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Abstract

In this work, four ultrafiltration (UF) membranes with molecular weight cut-offs (MWCOs) of 5, 15, 30 and 50 kDa, respectively, were fouled with 1 % BSA aqueous solutions and cleaned with different saline solutions. The influence of MWCO, membrane material and operating conditions on the cleaning efficiency was investigated. Saline solutions were able to clean the 5, 15 and 30 kDa membranes, but not the 50 kDa membrane. NaCl, NaNO$_3$, NH$_4$Cl and KCl were the most effective salts. The cleaning tests demonstrated that the higher the temperature of the saline solution was, the higher the cleaning efficiency was also. In addition, an increase in the crossflow velocity resulted in an increase in the hydraulic cleaning efficiency (HCE). However, there was an optimum value of salt concentration to clean the membrane effectively. Response Surface

Methodology was used to investigate the relationship between salt concentration and temperature in the cleaning process.

1. Introduction

In the last years, the purification and fractionation of whey proteins has grown in interest due to their nutritional, functional and biological characteristics and their therapeutic and food applications. The major whey proteins are β-lactoglobulin (βLG), α-lactalbumin (α-LA), bovine serum albumin (BSA) and immunoglobulin (Ig) [1]. Membrane separation technologies are being increasingly used in the food industry to fractionate and purify these proteins as an alternative to the conventional concentration and purification methods [2]. Among all the separation processes, ultrafiltration (UF) is one of the most used in the dairy industry. Its most well-known applications are milk dehydration and whey concentration [3].

Cleaning of UF membranes in the food industry is a key step of the global process. In most cases, cleaning needs to be carried out up to once a day to remove fouling from the membrane surface and to recover the permeability and selectivity of the membrane [4]. Some authors reported that more than 80 % of the total production costs in the dairy industry are attributed to the cleaning of the equipments [1]. For that reason, choosing the best cleaning agents and operating conditions to optimize the cleaning processes is necessary [3].

2

Membrane cleaning can be performed by means of physical, chemical and biological cleaning procedures. However, chemical methods are the most often used in the food industry [3]. Some of these chemical agents are acids, alkalis, surfactants, disinfectants or combinations of them. Choosing one or another depends on the feed composition, the type of foulants deposited on the membrane surface and the structure of the membranes [5]. However, in order to clean the membranes fouled with milk and whey, most of the studies in the literature recommend the same cleaning protocol: one alkali washing step followed by an acid washing step [2, 3, 6]. If the membrane separation characteristics are not the initial ones after this protocol, another washing stage based on disinfectants or surfactants, such as sodium hypochloride or sodium dodecyl sulphate, can be performed [1].

Nevertheless, these conventional cleaning operations may be aggressive for the membranes and may damage them more or less quickly, reducing the membrane lifetime and selectivity and increasing the productions costs (large energy and water consumption and long duration of the cleaning cycle) [4]. In addition, conventional cleaning agents have a negative impact on the environment when they are discharged as waste streams after the cleaning process. To overcome these problems, cleaning based on alternative techniques such as ultrasounds, saline solutions or electromagnetic fields are growing in interest in the last years [7, 8]. However, only a few papers are related to the cleaning of membranes by means of saline solutions [9]. In this case, previous studies [9] demonstrated that inert salts or even seawater can be effective for removing the natural organic matter deposited on a reverse osmosis membrane. Saline solutions cause changes on the cross-linked fouling layer due to the different concentration in the bulk solution and in the gel layer, breaking the integrity of the gel layer. Then, an ion-exchange reaction between salt ions and foulant molecules occurs, which results in the complete breakup of the gel layer. On the other

3

hand, several authors [10, 11, 12] reported the salting-out and salting-in capability of several anions and cations to decrease or increase protein solubility, respectively. They investigated the protein-protein interactions in different salt solutions at pH values above and below the isoelectric point (pI) of the proteins.

The aim of this work is to evaluate the ability of saline solutions to clean two flat-sheet polymeric UF membranes with molecular weight cut-offs (MWCOs) of 5 and 30 kDa and two monotubular ceramic UF membranes with MWCOs of 15 and 50 kDa fouled by BSA 1 % (w/w). The influence of MWCO, membrane material and operating conditions of the cleaning process (temperature, crossflow velocity and salt concentration of the cleaning solution) on the efficiency of the cleaning process was investigated. The optimal values of these operating conditions to achieve the highest cleaning efficiency were determined by means of a Response Surface Methodology (RSM) analysis.

2. Response Surface Methodology

A common method to study the performance of membrane processes is the "one-factor-at-a time" approach, which is based in the variation of one factor while the other factors are kept constant [13]. In some experimental set-ups, the number of factors to study is large. In the case of membrane technologies these factors usually are transmembrane pressure, crossflow velocity, time, temperature, solute concentration and pH. In this case, a traditional approach may result in a lot of experimental runs that require high energy, chemicals and time consumption. In addition, this approach ignores interaction effects between the considered factors and it is not appropriate to optimize the process [14]. To overcome these limitations, the RSM analysis can be used.

RSM is a combination of statistical and mathematical techniques widely used in the development, improvement and optimization of processes that contain a variable of interest (response variable) influenced by several variables, and it is used to evaluate the relative significance of these variables even in the presence of complex interactions. The objective of RSM is the investigation of the response variable over the entire factor space, determining the optimum operating conditions or a region where the response variable satisfies the operating specifications (near to its optimal value) [13, 14, 15].

3. Experimental

3.1. Materials

Fouling experiments were performed using a BSA aqueous solution with a concentration of 1 % (w/w) as a feed solution. BSA was supplied in powder (98% purity, A3733, Sigma-Aldrich) and feed solutions were prepared by dissolving BSA in deionized water until the desired concentration was achieved. BSA has a molecular weight of approximately 66 kDa and its isoelectric point is 4.9, according to the manufacturer. Feed solutions had a pH of about 7, thus BSA has mainly negative net charge on its surface. Its configuration is elliptic (11.6x2.7x2.7 nm) and it is one of the most widely used whey proteins to prepare model solutions for UF experiments [16, 17, 18]. According to the information provided by the manufacturer, BSA used in the experiments was prepared using a heat shock fractionation method followed by a lyophilization process to obtain BSA in a powder form.

5

After the fouling step, membranes were rinsed with deionized water to remove the loose protein deposit on the membrane surface. Then, the membranes were cleaned with several saline solutions (NaCl, NaNO$_3$, Na$_2$SO$_4$, KCl, and NH$_4$Cl). The chemicals were supplied by Panreac (Spain). The solutions were prepared dissolving the salts in deionized water with no pH adjustment. Different salt concentrations were used to study the influence of salt type and concentration on HCE.

Finally, the membranes were cleaned to recover their initial permeability conditions if it was necessary. In the case of the polymeric membranes, NaOH (Panreac, Spain) aqueous solutions with a pH of 11 at 45 ºC were used, while NaClO (Panreac, Spain) aqueous solutions with a concentration of 250 ppm (adjusting the pH at 11 with NaOH supplied by Panreac, Spain) were used to clean the ceramic membranes.

