Filtration characteristics of hollow fiber microfiltration membranes used in a specific double membrane bioreactor

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Keywords:
Membrane
Fouling
Microfiltration
Hollow fiber
Backpulse

A B S T R A C T

The performance of the microfiltration in a specifically designed membrane bioreactor operating under various transmembrane pressures with periodic backwashing was investigated for model media. These media were representative of some usual components of a fermentation medium: BSA solution ($2\,g\,L^{-1}$), yeast suspension ($8\,g\,L^{-1}$, dry mass) and a mixture of BSA/yeast ($2\,g\,L^{-1}/8\,g\,L^{-1}$). In this system, the separation was provided by a 0.1 μm polysulfone hollow fiber membrane. The net permeate fluxes observed for yeast/BSA mixture were proportional to the transmembrane pressure applied (ΔP) but were less than those obtained with water osmosis, showing that, in spite of the periodic backwash, a small amount of irreversible fouling remained. This fouling can be assumed to be due to internal fouling by protein and/or external fouling by a residual yeast cake. Moreover, the net permeate flux obtained with the yeast/BSA mixture was higher than that obtained with the BSA alone, showing that a thin yeast cake probably acted as a primary filtration layer that could protect the polysulfone membrane against protein fouling. These experiments enable operating recommendations to be made for the use of this specific bioreactor concerning the transmembrane pressure value and the possible addition of inert particles.

1. Introduction

Most microbiological processes use microbial consortia, especially in agro-food processes [1,2] and biological wastewater treatment [3,4]. The performance of these processes depends strongly on phenomena of interaction among the microorganisms and numerous works have tried to analyze the complex mechanisms of interactions among the microbial species involved [5,6]. However, the routine procedures used for tracking individual strains are generally time consuming and costly. In many cases, these procedures involve the isolation of the microorganisms and their growth in Petri dishes (for example) with a subsequent macroscopic identification and count of the microbial colonies, or fastidious microscopic observation. Frequently this kind of identification is incomplete and has to be followed by a second procedure.

The biomass concentration of each participating species in microbial consortia is the key parameter for the quantitative study of the interactions. Numerous works have shown the possibility of carrying out studies of mixed-population interactions. The basic idea comes from New Brunswick Scientific (Edison, NJ) with a system called EcoloGen [7], in which different strains or species are cultivated in separate chambers. Each culture chamber communicates with a central chamber through a flat membrane. Solutes, to which the membranes are permeable, diffuse between the central and the culture chambers and so all the cultivated species are brought into contact with all the dissolved substances. Mass transfer by diffusion resulting in a slow homogenization of the fluids between the chambers appears to be a major limitation to this approach. Petchanker and Ercoli [8] discussed the possibility of building a membrane reactor where the exchanges between the culture chambers were achieved by diffusion and convection (filtration) of the medium. However, their approach was theoretical and they only presented a mathematical model and a computer simulation of a microbial interaction.

This article presents a specific bioreactor previously designed to study microbial interactions [9–11]. In this process, the microbial species in two tanks are physically separated by a microfiltration membrane.

In order to give to the microorganisms a molecular environment in each compartment similar to the one that would be obtained if the microbial cells were cultivated in the same reactor, two criteria have to be considered: (i) the flow rates between compartments have to be sufficient with respect to the microbial kinetics and (ii) all the molecular compounds of the medium that have an effect on the microorganism behavior must pass through the membrane [9,10]. Three papers have shown the suitability of the double membrane bioreactor in a case of a protein-mediated killer interaction [10], even confronted with an existing mathematical model

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describing the population dynamics in the mixed killer/sensitive cultures [12,18] but no systematic study has been done on fouling and fouling effects on transmission.

Microfiltration membranes are widely used for carrying out solid–liquid size separation because of their technical advantages such as gentle conditions, no phase change, and therefore low energy expenditure, absence of additives, and compact design. However, due to membrane fouling, their potential has not been fully realized. One way to minimize fouling is to use an in situ cleaning technique such as backpulsing. Backpulsing involves a high-frequency reversal of the permeate flow by applying pressure on the permeate side. During the backpulse, a portion of the external cake may be lifted off the membrane and swept away by the inverse flow. Internal foulants may also be partially or completely removed. Backpulsing has shown considerable promise for a variety of microbiological applications. Kuberkar and Davis [13] show a total permeate improvement between 20 and 90% with backpulsing for a mix of BSA and yeast (*Saccharomyces cerevisiae*). Redkar and Davis [14] studied the backpulsing process for microfiltration of washed yeast suspensions. Under optimum conditions, flux enhancement of approximately 40-fold was observed.

One of the main technological advantages of this double membrane bioreactor is that the rapid flow inversion keeps a similar environment in the two reactors. The flow inversion creates an autocleaning process (backpulse). However, it can be assumed that the reversibility of the cake fouling on the membrane depends not only on the operating conditions (volume exchanged and compression level) but also on the specific properties of the culture medium.

Backpulsing has been applied to numerous multicomponent systems, such as fermentation broth [15,16], but no systematic studies have been done where the main usual components of the microbial culture media have also been individually studied under similar processing conditions.

Leading on the knowledge about conventional membrane processes, the aim of this work is to examine, in this very specific reactor, the membrane fouling and protein transmission during microfiltration of protein and protein-cell mixture with a high frequency of backpulsing, in order to develop a rational optimization of the operating conditions of this specific process.

2. Theorical considerations

2.1. Device concept

A scheme of the bioreactor concept is shown in Fig. 1. It is composed of two fermentors interconnected by a hollow fiber membrane module, which is immersed in the liquid of one of the fermentors. Liquid flow and mixing are induced by applying gas compression alternately to the headspace of each of the reactors. Compression is obtained by the admission of compressed filter-sterilized air through electro-valves controlled by the measurement of liquid levels in the reactors (conductivity probes). The resulting filtration swing allows the same mean liquid volume to be maintained in both tanks. An extra benefit of this swing is the limitation of fouling thanks to the resulting backpulsing.

In the system configuration used here, a solenoid valve allowed a temporization of the opening of the valves based on the signals of the liquid level sensors. The resulting parts of the cycle are labeled in Fig. 2.

