RESEARCH

Preliminary modeling of the perfusion culture of mammalian cells with a spinfilter as retention device

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ABSTRACT

Specific equations describing the behavior of cell growth and the filtration mechanism of a perfusion culture in stirred tank fermentors at 30 L scale were derived from basic equations for mass balance and mechanical energy in a spinfilter. These equations, when used for modeling the operation process in the Matlab package together with previously reported experimental data, yielded results similar to those of culture kinetics. The operational variables with the highest influence on the process were analyzed with the Matlab module, comparing them to a basal case using the spin rate of the spinfilter and the filtration area usually employed in the production runs as comparison parameters. Two additional comparisons were also performed, using cases with different filtration areas (smaller and higher than that of the basal case) in which the stirring rate was varied to analyze the behavior of the perfusion flow capacity during the run. The influence of the filtration area on the fermentation life was corroborated, with higher values for the latter as filtration area and spin rate of the filter increased.

Keywords: spinfilter, clogging, perfusion flow

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RESUMEN

Modelación preliminar del cultivo en perfusión de células de mamíferos en tanque agitado con *spinfilter* como dispositivo de retención. Partiendo de las ecuaciones básicas del balance de masa y de energía mecánica aplicado en el *spinfilter,* se llegó a las ecuaciones particulares que describen el comportamiento del crecimiento celular y al mecanismo de filtración en el cultivo en perfusión en los fermentadores de tanque agitado a escala de banco (30 L). Con las ecuaciones desarrolladas, los datos experimentales reportados en trabajos anteriores y utilizando el programa Matlab, se simuló el proceso de operación y los resultados fueron semejantes a los de la cinética del cultivo. Se analizaron las variables de operación que tienen una marcada influencia durante el proceso, utilizando el módulo del Matlab. Estos se compararon con un caso base, tomando como parámetros: la velocidad de agitación del *spinfilter* y el área de filtración normalmente empleados en producción. También se compararon con dos casos a dos niveles de áreas de filtración (menor y mayor que la del caso base) en los que se varió la velocidad de agitación para observar el comportamiento de la capacidad de flujo de perfusión durante la corrida. Se comprobó la influencia sobre el tiempo de vida de la fermentación (de la filtración), cuyo valor ascendió para la mayor área de filtración y la mayor velocidad de giro del *spinfilter*.

Palabras clave: spinfilter, colmatación, flujo de perfusión

*I*ntroduction

The culture of mammalian cells by perfusion in a stirred tank fitted with a spinfilter as a retention device is a methodology aimed at retaining the highest number of cells inside the fermentor, which therefore allows the obtaining of high cell densities and, consequently, high product concentrations in a relatively short period of time, with high volumetric flows in small-scale facilities [1]. The filtration time, in the case of the bioreactors of the Center for Molecular Immunology (Havana, Cuba) fitted with spinfilters for the perfusion culture of mammalian cells, is of 18 days. Filtration time, however, can be extended to 90 days according to literature [1-4]. Given that no mathematical models for this system have been published, it was decided to model its behavior as a tool to guide further efforts in extending culture time [5] or filtration or perfusion time by varying the spin rate of the spinfilter and its filtration area [2, 3].

*M*aterials and methods

Bioreactor

The fermentor used for the experimental runs (CMF 400, manufactured by Chemap AG) has a total volume of 41 L and an effective volume of 30 L, with a diameter of 0.27 m, a height of 0.7164 m and an effective height of 0.52 m. It has a propeller-type impellent with a diameter of 0.088 m [1, 3].

Spinfilters

The 41 L bioreactors used cylindrical stainless steel spinfilters (Chemap AG) with a diameter of 0.088 m

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and a height of 0.152 m [1, 3], fitted with a 15 µm pore size stainless steel mesh.

Cell line

The study used the NSO/H7 host cell line [1, 3].

Culture medium

The study used the PFHM II protein-free culture medium [1].

Results and discussion

Derivation of hydrodynamic equations

At the Center for Molecular Immunology, perfusion cultures are usually performed in a stirred tank, using a spinfilter as a separation device. A spinfilter is a rotatory cylinder spinning on its axis that allows the continuous separation of cultured cells from the culture media and, therefore, the obtaining of a clarified culture supernatant.