3.2. Membranes

Two monotubular ceramic membranes INSIDE CéRAM^{TM} (TAMI Industries, France) and two flat sheet polymeric membranes (Microdyn-Nadir, Germany) were used in the experiments. The ceramic membranes were 200 mm long with an internal diameter of 6 mm and an external diameter of 10 mm. They consisted of a TiO$_2$ support layer and a ZrO$_2$-TiO$_2$ active layer. The molecular weight cut-offs (MWCOs) of these membranes were 15 and 50 kDa, respectively. Polymeric membranes were polyethersulfone (PES) membranes with an effective area of 100 cm^2 and two different MWCOs (5 and 30 kDa).

3.3. Experimental set-up

6

All fouling and cleaning experiments were performed in a VF-S11 UF plant (Orelis, France). The main parts of the plant are (Fig. 1): a feed tank, a temperature regulating system, a variable speed volumetric pump, two manometers placed at both sides of the membrane module and a scale. The feed tank consists of a 10 L stainless steel tank with a double jacket, which contained the BSA or the cleaning solution. The temperature was kept constant during the experiments by means of the temperature regulating system. Crossflow velocity was controlled with a variable speed volumetric pump. The maximum operating pressure was 4 bar. Pressure drop across the membrane module was measured with two manometers. Finally, a scale (0.001 g accuracy) was used to gravimetrically determine the permeate flux.

Both the retentate and the permeate were recirculated back to the feed tank in order to keep the concentration of the feed solution constant, except in the case of the rinsing step.

3.4. Experimental procedure

3.4.1. Fouling experiments

Fouling tests were carried out with a 1 % (w/w) BSA aqueous solution at a transmembrane pressure of 2 bar and a crossflow velocity of 2 m·s^{-1}. The temperature of the fouling solution was set to 25 ºC. The duration of the fouling tests was 2 h. Those conditions were selected according to previous studies on whey ultrafiltration [19]. During the experiments, the permeate flux and the hydraulic resistance were monitored to check the fouling process and to ensure that reproducible values of flux and resistance were obtained in all runs.

7

Each fouling experiment was repited a minimum of 10 times and the maximum error among the runs was 10 %.

Permeate concentration of BSA during the fouling tests was measured by an UV-visible spectrophotometer (Hewlett-Packard 8453) at a wavelength of 278 nm. This was the wavelength of maximum absorbance for the BSA solution used as feed. Rejection coefficient was calculated as follows (Eq. 1):

$$\text{Rejection}\,(\%) = \left(1 - \frac{C_p}{C_b}\right)100 \qquad \text{Eq. 1}$$

where C_p is the permeate BSA concentration and C_b is the BSA concentration in the feed solution (1 % (w/w)).

At the end of the tests, the hydraulic resistance after the fouling step (R_f) was evaluated by means of Darcy's law (Eq. 2).

$$J = \frac{\Delta P}{\mu \cdot R} \qquad \text{Eq. 2}$$

where J is the permeate flux, ΔP is the transmembrane pressure, μ is the feed solution viscosity and R is the total hydraulic resistance.

3.4.2. Rinsing and cleaning experiments

Cleaning experiments were performed at three different temperatures (25, 37.5 and 50 º C) with different salt concentrations (0, 1.25, 2.5, 5, 7.5, 25 and 100 mM). Transmembrane pressure was 1 bar and crossflow velocity was varied between 1.2 and 4.2 m·s^{-1}. The pH of the saline solutions tested varied from 6.8 to 7. Each cleaning experiment was performed twice and the maximum error among the runs was 10 %. After the fouling and cleaning steps, the membranes were rinsed with deionized water at 25 ºC. Rinsing steps were carried out at the same experimental conditions of transmembrane pressure and crossflow velocity as those of the cleaning step. Low transmembrane pressures favour foulants removal and relax the compressible fouling layer formed in the fouling step [4].

Finally, if the initial permeability of the membranes was not recovered after the cleaning process, the ceramic membranes were cleaned with a NaClO aqueous solution at pH 11 and the polymeric membranes were cleaned with NaOH aqueous solutions at pH 11, as it was indicated in section 3.1 and it was recommended by the manufacturer.

3.5. Evaluation of membrane cleanliness

The efficiency of the cleaning protocol was determined following the method developed by Daufin *et al.* [20] and Matzinos and Álvarez [19]. These authors determined the hydraulic resistance of the membrane after each step (fouling, first rinsing, cleaning and second rinsing) when it was cleaned with sodium hydroxide solutions. They proposed the term "hydraulic rinsing efficiency" (HRE) to evaluate the efficiency of the first rinsing to restore the membrane permeability. The HRE is calculated as follows:

$$HRE = \frac{R_f - R_{r1}}{R_f - R_m} \cdot 100$$

Eq. 3

9

where R_f is the hydraulic resistance after the fouling step, R_{r1} is the hydraulic resistance after the first rinsing step and R_m is the hydraulic resistance of the new membrane.

These authors [19, 20, 21], used a similar equation (Eq. 3) to evaluate the efficiency of the entire cleaning process (after the second rinsing) to restore the initial membrane permeability:

$$HCE = \frac{R_f - R_{r2}}{R_f - R_m} \cdot 100 \qquad \text{Eq. 4}$$

where HCE is the hydraulic cleaning efficiency and R_{r2} is the hydraulic resistance after the second rinsing.

3.6. AFM measurements

The roughness of the membranes studied was measured by atomic force microscopy (AFM) using a Multimode atomic force microscope (Veeco, Santa Barbar, CA, USA) equipped with a NanoScope V controller. Measurements were performed at ambient conditions using the tapping mode of imaging. Roughness values were obtained from 5 μm x 5 μm samples and considering the average value of five areas of 1 μm x 1 μm. Among the different parameters to evaluate the membrane roughness, the Root Mean Square roughness (R_q) was selected. This is one of the most often used parameters to study membrane roughness. It represents the standard deviation of the height values (Z) of the surface within a specific area, according to the Eq. 5 [22]:

10

$$R_q = \sqrt{\frac{\sum \left(Z_i - Z_{avg}\right)^2}{N_p}} \qquad \qquad \text{Eq. 5}$$

where Z_i is the Z value currently measured, Z_{avg} is the average of the Z values and N_p is the number of points within the given area.

3.7. RSM analysis

After the cleaning processes, a RSM analysis was carried out to evaluate which values of the operating conditions resulted in the highest HCE. The RSM analysis was performed with the Statgraphics® software using a factorial design. The experimental data used for the statistical analysis is shown in Table 1. A relationship between the response variable (HCE) and the design variables (temperature, T_c, NaCl concentration, C, and crossflow velocity, v) was obtained. A Multiple Linear Regression analysis was applied to search for a model equation for HCE as a function of the operating conditions studied. Firstly, all the independent variables and their interactions were taken into account. Then, the coefficients of the regression model with p-values higher than 0.05 were neglected because they were not significant and a new regression analysis was performed.