The permeate flow and air supply lines were switched by electro-valves EV1 and EV2 which were controlled by the level probe in each tank. Pulsing was carried out at a frequency depending on membrane fouling but with a fixed filtration volume. Each step of the cycle (filtration and backwash) is described as follow: In the reactor A, $EV2_a$ was opened and $EV1_a$ was closed for 4 s before

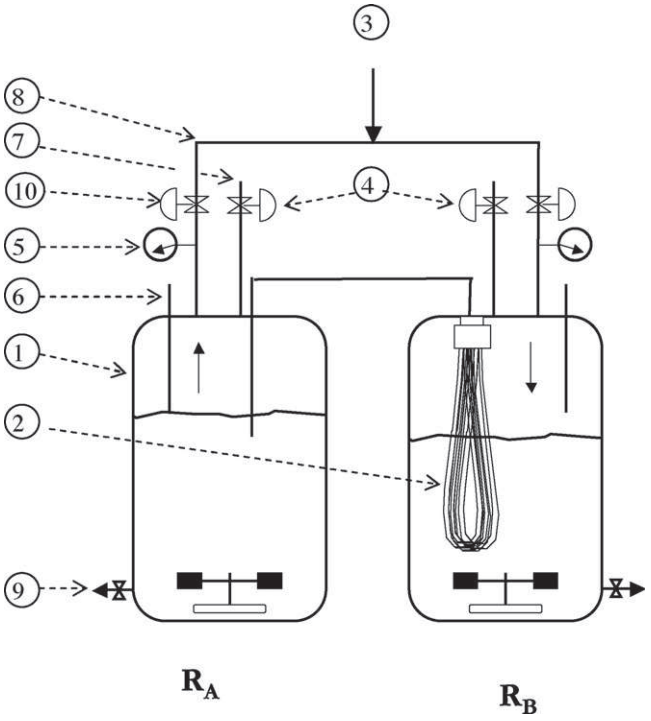

Fig. 1. Schematic design of the double-membrane bioreactor: (1) biological reactor, (2) membrane, (3) compressed air, (4) electro-valve EV1, (5) pressure gauge, (6) level gauge, (7) gas outlet, (8) air inlet, (9) sampling outlet, and (10) electro-valve EV2.

the application of the pressure. In parallel $EV2_b$ and $EV1_b$ of the reactor B are closed. This step named temporization ensures that pressure applied will be at the desired value and not in a transient state (Fig. 2b). Secondly, $EV1_b$ was opened and the suspension is filtered through the microfiltration membrane to reach the level imposed by the level probe in the reactor B. When the volume of filtration fixed by the level probe was reached, $EV1_b$ $EV1_a$ and $EV2_b$ were closed and $EV2_b$ was opened (temporization step). Finally $EV1_a$ was opened to begin the backwash step.

2.2. Filtration

- Forward step

A two-step process is considered here, comprising an inner to outer filtration stage followed by an outer to inner backward filtration step, where the filtrate is used to backwash through the membrane to remove the deposit. During the filtration phase the filtrate flow rate is given by conventional filtration theory based on Darcy's law. The whole flow resistance can be assumed to be composed of two parts: the fouling filtration resistance R_f and the resistance of the membrane such that R_m. The filtration flow rate is then given by

$$J = \frac{\Delta P}{\mu(R_m + R_f)} \tag{2.1}$$

The fouling filtration resistance R_f can be expressed as

$$R_f = R_r + R_{ir} \tag{2.2}$$

where R_r is the resistance caused by reversible fouling and the concentration polarization and R_{ir} is the resistance caused by irreversible fouling.

