification of tissue culture fluid removes carry-over DNA containing cells as well as cell debris. The two unit operations applicable are centrifugation and filtration. Centrifugal separation could be performed with tubular bowl centrifuge, chamber bowl centrifuge, or continuous discharge disk centrifuge. In general, major problems encountered with any centrifugation operation are heat generation, aerosol formation, and labor-intensive assembly. With the advent of membrane technology, many microfiltration systems are suitable for cell separation (*1-3*). The systems are available in configurations of hollow fibers, flat sheet, and spiral-wound cartridges. They offer ease of operation, rapid recovery of highly clarified supernatants, and good temperature control. They are also amenable to automation. In this investigation

we will compare several microfiltration methods with emphasis on a tangential flow unit consisting of a plate-and-frame design. The data obtained provided the basis for pilot- and process-scale operations.

EXPERIMENTAL

Fresh and aged cell culture fluid from large-scale fermentors of baby hamster kidney cells were used in this study. The tangential flow filtration unit, Sartocon II, was purchased (Sartorius Corp., Bohemia, New York). It is equipped with a 0.6 m^2 0.45 μm hydrophilic membrane module. The membranes are made from cellulose acetate and are bonded together with silicon. Recirculation was achieved with a Watson-Marlow 701S/R manual control peristaltic pump (Bacon Technical Industries, Inc., Concord, Massachusetts). When the unit is in operation, the liquid flows across the membrane through a thin channel containing turbulence-generating promoters. The transmembrane pressure causes some product to cross the membrane. The remaining unfiltered liquid flows out of the thin channel back into the original container, and the process is repeated. Figure 1 shows the equipment arrangement in the experiment. The mod-

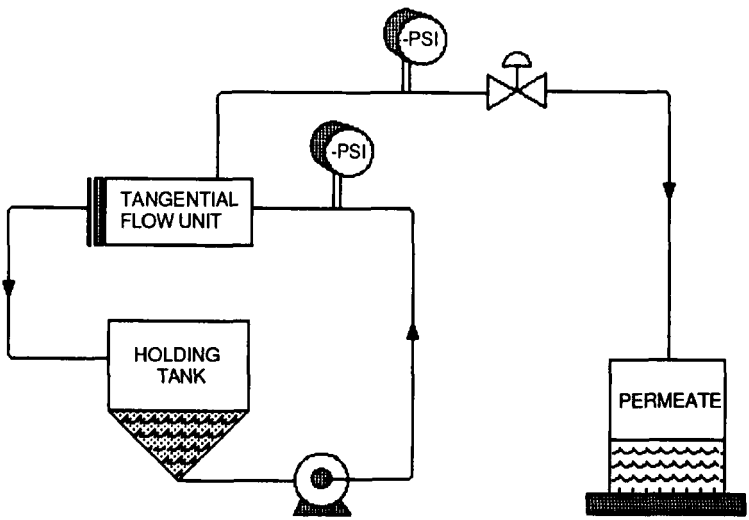

FIG. 1. Tangential flow system for mammalian cell separation.

ule, pump, and pressure gauges could be mounted on a mobile trolley which is stationed conveniently close to the fermentors. This would allow the cell separation step to be carried out in a cell containment area. After clarification, the permeate could either be sent directly to an ultrafilter or be stored in a tank for downstream processing.

Filter cartridges made of 0.4 μm polycarbonate pleated membrane were obtained from Nuclepore Corp., Pleasanton, California. The surface area of each filter is 1.7 m^2.

For cell count measurements an aliquot of 0.5 to 1.0 mL is withdrawn from a cell suspension and placed in a test tube. This aliquot is diluted with an equal volume of vital stain (Trypan Blue Dye) and mixed thoroughly. The sample is transferred to a Neubauer hemocytometer. Based on the principle that the dye is absorbed by dead cells but excluded by viable ones, the total cell population can be counted by using the low power of a microscope. This permits the calculation of viability or the percentage of viable cells in the sample.

Terg-a-zyme, urea, NaCl, Na hypochlorite, citric acid, and sodium borate were obtained from VWR Scientific, San Francisco, California.