3.8. Optimization method

After the RSM analysis, an optimization algorithm based on a pattern search was used to evaluate the values of temperature, NaCl concentration and crossflow velocity that maximize the HCE for each membrane. The optimization method was performed using the

11

"patternsearch" function of Matlab® software. "Patternsearch" finds the minimum of an objective function by means of a pattern search. As the aim of this work is to achieve the maximum of the model equations HCE_5, HCE_{15} and HCE_{30}, the objective functions selected for the "patternsearch" algorithm were the negative form of these equations ($-HCE_5$, $-HCE_{15}$ and $-HCE_{30}$). In addition, the maximum value of temperature was limited to 50 ºC and the maximum value of crossflow velocity was limited to 2.18 $m \cdot s^{-1}$ for the 5 and 30 kDa membranes and 4.2 $m \cdot s^{-1}$ for the 15 kDa membrane, as these were the higher values tested for these design variables.

4. Results and discussion

The values of R_m for the membranes used in the experiments were: $9.453 \cdot 10^{12}$, $5.001 \cdot 10^{12}$, $3.794 \cdot 10^{12}$ and $1.921 \cdot 10^{12}$ m^{-1}, for the membranes of 5, 15, 30 and 50 kDa, respectively. These values were taken as a reference to calculate HCE.

4.1. Fouling experiments

Fig. 2 shows the evolution of permeate flux with time for all the membranes tested at a transmembrane pressure of 2 bar, a crossflow velocity of 2 $m \cdot s^{-1}$ and a temperature of 25 ºC. The experimental data that corresponds to the membrane of 50 kDa show a sharp flux decline in the first minutes of operation. This is not observed for the membranes of 5, 15 and 30 kDa, which show a much lower flux decline rate. This can be attributed to the fact that the 50 kDa membrane shows a MWCO much closer to the molecular weight of BSA molecules (66 kDa) than the rest of the membranes. These results are in accordance with previous works. For example, Qu *et al.* [23] fouled three PES membranes of 10, 30 and

100 kDa with extracellular organic matter (EOM). The molecular weight distribution was divided into two fractions, a high molecular weight fraction (greater than 100 kDa) and a low molecular weight fraction (lower than 100 kDa). They observed that the membranes of 10 and 30 kDa showed the most severe fouling in terms of high relative flux reduction and the worse fouling reversibility. This was due to the fact that the MWCOs of these membranes and the molecular weight of low molecular weight EOM were more similar than in the case of the 100 kDa membrane. Therefore, low molecular weight EOM can penetrate inside the pores of the 10 and 30 kDa membranes and cause pore constriction. As a consequence, these membranes showed more severe fouling. These authors demonstrated that fouling is more severe when the difference between the membrane MWCO and the molecular weight of solute molecules is lower.

Fig. 3 shows the evolution of the rejection coefficient with time during the fouling step for all the membranes tested. As it can be observed, the rejection coefficient achieved steady-state values after about 100 min of operation for all the membranes tested. Although all the membranes had a rejection coefficient higher than 99 % at the end of the step, the 50 kDa membrane showed the lowest rejection coefficient. This can be explained taking into account the difference between the size of BSA molecules and the membrane pore size. That difference is larger for the low MWCO membranes (5 and 15 kDa) and thus, the values of rejection are higher than in the case of the 30 and 50 kDa membranes. This is in agreement with other authors [25, 26].

The evolution of the hydraulic resistance during the four steps (fouling, first rinsing, cleaning and second rinsing) can be observed in Fig. 4. The operating conditions for all the membranes in the cleaning and rinsing steps were the same. As it can be observed, the

13

original hydraulic membrane resistance was restored for the polymeric membranes (5 and 30 kDa) after the second rinsing step. However, the ceramic membranes were partially cleaned at the experimental conditions tested. The reason for this difference is the higher roughness of ceramic membranes in comparison with polymeric ones [24]. The roughness of all the membranes tested was measured by means of AFM and the values of R_q obtained were 0.487, 17.900, 1.657 and 27.133 nm for the membranes of 5, 15, 30 and 50 kDa, respectively. In order to increase the cleaning efficiency, higher crossflow velocities were considered to clean the ceramic membranes.

4.2. Cleaning experiments

4.2.1. Effect of the salt type

The 15 kDa membrane was used to investigate the effect of the salt type on the HCE. Fig. 5 shows the values of the HCE obtained when different saline solutions were used to clean this membrane at 25 ºC. The values of the HCE were also compared with the values of the HRE. As it can be observed, the highest values of HCE were obtained for chloride salts and NaNO$_3$. For these saline solutions the HCE was very similar, varying from 45.9 to 55.3 %. These values were considerably higher than the HRE (22.8 %). Therefore, these salts were able to remove part of the fouling layer at the operating conditions considered.

Among all the salts tested, Na$_2$SO$_4$ showed the lowest value of HCE (23.4 %) at the experimental conditions tested. This value is very similar to the value of HRE (22.8 %). Therefore, at 25 ºC and 100 mM no improvement on the cleaning efficiency was observed

14

when a Na_2SO_4 solution was used to clean the membrane fouled with a BSA aqueous solution of 1 % (w/w).

Lee and Elimelech [9] observed that saline solutions were able to clean reverse osmosis membranes fouled with organic matter. They indicated that saline solutions were able to decrease dramatically the foulant-foulant adhesion forces. Lee and Elimelech [9] performed the cleaning of reverse osmosis membranes fouled with alginates with the same saline solutions as the ones used in this paper to clean the UF membrane fouled with proteins. The differences between the hydraulic cleaning efficiencies obtained by Lee and Elimelech [9] at 25 ºC and the results shown in Fig. 4 can be due to the different membranes and feed solutions considered. Moreover, these authors used very low feed concentrations. They fouled reverse osmosis membranes with a feed solution of 0.02 $g \cdot L^{-1}$, while, in this paper, a concentration of 10 $g \cdot L^{-1}$ was used to foul UF membranes.

Several authors studied the effect of salts on protein-protein interactions. Tsumoto *et al.* [27] observed that, at the same concentration, several salts cause a decrease in protein solubility (salting-out effects) while others increase protein-solubility (salting-in effects). The effect of the type of salt was tested at a concentration of 100 mM. This effect was related by several authors to the surface tension of the salt solution (the higher the surface tension, the higher the salting-out effect) and to the type of interactions between the salt and the proteins. Tsumoto *et al.* [27] studied the preferential interactions of several salts with BSA. They demonstrated that Na_2SO_4 was a strong salting-out salt due to unfavorable interactions with BSA. Thus Na_2SO_4 enhances protein-protein and protein-surface interactions and decreases protein solubility.