- Backwash step

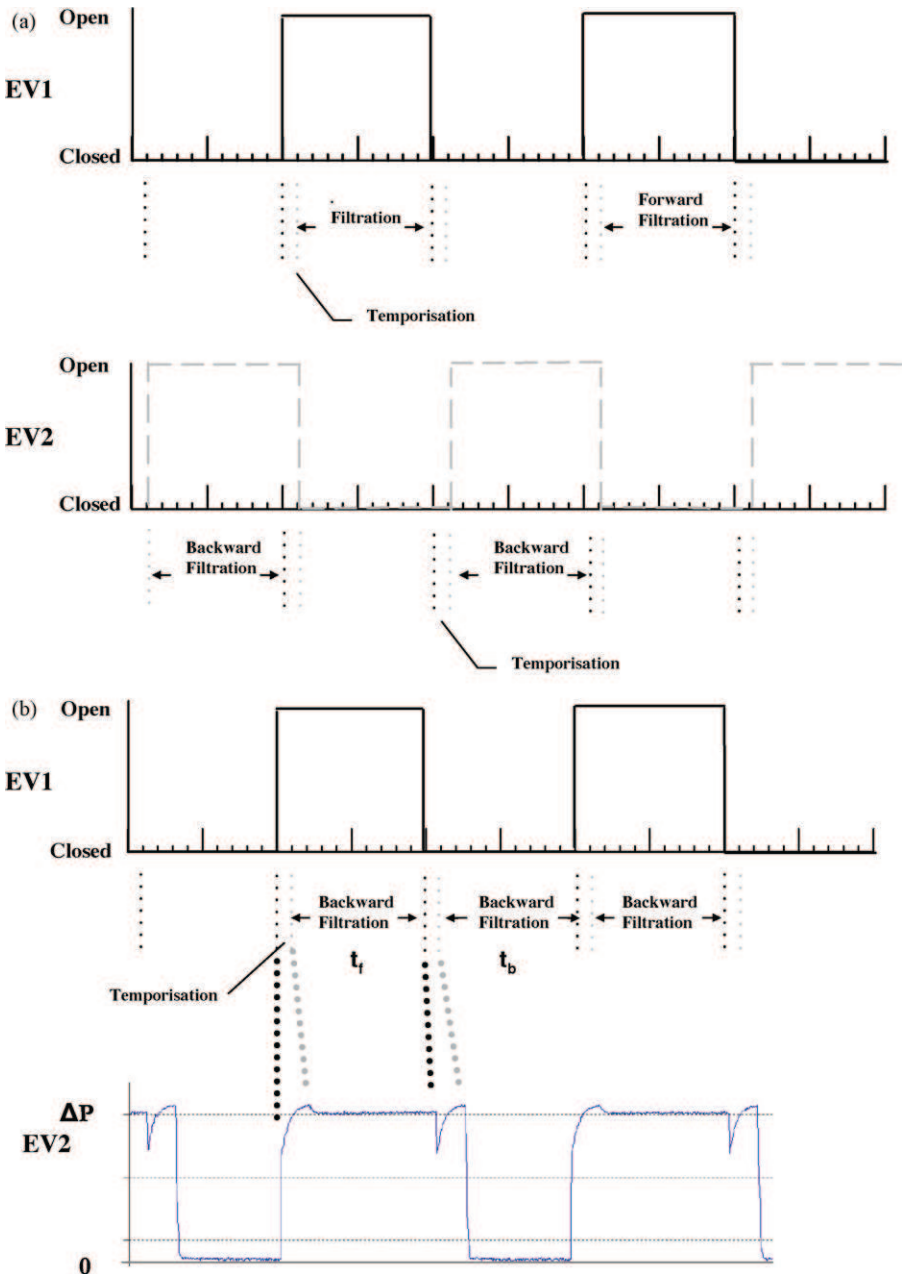

Fig. 2. (a) Control signal sent to valves (EV1 and EV2) to produce the forward and backward filtration of the cycle. (b) Control signal sent to valve EV1 to produce the forward filtration of the cycle and associated pressure acquisition curve in reactor A.

The time required for the backward filtration depends on the volume of filtrate selected (fixed) and the backwash flow rate

$$t_b = \frac{V_b}{Q_b} = \frac{V_b}{J_b} S \tag{2.3}$$

where Q_b is determined by the pressure applied during the backwash step ΔP_b and the resistance of the membrane:

$$Q_b = \frac{\Delta P_b}{\mu R_m} S \tag{2.4}$$

The pressure used for the backwash can be defined in terms of the pressure used for filtration:

$$\Delta P_b = \alpha \Delta P \tag{2.5}$$

In the case of the present work $\alpha = 1$.

For the overall process involving the two steps of forward and backward filtration, the total amount of filtrate produced is $V - V_b$ and the time to produce this amount is $t + t_b$, where V is the amount of filtrate produced in time t devoted to the forward filtration step and V_b is the amount of filtrate used in backwash in the time t_b. Then the productivity of the membrane system using a periodic backwash to remove the reversible component of the foulants layer [15,17,14,25] is given by

$$Q_{net} = \frac{V - V_b}{t + t_b} \tag{2.6}$$

$$Q_{net}\ (\mathrm{m^3\,s^{-1}}) = \frac{Q_f t - Q_b t_b}{t + t_b} \tag{2.7}$$

It should be pointed out that these last two equations could not be used in the atypical configuration described in this work and represents general case configurations when $V \neq V_b$.

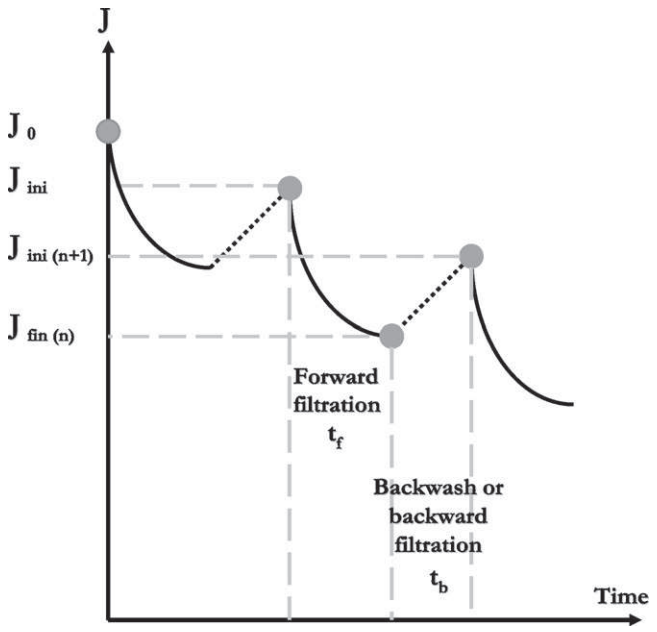

Fig. 3. Schematic representation of flux decline for each step of filtration with corresponding filtration time.

Nevertheless, the net flux is expressed as

$$J_{net}\ (\mathrm{L\,h^{-1}\,m^{-2}}) = \frac{\left(\int_0^{tf} J_f(t)\,\mathrm{d}t - \int_{tf}^{tf+tb} J_b(t)\,\mathrm{d}t\right)}{t_f + t_b} \quad (2.8)$$

where J_{net} is the net permeate flux of the microfiltration membrane operating with periodic backpulse corresponding to the average permeate flux per cycle; J_f is the permeate forward flux ($\mathrm{L\,h^{-1}\,m^{-2}}$), J_b is the backpulse flow rate ($\mathrm{L\,h^{-1}\,m^{-2}}$), t_f and t_b are the duration of respectively the forward filtration and backward filtration time (h) (Fig. 3).

3. Materials and methods

3.1. Materials

3.1.1. Yeast and BSA solutions

Yeast suspensions and BSA solutions were prepared by adding washed yeast (*S. cerevisiae*, commercially available Lesaffre's active dry yeast) and BSA (Sigma, heat shocked fractionate, fraction V powder, batch #066K0708) in appropriate concentrations to buffer. Phosphate buffer saline (PBS) buffer was used as the suspending solution. The buffer solution (pH 7.1) was prepared fresh for each experiment by mixing 80 g of NaCl (Panreac, batch #0000080666), 2 g KCl (Prolabo, batch #M065), 14.4 g of Na_2HPO_4 (VWR, batch #06J160009), and 2.4 g of KH_2PO_4 (VWR, batch #0503464) in 10 L of osmosis water.

Before use, the yeast suspension was hydrated in PBS and washed by centrifugation (Beckman, model Avanti 20 g) for 10 min at 5000 rpm. The supernatant was then discarded and the pellet was suspended in PBS. The washing procedure was repeated three times for each suspension.

Protein solution was prepared by dissolving 4 g of BSA powder (heat shocked fractionate fraction V) in 2 L of osmosis water. As this powder is produced by a process that involves heating and spray drying, some of the protein content is denatured and the solution can present some aggregates.

