RESEARCH

Mechanism of clogging by exchange flow of a spinfilter as filtrating centrifuge

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ABSTRACT

Perfusion cultures usually reach high cell densities and, therefore, require a spinfilter for cellular retention. But spinfilters may clog prematurely so we decided to study the process by pumping a suspension culture through the filter, so that the cells are trapped inside and the clarified flow-through can be measured by collecting it in a measuring cylinder until partial or total obstruction of the filter, which in this case is used as a filtrating centrifuge. Filtrate volume with time was measured at several cellular concentrations and rotational speeds, calculating the behavior of specific resistance for the apparent cake, the resistance of the filtration medium, cake porosity and hydrodynamic regime. The results were used to adjust the filtration profile.

Keywords: clogging, exchange flow, spinfilter

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RESUMEN

Mecanismo de colmatación por el flujo de intercambio en el filtro rotatorio como centrífuga filtrante. El filtro rotatorio (*spinfilter*) se emplea para la retención celular en el cultivo en perfusión, con altas densidades celulares. Debido a su obstrucción precoz, se precisó el estudio de este sistema, introduciéndole el caldo de cultivo (suspensión) por el interior del filtro, para obtener la suspensión clarificada por el exterior de este, y que las células queden retenidas en su interior. El líquido claro se recogió en una probeta, hasta que el filtro se obstruyó parcial o totalmente. Por lo tanto, el filtro rotatorio se usó como una centrífuga filtrante, en la que se fue midiendo el volumen de filtrado con respecto al tiempo, para varias concentraciones de suspensión y de velocidad de giro del dispositivo. Se le ajustó el mecanismo de filtración que controla el proceso. Para el ajuste del perfil de filtración, se determinó la influencia de la concentración de la suspensión y de la velocidad de giro del filtro rotatorio, y se obtuvo el comportamiento de la resistencia específica (de la torta aparente), de la resistencia del medio filtrante, la porosidad de la torta y el régimen en que ocurre el proceso hidrodinámico de separación.

Palabras clave: colmatación, flujo de intercambio, filtro rotatorio

*I*ntroduction

The retention methods most widely applied at the industrial scale are those employed in suspension systems [1], since their scale-up bottlenecks and problems are easier and better known [2] than those of other alternatives [3]. Perfusion processes in general can be classified depending on the method used to retain the cells inside the reactor, ranging from the use of exclusion barriers [4, 5] to vesicles or beads of a porous material used to entrap the cells within a separate phase from that of the culture medium [6, 7], or the use of a device coupled to the fermentor, such as a spinfilter or an external rotary filter [8-14]. The latter method is the only one that can be potentially scaled-up to cultures of hundreds or thousands of liters to obtain dozens or hundreds of kilograms of the final product [3, 15]. In spite of their widespread applicability some operational aspects of spinfilters remain unstudied. For instance, the process of clogging of spinfilters has not been well examined, and the most important variables affecting it are not known. At the same time, neither the hydrodynamic regimen under which they operate (laminar *vs.* turbulent), nor the relationship between the hydrodynamic process and culture kinetics or the compressibility of the apparent cake on the surface of the membrane of the device, are sufficiently know. This may be determined through experimentation, calculating the specific resistance of the cake, the resistance and porosity of the filtration medium and measuring the drop in pressure. It is therefore important to determine the most probable mechanism of clogging operating during spinfilter occlusion, so as to better model this culture regime through research.

*M*aterials and methods

Materials

Filtration system

The filtration system is composed of a mechanical stirrer (Alka, Germany) and a spinfilter (B. Braum, Germany) protected by a glass case. The filter has a cylindrical shape (diameter of 8.5 cm and height of 26 cm) and is made of stainless steel, with a 10 µm pore size mesh of the same material. The spinfilter is coupled to the stirrer through a shaft at the upper part of the filter structure [6].