15

Hofmeister [10, 11] investigated the effect of salts on protein precipitation at high salt concentrations. A ranking of the effectiveness of various cations and anions to precipitate proteins was named as Hofmeister series. The strongest effectiveness was observed for SO_4^{2-}. Moreover, anions appear to have a greater effect on protein solubility than cations. Zhang [12] demonstrated as well that SO_4^{2-} is the most salting-out anion among those included in the series. This is in agreement with the low value of HCE obtained when the cleaning step was performed with Na_2SO_4 at a concentration of 100 mM. Zhang [12] reported that at pH above the protein isoelectric point, when the protein is negatively charged, multivalent cations can neutralize the net protein charge, weakening the electrostatic intermolecular interactions more effectively than monovalent cations, and decreasing protein solubility. On the other hand, Cl^- and NO_3^- are able to specifically bind to the proteins surface more strongly than monovalent cations. Therefore, the repulsive intermolecular interactions increase, thus reducing protein-protein interactions, and raising protein solubility. This can explain the higher value of HCE observed for chlorides and nitrates.

Among the salts with a high value of HCE, NaCl was chosen to continue this work, due to the lower pollutant character and cost compared to the other salts tested.

4.2.2. Effect of salt concentration

Fig. 6 shows the values of HCE obtained when NaCl solutions at different concentrations were used to clean the 5, 15 and 30 kDa MWCO membranes at two different temperatures: 25 and 50 ºC. At 50 ºC, it can be observed that HCE increased as NaCl concentration increased up to 2.5 mM for the 15 kDa membrane and up to 5 mM for the 5 and 30 kDa

16

membranes. At these experimental conditions, HCE values of 100 % were achieved for all the membranes. At 25 ºC the HCE observed was lower and it increased with concentration up to 2.5 mM, however, a further increase of concentration resulted in a decrease of the HCE.

As other authors explained [28, 29, 30], at low salt concentrations the surface tension decreases when salt concentration increases. However, at high salt concentrations the surface tension increases linearly with concentration. According to Tsumoto *et al.* [27], a decrease in the surface tension results in an enhancement of the salting-in effects of the saline solutions. Therefore, the salting-in effects are better observed at low salt concentrations.

On the other hand, HCE cannot increase with salt concentration if the physical conditions for the mass transport of the protein deposits removed from the gel layer to the bulk solution are not the optimal. There is an optimal salt concentration to carry out the cleaning process. Up to this concentration, when salt concentration increases, HCE increases. But above this concentration HCE does not increase with salt concentration or it can even decrease. This may be due to the fact that membrane fouling due to the accumulation of salt molecules on the membrane surface or inside its pores may also occur. In this case, fouling and cleaning mechanisms become competitive. This is in agreement with the results reported by Lee and Elimelech [9] and Cabero Cabero [31]. The increase in HCE with an increase of salt concentration is greater at high temperatures. This is due to the effect of temperature on protein deposits. This effect is commented in section 4.2.3.

17

Fig. 7 shows the values of the HCE when the 50 kDa membrane was cleaned with NaCl solutions at different concentrations at 50 ºC. As it can be observed, HCE increased as NaCl concentration increased up to a certain value (7.5 mM) and afterwards it decreased. However, the maximum value of HCE for the 50 kDa membrane was 79.19 %, while in the case of the 15, 5 and 30 kDa membranes (Fig. 6), a 100 % HCE was achieved at low salt concentrations (2.5 and 5 mM, respectively). Therefore, the 50 kDa membrane was not completely cleaned with NaCl solutions at the experimental conditions tested. This can be due to the more severe fouling that was observed for this membrane as it was commented in section 4.1. In this case, the temperature of 25 ºC was not considered as even lower HCE could be expected.

4.2.3. Effect of cleaning solution temperature

Fig. 8 shows the values of HCE for the 5, 15 and 30 kDa membranes when the cleaning step was performed at different temperatures and a NaCl concentration of 5 mM. According to Fig. 6, 100 % of HCE was achieved for all the membranes at 50 ºC, but not in the case of 25 ºC. Thus, an intermediate temperature (37.5 ºC) was considered as well to investigate the effect of the cleaning solution temperature on the HCE. As it can be observed, the higher the temperature of the cleaning solution is, the higher the HCE is, achieving an efficiency of 100 % when the cleaning was carried out at 50 ºC for the 5, 15 and 30 kDa membranes. The increase of HCE when temperature varies from 25 to 37.5 ºC is higher than the increase of HCE when temperature increases from 37.5 to 50 ºC (about 85 % in the first case and 9 % in the second case). This effect can also be observed in Fig. 6. In this case, HCE was plotted as a function of concentration for two different

18

temperatures (25 and 50 ºC, respectively). As it can be observed, for each concentration HCE increases with temperature.

When the temperature of the saline solution increases, the surface tension decreases [32, 33]. The decrease in the surface tension is caused by the adsorption of hydrophilic ions from the air/water surface [34]. According to several authors, surface tension decreases linearly with temperature. As it was reported by Tsumoto *et al.* [27], the higher the surface tension, the stronger the salting-out effects of the salt.

Temperature has also an effect on protein solubility. In general, protein solubility increases with temperature up to 50 ºC. However, when the temperature of the solution is high enough during a certain time, the protein is denatured. Proteins are denatured due to the effect of temperature on the non-covalent bonds involved in the stabilization of secondary and tertiary structure. Denaturation decreases protein solubility compared to the native protein [35].

In addition, in mass transfer processes, the diffusivity coefficient increases as temperature rises. Therefore, the rate of transfer of solute molecules from the membrane surface towards the bulk solution is greater. Moreover, high temperatures can weaken the structural stability of the fouling layer, swelling it and facilitating its removal from the membrane surface. An increase in temperature may also increase the rate of the interaction between the salt and the deposited proteins [9].

For all these reasons, the most convenient temperature to carry out the cleaning of the 5, 15 and 30 kDa membranes fouled with BSA solutions is about 50 ºC (the highest temperature tested).

Fig. 9 shows the values of HCE obtained for the 50 kDa membrane when the cleaning step was performed at different temperatures (50, 60, 70 and 80 ºC) and a NaCl concentration of 7.5 mM. According to Fig. 7, at 50 ºC the highest HCE was obtained at a NaCl concentration of 7.5 mM for this membrane. Due to the effect of temperature on the HCE that was observed for the other membranes (5, 15 and 30 kDa), it was expected that an increase in temperature resulted in an increase in HCE for the 50 kDa membrane as well. From Fig. 9 it can be observed that HCE increases with temperature. However, the maximum value of HCE was 90.5 % at the highest temperature tested (80 ºC). Thus, the 50 kDa membrane was not completely cleaned despite the high temperatures considered. The reason for that is the more severe fouling due to the penetration of BSA molecules in the porous structure of this membrane in comparison with the membranes of lower MWCO (5, 15 and 50 kDa).

4.2.4. Effect of crossflow velocity

Fig. 10 shows the results of HCE for the 5, 15 and 30 kDa membranes when different crossflow velocities were tested at the optimal conditions of NaCl concentration (2.5 mM for the 15 kDa membrane and 5 mM for the 5 and 30 kDa membranes) and temperature (50 ºC). In the case of the ceramic membrane (15 kDa), higher crossflow velocities were tested due to the higher roughness of this membrane in comparison with the polymeric ones.