In order to check the homogeneity of the fluid models, laser granulometry and dynamic light scattering (DLS) measurements were performed on the yeast suspensions and on the protein solution using a Malvern Mastersizer 2000 and a Malvern NanoS system, respectively. Figs. 4 and 5 provide information on the colloidal protein size distribution and the yeast apparent particle diameter, which appears to be bimodal for the BSA solution with two groups of particles corresponding to mean apparent diameters of 6 and 200 nm. The ~6 nm mean diameter may represent the size of BSA monomer, whereas the characteristic diameters of 200 nm are, most likely, protein aggregates. The DLS measurements could show the presence of different classes of particles but not their proportions. The light intensity depends on the size of the particles.

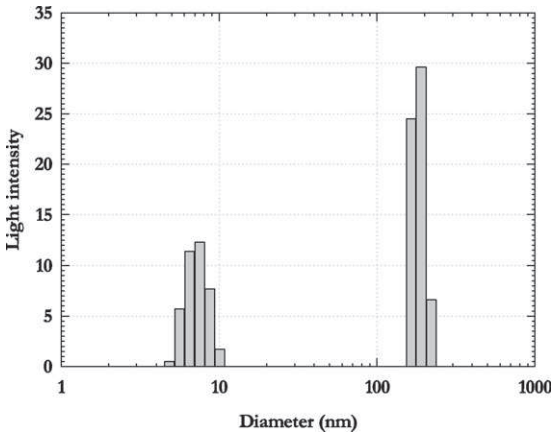

Fig. 4. Typical data of apparent particle diameter in protein solution (2 g L^{-1} of PBS) using dynamic light scattering. DLS measurement angle 60°.

3.1.2. Bioreactor device and membranes

The current device is composed of two 2-L fermentors (Setric Genie Industriel, Toulouse, France) interconnected by an hollow fiber membrane module from Polymem (Toulouse, France) with a specified design immersed in one of the fermentors.

Fibers were U-shaped and were held together with an epoxy-resin at their upper part. The upper part of the bundle was contained in a stainless steel punched receptacle and the filtering part immersed freely in the liquid. Fibers were made of polysulfone and had a nominal pore diameter of 0.1 μm, while the internal and external diameters were 0.25 and 0.43 mm, respectively. Corresponding maximal filtration areas were 0.3 and 0.45 m^2, respectively.

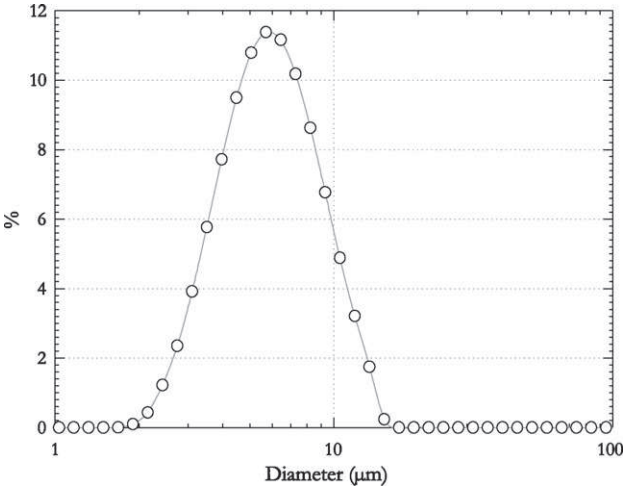

Fig. 5. Typical data of apparent particle diameter in yeast suspensions (8 g L^{-1} of PBS) using laser granulometry.

Each fermentor is connected to an acquisition card which can control the operating parameters (stirring rate, pH, temperature, partial pressure of dissolved oxygen).

3.2. Methods

All experiments were performed at $T = 25\,^{\circ}C$ and repeated twice for each condition. An average transmembrane pressure drop was maintained for both forward and backward filtration with the permeate side open to the atmosphere during each cycle. Each run began by filtering osmosis water and buffer solution through the clean membrane until the permeate flux was stable (usually after a period of 10 min). At this point, a switch was made to the different BSA, BSA/yeast and yeast suspensions. A sample of yeast suspension was withdrawn from the reactor and centrifuged three times at 5000 rpm with a PBS buffer wash between each centrifugation. Biomass concentration was then estimated by cell dry mass determination after filtration (0.2 μm pore size filter) and drying to constant weight under partial vacuum (24 h, 200 mmHg, 60 °C). The filter masses were measured once again after 48 h to check their stability. All yeast concentrations were expressed as dry cell mass (DCM) of yeast (after washing) per unit volume of solution.

The protein concentration was measured through UV light absorbance at a wavelength of 595 nm using the micro-Bradford method (Biorad) and a Synergy HT spectrophotometer. Each sample was diluted to 1/10 to come within the measuring range ($10–1000\ \mu g\ mL^{-1}$) and tested in triplicate. The accuracy and precision of this protein assay is entirely dependent on the accuracy and precision of the pipetting and was evaluated at better than 1%.

Filtration experiments were carried out for BSA, yeast, and mixtures of BSA and yeast as follows:

- The suspensions and/or mixtures were placed in reactor A (RA–Fig. 1) and the phosphate buffer placed in reactor B (RB—Fig. 1).
- The hollow microfiltration module was placed in reactor B.
- Each cycle was a forward filtration (from the internal face of the hollow fiber microfiltration membrane module to the external face) followed by a backward filtration (form the external face of the hollow fiber microfiltration membrane module to the internal face).
- The experiments were then run for a period of 3600 s.

The values of the key parameters are given in Table 1.

Eqs. (3.1) and (3.2) shows the way to determine respectively the forward and backward permeate flux in this process:

$$J_f = \frac{V_f}{t_f \times S_{fi}} \tag{3.1}$$

Table 1
Summary of parameter values used for the experiments.