Volume measurement device

A 2 L measuring cylinder, used as the supporting base of the spinfilter, is employed to determine volume. The cylinder collects the cell suspension filtrate from

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inside the spinfilter after its passage through the cell cake and the filter mesh [6].

Cell line for the formation of the filtering cake

Here we used the NSO/H7 cell line [6].

Device for the inclusion of the suspension in the spinfilter

A peristaltic Watson Marlow pump (model 323) was used. This pump has a 3-roller head and a digital control system [6] (Figure 1).

Methods

Measurement of filtration rate

Filtration rate was measured with the filter operating at 3 different rotational speeds (100, 200 and 300 rpm) for cell suspensions at concentrations of 1 x 10^6, 3 x 10^6, 6 x 10^6 and 9 x 10^6 cells/mL [6] in order to examine the mechanism of clogging and calculate hydrodynamic variables such as the specific resistance of the cake, the resistance of the filtering medium and porosity (Figure 2).

Discussion

Experimentation

Several different events occur simultaneously during perfusion culture with a spinfilter [8]. Some authors [8] have examined whether the exchange flow through the membrane (F), in itself, has any influence on the clogging process of the spinfilter during fermentation. Therefore, our approach is not to study its influence, but to use it for modeling the clogging regime. Further research will address its effect on culture performance (Figure 3).

The experiment measured the clarified volume at fixed time intervals [16], graphically representing the values of 1/V against 1/t (obtained during the experiment). These two parameters showed a linear correlation in the fitted curve (Figure 4).

A general differential equation for the operation of centrifugal filtration was used [16-18] due to the exchange flow that characterizes centrifugal operation. This flow affects the operation and clogging of the membrane, for which reason its main component was further investigated.

$$\frac{d^2t}{dV^2} = K\left(\frac{dt}{dV}\right)^n \qquad (1)$$

Where:

t: time (s)

V: volume (mL)

K: constant (mL^2/s)

In equation 1 the order n = 3/2 was fixed to obtain a curve of $^1/_V$ (mL^{-1}) against $^1/_t$ (s^{-1}), representing the standard blocking mechanism, from which we obtained:

$$\left(\frac{1}{V}\right) = \frac{1}{q_0}\left(\frac{1}{t}\right) + \frac{K}{2\,(q_0)^{1/2}} \qquad (2)$$

Where:

q_o: constant (mL/s)

$\frac{1}{q_0}$: slope and $\frac{1}{q_0} = \frac{\mu Rm}{(-\Delta P)S}$

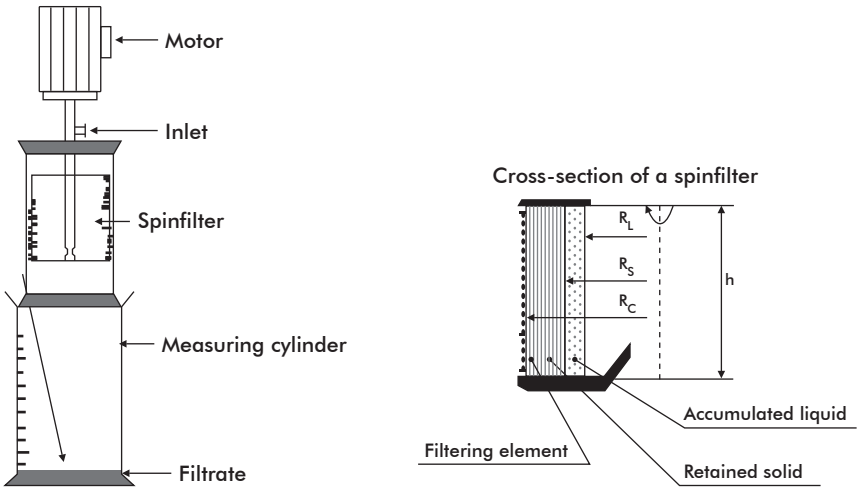

Figure 1. Measuring device. R_l: Radius from the spindle to the surface of the accumulated liquid layer, R_s: Radius from the spindle to the surface of the cake, R_c: Radius from the spindle to the surface of the filtering mesh.