As it can be observed, when crossflow velocity increases HCE increases for all the membranes tested, achieving a HCE of 100 % at the highest crossflow velocity tested (4.2 m·s^{-1} for the 15 kDa membrane and 2.18 m·s^{-1} for the 5 and 30 kDa membranes). When crossflow velocity increases, the shear stress generated also increases and it can cause the erosion and removal of the protein deposit from the membrane surface [36, 37].

The effect of the crossflow velocity on the HCE was not tested for the 50 kDa membrane. The reason is that 4.2 m·s^{-1} is the highest crossflow velocity that can be reached in the UF plant. As it was previously shown, the HCE for this membrane was lower than 100 % at this crossflow velocity. Taking into account the results shown in this section, even lower values of HCE were expected if the crossflow velocity is decreased.

4.2.5. Statistical and optimization analysis

The efficiency of the cleaning process is influenced by operating conditions such as temperature, transmembrane pressure, crossflow velocity, the nature of the cleaning agent and its concentration, pH and ionic strength [4]. In this work, three of these operating conditions (temperature, NaCl solution and crossflow velocity) were varied.

Fig. 11 shows the surface contours for the response variable (HCE) as a function of the operating conditions of temperature and NaCl concentration for the membranes of 5, 15 and 30 kDa studied. Crossflow velocity was set at 2.18 m·s^{-1} for the 5 and 30 kDa membranes and at 4.2 m·s^{-1} for the 15 kDa membrane. The grey colour in the lower left corner shows the most unfavourable conditions, because lower values of HCE were

21

obtained (about 40 %). On the other hand, the black colour in the upper right corner represents the highest values of HCE achieved (greater than 95 %). At temperatures higher than 42-45 ºC and NaCl concentrations higher than 2.6-3 mM, the HCE was observed to be higher than 95 % for the three membranes considered. It is important to note that the higher the temperature of the cleaning solution was, the higher the HCE was for all the membranes tested. However, there was an optimal value of NaCl concentration to maximize the value of HCE, because further increases in salt concentration did not result in higher values of HCE.

In addition, mathematical relationships between the values of HCE and the operating conditions were obtained for the 5, 15 and 30 kDa membranes (Eqs. 6, 7 and 8, respectively). The statistically significant factors were the same for the 5 and 30 kDa membranes. The regression coefficients for each equation were 0.979, 0.893 and 0.962, respectively. Table 2 shows the results of the ANOVA.

$$\text{HCE}_5\ (\%) = a + b \cdot T_c + c \cdot C - d \cdot C^2 + e \cdot v^2 \qquad \text{Eq. 6}$$

$$\text{HCE}_{15}\ (\%) = a + f \cdot T_c \cdot C - d \cdot C^2 + g \cdot v \qquad \text{Eq. 7}$$

$$\text{HCE}_{30}\ (\%) = a + b \cdot T_c + c \cdot C - d \cdot C^2 + e \cdot v^2 \qquad \text{Eq. 8}$$

where HCE_5, HCE_{15} and HCE_{30} are the hydraulic cleaning efficiencies for the membranes of 5, 15 and 30 kDa, respectively, T_c is the temperature of the cleaning solution (ºC), C is the NaCl concentration (mM), v is the crossflow velocity (m·s^{-1}) and a, b, c, d, e, f and g are the estimated coefficients for each statistically significant parameter.

The results of the pattern-search optimization method are shown in Table 3. According to them, the optimal values of the design variables were: a cleaning solution temperature of 50 ºC for all the membranes tested, a crossflow velocity of 2.18 m·s^{-1} for the polymeric membranes (5 and 30 kDa), a crossflow velocity of 4.2 m·s^{-1} for the ceramic membrane (15 kDa) and NaCl concentrations of 4.61, 4.56 and 4.44 for the 5, 15 and 30 kDa membranes, respectively.

5. Conclusions

Different saline solutions were tested to clean the 15 kDa membrane fouled with a 1 % (w/w) BSA aqueous solution. The highest values of HCE were achieved when the cleaning was performed with NaCl, KCl, NaNO$_3$ and NH$_4$Cl solutions. The lowest value of HCE was obtained when Na$_2$SO$_4$ solutions were used. NaCl was selected to be used as cleaning agent because of its lower cost and environmental impact.

The cleaning solution concentration, temperature and crossflow velocity had a great effect on HCE. The results obtained demonstrated that the higher the temperature of the cleaning solution was, the higher the HCE was. In addition, as crossflow velocity increased, HCE also increased. However, when salt concentration increased up to a certain value (2.5 mM for the 5, 15 and 30 kDa membranes) HCE increased as well, but a further increase in NaCl concentration did not result in higher values of HCE or could even cause their decrease. Saline solutions were able to clean the 5, 15 and 30 kDa membranes. However, they were not effective to completely clean the 50 kDa membrane. This can be attributed to the more intense fouling observed for this membrane.

23

According to the results of the RSM analysis and the results of the pattern-search optimization method, the best operating conditions to clean the 5, 15 and 30 kDa membranes were a cleaning solution temperature of 50 ºC, crossflow velocities of 2.18 m·s^{-1} for the 5 and 30 kDa membranes and 4.2 m·s^{-1} for the 15 kDa membrane and NaCl concentrations of 4.61, 4.56 and 4.44 for the 5, 15 and 30 kDa membranes, respectively. The selected experimental conditions resulted in the maximum values of HCE$_5$, HCE$_{15}$ and HCE$_{30}$ (about 100 %).

An equation to relate HCE with the operating conditions was obtained by means of a Multiple Regression Analysis for the low MWCO membranes. For the polymeric membranes, the statistically significant factors were the same for both membranes.

Acknowledgements

Nomenclature

List of symbols

a   Model equation coefficient (%)

b   Model equation coefficient ($°C^{-1}$)

c   Model equation coefficient (mM^{-1})

C   NaCl concentration (mM)

C_b BSA concentration in the feed solution ($g·L^{-1}$)

C_p Permeate BSA concentration ($g·L^{-1}$)

d   Model equation coefficient (mM^{-2})

e   Model equation coefficient ($m^{-2}·s^2$)

f   Model equation coefficient ($°C^{-1}·mM^{-1}$)

g   Model equation coefficient ($m^{-1}·s$)

J   Permeate flux ($m^3·m^{-2}·s^{-1}$)

N_p Number of points within the given area (dimensionless)

ΔP Transmembrane pressure (bar)

R   Total hydraulic resistance (m^{-1})

R_m Resistance of the new membrane (m^{-1})

R_f Resistance after the fouling step (m^{-1})

R_{r1} Resistance after the first rinsing step (m^{-1})

R_c Resistance after the cleaning step (m^{-1})

R_{r2} Resistance after the second rinsing step (m^{-1})

R_q Root mean squared roughness (nm)

t   Filtration time (s)

T_c Temperature of the cleaning solution (°C)

v Crossflow velocity ($m·s^{-1}$)

Z   Height values of the surface sample (nm)

Z_i Z value currently measured (nm)

Z_{avg} Average of the Z values of the sample (nm)

