Changes in structure and performance during diafiltration of binary protein solutions due to repeated cycles of fouling / alkaline cleaning

M. Carmen Almécija[a,*], Antonio Guadix[a], José I. Calvo[b,†], Emilia M. Guadix[a]

[a] Department of Chemical Engineering, University of Granada, 18071 Granada, Spain

[b] SMAP (Group of Surfaces and Porous Materials), Department of Applied Physics, University of Valladolid, 34071 Palencia, Spain

* mcalmeci@ugr.es

† Corresponding author. Tel.: +34 979108373; E-mail address: jicalvo@termo.uva.es

Highlights:

-Effect of temperature on NaOH cleaning during protein filtration has been studied.
-50ºC was the temperature with better cleaning and protein separation efficiency.
-LLDP has been used to analyze structural changes induced by fouling-cleaning steps.

Abstract

The purpose of this work was to evaluate the effect of the temperature (50 and 60ºC) of a NaOH cleaning solution during the diafiltration of a binary mixture of bovine serum albumin and β-lactoglobulin, through a 300 kDa tubular ceramic membrane along repeated operational cycles. To this aim, final permeate volume, membrane and fouling resistances and individual protein concentration were analyzed. At the end of each individual study, the membranes were characterized by liquid-liquid displacement porosimetry. As a result, 50ºC was found to be the most appropriated temperature due to its higher capability to restore the initial membrane resistance and the higher efficiency achieved in terms of protein separation. Both conditions fulfilled without altering the structural properties of the membrane as given by porosimetric analysis. In contrast, a great fouling resistance involving null protein transmission occurred when cleaning was performed at 60 ºC.

Keywords: ceramic membrane; chemical cleaning; fouling; liquid–liquid displacement porosimetry; protein fractionation.

1. Introduction

In the last years, extensive research has been focused on the separation and purification of proteins by membrane technology [1]. In particular ultrafiltration (UF), has found a widespread application in the concentration and desalting of proteins [2]. This process is able to achieve high productivity and concentrate purity simultaneously [3, 4]. Moreover, selectivity towards individual proteins in a complex mixture (a usual drawback when Ultra- filtering non model proteins) can be enhanced through the application of high performance tangential flow filtration (HPTFF), where a proper choice of pH and ionic strength maximizes differences in the effective hydrodynamic volume of the different proteins [5, 6].

However, membrane operation (not only UF) is generally characterized by a progressive decrease in permeate flow and protein transmission along the time [7], which diminishes the strong potential of membranes as a choice separation method for the biotechnological processes. Very often the proteins or other solutes filtered or retained in the membrane lead to adhesion onto the membrane surface, directly or forming aggregates. This phenomenon, mostly irreversible and usually termed as membrane fouling, causes a progressive and substantial decrease of membrane performance characteristics with time. In any case, fouling is usually reverted by periodic chemical and physical cleaning, which ideally intends torestore the original filtration characteristics of the virgin membrane [8]. That cleaning increases the membrane lifetime, a critical factor due to the considerable costs rise associated to membrane replacement. Membranes in industry are regularly maintained and cleaned in place (CIP), a practice comprising several steps as: (i) emptying the filtration system from both sides of the membrane, usually followed by backflushing; (ii) chemical cleaning after addition of several cleaning agents, and; (iii), disinfection and final water rinsing, especially in biotechnological, food or pharmaceutical industries, [9]. For those later industries, even sterilization should be required in most cases.

Therefore, from a practical point of view, the operation of membrane plants takes place in a

cyclic mode including filtration and cleaning steps. In the case of proteins, NaOH solutions are mentioned in the literature for their capability of removing protein deposits, which is improved with the concomitant action of a surfactant [10].

Although a considerable amount of research has addressed the problem of membrane cleaning optimization [11-12], studies of membrane cleaning have always been a complementary aspect, paying much more attention to a deeper knowledge of fouling. Only a few quantitative works have been published studying the effect of repeated fouling and cleaning cycles on the performance of ultrafiltration membranes [13-15].

For example, Weis and Bird [16] and Weis et al. [11, 17] studied the influence of fouling and cleaning processes over a number of operational cycles upon polymeric (polyethersulphone, polysulphone and regenerated cellulose) UF membranes fouled with products related to the pulp and paper industries, using formulated cleaning agent (P3 Ultrasil 11), sodium hydroxide or nitric acid as chemicals. Authors concluded that over long term operation, as the membrane surface became irreversibly fouled, physico-chemical interactions between cleaning agent and foulant were the dominant factors in determining the cleaning performance while membrane material, including its porosity and surface roughness, was less determining.

Blanpain-Avet et al. (2004) investigated the effect of 10 repeated fouling and cleaning cycles upon the membrane and cleaning performance of a 0.1 μm tubular ceramic microfiltration (MF) membrane with a whey protein concentrate as foulant. Their results showed that sodium hydroxide recovered flux whereas nitric acid had a negative effect on membrane resistance. They also found a slight increase on protein retention over the last few cycles indicating a change in membrane selectivity, although the cleaning efficiency did not decrease with cycles.

Mourouzidis-Mourouzis and Karabelas [18, 19] studied the fouling of MF ceramic membranes

employed in successive whey protein filtration cycles with intermediate backwashing. Their results indicated that irreversible fouling occurred mostly during the first cycle and did not significantly increased later on whereas reversible fouling developed in each cycle, mainly during the first minutes of filtration.

Zapata-Montoya et al. [20] studied the evolution of permeate flux, transmission of protein and membrane resistances, for a 50 kDa tubular ceramic membrane, along 50 cycles comprising milk ultrafiltration, alkaline and acid cleaning. Permeate fluxes and protein transmission did not suffer significant changes during the cycles. However, membrane resistance increased, mainly in the first operational cycles, which suggests the formation to some extent of a "chemically" irreversible fouling. Finally alkaline cleaning was able to reduce membrane resistance in one order of magnitude respect to that obtained just after finishing ultrafiltration, whereas acid cleaning decreased only 10% the membrane resistance value after alkaline cleaning.

According to the referred literature, it is worth to study the variables involved in the cleaning procedure of the operational cycles in order to detect any adverse effects caused by the cleaning agents on the expected membrane working lifetime [21, 22]. In particular, temperature is a critical variable because of its narrow margin of effective use, which results in poor cleaning performance at low temperature values and corrosive action at high ones.

Generally, an elevated temperature results in better membrane cleaning. However, it must keep in mind possible risks for membrane material stability and the increase of attraction between foulant layers which leads to stronger attachment to membrane surface and consequently more difficult to breakup [9].

In any case, the efficiency of the cleaning step should lead to detectable changes in the membrane structure along time, due to deposition of subsequent fouling layers and corresponding reduction on the membrane mean pore size and pore size distribution (PSD). These changes can be detected with the aid of porosimetric techniques through an autopsy of the used membrane after a gentle number of fouling/cleaning cycles.

The purpose of this research work was to study the effect of the temperature of the alkaline solution employed as cleaning agent in repeated cross-flow filtration and cleaning stages. Two temperatures were chosen for this study (50 ºC and 60 ºC), both in the range of temperatures often used to clean membranes after protein UF. In the preliminary work of this research, a wider range of temperatures was assayed in our laboratory. Nevertheless, these experiments were not included in the manuscript since, after a short number of cycles they conducted to improper results. For temperatures below 50 ºC, membrane cleaning was not effective at all. On the other hand, for temperatures above 70 ºC, membrane erosion was very noticeable.

2. Experimental

Two main factors (permeability and selectivity) are to be considered to check the success of a certain membrane for some industrial process. In that sense, present study will start accounting for possible changes in flux along time, due to an increase of fouling resistance. Attending to selectivity, protein transmissions were monitored during the diafiltration of a protein binary solution made of bovine serum albumin and β-lactoglobulin through a tubular ceramic membrane with molecular weight cut off (MWCO) of 300 kDa. Moreover, membrane and fouling resistances were measured after each stage during the operational cycles. Finally, at the end of each protocol, the membrane PSD was characterized by liquid- liquid displacement porosimetry (LLDP) to check possible changes in membrane structure. LLDP has proven to be reliable on characterizing from structural point of view membranes in the UF range, [23], but also the information it provides can be used to estimate functional performance related parameters, as permeability or MWCO, [24].