Modeling a perfusion culture in a stirred tank with a spinfilter presents a number of challenges, derived from the simultaneous operation of different phenomena such as centrifugal effects, axial and sweeping forces, among others [3, 6, 7]. However, the system can still be analyzed by decomposition into individual parts, modeling: 1) Its behavior as a rotatory filter; 2) Its behavior as a filtrating centrifuge and 3) Elements of mechanic energy balance in the interface of the outer and inner surface of the mesh (Figure 1).

By simultaneously using the equations corresponding to each individual part according to figure 1 and applying the filtration mechanism n = 3/2 [8, 9], the equation of Cozzeny-Karman [10] and the equation of Bernoulli [3, 8, 11, 12], the following is obtained:

$$Fp = q^*_0 \left[\frac{1}{1+\frac{q^*_0 Ks^* tp}{2}}\right]^2 \left[\left[\frac{\rho ss}{\rho^* ss}\right]\left[\frac{\varphi^*}{\varphi}\right]-1\right]$$

Fp: perfusion flow (L/h)
q_0*: constant (mL/s)
Ks: separation power of the spinfilter
tp: time of perfusion (s)
ρ_{SS}: Density of the suspension (kg/m^3)
φ: viscosity of the fluid (Pa-s)
The asterisk indicates the exit flow.

Therefore:

$$Fp = f(X(t), n, S, V, t...)$$

Where:
$X(t)$: cell density in relationship to elapsed time
n: spin rate of the spinfilter
S: filtration area
V: volume of the bioreactor (L)
t: time (h)

Biomass balance equations

Previous work on this subject [1] dismissed the influence of the exchange flow through the filter (F) in order to simplify the calculations; however, this variable has a large influence on the process of clarification and the operation time [13]. Therefore, it was decided to include this variable in the biomass balance corresponding to this system (Figures 2 and 3).

Modeling the E1 exponential growth phase

Modeling the exponential growth phase (E1) is simple, since it involves the same equations used for discontinuous cultures [1, 3, 14, 15]:

$$X(t) = X_0 e^{\mu MAX(t-t_0)}$$

Where:
X_0: starting biomass concentration in the bioreactor (cells/mL)
µMAX: maximum specific growth rate (h^{-1})

For edge conditions:

$$X_0 = 0.5 \times 10^6 \text{ cell/mL};\ X(t_1) = 10^6 \text{ cell/mL};\ t_0 = 24h \text{ and } t = t_1$$

Modeling the E2 exponential growth phase under continuous flow

Exponential growth can be modeled in this case with the following differential equation:

$$\mu_{MAX} XV = X_s FP + V\frac{dX}{dt}$$

Where:
Xs: biomass concentration in the spinfilter (cells/L)