Greek letters

μ Feed solution viscosity (kg·m^{-1}·s^{-1})

Abbreviations

AFM   Atomic force microscopy

BSA   Bovine serum albumin

EOM   Extracellular organic matter

HCE   Hydraulic cleaning efficiency

HRE   Hydraulic rinsing efficiency

MWCO   Molecular weight cut off

PES   Polyethersulfone

pI   Isoelectric point

RSM   Response surface methodology

UF   Ultrafiltration

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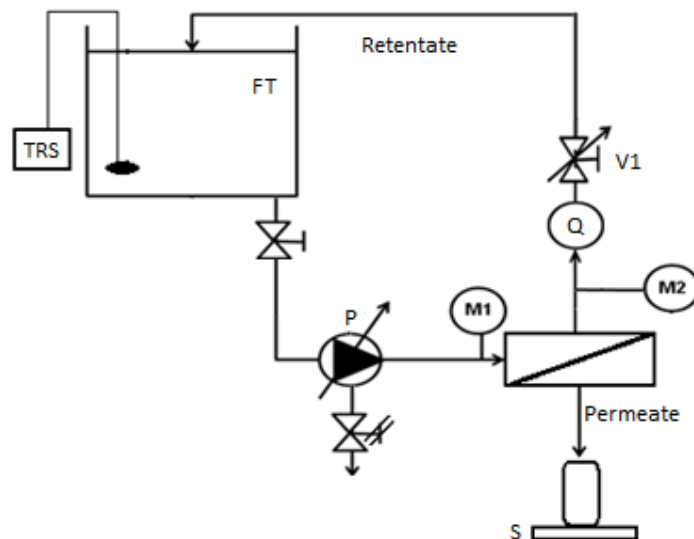
Fig. 1. Pilot plant used in the experiments (TRS: temperature regulating system; FT: feed tank; P: pump; M1 and M2: manometers; S: scale; V1: regulating pressure valve).
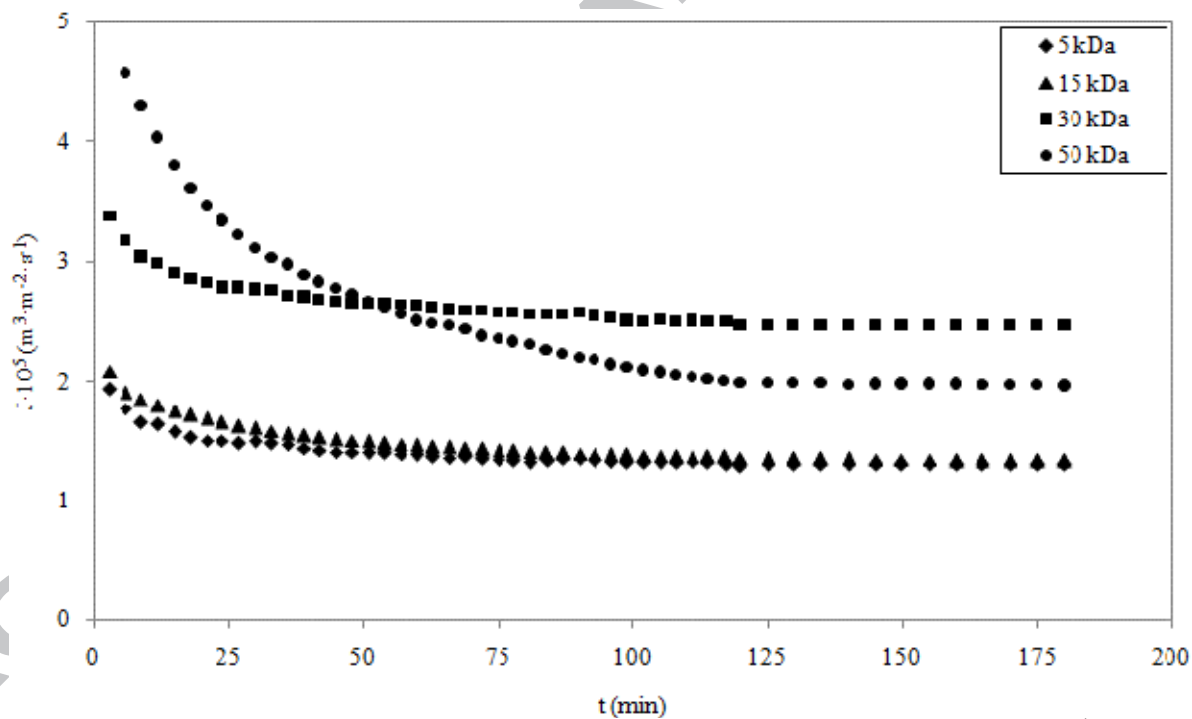