2.1. Materials

As a filtration solution, it was employed a binary mixture of bovine serum albumin (BSA) and β-lactoglobulin (BLG), both with reagent purity and received from Sigma-Aldrich (St. Louis

MO, USA). Selection of the proteins in this study was because both are different enough (respective molecular weights of 18.4 kDa for b-lactoglobulin and 66.5 kDa for BSA) to be effectively separated in appropriated conditions of filtration.

Protein solutions were prepared at a concentration of 0.125 g/L on each protein. Solution pH was adjusted to 5 by addition of appropriated amounts from a 37 % concentrated solutions of HCl. At such value, very close to the IEP of both proteins (4.9 for BSA and 5.2 for BLG), it was found maximum selectivity for BLG, [25].

The membrane selected was a tubular ceramic Céram Inside module (TAMI Industries, Lyon, France) made of ZrO_2–TiO_2, 25 cm long and with a filtration area of 47 cm^2. The nominal molecular weight cut-off of the membrane was 300 kDa, which was found previously to allow the transmission of significant amounts of β-lactoglobulin (18.4 kDa) while retaining most of the bovine serum albumin (66.5 kDa) [25].

Three membranes were used in this study: two of them, A and B, served to 30 operational cycles with alkaline cleaning at 50 ºC and 60 ºC, respectively, while an extra membrane served as control.

In a previous paper by some of the authors, [26], milk was filtered with a 50 kDa ceramic membrane for 50 cycles, although no significant variations were observed after the first 30 cycles. Based on that, 30 cycles were considered appropriate for this work, being even higher than used in other previous works, [13, 17].

A process flow diagram is shown in Fig. 1:

Figure 1

2.2. Experimental procedure

The initial resistance of the membrane, R_0, was determined as the inverse of the slope of water permeate flux vs. transmembrane pressure plot (see Eq. (1)). In this determination, demineralized water at 30 ºC was used.

$$J = \frac{\Delta P_m}{R_0}$$

Eq. (1)

Two virgin ceramic membranes (A and B) were submitted to 30 operational cycles each, which were performed in consecutive days. Each cycle comprised four stages:

(a) Ultrafiltration: 1 L of solution was cross-flow ultrafiltered for 4 hours in a continuous diafiltration mode under the following conditions: transmembrane pressure 1 bar, temperature 30 °C and cross-flow velocity 3.5 m/s.

(b) Initial water rinse: After the passing of 10 L of demineralized water at 30 ºC, it was determined the fouling resistance, R_F, as indicated in next equation:

$$J = \frac{\Delta P_m}{R_F}$$

Eq. (2)

(c) Cleaning protocol: 2 L of a solution of 20 g/L NaOH + 2 g/L sodium dodecyl sulphate (SDS) was circulated for 30 min at 1 bar of transmembrane pressure and a cross-flow velocity of 3.5 m/s. The only difference in the experimental protocol applied to samples A and B was the cleaning temperature. This was selected as 50 ºC for membrane A being 60 ºC for membrane B.

(d) Final water rinse: demineralized water, in enough amount to reach neutrality in the permeate side, was pumped through the membrane, which was followed by the determination of membrane resistance with pure water, which will be termed clean membrane resistance, R_C, see Eq. (3).

$$J = \frac{\Delta P_m}{R_c}$$

Eq. (3)

2.3. Determination of protein concentration

Individual protein concentrations of β-lactoglobulin and BSA were determined by reversed-

phase high-performance liquid chromatography (RP-HPLC) using the method described by Elgar et al. [27] and extended by Palmano and Elgar [28]. The HPLC system (Waters, Milford MA, USA) consisted of an Alliance Separation Module 2690 interfaced with a M-474 absorbance detector and a Millenium data acquisition and manipulation system.

A 1 mL Resource RPC column (Amersham Biosciences, Uppsala, Sweden) was operated at room temperature at a flow-rate of 1 mL/min. The detection was by absorbance at 214 nm. The solvents used were: (A) 0.1% (v/v) trifluoroacetic acid (TFA) in Milli-Q water and (B) 0.09% (v/v) TFA, 90% (v/v) acetonitrile in Milli-Q water. The column was equilibrated in 80% solvent A. The gradient employed is shown in Table 1.

2.4. Determination of pore size distribution

After the completion of the 30 operational cycles, both membrane samples employed (A and B) were removed from the filtration setup and two pieces of each were cut (around 4 cm long and corresponding to non consecutive zones of the tube and none placed at the membrane edges). Both samples of each membrane after sealed with an enamel painting were analyzed using a liquid-liquid displacement porosimeter (LLDP) which provides the whole PSD, mean pore radius, limit permeability, porosity and an estimation of the MWCO, [29-31]. This analysis was also carried out for two pieces of a virgin membrane for comparison.

The porosimetric mixture was (1/1 v/v) water-isobutanol mixture, which presents a surface tension of 1.9 mN/m and a dynamic viscosity of 4.3 mPa·s at 25 ºC. After gentle mixing and further separation, the aqueous and organic phases were employed as the displacing and wetting liquids, respectively.

Experimentally, LLDP consist in a steeply increment of the transmembrane applied pressure over a bathed membrane and the determination of the flux through the sample at such applied pressure. After completion of a porosimetric run, a curve flow vs. pressure is obtained from which, successive increments of permeability are attributed to the successive opening of smaller

pores (according to Cantor equation applied pressure and size of the pores therefore opened are inversely proportional). Then, number of pores yet opened in each incremental step can be evaluated from application of an appropriated transport model for the liquid fluid inside the pores. Attending the characteristics of the membranes used, Hagen-Poiseuille model for convective transport inside capillary tubes is customarily used for determining the amount of pores leading to such permeability increment. Detailed description of calculation procedure can be found in [32].

3. Results and discussion

As mentioned above, three membranes were used in this study: two of them, A and B, used in normal operation up to 30 operational cycles including alkaline cleaning after each cycle at 50 ºC and 60 ºC, respectively, while an extra membrane served as control.

3.1. Fluid dynamics

The initial resistances of the membranes, R_0, were $(7.78 \pm 0.15)\cdot 10^8$ kg/(m^2·s) for membrane A and $(7.40 \pm 0.10)\cdot 10^8$ kg/(m^2·s) for membrane B. This small difference indicates that both membranes were in similar condition before being employed, in fact, they belong to the same manufacture batch. Therefore, any variation detected in the experiments, was due to the different cleaning temperatures.

The fluid-dynamic behavior of each membrane during the operational cycles is shown in Figs. 2-5. In Fig. 2 the total permeate volume collected at the end of each cycle it is represented, for both membranes, versus the number of cycles (1-30). A different behavior was found for each temperature. At the lowest cleaning temperature, 50 ºC, permeate volume was almost constant across the first 20 cycles (with a mean permeate volume around 1.06 L), followed by a slight decrease in the last ten cycles to about 0.89 (a reduction about 16.5%). On the other hand a greater reduction was observed for the highest temperature treatment, 60 ºC. In this case, a more or less constant reduction rate was observed from around 0.95 L corresponding

to the first 5 cycles to about 0.55 L of the last 5-6 (leading to an accumulated reduction over 40 %).

In Fig. 3, it is plotted the time evolution of the permeate volume for two extremal cycles (cycles 1 and 30). In all cases, a linear relationship between permeate volume and filtration time was obtained. This linearity is caused by the working mode, diafiltration, where the negative effects of fouling of permeate flow during the filtration is balanced by a decreasing protein concentration in the solution. But the important fact is the slope of such straight lines (which is equal to the mean permeate flow). It can be seen that for both membranes, such slope decreases from cycle 1 to cycle 30, being this decrement clearly attributed to irreversible fouling. For 50 ºC, the mean permeate flow through membrane A decreased from 0.282 to 0.211 L/h (almost 25 % reduction), while for membrane B (60 ºC) a greater decay took place, from 0.268 to 0.150 L/h (45 % lower in this case).

Fig. 4 represents the ratio between the fouling resistance, R_F, measured after diafiltration for each cycle and membrane, and the corresponding initial membrane resistance, R_0. Also the behavior of this ratio is different for each temperature, being fairly constant (around 8 and increasing very slowly) for the membrane cleaned at 50 ºC. While, for 60 ºC, this ratio increased continuously from an initial value of 5 to a final value of 16 after 30 cycles.

With respect to the resistances of the membranes after cleaning step, R_C, their normalized values are plotted in Fig. 5, again versus the number of cycles of filtration accomplished. For membrane A (cleaning temperature of 50 ºC), this value remained practically constant around 1 during all the cycles. This involves a proper performance of the cleaning procedure, which restitutes the clean resistance to the original (100 % recovery of initial permeability). However, at 60 ºC, after 7 cycles, the membrane resistance increased 29% respect to the initial value. Afterwards, a sharp reduction took place, R_C/R_0 reaching a final value of 0.93.