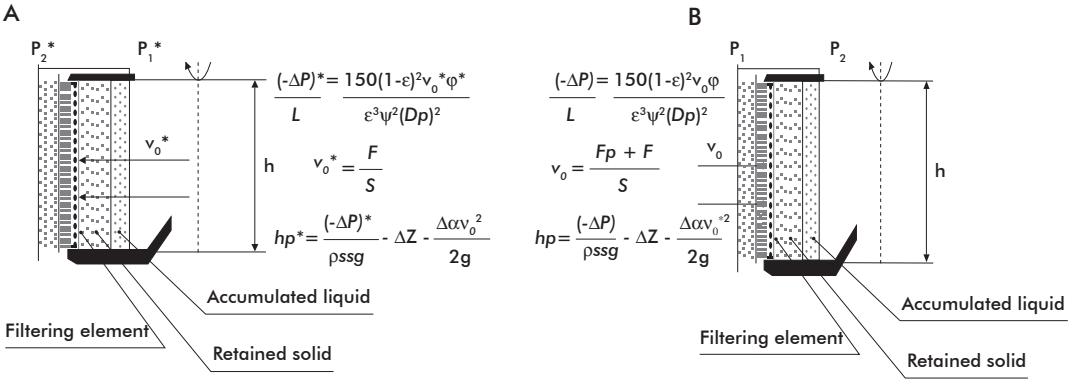

Figure 1. Longitudinal section of the spinfilter and direction of the flow speed at the surface of the mesh. A) Flow speed exiting the interior of the mesh. B) Flow speed entering the interior of the mesh. The asterisk indicates the exit flow. The equations describing the pressure gradient between both surfaces of the filter (-ΔP/L), the fictional fluid speed (v_0) and energy loss are shown. ΔZ, thickness of the filtering medium; -ΔP, pressure drop through the cake and the filtering medium (Pa); ε, porosity of the filtering medium; V, volume of the bioreactor (L); φ, viscosity of the fluid (Pa-s); Dp, particle diameter (m); F, cell exchange flow through the mesh (L/h); S, filtration area (m^2); ρss: density of the suspension (kg/m^3); g, gravitational acceleration (9.81 m/s^2); Δα: characteristic of the path followed by the fluid; hp, loss of energy through the mesh (m).

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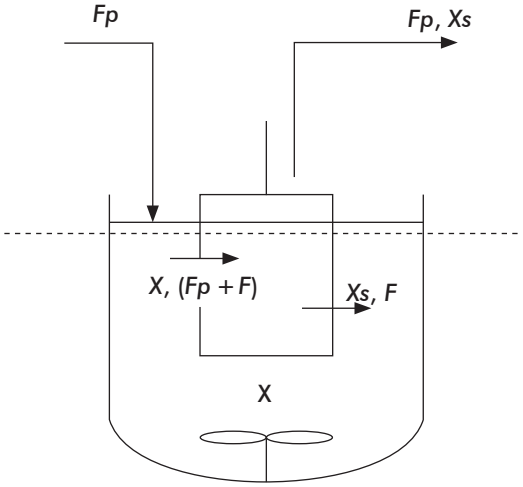

Figure 2. Flow distribution in the system.

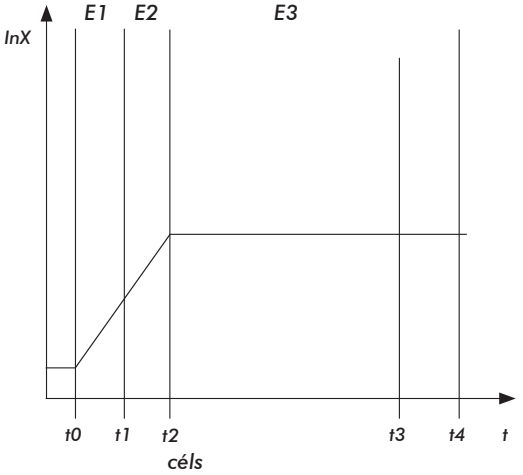

Figure 3. Culture kinetics and duration of each phase

Taking into account that feeding and extraction are described by the same function as that of perfusion flow capacity through the membrane, and assuming that the system reaches 90% retention instantaneously upon starting the perfusion, the differential equation can be arranged to depend on biomass concentration in the bioreactor [3]:

$$\left[C1 - \frac{C^*2(\beta\text{-}1)(C5+C6e^{C3X})^2 e^{C3X}}{(C4+C2e^{C3X})(C7+(C8+C9t)(e^{C3X}))^2}\right]X = \frac{dX}{dt}$$

Where:

$C1 = \mu_{MAX} = 0.003\ h^{-1}$

$C^*2 = 8.561 \times 10^{-4}\ (g/cell)$

$C3 = 0.02314\ (mL/10^6\ cells)$

$C4 = \rho$; ρ: density of the pure liquid (kg/m^3)

$C5 = 2\ \rho q^*_0 (1\text{-}H)$; H: humidity of the cake

$C6 = 0.0074 \left[(1\text{-}H)\left[1 - \frac{\rho}{\rho_p}\right] - 1\right]$; ρp: particle density (kg/m^3)

$C7 = 2\rho\ (1\text{-}H)\ \sqrt{q^*_0}$

$$C8 = 0.0074\left[(1\text{-}H)\left[1 - \frac{\rho}{\rho_p}\right] - 1\right]\sqrt{q^*_0}$$

$$C9 = 1.874 \times 10^{-4}\left[\frac{(1\text{-}H)}{pVu\ \eta Dn^2}\right]\left[\frac{\alpha\varphi^*}{S}\right]$$