Fig. 2. Evolution of permeate flux with time during fouling experiments at 2 bar, 2 m·s^{-1} and 25 ºC.
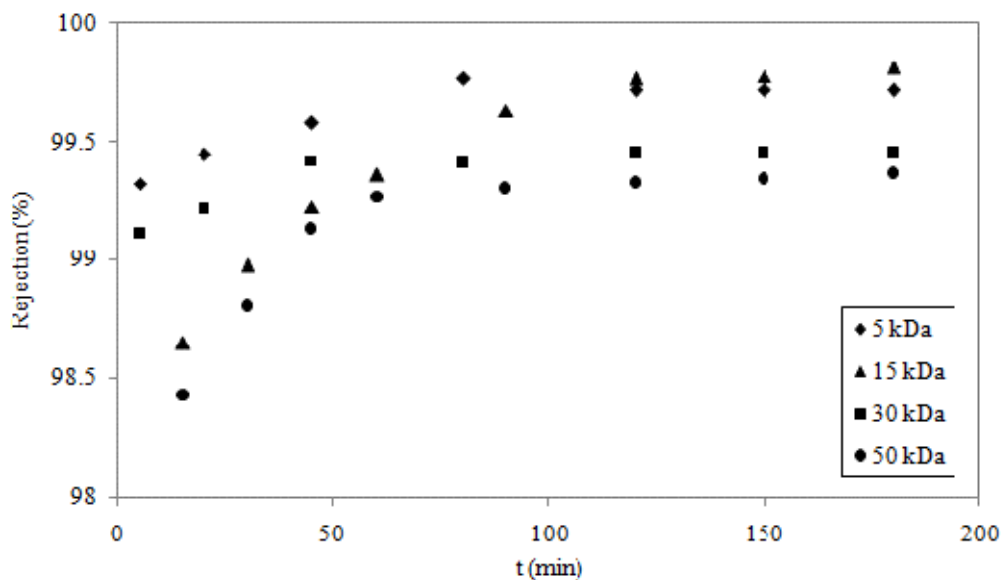
Fig. 3. Evolution of rejection with time during the fouling step for each membrane.
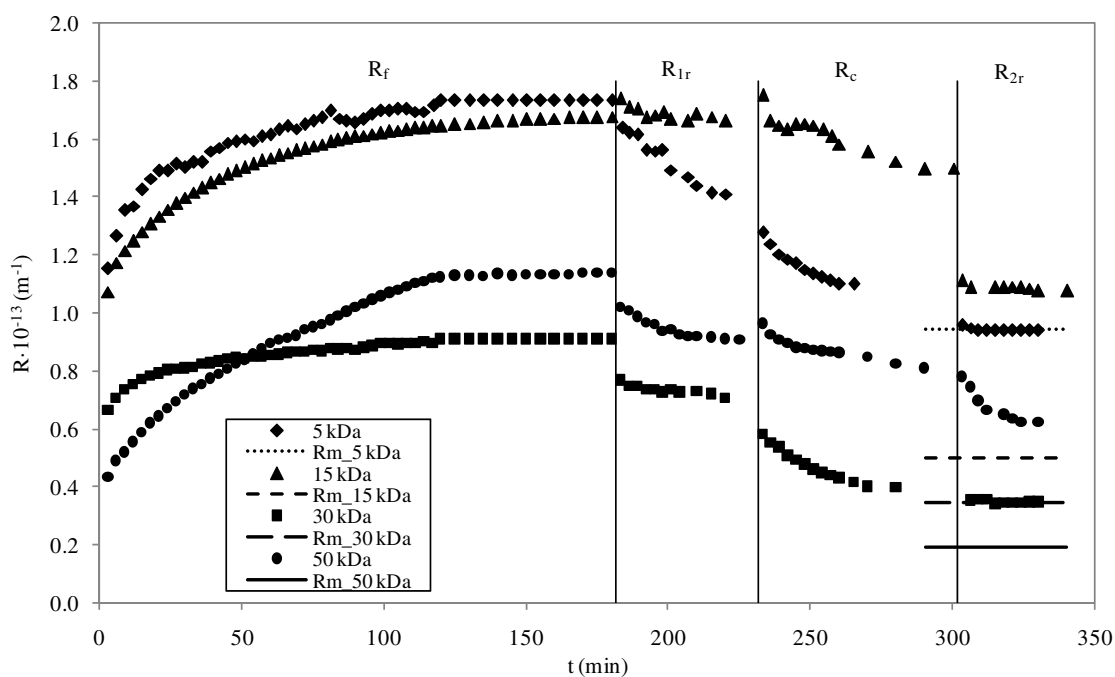
Fig. 4. Evolution of total hydraulic resistance with time for each membrane (25 ºC, 2 bar and 2 m·s^{-1} in the fouling step; 25 ºC and 1 bar in the rinsing steps and 50 ºC and 1 bar in the cleaning step. Crossflow velocity was 2.18 m·s^{-1} for all the membranes).
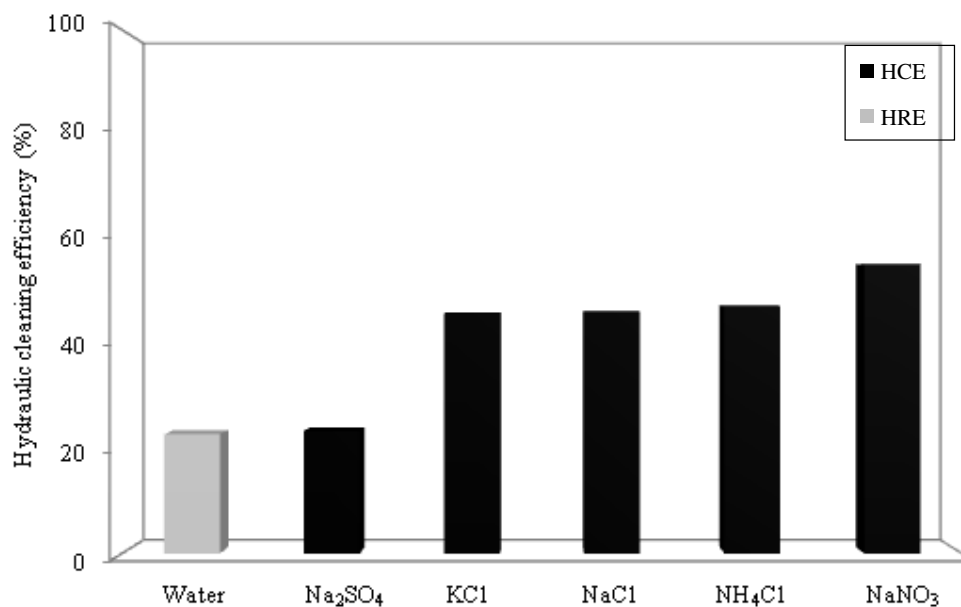
32

Fig. 5. Influence of the type of saline solution on the values of HCE (black bars) and comparison with the value of HRE (grey bar) (membrane MWCO: 15 kDa; temperature: 25 ℃; concentration: 100 mM; crossflow velocity: 4.2 m·s^{-1}).
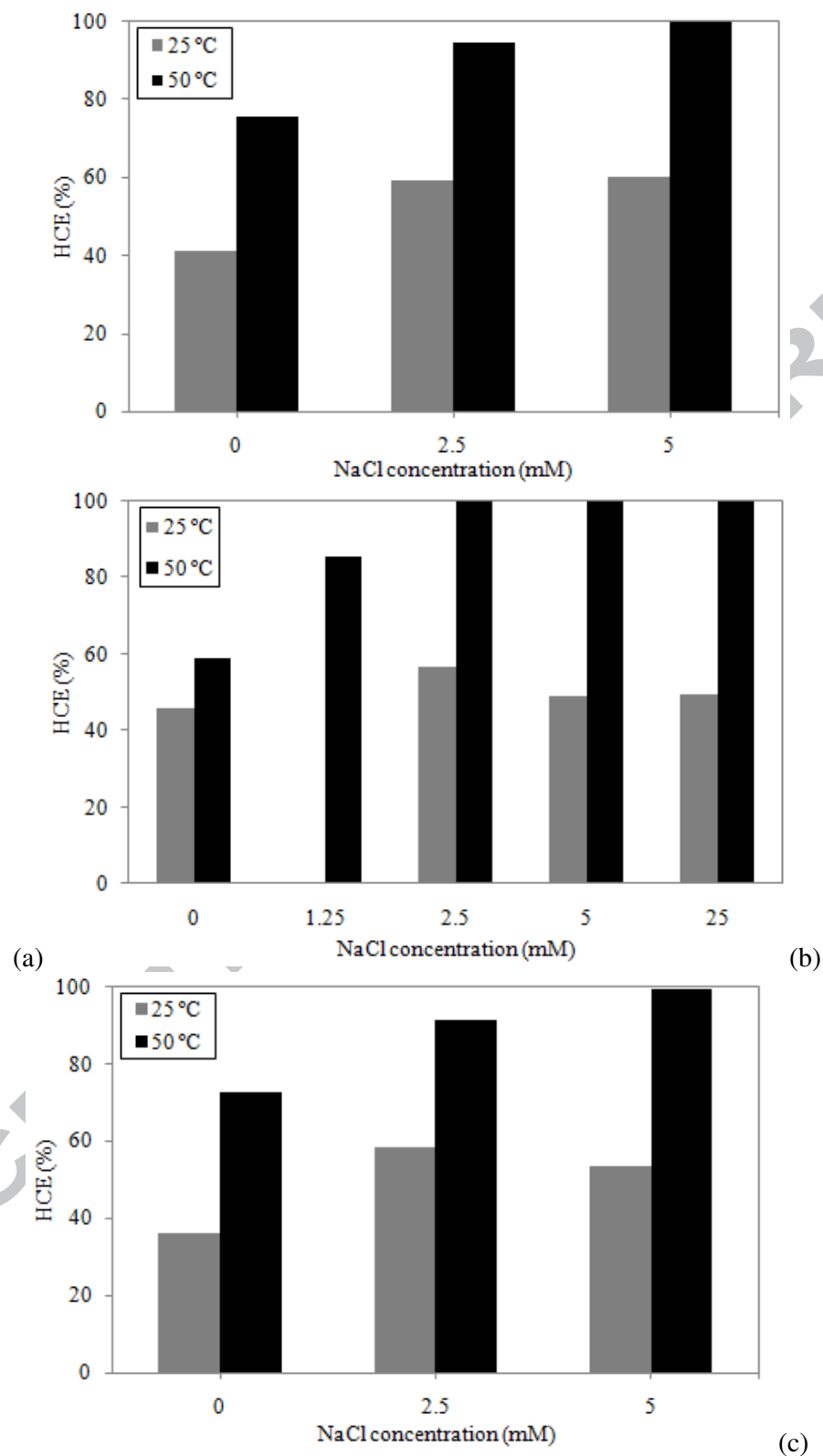
(a)