According to this last value of normalized resistance, it seems that membrane B initially did not achieve an effective cleaning, which results in a final permeability lower than the original one.

Nevertheless as cleaning continues along more operation cycles, this initial worsening is followed by a continuous increment of cleaning performance. Values of the ratio Rc/R0 lower than 1 may be due to pore erosion provoked by the cleaning agents. Nevertheless, such erosion does not seem to be very dramatic since the lowest ratios are around 0.9. So it could be concluded that 60 ºC cleaning protocol gives, after enough number of cycles, a better restoration of the initial conditions than 50 ºC procedure. In any case this asserts must be checked with the findings coming from the rest of analysis.

3.2. Protein transmission

Any cleaning protocol used in a certain membrane process should be able to restore the initial permeability of the membrane, but also to maintain the selectivity for the filtered species, which is the final reason of using membrane process for separation. In this process it was used HPTFF diafiltration to separate a mixture of two model proteins (BSA and BLG) having not too different sizes. The goal is to maintain the differential selectivity of the process for both proteins after a certain number of operational cycles which could affect the membrane properties leading to undesirable changes in process performance and most important to loss the ability to separate both proteins. So the discussion will be stated in terms of transmission

of individual proteins through the membrane. Accordingly, both retentate (Y_R) and permeate yields (Y_P), were defined as the ratio between mass of protein in the retentate or permeate, respectively, and the mass of protein in the initial feed.

For membrane A, in Fig. 6 and Fig. 7, it can be seen the time evolution (measured every hour up to 4 h) of the retentate and permeate yield, respectively, for BSA (a) and BLG (b). BSA in the retentate (Fig. 6a) showed a quite unpredictable and oscillating trend with the number of cycles. Average values of yield were 0.88 after 1 h and 0.74 after 4h. This apparent loss of BSA was not totally due to transmission, since, at most, permeate yield for this protein (Fig. 7a) achieved a value of 0.09 at cycle 16. This could be justified by the adsorption of a significant

amount of BSA onto the membrane surface during the diafiltration. In the case of BLG, after initial acute oscillations (first 5 cycles) similar to that found in BSA, both retentate (Fig. 6b) and permeate yield (Fig. 7b) presented almost constant values. In this sense, mean retentate yield decreased from 0.68 after 1 h to 0.28 at the end of the cycles, while for their respective values in the permeate, a increase from 0.06 to 0.27 was detected. This could represent a BLG adsorption by the membrane ranging from 26 % to 45 %.

In any case the high amount of BSA retained in the membrane could be a bit surprising according to the relative sizes of molecule (3.5 nm at pH 5, [33]) and membrane mean pore size (15.4 nm, see table 2). This may be due to the formation of protein aggregates since the working pH is close to the isoelectric point. Such full retention of BSA by a 300 kDa membrane was also found by Almecija et al., [34], in the ultrafiltration of bovine whey.

It is also noteworthy that in the case of the protocol which used the cleaning temperature of 60 ºC (membrane B), neither BSA nor BLG passed through the membrane, in any of the cycles of filtration; which involves null protein concentration in cumulated permeates. Therefore, only evolution of protein yield in the retentate can be plotted (see Fig. 7). In this figure it was observed that the amount of each protein decreased with time for all the cycles. For example, at cycle 30, only 53 % of the BSA and 24 % of the BLG remained in the final retentate. Moreover, the decrease of protein amount was sharper for BSA as the number of cycles increased, while maintained more constant for the case of BLG. In both cases, the loss of the complementary amount of protein should be due to adsorption phenomena (since no transmission was detected, all the protein should remain in the retentate). This adsorptive behavior was more extended than in the case of membrane A.

In order to evaluate the degree of fractionation achieved by the diafiltration process, a separation efficiency (E) was defined. This variable was only calculated for membrane A, since no actual separation occurred for membrane B. Since the process was designed to have more BLG than BSA passing through the membrane, we can define the efficiency of the

separation as how greater is the concentration of BSA in the retentate compared with that of BLG. Similarly we can say that the process will be efficient if concentration of BLG in the permeate is clearly higher than that of BSA. Accordingly, for BSA, efficiency (Fig. 8a) was the ratio between the concentrations of BSA and BLG in the final retentate divided into the same ratio in the initial feed. Efficiencies were around a mean value of 2.6, with some high values up to 4.0 in the central cycles. With respect to BLG, its efficiency (Fig. 8b) was the ratio between the concentration of BLG and BSA in the final permeate, divided by the same ratio in the feed. Although values higher than 10 were obtained in the initial cycles; the BLG efficiency oscillated afterwards around 4.5. As a result, taking into account that BLG transmission took place and no significant amount of BSA passed through the membrane, significant efficiencies in the fractionation of the mixture were achieved and maintained along the cycles assayed.

3.3. Membrane characterization

After the proper analysis of data coming from LLDP, the contribution of the different pore size intervals to the total permeability (in percentage), the mean pore radius and the limit permeability were calculated. These data were obtained from the porosimetric curve (membrane volume flow against applied pressure), using the Cantor equation and the Hagen-Poiseuille convective transport model, assuming that the pores are cylindrical [32], to convert permeability increases in number of pores opened.

Results arising from LLDP analysis are presented in Table 2, which summarizes the asymptotical permeability (maximum permeability to the displacing liquid achieved in the LLDP analysis), the mean pore radius, the membrane porosity (calculated supposed an active layer thickness of 4.5 μm) and the result of the MWCO estimation from pore size distribution, [24]. While Fig. 10 presents an example of the pore size distribution for each membrane analyzed (virgin membrane and samples A and B) in terms of contribution of each pore class to the whole membrane permeability (results for each membrane have been normalized to their

limit permeability).

According to Fig. 10, the pore size distribution of the virgin membrane is slightly shifted to bigger pore sizes in the case of membrane A. Obviously pores of bigger pores cannot appear after using the membrane unless chemical cleaning should lead to destruction of membrane material and consequently to pore enlargement. But this is a fact hardly expected when using ceramic membranes, which are strongly recognized to be stable under strong acid/basic environments. The reason of such shifting must be attributed to the effect of irreversible fouling. This fouling should be more intense in the case of smaller pores of the membrane which become irreversibly clogged, even after repetitive cleaning steps. Then, the remaining distribution maintains the part of the distribution corresponding to biggest pores with a small loss of porosity (see table 2). Regarding the permeability, this loss due to fouling is very small (in fact membrane A presents a permeability slightly higher than virgin one, but reasonable taking into account the relative errors and the usual sample to sample variability).

In the case of membrane B, again quite different results are found. In this case the pore size distribution is clearly shifted to lower pore sizes, which means the biggest ones become irreversible fouled in a way that cleaning steps are not able to restore. Here the porosimetric results can be interpreted in terms of inadequate choice of cleaning temperature. Working at 60 ºC, the cleaning seems to be clearly ineffective leading to a general increase of irreversible fouling (as it was observed from plots of fouling resistance versus cycles of operation). This fouling affects the whole population of pores and causes strong diminution of porosity and moreover, of permeability (45 % of the original one). Nevertheless, the part of the distribution corresponding to biggest pores suffers much strongly this fouling surely due to the effect of temperature which stimulates the protein denaturation and consequently the formation of stronger attachment of big protein aggregates to the membrane surface. This causes the resulting shift to lower values of the pore size distribution.

Finally it is worth noting the results for the MWCO estimation presented in last column of Table 2. Since this is a rough estimation of the actual cut-off value for retention of dextran, accordance between value quoted for virgin membrane (211 kDa) and nominal one (300 kDa) is remarkable. In the case of results for membrane cleaned at 50 ºC this agreement is even better (268 kDa). But his improvement is mostly related with the shifting yet commented to higher pore sizes, then it is expected that MWCO (which roughly can be assumed to correspond to the 90 % biggest pores in the population) also should increase as mean pore

size does. While, for membrane B protocol (60 ºC cleaning) cut-off value reduces strongly (116 kDa) indicating (similarly to that showed in Fig. 9) the higher contribution of very small pores and the almost nil contribution of the biggest ones (such contribution in membrane B is substantially lowered).