Where: η: filling coefficient, D: spinfilter diameter (m), α: specific resistance of the apparent cake (m/kg).

$$C10 = \frac{C1}{C9}$$

$$C11 = \frac{C5\sqrt{C2}}{C9}$$

$$C12 = \frac{C6\sqrt{C2}}{C9}$$

$$C\ 2 = \frac{C^*2\ (\beta\ -\ 1)}{V}$$

$$\beta = e^{0.007}$$

solving:

$$X(t) = X_k e^{\left[C10(C8+C9t) + \frac{C12}{C2(C8+C9t)}\right]}$$

Where:

X_k: cell density at perfusion start (around 10^6 cell/mL)

And for the perfusion flow capacity:

$$Fp(X(t),t) = \left[\frac{C^*2(\beta\text{-}\ 1)(C5+C6e^{C3X})^2 e^{C3X}}{C4+C2e^{C3X})(C7+(C8+C9)(e^{C3X}))^2}\right]$$

For edge conditions:

$X(t_1) = 10^6\ cells/mL$, $X(t_2) = 9 \times 10^6$ cells/mL

Modeling the phase of limited growth under continuous flow (stationary phase), E3

During the third phase the stationary state (regarding biomass) is finally reached; that is, biomass remains constant. Therefore:

$$\mu XV = X_s Fp$$

$$\mu = 0.9Ds = 0.9\left[\frac{Fp}{V}\right]$$

And for the perfusion flow:

$$Fp(X(t),t) = \left[\frac{C^*2(\beta\text{-}\ 1)(C5+C6e^{C3X})^2 e^{C3X}}{C4+C2e^{C3X})(C7+(C8+C9)(e^{C3X}))^2}\right]$$

In this phase perfusion flow capacity depends only on time, and therefore:

$$Fp = Fp(t)$$

For edge conditions:

$$X(t_3) = X(t_2) = 9x10^6\ cells/mL$$

$$t = t_2,\ Fp = Fp\ (t_2),$$

$$t = t_3,\ Fp = Fp\ (t_3)$$

Limitations of the model

These models have some limitations, since upon starting perfusion 1) the spinfilter is assumed to reach instantaneously a retention of 90%; 2) extraction flow is described by the same function as perfusion capacity through the mesh of the filter; 3) The study was performed only for 3 discrete spin rates of the spinfilter and the same number of different filtration areas; 4) The small-scale study was performed with only one spinfilter mesh; 5) The influence of the spin rate of the impellent over perfusion flow capacity is

not taken into account; 6) The influence of the pressure inside the bioreactor on perfusion flow capacity is not taken into account; 7) The washing phase of the fermentor was not modeled and 8) The determination of limiting substrate was not performed, and neither $\mu = f(S, \mu_{MAX})$ nor O_2 balance nor product formation were adjusted.

Biomass simulation

The simulation of cell density with time in the three growth phases was performed with the real filtration area ($S = 4.2 \times 10^{-2}$ m^2) and the spin rate of the spinfilter (200 rpm), using time (from t = 0 to X(t) = 9×10^6 cells/mL) as input parameter [16, 17]:

Figure 4 shows the stages of exponential growth and exponential growth under constant flow, which for this model fall midrange between the actual run data (Figure 4C), revealing practically the same maximum specific growth rate. In other words, the curve of the model has the same slope as those of the actual runs, with the exception of run 3231TA-0207, which differs in this aspect due to a longer adaptation phase in comparison to the others. Something similar is observed for the stationary phase; although the actual run data follow a curve characterized by a fall that is not present in the model. While this difference is expected, given that the model did not take into account the washing phase that takes place upon complete clogging of the spinfilter, it still constitutes a weakness of the model.

Simulation of perfusion flow capacity

This simulation used as input variables the variation of cell density, the spin rate of the spinfilter, the area of the spinfilter and the operation time, setting the perfusion flow capacity of the system as output variable. Three discrete values for the filtration area were analyzed: that corresponds to the actual filter, and both larger or smaller values, varying on each case the spin rate of the spinfilter (100 rpm, 200 rpm and 300 rpm) (Figure 5) [18, 19].