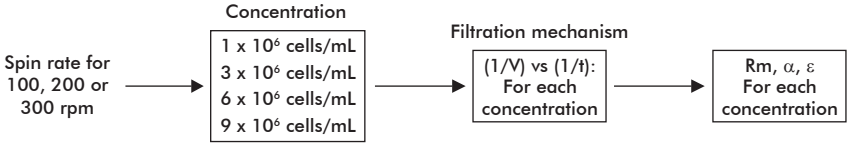

Figure 2. Experimental design for the determination of the filtration mechanism and the hydrodynamic variables.

$\frac{K}{2\,(q_0)^{1/2}}$: Intercept and $K = \frac{\mu C\alpha}{\Delta PS^2}$

being:

μ: viscosity (Pa-s)

Rm: resistance of the filtration medium (m^{-1})

C: ratio of solids in the cake / filtrated volume (kg/m^3)

α: specific resistance of the cake (m/kg)

$-\Delta P$: pressure differential (Pa)

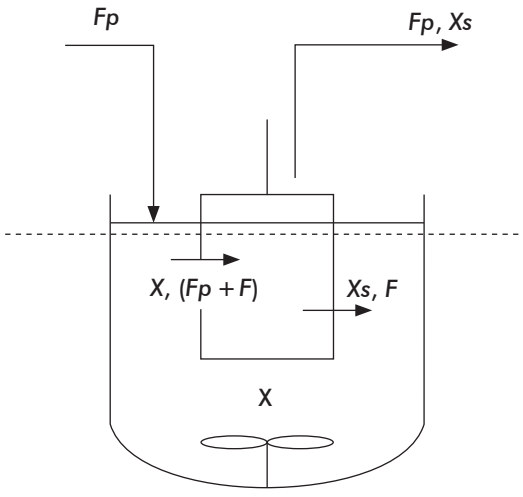

Figure 3. Flow distribution in a perfusion culture system. F: exchange flow (L/h); Fp: perfusion flow; Xs: biomass concentration in the spinfilter.