Therefore, porosimetric results show that the cleaning temperature of 50 ºC does not alter significantly the membrane properties, since the porosimetric parameters of this membrane were similar to the virgin membrane. On the contrary, the membrane cleaned at 60 ºC showed a different behaviour, with smaller pore sizes and lower limit permeability. However, these data were in contrast with the permeability obtained with demineralized water at the end of

the protocol, since it resulted similar to the permeability of the virgin membrane, as it was indicated in the analysis of the membrane resistances. This contraposition suggests that the cleaning at 60 ºC could lead to opening pores with size smaller than 2 nm, pores that indeed are not detectable with the maximum pressure achievable at the LLDP setup used, but contributing anyway to increase the water permeability.

These findings are consistent with the fact that alkaline cleaning solutions at temperatures over 50 ºC could lead to a fast hydrolysis of proteins and other organic residues found in the feed stream [35], and then increasing the attachment strength of these proteins to the membrane surface and reducing sensibly the cleaning efficiency.

Finally, the porosimetric results are in accordance with those coming from protein transmission analysis. Higher temperature protocol should lead, as commented to higher protein hydrolysis increasing sharply the retention of BSA (which having bigger size and more groups sensible to act as adsorption sites when denaturalized is then prone to form bigger aggregates which are totally retained and also reduce the pore size of the membrane by pore clogging).

Regarding the strong change in fluidodynamic of the membrane when the cleaning temperature was raised by only 10 ºC, it is clear that supposed benefits of higher temperature cleaning in terms of resistance restoration are not confronted with porosimetric and retention results. It is clear that cleaning at 60 ºC results in higher fouling along time and almost nil transmission of BSA and BLG through the membrane, being this fouling more strongly attached to the membrane surface and resulting in important changes in the membrane structure with smaller pores present in the pore size distribution, clogging of the biggest pores and a lower final permeability.

4. Conclusions

A different behavior of the ceramic membrane was observed depending on the cleaning temperature applied. Regarding the fluidodynamics, almost constant values along the cycles assayed were obtained for the temperature of 50 ºC, with final permeate volumes around 1 L, membrane resistances after cleaning similar to the initial one and fouling resistances around 8.15 times the initial membrane resistance. In contrast, when the cleaning temperature was raised 10 ºC, although the membrane resistance was restored to its initial value, new pores could have been opened, involving new protein adsorption points, which could explain the constant increase in fouling resistance up to 16 times the initial membrane resistance, and therefore, the decrease of the final permeate volume with the number of cycle down to 0.49 L. With respect to protein transmission, null protein concentration in cumulated permeates were found for 60 ºC, probably due to the already mentioned fouling. For the cleaning

temperature of 50ºC, BSA was preferably retained with a mean value of 74% of the initial BSA, whereas the amount of BLG in the cumulated permeate remained constant along the cycles but increased with time during diafiltration, achieving 26.43 % of the initial BLG after 4 h.

The analysis by LLDP showed that pore size distributions, mean pore radius, limit permeability and cut-off estimation for the membrane after 50 ºC protocol was very similar to the virgin membrane ones. On the contrary, the membrane cleaned at 60 ºC presented smaller pore sizes in a distribution clearly skewed to the left of the axis, which also leads to lower MWCO (as estimated from LLDP data) and lower limit permeability.

As a conclusion, 50 ºC could be an appropriate alkaline cleaning temperature in BSA-BLG fractionation processes since it restores the membrane resistance to the initial value after each operational cycle, efficiencies in the fractionation of this mixture were achieved and maintained along the cycles and it does not alter the membrane porosimetric characteristics. However, a temperature of 60 ºC could not be employed in the practice, because of the
intense alteration of the pore size distribution of the membrane, which causes the absence of protein transmission, and therefore, null protein fractionation.

Finally it has been showed how a structural characterization technique (LLDP) can be used to discriminate optimal cleaning conditions among quite similar values.

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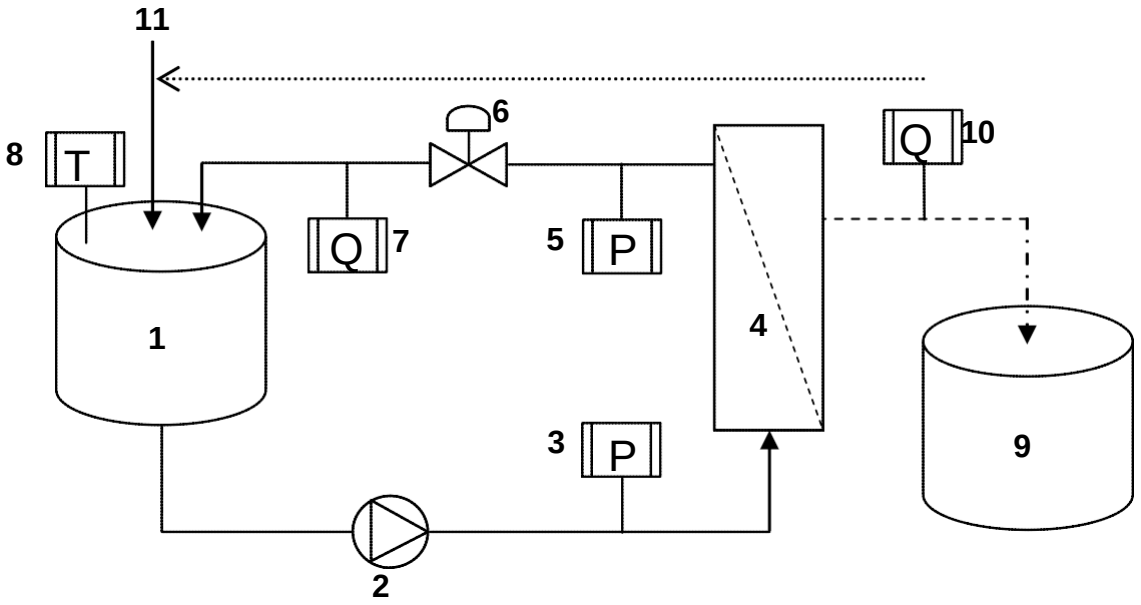

Fig. 1. Process flow diagram: Feed tank (1), pump (2), pressure gauge (3, 5), membrane module (4), valve (6), retentate flow meter (7), temperature probe (8), permeate tank (9), permeate flow meter (10), feed (11).

Figure 2

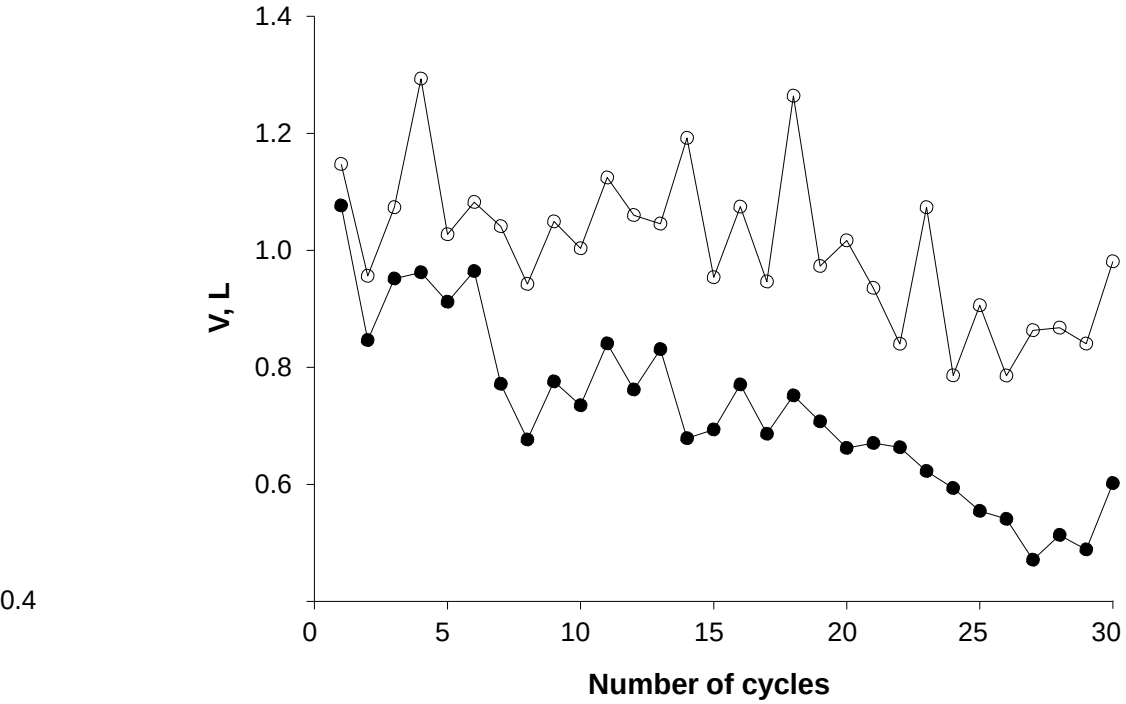

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Fig. 2. Final permeate volume as a function of the number of cycles for membrane A (○) and B (●).