A similar behavior was observed at the beginning (Figure 6A, B and C) if the fixed area is taken into account, since an increase of the spin rate of the rotatory filter results in an increase of both perfusion flow capacity and filtration time, with a non-linear dependence according to equation (4) [3]. The perfusion flow from the pump (Fp_B), ranging from 7.5 to 30 L/day, would be added to each Fp value corresponding to the spin rate of the spinfilter. Although literature mentions this behavior [2, 13, 20-22], this phenomenon is not described analytically, and therefore the results reveal a direct influence of the spin rate on perfusion flow capacity. The effect probably derives from an enlarged

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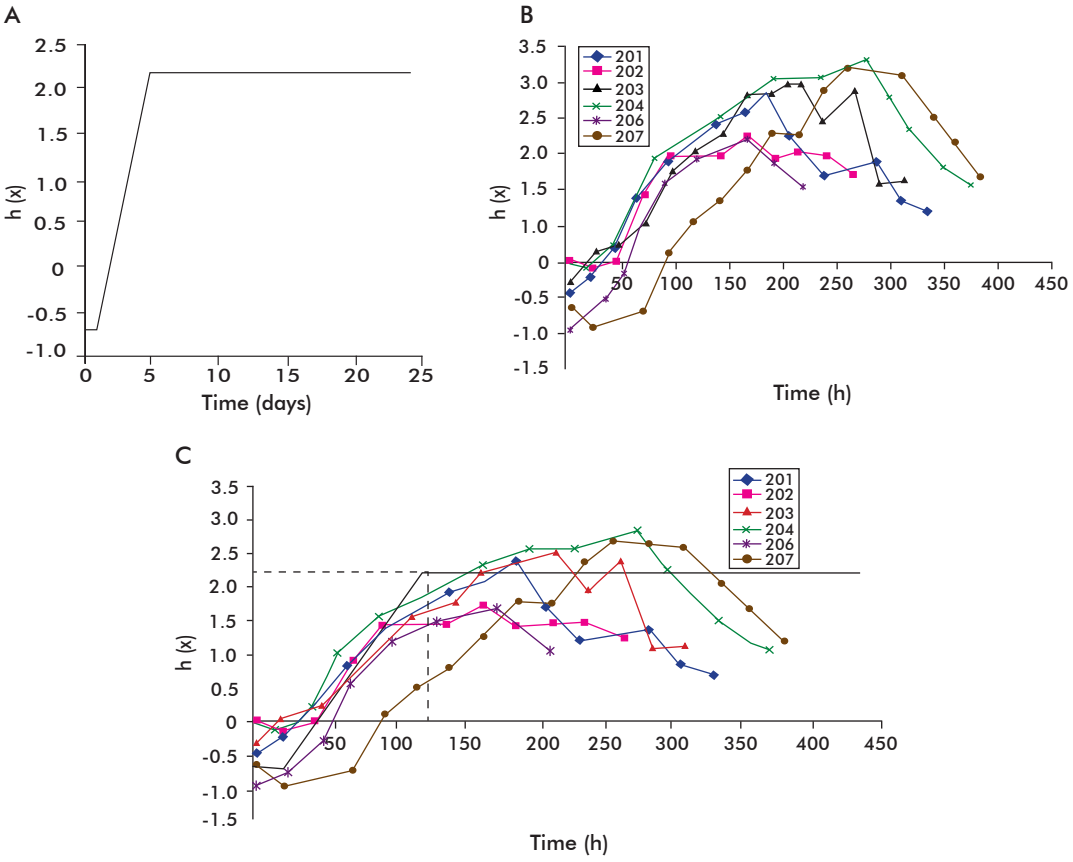

Figure 4. Description of growth kinetics A) Simulation of actual conditions B) Actual runs: 3231TA-0201, 3231TA-0202, 3231TA-0203, 3231TA-0204, 3231TA-0206 and 3231TA-0207. C) Superposition of the simulation with the actual data. The discontinuous line indicates the values of time and the natural logarithm of the cell concentration separating the exponential growth phase under constant flow from the stationary phase (t = 120 h, ln(x) = 2.25).