Membrane characteristics
Hollow fiber (polysulfone) I.D. 0.23 mm, $L = 15$ cm, Nom. Pore size: 0.1 μm
Feed conditions
BSA in PBS
Concentration: $2\ g\ L^{-1}$
pH 7.1, $T = 25\,^{\circ}C$, $\Delta P = 0.4$ bar
Yeast in PBS
Concentration: $8\ g\ L^{-1}$
pH 7.1, $T = 25\,^{\circ}C$, $\Delta P = 0.4$ bar
Mix yeast–BSA in PBS
Concentration: BSA = $2\ g\ L^{-1}$, yeast = $8\ g\ L^{-1}$
pH 7.1, $T = 25\,^{\circ}C$, $\Delta P = 0.2, 0.4, 0.6, 0.8$ bar
Process operating parameters
Filtration transmembrane pressure (forward and backward): 0.2, 0.4, 0.6, 0.8 bar
Volume of filtration: 400 mL
Agitation: 300 rpm

$$J_b = \frac{V_f}{t_b \times S_{fe}} \tag{3.2}$$

with V_f the selected filtrate volume (400 mL) determined according to previous studies [10], t_f and t_b are respectively the forward filtration time the backward filtration time. These last two parameters are recorded and included in Eqs. (3.1) and (3.2) to calculate the forward and backward fluxes. S_{fi} is the total inner filtration surface of the hollow fiber microfiltration module ($0.3\ m^2$). As it could be observed in Fig. 1 the module is partially immerged and a small part of the outer filtration surface is not immerged. Experiments have been done to evaluate the flux loss between up level position (before filtration) and down level position (after filtration of V_f). A small difference inferior to 10% have been observed (data not shown). Accordingly, the outer filtration surface S_{fe} was evaluated at $0.4\ m^2$.

As previously mentioned, the net permeate flux is then calculated as the time average of the permeate forward flux; J_f and backward permeate flux J_b:

$$J_{net}\ (L\,h^{-1}\,m^{-2}) = \frac{\left(\int_0^{tf} J_f(t)\,dt - \int_{tf}^{tf+tb} J_b(t)\,dt\right)}{t_f + t_b}$$

4. Results and discussion

The purpose of this paper was to study the joint effect of transmembrane pressure and fluid characteristics on process performances in order to deduce pilot procedure recommendations for cases of complex fluid separation.

4.1. Effect of transmembrane pressure on filtration characteristic

4.1.1. Net flux J_{net}

The aim of this part was to examine the performance of the atypical filtration mode previously described, in terms of net flux permeate during filtration of model mixtures of BSA and yeast and to try to link the effects of transmembrane pressure and fluid characteristics. The influence on the net flux decline determined thanks to Eqs. (3.1), (3.2) and (2.8) due to each operating parameter was analyzed separately by studying the effect of transmembrane pressure ΔP on the net permeate flux for the $2\ g\ L^{-1}$ protein solution filtered together with $8\ g\ L^{-1}$ yeast (Fig. 6). Each dot in Fig. 6 is the net flux J_{net}, i.e. the productivity for one cycle, pulse and back.

The net filtration flux behaves as conventional flux, and it declined rapidly within the first 600 s for transmembrane pressures

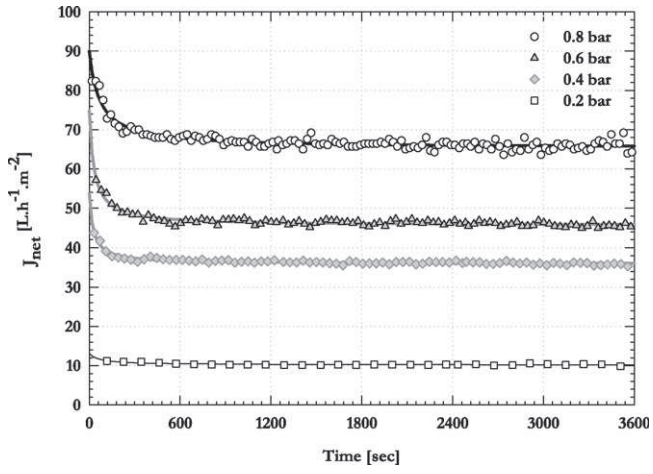

Fig. 6. Net permeate flux obtained for a BSA/yeast mixture ($2\ g\ L^{-1}$ BSA, $8\ g\ L^{-1}$ yeast) for transmembrane pressures of 0.2, 0.4, 0.6 and 0.8 bar.

of 0.4, 0.6 and 0.8 bar. No flux loss was observed for a ΔP of 0.2 bar. Further decline was quite small, and the flux value after 3600 s could be considered as the steady-state value, J_{ssnet}. It was noted first that the difference between each of the plots of the graph, i.e. the difference between the initial net permeate flux at the beginning of a series of filtration cycles and the initial net permeate flux of each subsequent filtration cycle, after a backward filtration, represented the net flux reduction due to two kinds of fouling. Indeed the volume of filtrate during the backwash step is fixed and has been determined in previous studies by Salgado [10] to be optimal for a minimal mixing time. But it may happen that cleaning is not complete and, in particular for hollow fiber microfiltration membrane, internal and residual particulate fouling can progressively build up to affect filtration performances. This latter kind of fouling can be present at the membrane level at the end of backwash filtration stage but could not be considered as irreversible fouling in a hydraulic point of view [23]. The resistance of the membrane R_m will increase from cycle to cycle due to internal fouling and, consequently, the time t_b will also increase, decreasing proportionally the backwash filtration flux then promoting residual particulate fouling, reducing the efficiency of the backwash. Moreover the backward filtration should provide a fouling too, given that protein must be mixed in the entire volume of the two tanks as we will discussed later.

In conventional microfiltration, two regions could be defined in the correlation of ΔP and steady-state flux. In the first region, the flux depended on ΔP and is determined by membrane properties. For the second region, the ΔP-independent flux could be explained as the balance between the flux and the lift velocity (back-transport velocity) generated by the shear stress due to the flow velocity gradient over the membrane. In this describe operating mode, Fig. 7 shows the effect of the transmembrane pressure ΔP on the steady-state net flux (J_{ssnet}) for the BSA/yeast mixture and for osmosis water. If there was no fouling, i.e. the backward filtration of each cycle removed all the foulants deposed on and/or in the membrane during the forward filtration, the steady-state net permeate flux should be close to that observed with osmosis (clear) water. The steady-state net permeate flux observed for the mixture of yeast and protein was proportional to transmembrane pressure ΔP but lower than that observed with osmosis water. A small amount of remaining foulants (yeast and protein aggregate for residual particulate fouling and/or protein for internal fouling) could be responsible for this. Due to the very short period of filtration, the conditions describe here are unsteady and an assumption

