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Mathematical model for simultaneous microfiltration and ultrafiltration of *Haemophilus influenzae* type b to cell separation and polysaccharide recovery

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ABSTRACT

The cell separation from the culture broth through tangential microfiltration to isolate poly-ribosyl-ribitol-phosphate polysaccharide (PRP), which is used in the vaccine of *Haemophilus influenzae* type b (Hib), represents an alternative to centrifugation. An inconvenient of microfiltration is the great amount of buffer used to diafilter the culture broth, which may dilute the product. The PRP is unstable due to various factors including concentration. To overcome this problem, an ultrafiltration system functioning simultaneously and in line with the microfiltration system concentrates the product, avoiding losses. Besides, the UF permeate stream can be recycled and used as diafiltration buffer in the microfiltration feed tank, reducing the use of buffer. Since two steps are merged into one, the time required for the global process would reduce. This study presents a mathematical model based on mass balance for a MF–UF simultaneous system for Hib cell separation, with the objective of optimizing its conditions. This model showed fair proximity to the obtained experimental data. Differences obtained in the recycle and no recycle systems indicate that the best option should be evaluated according to product characteristics. For PRP recovery, a recycle system is the most appropriate, however this model can be extended to any target molecule in such a system.

1. Introduction

Tangential flow filtration technology (TFF) is an important tool ubiquitous in industries such as water treatment, food and beverage. In addition, membrane technology has played an important role in biomedicine such as: hemodialysis, to treat chronic kidney failure or, plasmapheresis for plasma collection or therapeutic removal of pathogens of the blood [1–4].

On the subject of pharmaceutical application of TFF, Tanizaki et al. established a purification process of bacterial polysaccharide produced by *Neisseria meningitidis* serotype C in which a tangential ultrafiltration was introduced in the final step of purification in order to remove residues from enzymatic treatment (nuclease and proteases) [5]. The same idea was adopted by Takagi et al. and Albani et al. to purify polysaccharide produced by *Haemophilus influenzae* type b (Hib), using tangential ultrafiltration in the first step of purification to remove molecules with less than 100 kDa from the culture medium and metabolites produced by the microorganism [6,7], and it was also used in the last step of purification as proceeded by Tanizaki et al. Then, Albani et al. developed a scalable purification process of polysaccharide basically based on tangential filtration where a microfiltration membrane was used in order to separate insoluble material from the ethanol precipitation steps [8].

The capsular polysaccharide of Hib, which is released into the supernatant, is composed by units of ribosyl-ribitol, linked by a phosphodiester group and known as poly-ribosyl-ribitol-phosphate (PRP). This polysaccharide is used in the vaccine formulation for *H. influenzae* type b to prevent meningitis [9]. The chemical structure of PRP contributes for its natural instability where the hydroxyl group at the carbon 2 of ribose is located close to the phosphate group, which in alkaline milieu undergoes transesterification and consequent depolymerization [10,11].

Currently, PRP is isolated from *H. influenzae* type b cells by using centrifugation due to the low recovery found using tangential microfiltration; probably a consequence of the viscosity caused by the polysaccharide and the nature of the cake formed. Nevertheless, an alternative to centrifugation is desirable once this operation consumes much energy. On the other hand, besides the previous problems indicated for tangential microfiltration, the high amount of buffer used to diafilter the culture dilutes the material and renders PRP in a low concentration, which may contribute for its autohydrolysis. The stability of this molecule is dependent on a fine control of pH, temperature, and ionic strength; which affect its recovery [11]. A simultaneous UF tangential filtration system would be appropriate

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for PRP recovery and to avoid autohydrolysis, since it concentrates the product.

A similar technique was used by Cheang and Zydney for fractionation of whey protein to isolate β-lactoglobulin and α-lactalbumin through a two-step ultrafiltration process, which reached an yield as high as 95% for the α-lactalbumin [12].

In order to make the cell separation possible by using tangential microfiltration and also to improve the recovery of polysaccharide in the first step of the downstream process, this study intends to evaluate two MF–UF simultaneous systems for Hib cell separation based on a product mass balance simulation, allowing the determination of the process optimal conditions.

2. Theory

Filtration processes are often modeled by the rejection of a specific solute in regard to a specific membrane at set conditions. This relationship can be defined as follows [13]:

$$r = 1 - \frac{C_{permeate}}{C_1} \tag{1}$$

If we have the concentration of the target molecule on the permeate flow crossing the membrane and directed to the permeate stream Q, the mass balance obtained would be

$$\frac{dM}{dt} = -QC_{permeate} \tag{2}$$

$$\frac{dM}{dt} = -QC_1(1-r) = \frac{dC_1}{dt}V_1 + \frac{dV_1}{dt}C_1 \tag{3}$$

To calculate the rejection coefficient, the experimental data of a single step at constant volume can be integrated and plotted according to

$$\int \frac{dC_1}{C_1} = -\frac{Q}{V_1}(1-r)\int dt \tag{4}$$

$$\ln(C_1) = -\frac{Q}{V_1}(1-r)t + \ln(C_1|_{t=0}) \tag{5}$$

Thus, $-(Q/V_1)(1-r)$ would be the slope of the curve of $\ln(C)$ against time.