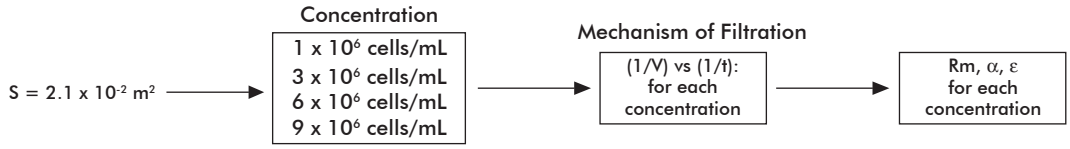

Figure 5. Logical direction of the simulation of perfusion flow capacity (Fp) and the filtration time of the spinfilter during culture, depending on cell density (X(t)), time (t), filtration area (S) and spin rate of the spinfilter (n).

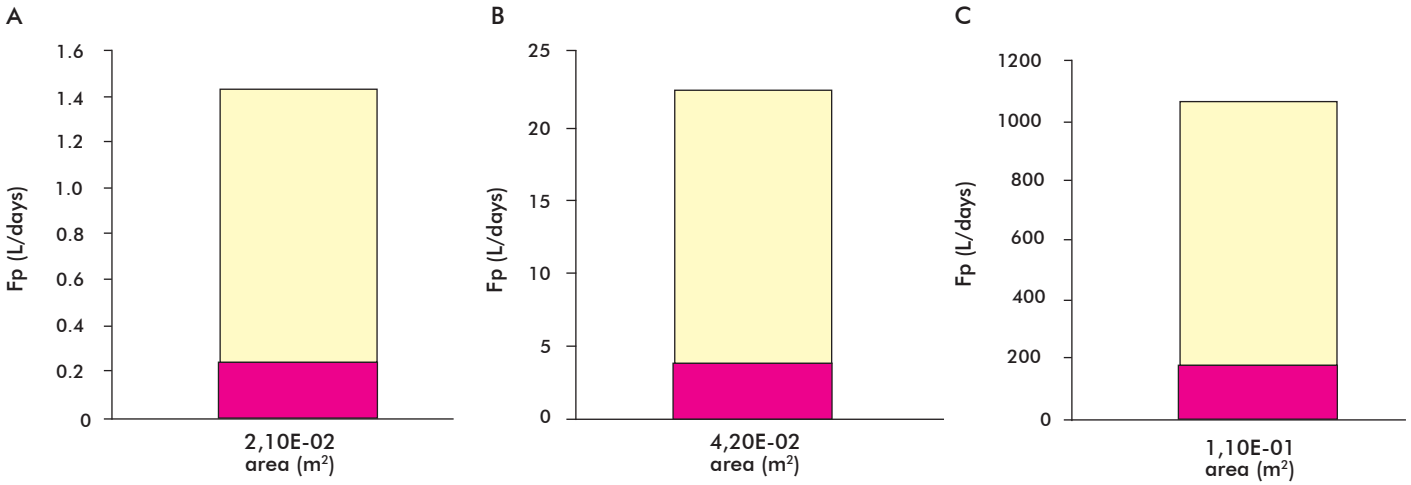

Figure 6. Dependence of perfusion flow capacity on the spin rate of the spinfilter. A) S= 2.1 x 10^{-2} m^2, B) S = 4.2 x 10^{-2} m^2, C) S =11.04 x 10^{-2} m^2.

zone of laminarity (Figure 7) due to the larger spin rate of the filter [8, 10, 13, 22], which allows a sweep of the cellular profile that approaches the membrane to occlude it, due to the drag of the exchange flow, that appears due to the centrifugal action of the filter itself on the cellular profile.

If the filtration area increases while the spin rate is kept constant, perfusion flow capacity increases almost exponentially (Figure 8). This effect is also described in literature [2, 13, 20-22] although it is not specified analytically, and constitutes another direct result of the influence of the spin rate of the spinfilter and the area on perfusion flow capacity. This must also be a result of the increase in the zone of laminarity (Figure 7) as the spin rate of the filter increases [8, 10, 13, 22] and of the filtration area, allowing a larger sweep of the cellular profile in spite of the drag due to the exchange flow, appearing due to the centrifugal action of the filter itself on the cellular profile.