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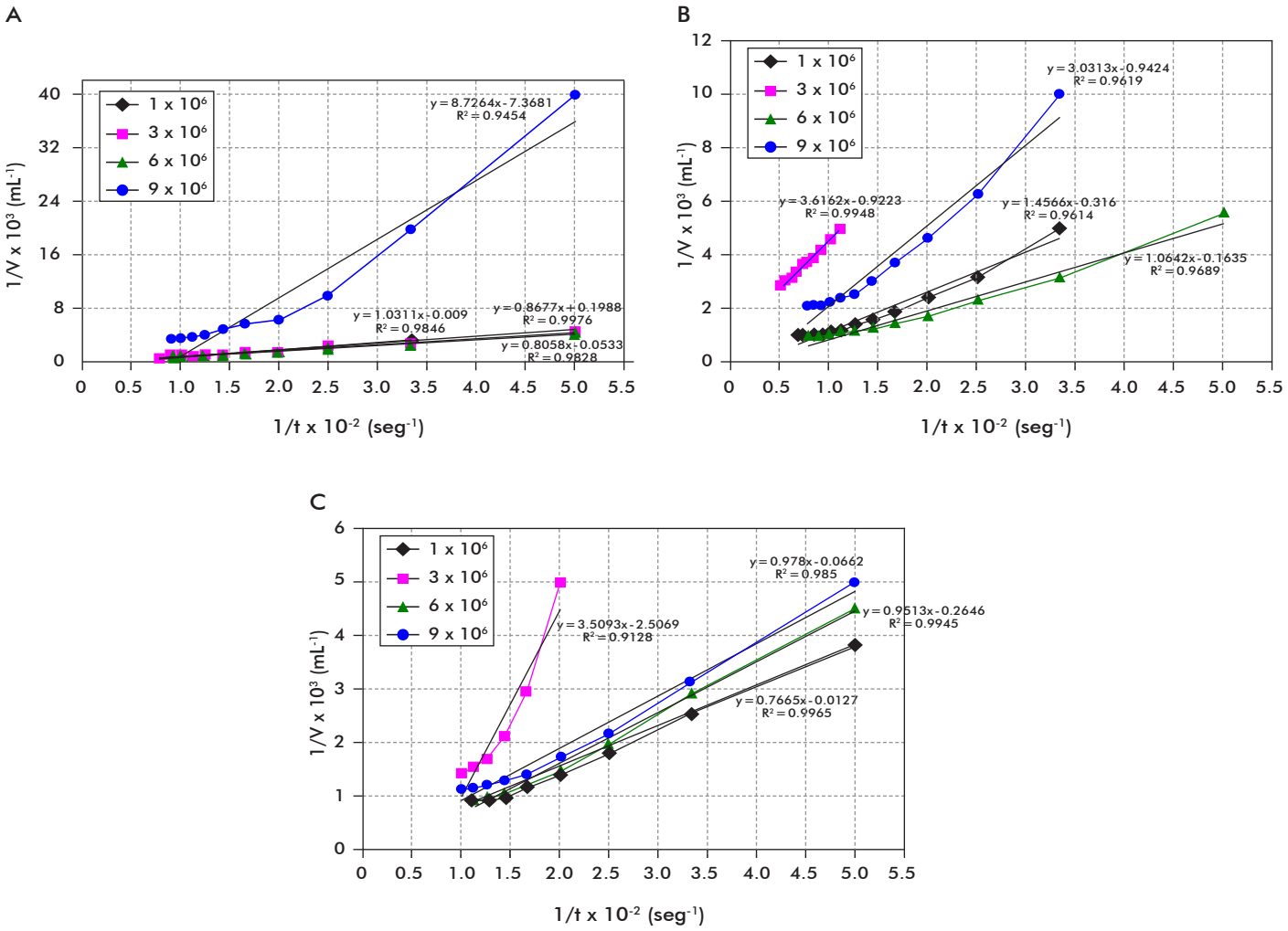

Figure 4. Fitting of the $^{1}/_{V}$ (mL^{-1}) against 1/t (s^{-1}) model of the spinfilter for spin rates of 100 rpm (A), 200 rpm (B) and 300 rpm (C).

S: filtration area (dm^2)

Therefore, from figure 4 and equation 2, we determined that the main mechanism is that of standard blocking [17, 18].

Since centrifugal force is the main component of the separation mechanism of a spinfilter, it was used to calculate the pressure drop (-ΔP) in equation 3 [16]:

$$(-\Delta P) = \frac{\rho\, Vu\, \eta\, Ks\, g}{S} \qquad (3)$$

Where:

ρ: density of the pure liquid (kg/m^3)
Vu: useful volume of the spinfilter (L)
η: filling coefficient
Ks: separation power of the spinfilter
g: gravitational constant (9.81 m/s^2)

The slopes and intercepts derived from the equation describing the trend line in the $^{1}/_{V} = f(^{1}/_{t})$ graphs from figure 4 were then used to calculate the hydrodynamic variables: resistance of the filtering medium (Rm), specific resistance of the cake (α) and porosity (ε), which can be used to characterize the regime under which the filtration process operates.

Behavior of the resistance of the filtering medium, the specific resistance of the cake and porosity

Once the values for the variables were obtained, the values of Rm = *f*(n), Lnα = *f*(n) and ε = *f*(n) were correlated, including the value of cellular concentration for each curve. In this case, n is the exponent. Figure 5 shows the behavior of the cake.