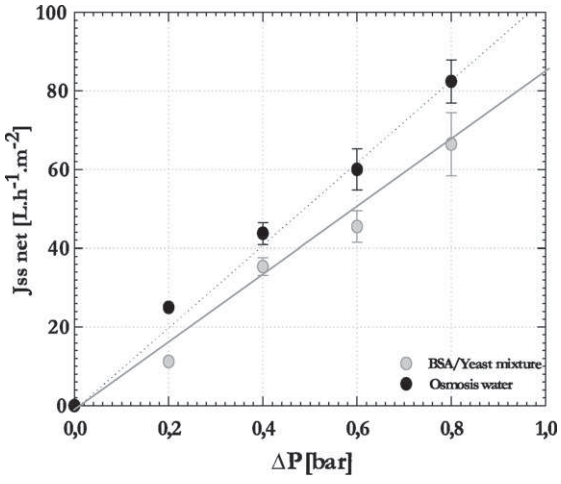

Fig. 7. Effect of transmembrane pressure, ΔP, on steady-state flux, J_{ssnet}, obtained for a BSA/yeast mixture (2 g L^{-1} BSA, 8 g L^{-1} yeast) for pressures of 0.2, 0.4, 0.6 and 0.8 bar.

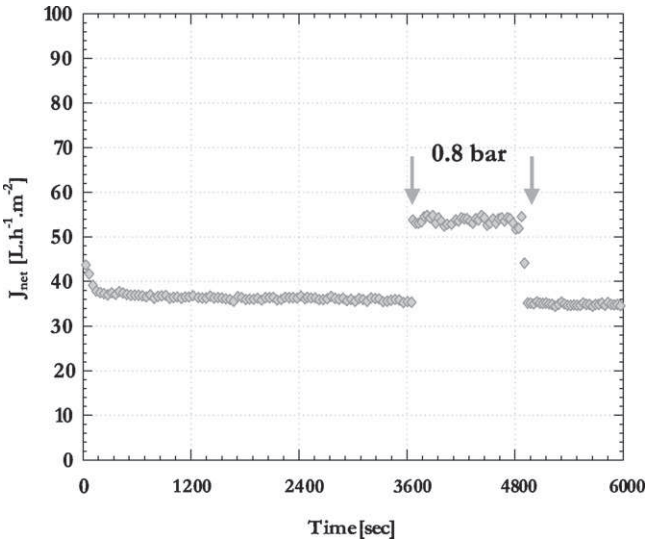

Fig. 8. Net permeate flux obtained for a BSA/yeast mixture (2 g L^{-1} BSA, 8 g L^{-1} yeast) for a variable pressure of 0.4 and 0.8 bar.

on the compressibility of the fouling cake would be premature. Nevertheless, in terms of process performance, this discrepancy is small and the linearity between ΔP and J_{ssnet} is conserved. To go deeper into this observation, the transmembrane pressure was doubled after 3600 s of experiment for 1200 s then was returned to the initial ΔP value. Fig. 8 shows that the net flux after this period was the same as before but the net flux at 0.8 bar was below the values recorded for an entire experiment performed at 0.8 bar. In this atypical filtration mode, the irreversible fouling due to internal fouling by protein and/or external fouling by a yeast cake is a small proportion of the fouling. Another phenomenon could occur and explain the latter observation. If any, construction of the fouling cake is known to be depending on the transmembrane pressure applied [26]. This could explain the differences between the net flux for an experiment at 0.8 bar from the beginning to the end and a short augmentation in the ΔP after a period at a lower pressure on a cake still built up under other pressure conditions.

4.1.2. Protein transmission

As discussed below, in this bioreactor all the molecular compounds of the medium that have an effect on the microorganism's behavior must not be retained by the membrane [9,10] and protein (BSA) was chosen to evaluate the rate of homogenization between the compartments. It should be pointed out that the values obtained at 3600 s in reactor A, added to those obtained in reactor B do not exactly match the values at $t = 0$ (Fig. 10). The estimation of these concentration values takes into account both the retention by the membrane and other protein losses such a those due to forward filtration and deposition on the pilot surfaces. Nevertheless, as shown in Fig. 9, the overall homogenization rate was reached at 240 s for each ΔP applied to the system. The feed concentration in each reactor did not fall notably thereafter for the remaining duration of the experiment. These observations are consistent with the literature [21] for dead end filtration as well as cross flow filtration [20], where protein rejection is below 15% although a fouling cake is present.

In this first part, the effect of the transmembrane pressure on the net flux decline for a mixture of yeast and protein has been studied. It points out that very little fouling occurs even when a long backward filtration occurs during each cycle, and this cake has a weak/negligible effect on protein transmission under all the conditions checked.

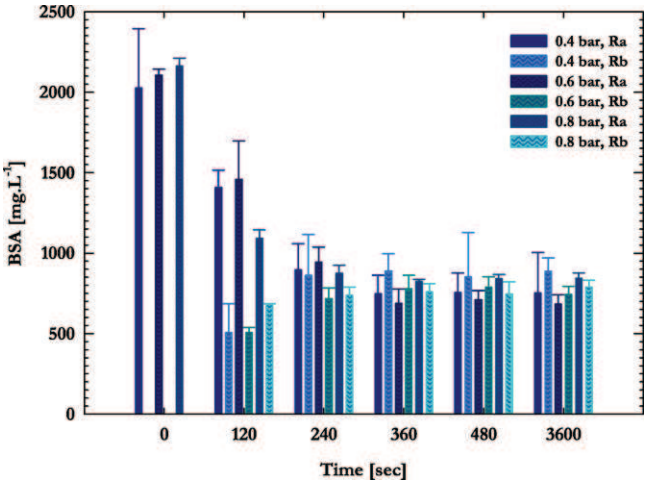

Fig. 9. Amount of protein vs. time in each tank for 0.4, 0.6 and 0.8 bar.