Figure 3a

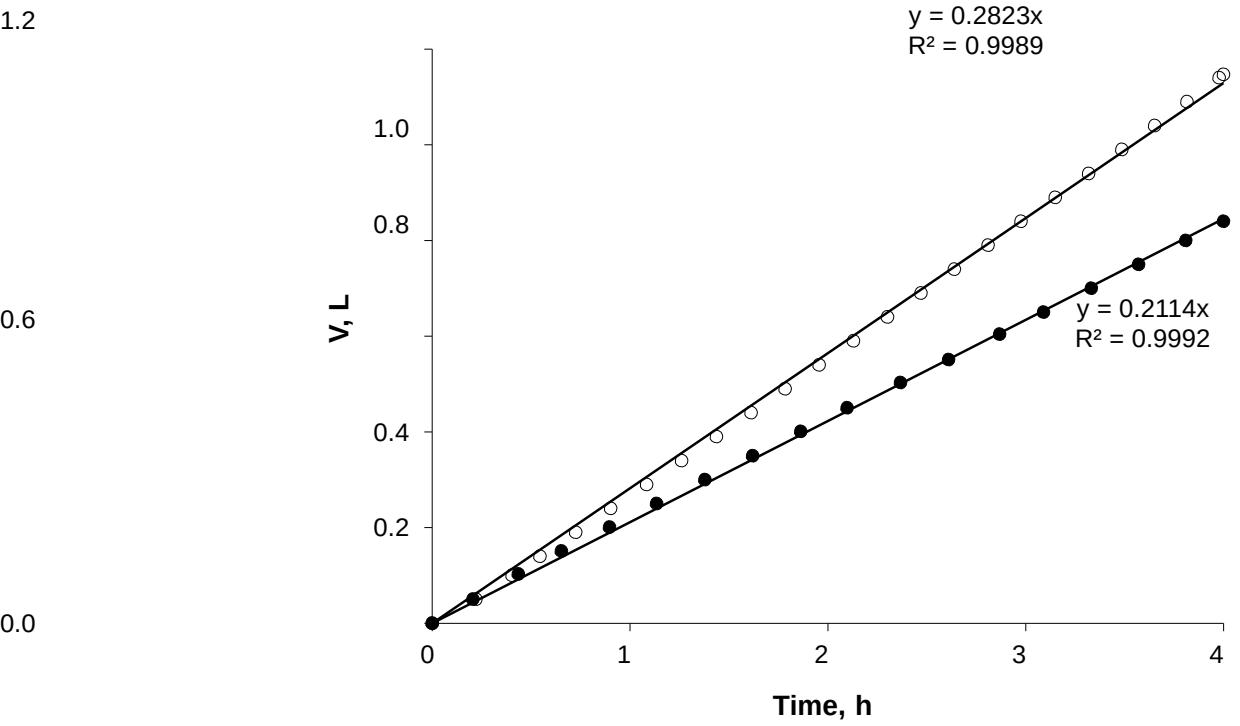

Figure 3b

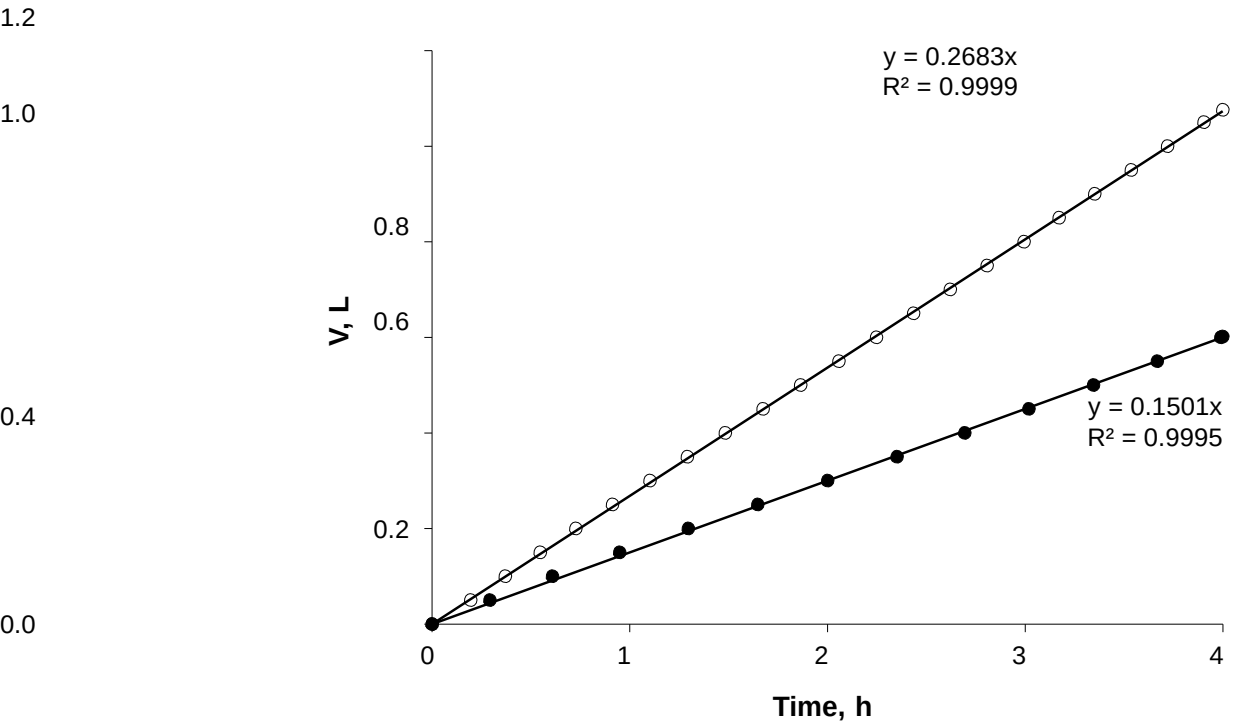

Fig. 3. Time evolution of the permeate volume for 1 (○) and 30 (●) operational cycles for membrane A (a) and B (b).

Figure 4

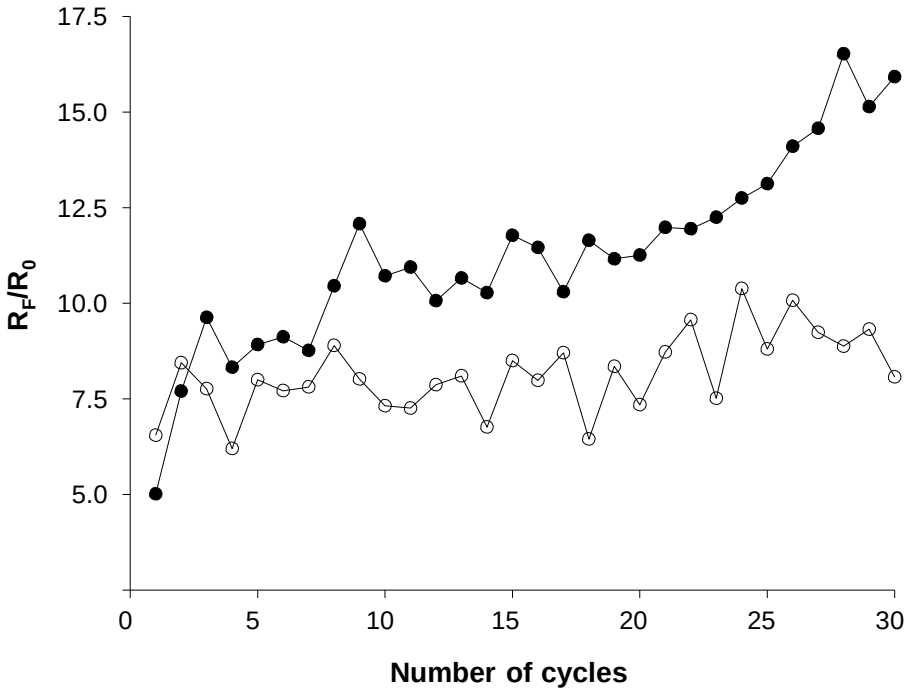

Fig. 4. Evolution of the normalized fouling resistance with the number of cycles for membrane A (○) and B (●).