First a system with no recycle of UF permeate is considered (Fig. 1). Applying a mass balance to $V1$, we obtain

$$\frac{dM_1}{dt} = -Q_1C_1(1-r_1) \tag{6}$$

$$\frac{dC_1}{dt}V_1 + \frac{dV_1}{dt}C_1 = -Q_1C_1(1-r_1) \tag{7}$$

As $dV_1/dt = Q_d - Q_1$

$$\frac{dC_1}{dt} = \frac{Q_1C_1r_1 - Q_dC_1}{V_1} \tag{8}$$

Now considering $V3$

$$\frac{dM_3}{dt} = +Q_2C_2(1-r_2) \tag{9}$$

$$\frac{dC_3}{dt} = \frac{Q_2C_2(1-r_2) - \frac{dV_3}{dt}C_3}{V_3} \tag{10}$$

Given the fact that $dV_3/dt = Q_2$, then

$$\frac{dC_3}{dt} = \frac{Q_2[C_2(1-r_2) - C_3]}{V_3} \tag{11}$$

Finally, for $V2$

$$\frac{dM_2}{dt} = Q_1C_1(1-r_1) - Q_2C_2(1-r_2) \tag{12}$$

$$\frac{dC_2}{dt}V_2 + \frac{dV_2}{dt}C_2 = Q_1C_1(1-r_1) - Q_2C_2(1-r_2) \tag{13}$$

If $dV_2/dt = Q_1 - Q_2$,

$$\frac{dC_2}{dt} = \frac{Q_1C_1(1-r_1) - [Q_2(2-r_2) - Q_1]C_2}{V_2} \tag{14}$$

When $Q_1 = Q_2 = Q_d$, the system is in equilibrium; thus $dV_1/dt = 0$ and $dV_2/dt = 0$.

$$\frac{dC_1}{dt} = \frac{-Q_1C_1(1-r_1)}{V_1} \tag{15}$$

$$\frac{dC_2}{dt} = \frac{Q_1C_1(1-r_1) - Q_2C_2(1-r_2)}{V2} \tag{16}$$

However, in other situations – e.g. concentration of $V1$ – is not possible to assume $dV_1/dt = 0$. Moreover, due to the fact that this is a dynamic equilibrium, it is difficult to maintain $Q_1 = Q_2$, therefore V_2 is likely to undergo variations.

Starting the mass balance from $V1$ for the system with recycled UF permeated stream (Fig. 2)

$$\frac{dM_1}{dt} = -Q_1C_1(1-r_1) + Q_2C_2(1-r_2) \tag{17}$$

$$\frac{dC_1}{dt}V_1 + \frac{dV_1}{dt}C_1 = -Q_1C_1(1-r_1) + Q_2C_2(1-r_2) \tag{18}$$

As $dV_1/dt = Q_2 - Q_1$

$$\frac{dC_1}{dt} = \frac{Q_1C_1r_1 + Q_2[C_2(1-r_2) - C_1]}{V_1} \tag{19}$$

Secondly, we study the behavior in $V2$

$$\frac{dM_2}{dt} = Q_1C_1(1-r_1) - Q_2C_2(1-r_2) \tag{20}$$

Due to the fact that $dV_2/dt = Q_1 - Q_2$

$$\frac{dC_2}{dt} = \frac{Q_1[C_1(1-r_1) - C_2] + Q_2C_2r_2}{V_1} \tag{21}$$

If we consider r, Q and V to be constant, we obtain a system of differential equations

$$\frac{dC_1}{dt} = \frac{-Q_1C_1(1-r_1) + Q_2C_2(1-r_2)}{V1} \tag{22}$$

$$\frac{dC_2}{dt} = \frac{Q_1C_1(1-r_1) - Q_2C_2(1-r_2)}{V2} \tag{23}$$

where if Q_1 and Q_2 are constant and equal, and the rejection coefficients are considered constant, it can be integrated as a system of first order linear equation [14].

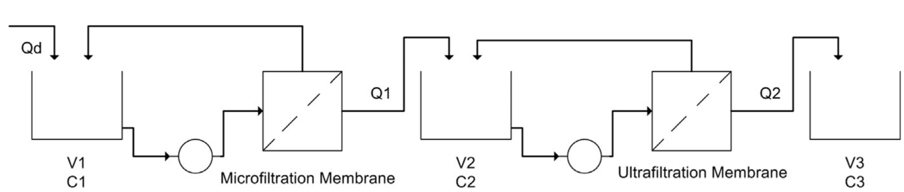

Fig. 1. Scheme of mass balance for a microfiltration and ultrafiltration simultaneous system with no recycle of the UF permeate stream.