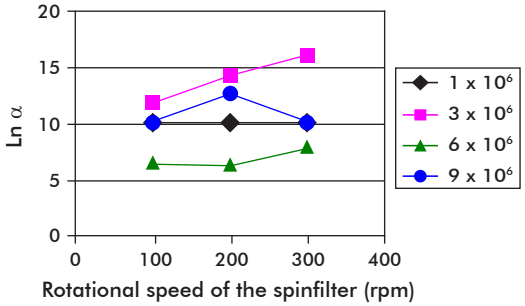

Figure 5. Variation of the specific resistance of the cake depending on the rotational speed of the spinfilter (rpm) and the cellular concentration.

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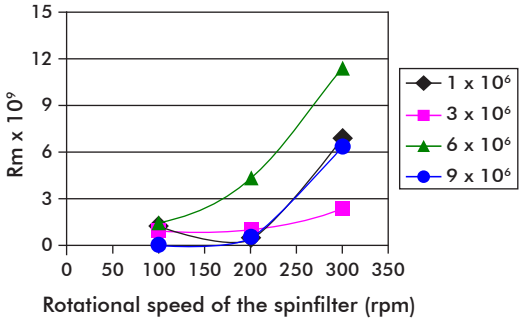

Figure 6. Variation in the resistance of the filtering medium depending on the rotational speed of the spinfilter (rpm).

The figure demonstrates that the specific resistance of the cake increases with the rotational speed of the filter, implying that it is directly proportional to the pressure of the system. Therefore, the (apparent) cake formed on the surface of the membrane is compressible, which had not been clearly determined before. This is not, however, the behavior observed when the cellular concentration is 1 x 10^6 cells/mL; this may arise from the existence of a local optimum whose determination is not among the goals of this study.

An analysis of the behavior of the filtering medium (Rm) also shows an increase as the rotational speed of the filter increases (Figure 6). This is due to the presence of a centrifugal force, which produces a laminar flow zone inside the filter (Figure 7). The width of this zone is directly related to the rotational speed, and it is a main factor contributing to the resistance of the filtering medium in the system.

Porosity, on the other hand, decreases with increasing rotational speed (Figure 8), due to the compaction of the cake with the increase in the pressure inside the system. A combination of these variables to find optimum points is possible, since porosity becomes larger with increases in spin rate and cell density. This behavior, however, is not observed at 1 x 10^6 cells/mL, which we attribute to the possible existence of a local optimum at this point (not pursued any further since it falls outside the scope of our investigation).
The variations in the flow regime as the fluid goes through the filter mesh were also studied, in relation to the development of the laminar flow regime (Table 1). The first ideas regarding this topic have not been clearly expressed in the literature [19, 20].

It is verified that the filtration mechanism for the spinfilter corresponds to n = 3/2 with:

$$\left(\frac{1}{V}\right) = \frac{1}{q_0}\left(\frac{1}{t}\right) + \frac{K}{2\,(q_0)^{1/2}} \qquad (2)$$

In terms of flow, we obtain [18]:

$$F = \frac{q_0}{\left[1 + \frac{q_0 K^* t}{2}\right]^2}, \text{ where } K^* = \frac{K}{2\left[q_0\right]^{1/2}} \qquad (4)$$

Therefore, the important effect of time on the exchange flow through the mesh (F) is observed, as evidenced by its rapid decrease in the course of time.

Equation 4 represents the model for the exchange flow through the mesh (F) in spinfilters. This effect