Figure 5

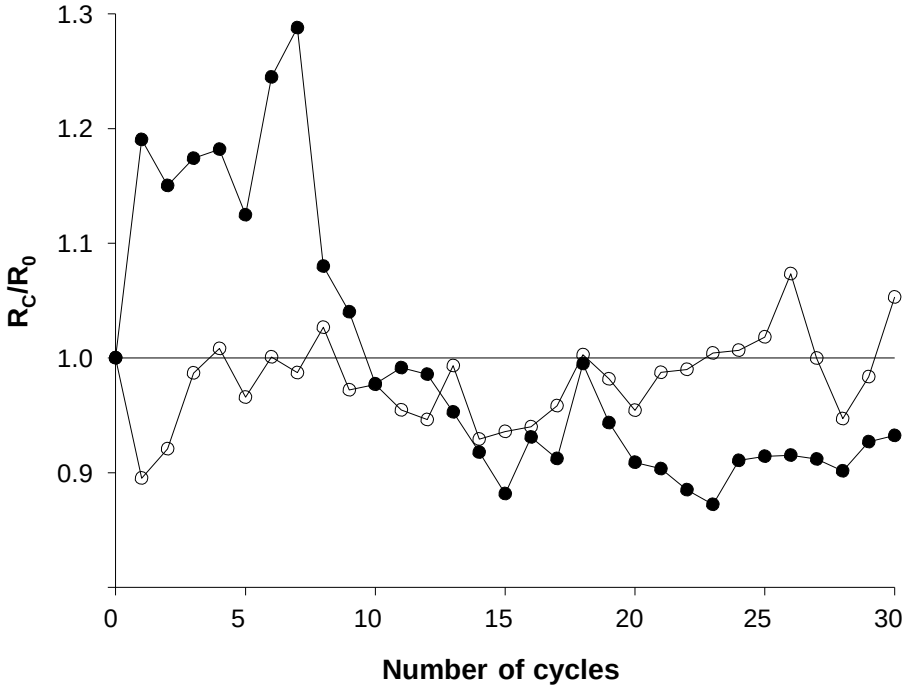

Fig. 5. Evolution of the normalized membrane resistance with the number of cycles for the protocol at 50 ºC (○) and 60 ºC (●).

Figure 6a

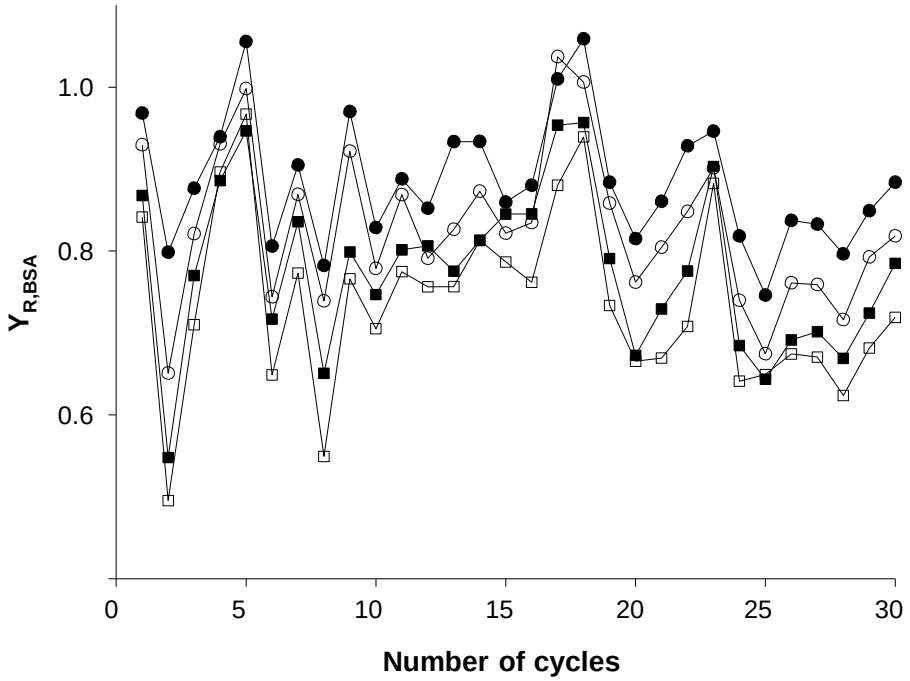

Figure 6b

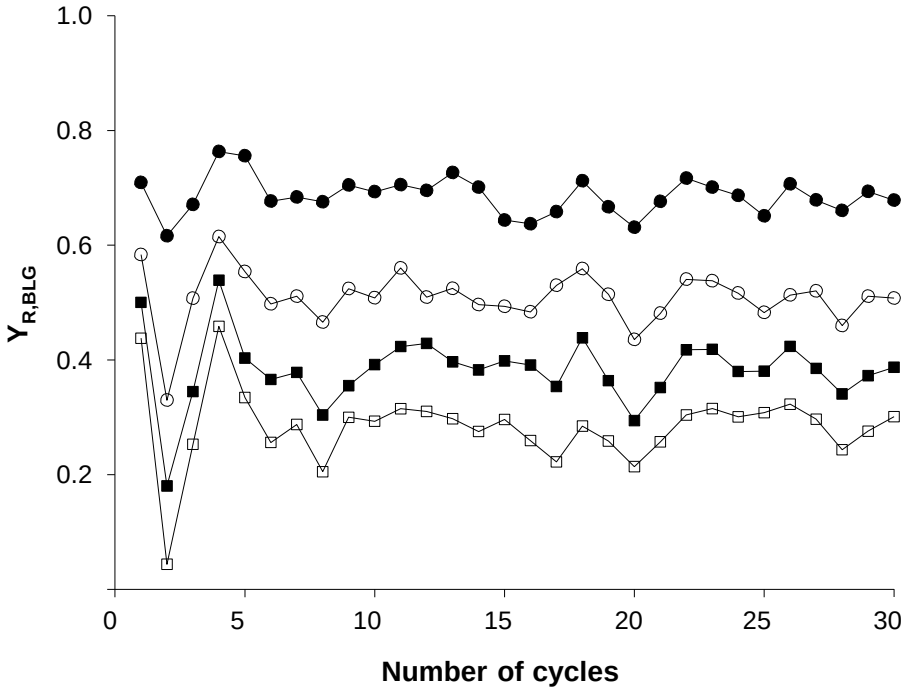

Fig. 6. Evolution of the retentate yield of BSA (a) and BLG (b) with the number of cycles for membrane A. Series represent diafiltration times of 1 (●), 2 (○), 3 (■) and 4 h (□).