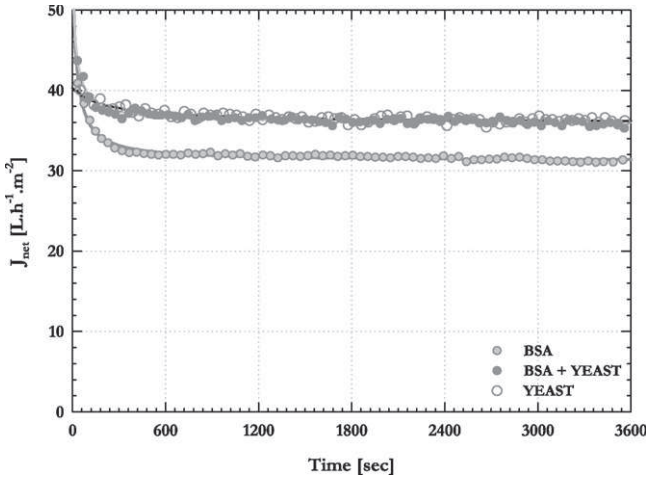

Fig. 11. Net permeate flux for mixture of 2 g L^{-1} of BSA and 8 g L^{-1} of yeast compared with a solution of BSA and a suspension of yeast (0.4 bar).

4.2. Effect of fluid characteristics on filtration performance

4.2.1. Net flux J_{net}

The effect of operating conditions on the net flux decline for a mixture of yeast and protein demonstrated that fouling was slight even when a long backward filtration was applied during each cycle. The influences of fluid characteristics were then evaluated for a fixed operating condition by studying the effect proper to each of the components of the BSA/yeast mixture used.

Fig. 10 shows the net flux evolution over a filtration period of 3600 s for a solution of BSA (2 g L^{-1}), a suspension of yeast (8 g L^{-1}), and a mixture of BSA/yeast at a ΔP of 0.4 bar.

For the yeast suspension, the flux declined rapidly within the first 600 s and then seemed to reach a steady state with J_{ssnet} = 36 L h^{-1} m^{-2}. Since yeast cells are much larger (7 μm) than the membrane pores (0.1 μm) they can form an external cake on top of the membrane. For every cycle of forward/backward filtration, the backpulse can be expected to cause the elimination of much of the external cake. The reverse permeate flow during each cycle lifts the cake off the membrane by applying hydrodynamic viscous drag which is resisted by the adhesive force. Two kinds of fouling cakes can be distinguished in the literature [13]: mobile or loose cake and adhesive/compact cake. The backward filtration is expected to clean the membrane for the first kind of cake and the results shown in Fig. 11 agree with this assumption. The flux lost is just 14.3% compared to J_{ssnet} at t = 0 (Table 2) and the recovery of the permeability after a cycle of washing with osmosis water is more than 90%.

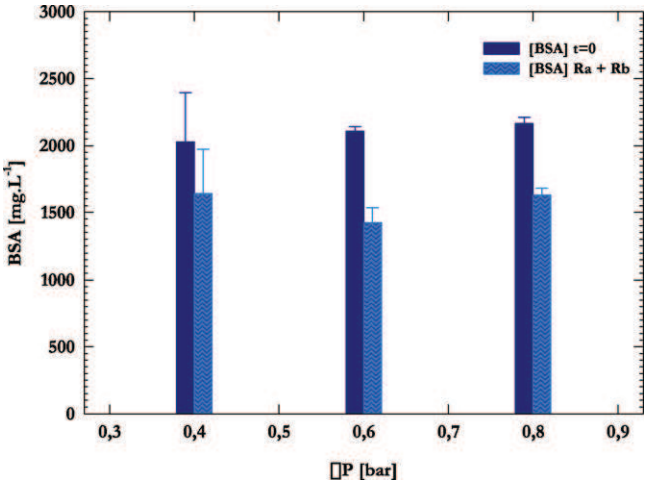

Fig. 10. Amount of protein at t = 0 s in Ra and at t = 3600 s for Ra + Rb.

With the BSA solution, the net flux decline was greater and J_{ssnet} = 31.8 L h^{-1} m^{-2} after a period of 3600 s. Many authors have shown that the main mechanisms involved in the primary BSA/protein fouling come from aggregates, which block pores and serve as nucleation sites for the deposition of additional proteins [13,19,20]. Backpulse during each cycle of filtration is able to remove a small portion of foulants, including aggregates and BSA monomer clogged inside the pores. Nevertheless, in terms of performance, for a high protein concentration compared to values found in the literature [19,22], the net flux decline is just 22.5% (Table 2).

Güell et al. showed that protein fouling of a microfiltration membrane could be reduced by forming a thin cake layer of yeast on the membrane surface, either during or before protein filtration [19]. One of their observations was a significantly higher flux in the presence of yeast cake than in its absence. Figs. 11 and 12 respectively show the resulting fluxes in the cases of a BSA/yeast mixture and a plot of a prefiltered yeast suspension before addition of BSA versus time, in the same operating conditions as described above. Clearly, for the BSA/yeast mixture, the net permeate flux is higher than for BSA alone and similar to that with yeast alone. (The J_{ssnet} after a period of 3600 s is 35 L h^{-1} m^{-2}.) According to the literature [19,13], when yeast cells are present in suspension in the feed mixture, the net flux decline is smaller. In Fig. 12, no significant net flux decline was observed after the addition of BSA. Small amounts of yeast present on the membrane surface produced a dynamic layer on the top of the membrane which avoided direct clogging of the membrane porous material by the protein aggregates involved in the nucleation and additional adsorption of protein monomer.

Table 2
Initial flux with osmosis water (J_{0net}, L h^{-1} m^{-2}) on clean membrane, steady-state net permeate flux J_{ssnet} (L h^{-1} m^{-2}), percentage of flux lost (referred to initial flux t = 0) and irreversible percentage loss (referred to initial flux with osmosis water) for ΔP = 0.4 bar.