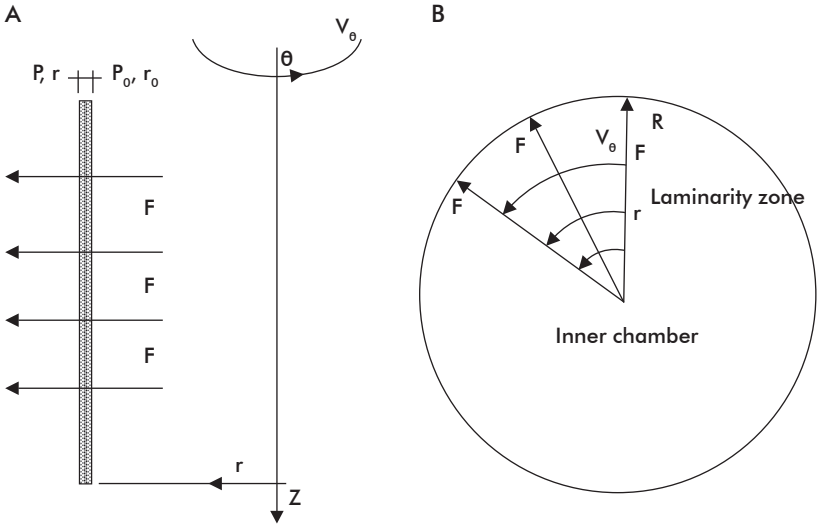

Figure 7. Resistance of the laminarity zone to the exchange flow (F). (A) Longitudinal section of the spinfilter. (B) Transversal section of the spinfilter.

together with the perfusion flow F_p (crossing the filter membrane in the opposite direction to F, Figure 3 [21]) will be analyzed in future papers.

Conclusions

It was determined that the mechanism that best fitted the operation of centrifugal filtration in a spinfilter is that of an order n = 3/2 differential equation at constant pressure drop (-ΔP); that is, pressure drop does not depend on the concentration of the suspension, but on rotational speed, as shown in equation (3) [12, 17] where -ΔP is directly proportional to the rotational speed and the density of the pure liquid. Analyzing the trend of the graphs plotting porosity against rotational speed where ε = *f*(n), it was observed that porosity tends to decrease with rotational speed, implying that the cake formed on the mesh of the spin filter is compressible. This agrees with the literature, where compressible cakes are defined as those whose porosity decreases with higher pressure drops [17], resulting in an increase in hydraulic pressure. Lastly, it was determined that during the clogging of the spin filter the process operates under a laminar flow regimen. Although the presence of such a regime in this case had been previously suspected, and is in fact predicted by the available literature [19, 21], no true data had been collected thus far to support this assumption.

Table 1. Variation in the Re number depending on rotational speed and concentration

Rotational speed	Xs (x 10^6cells/mL)	Re
100 rpm	1	8.38 x 10^{-8}
	3	1.91 x 10^{-5}
	6	2.25 x 10^{-8}
	9	4.53 x 10^{-9}
200 rpm	1	8.82 x 10^{-8}
	3	4.20 x 10^{-6}
	6	8.98 x 10^{-7}
	9	6.75 x 10^{-6}
300 rpm	1	1.27 x 10^{-7}
	3	2.46 x 10^{-5}
	6	1.62 x 10^{-6}
	9	1.85 x 10^{-3}

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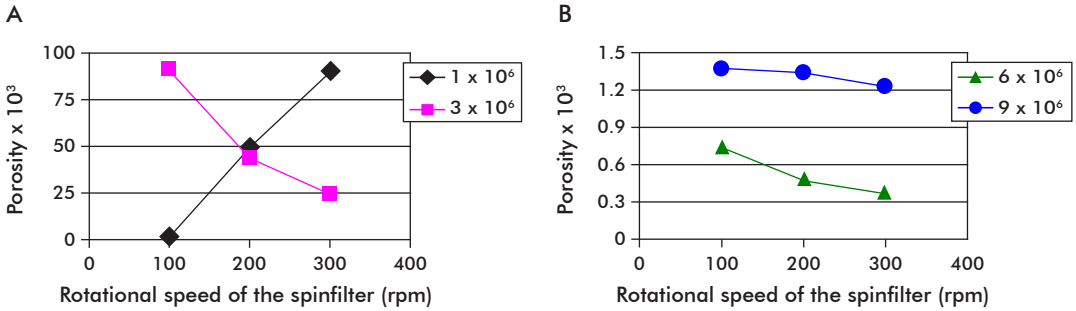

Figure 8. Variation in porosity depending on the rotational speed of the spinfilter (rpm), 1 x 10^6 y 3 x 10^6 cells/mL (A); y 6 x 10^6 and 9 x 10^6 cells/mL.

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