Figure 7a

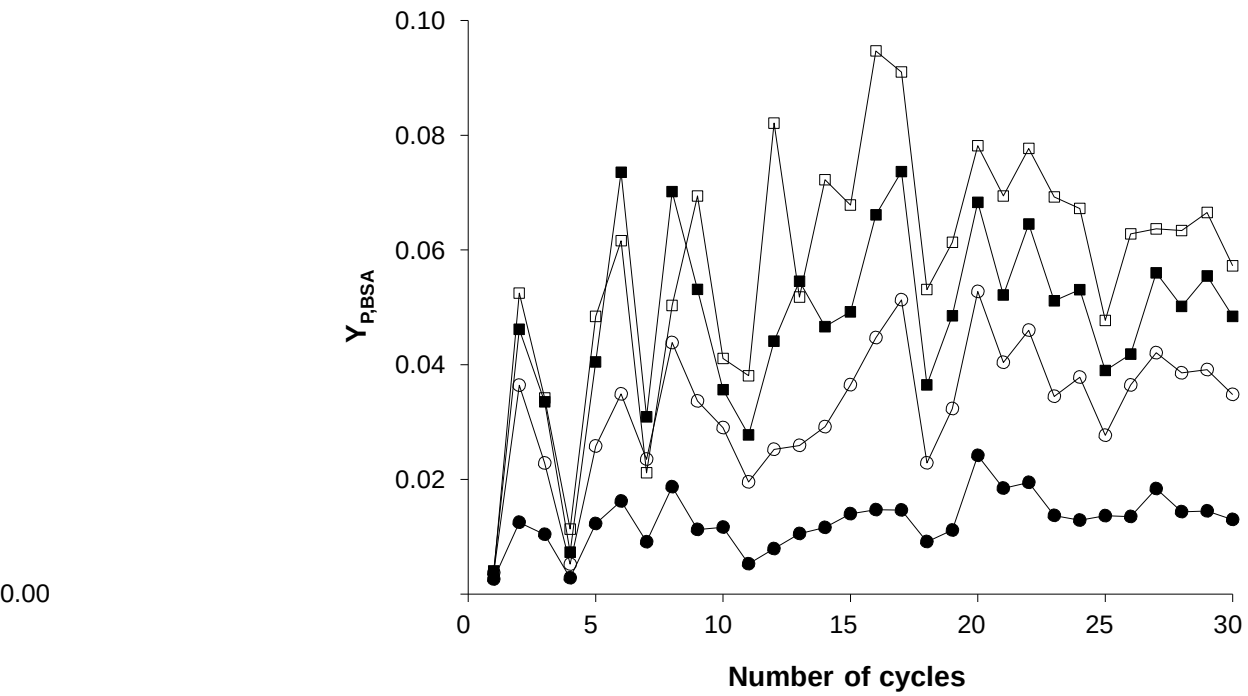

Figure 7b

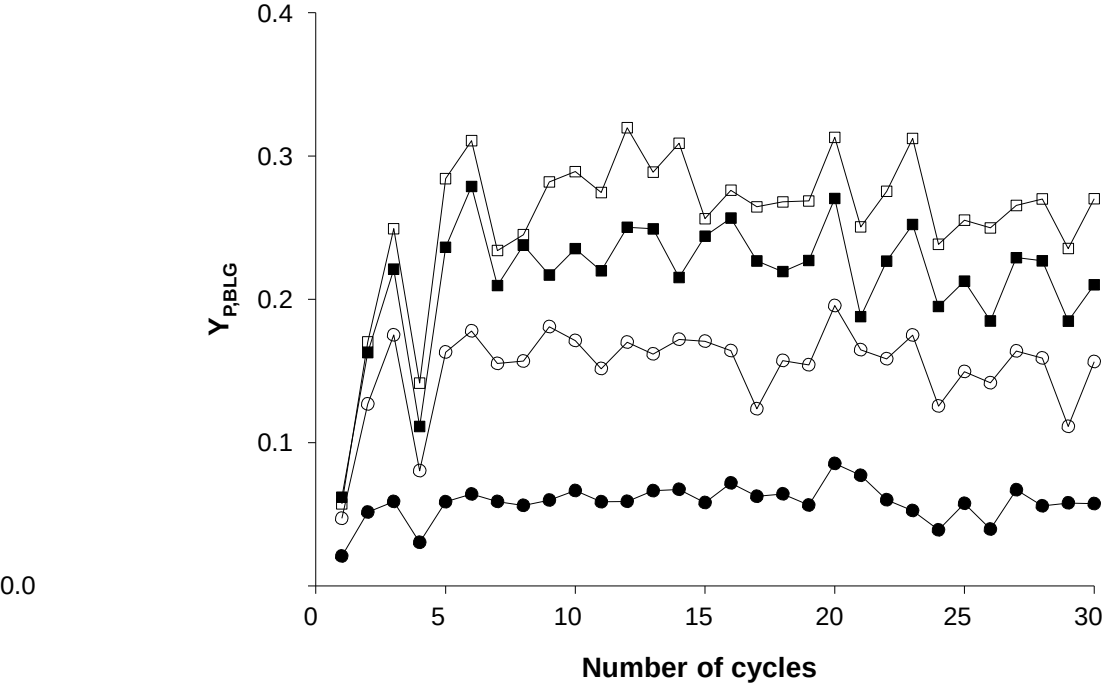

Fig. 7. Evolution of the permeate yield of BSA (a) and BLG (b) with the number of cycles for membrane A. Series represent diafiltration times of 1 (●), 2 (○), 3 (■) and 4 h (□).

Figure 8a

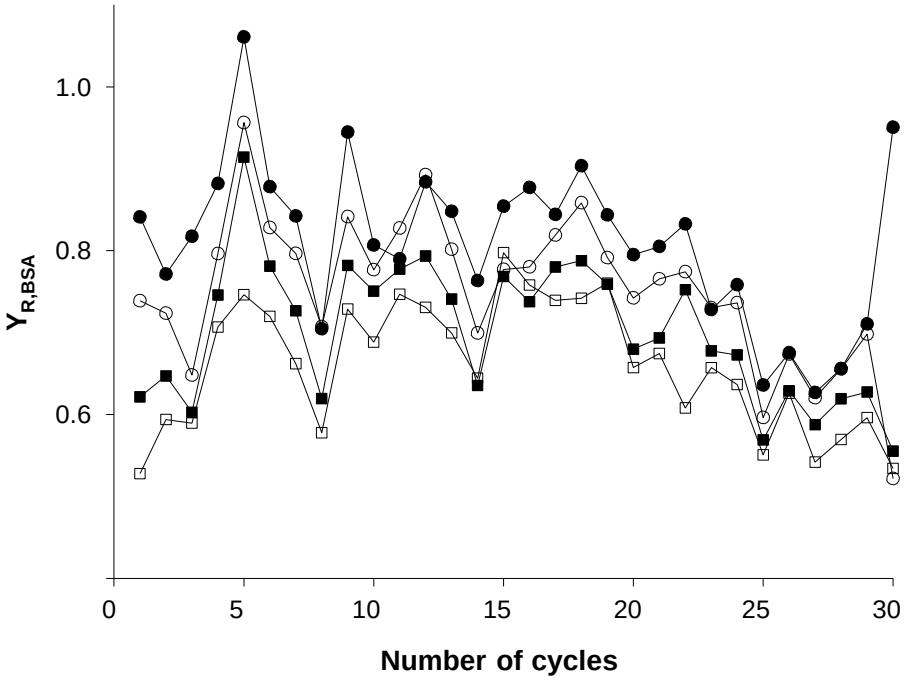

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Figure 8b

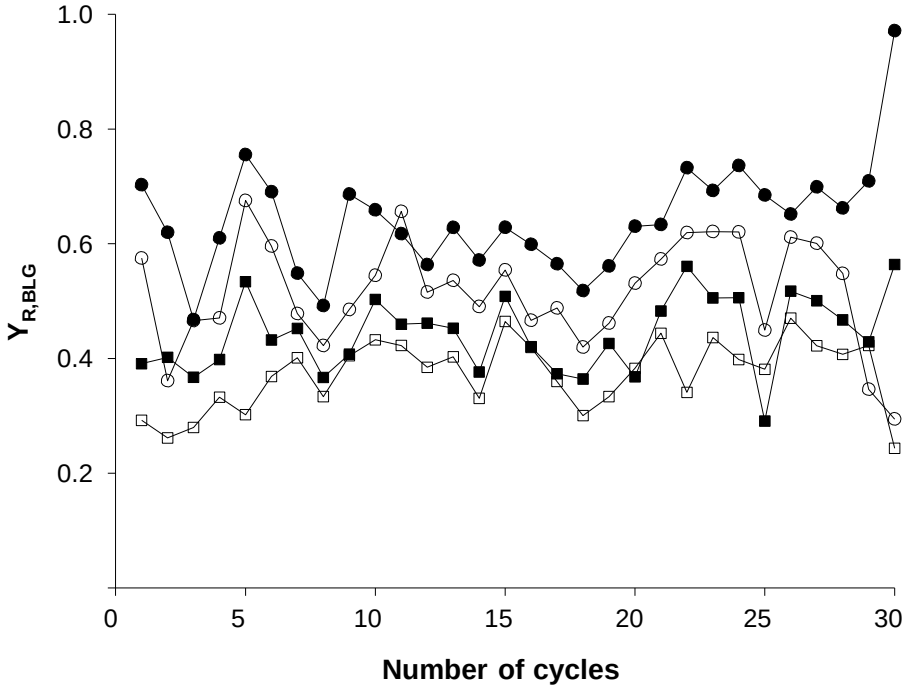

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Fig. 8. Evolution of the retentate yield of BSA (a) and BLG (b) with the number of cycles for membrane B. Series represent diafiltration times of 1 (●), 2 (○), 3 (■) and 4 h (□).