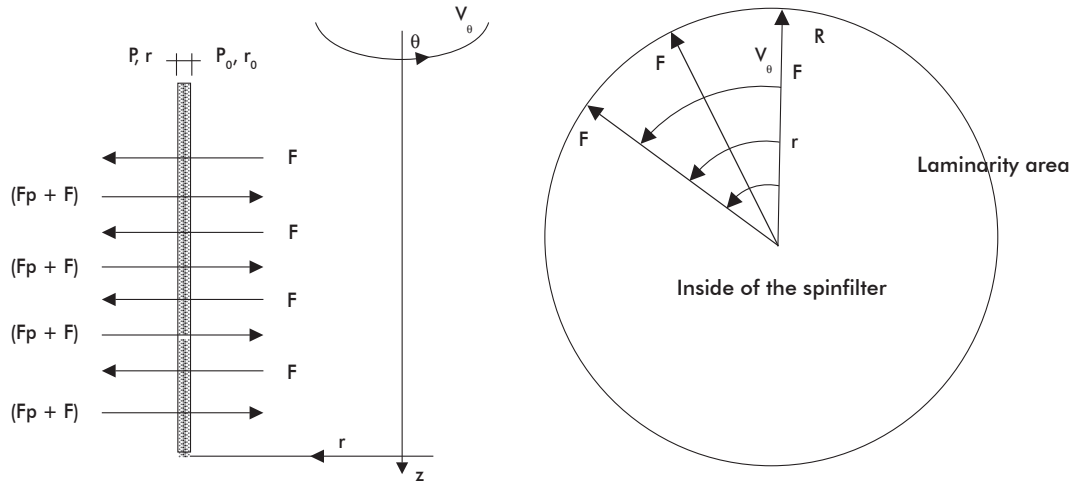

Figure 7. Resistance of the zone of laminarity to the exchange flow (F). A) Longitudinal section of the spinfilter. (Fp+F)- perfusion flow capacity; r, z- direction of the coordinate axes of the reference system; v_θ- spin rate of the spinfilter. B) Cross-section of the spinfilter. F- exchange flow through the membrane; R- radius of the spinfilter; P,P_0- pressure in both sides of the surface of the membrane; r_0, r- inner and outer radius of the spinfilter.

Conclusions

This work obtained mathematical models that predict the behavior of perfusion cultures of mammalian cells in stirred tanks for each of their growth stages, coupling kinetic and hydrodynamic equations [1-3, 9]. These novel results, which have no precedents in literature, allowed the analysis of the influence of the spin rate of the filter and the filtration area on the perfusion flow capacity of the system. In spite of the limitations of the models, it was possible to determine that an increase in spin rate at a constant filtration area results in an increase in the perfusion capacity of the system, which is even larger if the filtration area also increases, with a concomitant increase in filtration or perfusion times [2, 3, 13, 20].

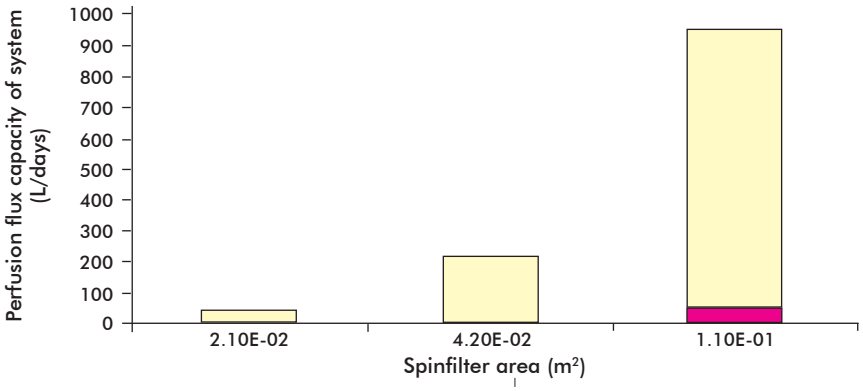

Figure 8. Dependency of perfusion flow capacity on the filtration area.

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