RESULTS AND DISCUSSION

Effects of Transmembrane Pressure

Fluid dynamics of filtration can be expressed in equation form as (*4*)

$$J = \frac{P}{(R_F + R_g)u} \tag{1}$$

where J = volumetric flux or volume of filtrate per unit area
P = pressure drop across the filter
R_F = resistance of the filter medium
R_g = resistance of the gel layer on the filter medium
u = viscosity

Pressure and volume flux are linearly related when water is tested, since it passes freely through the membrane. When tissue culture fluid is filtered, mammalian cells will slowly accumulate on the surface and R_g becomes an important factor on the flux. The reduction in flux when

water and tissue culture fluid are compared, as shown in Fig. 2, is a function of the resistance from the polarized layer. This layer acts as a hydrodynamic barrier which reduces the effective pressure driving force. Based on the slopes of the two sets of data in Fig. 2, pure water flux and tissue culture flux were calculated to be 0.66 and 0.40 $L/m^2 \cdot min/psi$, respectively. The higher reduction in flux seen in tissue culture fluid at increased pressure indicates that pressure enhanced the formation of a gel layer consisting of mammalian cells.

Effects of Cell Concentration on Flux

When cell concentration increases significantly on the surface, volumetric flux can be described by film theory. It relates the permeate flux J to the solute concentration by the following relationship (*5*):

$$J = k \ln (C_w/RC_b - 1/R + 1) \quad (2)$$

where k is the local mass transfer coefficient for the cells between the bulk solution and the membrane surface, C_w is the concentration at the wall, C_b is the concentration in the bulk solution, and R is the rejection coefficient.

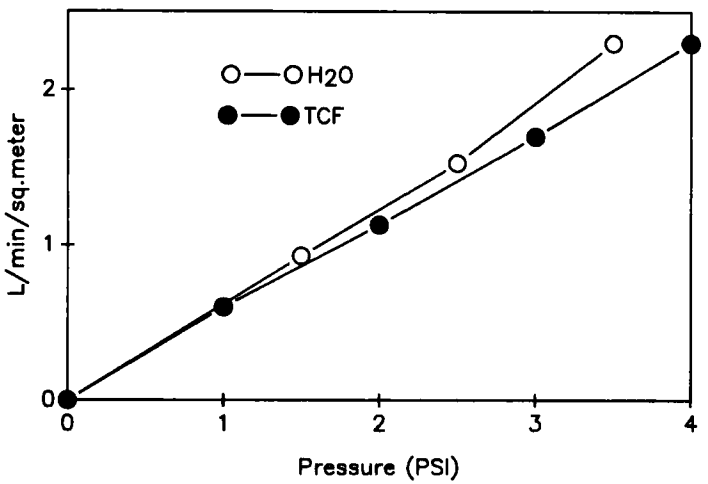

FIG. 2. Permeate flux vs transmembrane pressure.

For complete rejection of cell ($R = 1$), Eq. (2) becomes

$$J = k \ln (C_w/C_b) \tag{3}$$

In the experiment the module is tested with tissue culture fluid containing a range of cell concentrations. The permeate flux is plotted against the total cell concentrations in Fig. 3. The mass transfer coefficient calculated by best fit by using the nonlinear least squares iterative procedure is 2.5 L/$m^2 \cdot$ min. (Standard error is 25% of the parameter value.) This compares with 0.4 L/$m^2 \cdot$ min for bacterial cells at cell concentrations of less than 1% dry weight. (*3*). The higher mass transfer seen with mammalian cells (20 μm in size) vs bacterial cells (less than 10 μm in size) could be explained by (a) lower osmotic pressure from mammalian cells as predicted by the Van't Hoff equation for an ideal solution (*6*), and (b) larger cells enhance the momentum which dislodges the cells accumulating on the surface.

Extrapolation of permeate flux vs cell concentration data to zero flux can be used to derive an optimal operating concentration for diafiltration when removal of low molecular weight contaminants is desirable. This is the subject of an ongoing research effort.