Figure 9a

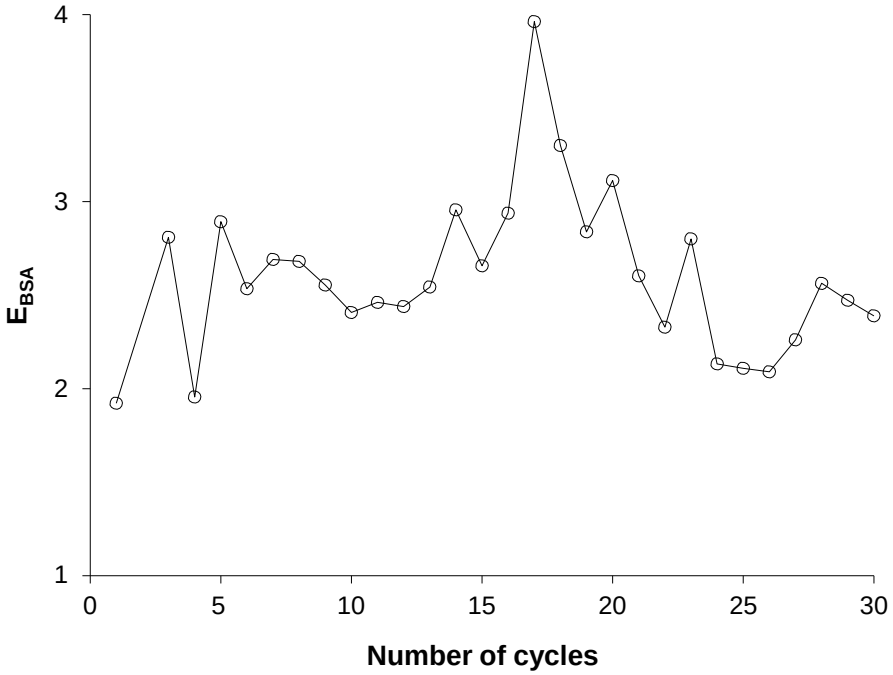

Figure 9b

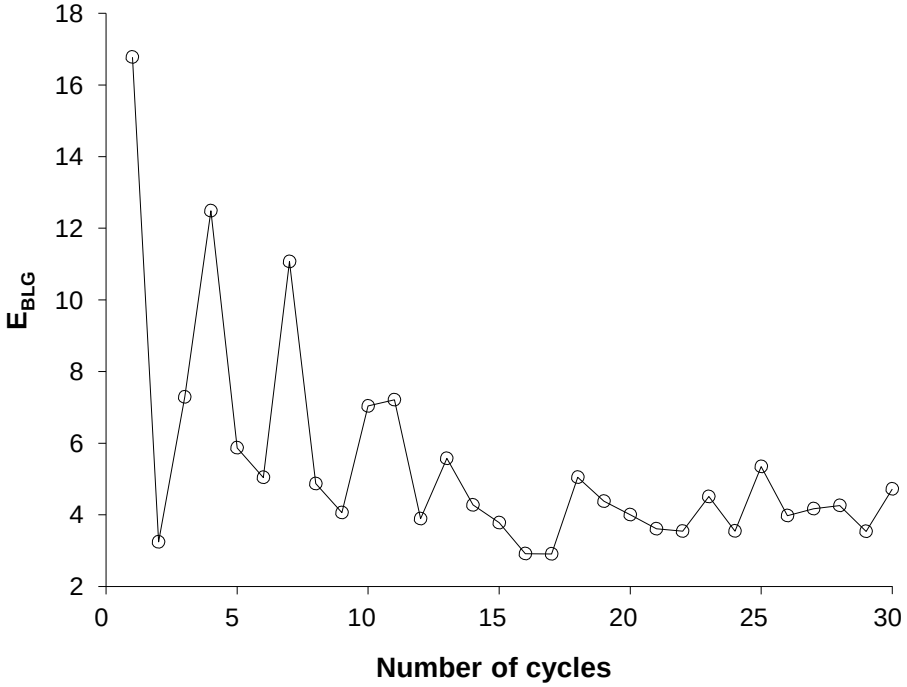

Fig. 9. Efficiency on the final separation of BSA (a) and BLG (b) as a function of the number of cycles for membrane A.

Figure 10

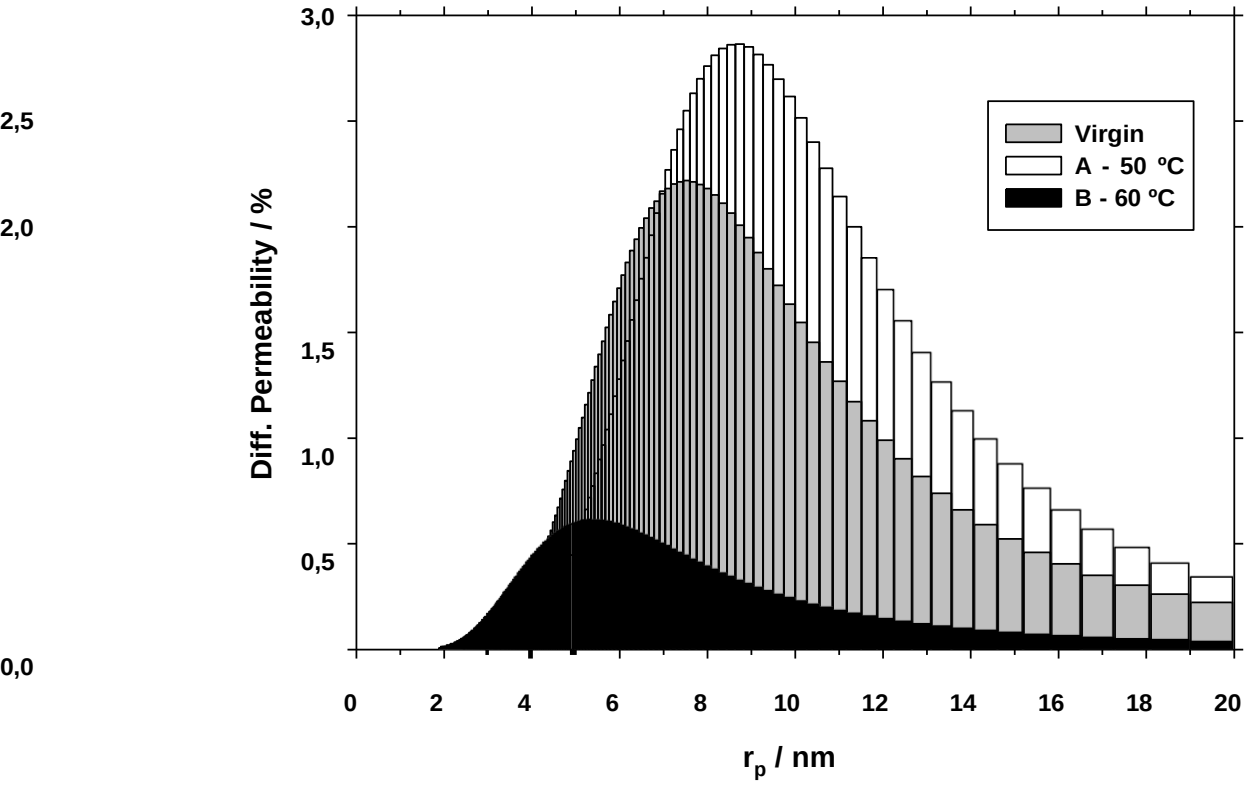

Fig. 10. Pore size distribution from permeability data for the virgin membrane, membrane A (50 ºC) and membrane B (60 ºC).

Table 1. Gradient employed in the analysis of β-lactoglobulin and BSA by RP-HPLC.

t (min)	Solvent B (%)
0-1	20
1-6	20-40
6-16	40-45
16-19	45-50

19-20	50
20-23	50-70
23-24	70-100
24-25	100
25-27	100-20
27-30	20

Table 2. Results of LLDP analysis for: virgin membrane, membrane A and membrane B.

Membrane	**Limit Permeability** $m \cdot Pa^{-1} \cdot s^{-1} \cdot 10^{10}$	**Mean Pore Radius** **nm**	**Porosity** **%**	**MWCO** **kDa**
Virgin	3,47 ± 0,05	7.7 ± 0,3	67 ± 12	211 ± 50
A - 50ºC	3,56 ± 0,21	8.8 ± 0,1	56 ± 6	268 ± 20
B - 60ºC	1,55 ± 0,09	5.5 ± 0,1	48 ± 8	116 ± 15