	J_{0net}	J_{ssnet}	% lost	J_{net} after water cleaning	% irreversible
Osmotic water	45 ± 1	45 ± 1	0	45 ± 1	0
Yeast	42.8 ± 1	36 ± 1	15.9	40 ± 1	6.5
BSA	42.8 ± 1	31.8 ± 1	25.7	38 ± 1	11.2
BSA/yeast	43.4 ± 1	35 ± 1	19.3	40 ± 1	7.8

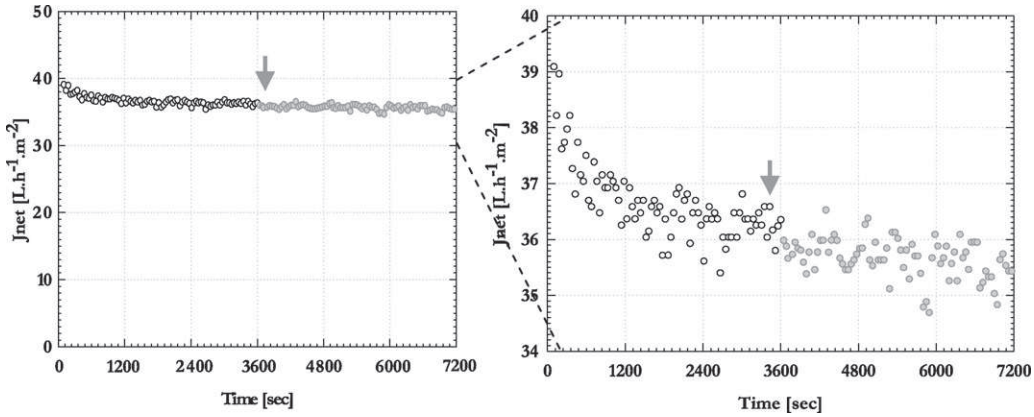

Fig. 12. Effect of adding BSA 2 g L^{-1} (arrow) to the filtration of 8 g L^{-1} of yeast. Net permeate flux decline (0.4 bar).

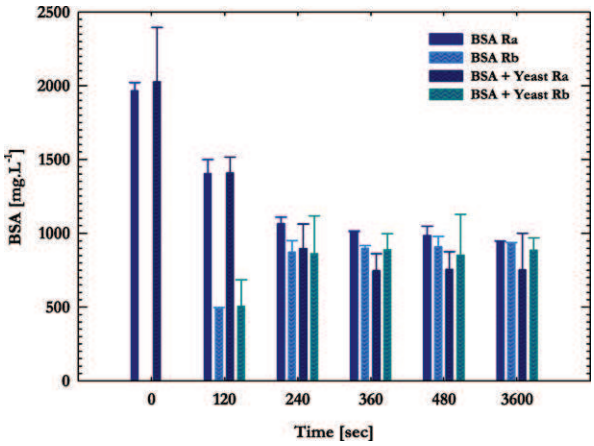

Fig. 13. Amount of protein vs. time in each tank for BSA and BSA/yeast mixture at 0.4 bar.

4.2.2. Protein transmission

As discussed above, the net flux decline with the BSA solution is greater than with the BSA/yeast mixture, probably because residual yeast forms a prefilter on the membrane surface. Moreover, as shown in Section 4.1, the overall homogenization rates of BSA solutions for each ΔP are not notably different. Although the net flux was higher when yeast was added to the solution of BSA as seen in Fig. 11, no difference was observed between the two solutions in terms of overall homogenization rate (240 s) and the feed concentration did not exhibit notable reduction of concentration up to the end of the experiments (Fig. 13).

Thus, the effects of the fluid characteristics for a fixed operating condition were evaluated. The experiments showed that a small amount of yeast on the membrane was able to reduce the net flux decline but had no noticeable effect on protein transmission and homogenization rate. These observations are convenient for the device's utility.

5. Conclusions

In order to rationally optimize the operating conditions of the process, this work examined membrane fouling and protein transmission during microfiltration of protein and protein-cell mixture with a high frequency of backpulsing. Although this process provides an atypical filtration mode using a long backward filtration time (compared to those presented in the literature), little membrane fouling occurred when protein, yeast suspension or protein/yeast mixture were filtered. This fouling showed itself as a decline in the net permeate flux of less than 20% of its initial value for each operating condition tested. In terms of protein transmission, no noticeable differences were observed for the overall mixing time and the concentrations were able to reach the same values in both reactors in 240 s. The net permeate flux at steady state was found to be proportional to the transmembrane pressure, showing that the flux, within the design range and for this type of microfiltration module, is predominantly determined by membrane properties.

In terms of protein transmission, no differences were observed for the different conditions tested. So, as far as the driving process is concerned, with high protein and biomass concentrations, higher transmembrane pressure will permit lower net flux decline and higher transmission performance. Net flux decline in the case of a BSA solution was found to be greater than for a mixture of BSA/yeast, linking with theory found in the literature. A thin yeast cake on the primary membrane could retain protein aggregates and reduce protein fouling.

To sum up the last conclusions and to give some operating recommendations for the use of this specific bioreactor, it is clear that enhancing transmembrane pressure leads to higher net flux and will allow higher soluble compounds transmission between the two tanks. As far as the fouling by soluble compounds of a similar size to the pores of the membrane is concerned, we could do some assumptions about adding inert particles to prevent net flux decline. Indeed this study and some authors [19] showed that yeast prevent internal fouling of small colloids, more precisely proteins monomers, by forming a prefilter entrapping these particles. Adding inert particles to the filtration–fermentation process could enhance the overall mixing rate by reducing internal fouling and improving net flux.

The study of this atypical process, involving rapid forward and backward filtration, shows interesting performances transposed to the field of application chosen. Indeed, as we discussed in the first part, as long as the interest compounds concentration equality is respected, this bioreactor could be used in the study of the behavior and interaction phenomena of microbial in dairy and oenological consortium involving protein and biomass at concentration levels closed to these used in this experiments [18,24].

Acknowledgements

The authors would like to thank Christophe Ellero for his technical support and Susan A. Becker for revising the English.

Appendix A. Nomenclature

ΔP	transmembrane pressure (bar)
EV1	electro-valve 1

EV2	electro-valve 2
J_{net}	net permeate flux ($L\,h^{-1}\,m^{-2}$)
J_{ssnet}	steady-state net permeate flux ($L\,h^{-1}\,m^{-2}$)
J_f	permeate forward flux ($L\,h^{-1}\,m^{-2}$)
J_b	permeate backward flux ($L\,h^{-1}\,m^{-2}$)
R_f	fouling resistance (m^2)
R_{ir}	irreversible fouling resistance (m^2)
R_m	membrane resistance (m^2)
R_r	reversible resistance (m^2)
t_f	duration of forward filtration (s)
t_b	duration of backward filtration (s)
S_{fi}	total inner forward filtration surface (m^2)
S_{fe}	total outer backward filtration surface (m^2)

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