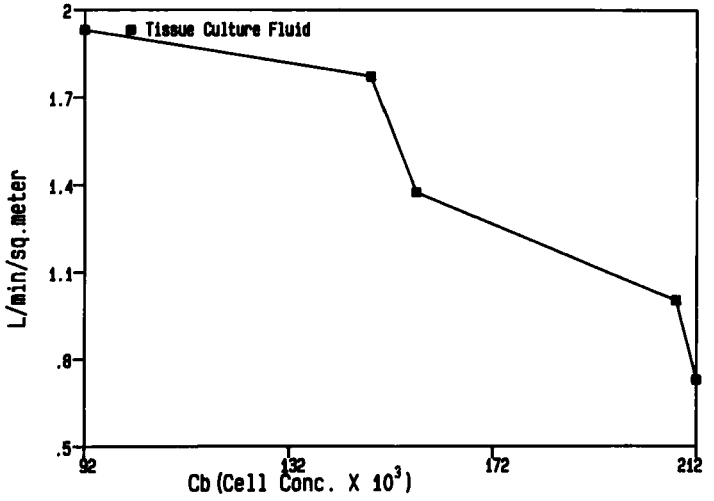

FIG. 3. Permeate flux vs cell concentration in tissue culture fluid.

Effects of Transmembrane Pressure on Cell Viability

Previously, Mourot et al. (*2*) demonstrated that an aseptic concentration of living microbial cells by cross-flow filtration preserves cell viability. In this study, samples were collected for cell counts under five transmembrane pressures (Fig. 4). An insignificant loss in viability was noted from an initial viability of 45%. During cell separation by tangential flow where the shear rate could be more than 10,000 s^{-1}, the principal concern is the excessive disruption of DNA-containing cells in the tissue culture fluid. DNA assay of the permeate (data not published) confirmed the viability observations.

Membrane Regeneration

As part of the experimental program, an investigation was made of methods by which membranes could be cleaned when fouling reduces the flux to minimal values. The following cleaning agents failed to restore the filter: (a) 4 *M* urea; (b) 2 *M* NaCl; (c) 0.25% Na hypochlorite buffered by citric acid and Na borate. Attempts to use 2000 ppm of Tween 80, a nonionic detergent, as suggested previously, also were unsuccessful (*7*). Further ex-

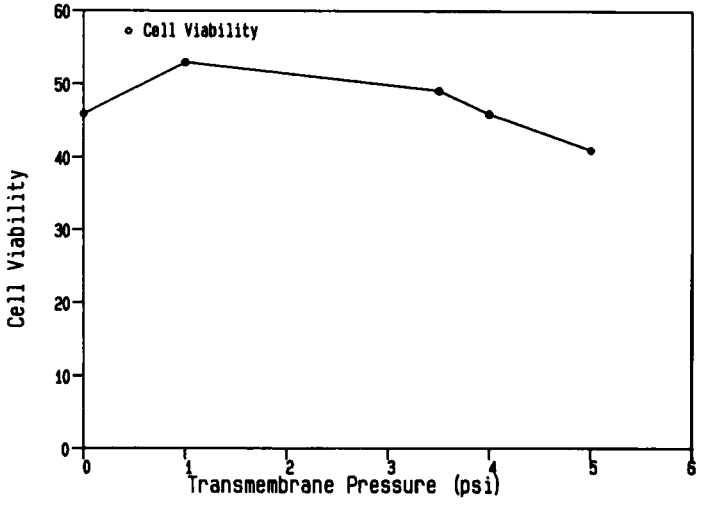

FIG. 4. Cell viability vs transmembrane pressure.

perimentation shows that a cleaning procedure using a 0.3% Terg-a-zyme solution is effective in restoring a seemingly plugged filter. In this procedure the solution was recirculated through the membrane at 50°C for 16 h. Alternatively, restoration was achieved by soaking the membrane in a pouch containing the Terg-a-zyme solution. A constant temperature (50°C) was maintained with a recirculating water bath.

Tangential Flow Filtration vs Dead End Filtration

Figure 5 shows the filtration profiles of tangential flow filtration vs dead end filtration (Nuclepore 0.4 filter cartridge). In this experiment an 80-L batch of tissue culture fluid was used in each of the filters investigated. Experience with dead end filters shows that the filtration rate of water or buffer is always proportional to the pressure differential. With a fluid-containing suspension of cells or cell debris, however, although increasing the initial pressure differential always resulted in initial faster filtration, deterioration of the rate developed rapidly; the quantity filtered at high pressure is always less than that at low pressure. Accordingly, dead end filtration of tissue culture fluid is started with a minimum pressure

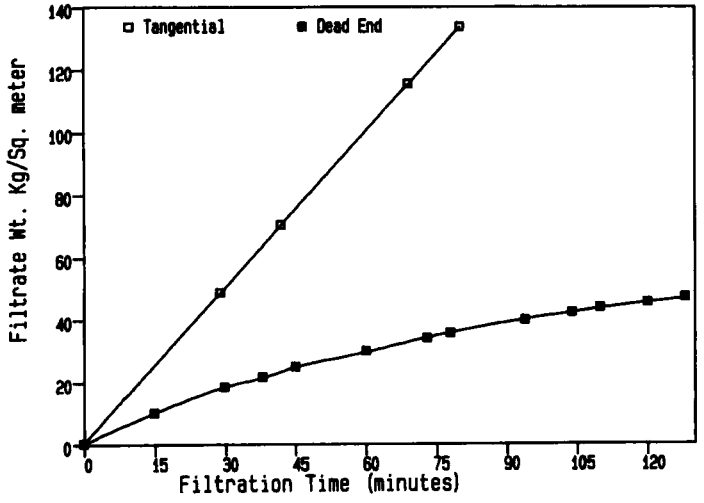

FIG. 5. Cell separation by membrane filtration.

(less than 1 psi). The pressure drop monitored over the course of the experiment under a constant pump setting indicated a slow rise to a final value of 6.5 psi. The nonlinear relationship between the filtrate collected and the filtration time resulted from the backpressure generated by gradual blocking of the pores in the filter.

With the tangential flow system, in contrast, the transmembrane pressure (4 psi) remained constant and the filtrate weight increased proportionately with time. It is also apparent that higher throughput per unit area was obtained due to a high recirculation rate which prevented particles from collecting on the surface.

Comparisons with Other Cell Separation Systems

An experiment with a dynamic 0.45 μm (Membrex Benchmark) rotary biopurification system (Membrex, Garfield, New Jersey) indicated a steep decline of flux after the first 15 min (unpublished data). The crossflow effect in this system is mainly derived from the spinning of the inner surface filter which forms the so-called Taylor vortices (*8*). In this context, the shear effects from a maximum rotation speed of 4000 rpm did not overcome the rapid buildup of solutes.

Another system under consideration is a ceramic hollow fiber ultrafiltration cartridge (0.45 μm) (Romicon, Lexington, Massachusetts). Good recirculation could be maintained, yet plugging occurred almost instantaneously. Our observation confirms a previous report that this configuration is not suitable for cell separation (*2*).

CONCLUSION

The technical feasibility of application of the tangential flow process to mammalian cell separation has been satisfactorily confirmed by the studies reported here. The efficiency of the process is governed by transmembrane pressure and interactions of cell concentrations with the membrane which is subjected to fouling. Regeneration of the membrane with 50°C Terg-a-zyme solution was effective whereas other chemical treatments failed. Removal of residual Terg-a-zyme is a valid concern since it may affect downstream processing. Measures to guard against fouling are therefore an important consideration during large-scale operations.

It is clear that the economics of the process are attractive if the filters are reusable numerous times under proper operating conditions. Experience has shown that the results from pilot-scale operations are not necessarily transferable to production-scale operations. The regeneration of a tangential flow unit and its subsequent use must meet good manufacturing procedures requirements in the pharmaceutical industry. Consequently, our ongoing efforts are aimed at confirming the suitability and cost effectiveness of this system or similar systems in the market (e.g., Millipore Prostack, Amicon tangential flow system).

Acknowledgments

The authors wish to acknowledge the technical contributions from Sartorius Inc. during start-up of this project. Discussions with Dr. G. Mitra, Director, Protein Purification Research, are also appreciated.

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Received by editor June 14, 1989