(b)

(c)

Fig. 6. Influence of NaCl concentration on the values of HCE for the membranes of 5 kDa (a), 15 kDa (b) and 30 kDa (c), when the cleaning solution temperature is 25 ºC (grey bars) and 50 ºC (black bars) and the crossflow velocity is 2.18 m·s^{-1} for the 5 and 30 kDa membranes and 4.2 m·s^{-1} for the 15 kDa membrane.
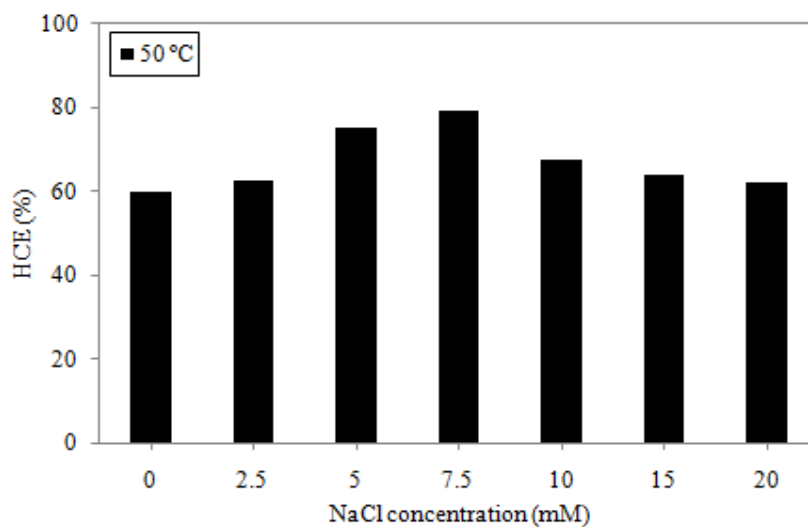
Fig. 7. Influence of NaCl concentration on the values of HCE for the membrane of 50 kDa, when the cleaning solution temperature is 50 ºC and the crossflow velocity is 4.2 m·s^{-1}.
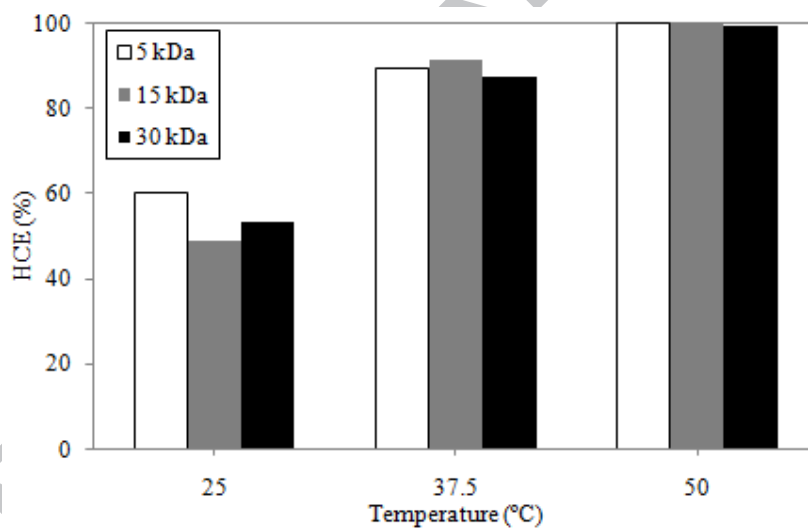

Fig. 8. Influence of temperature on the values of HCE for the membranes of 5 kDa (white bars), 15 kDa (dark grey bars) and 30 kDa (black bars), when NaCl concentration is 5 mM and the crossflow velocity is 2.18 m·s^{-1} for the 5 and 30 kDa membranes and 4.2 m·s^{-1} for the 15 kDa membrane.
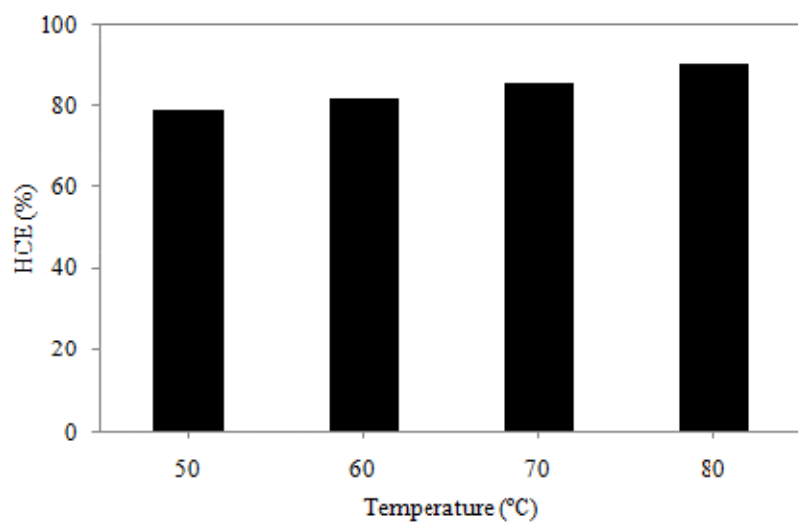
35

Fig. 9. Influence of temperature on the values of HCE, when NaCl concentration is 7.5 mM and crossflow velocity 4.2 m·s^{-1} for the 50 kDa membrane.
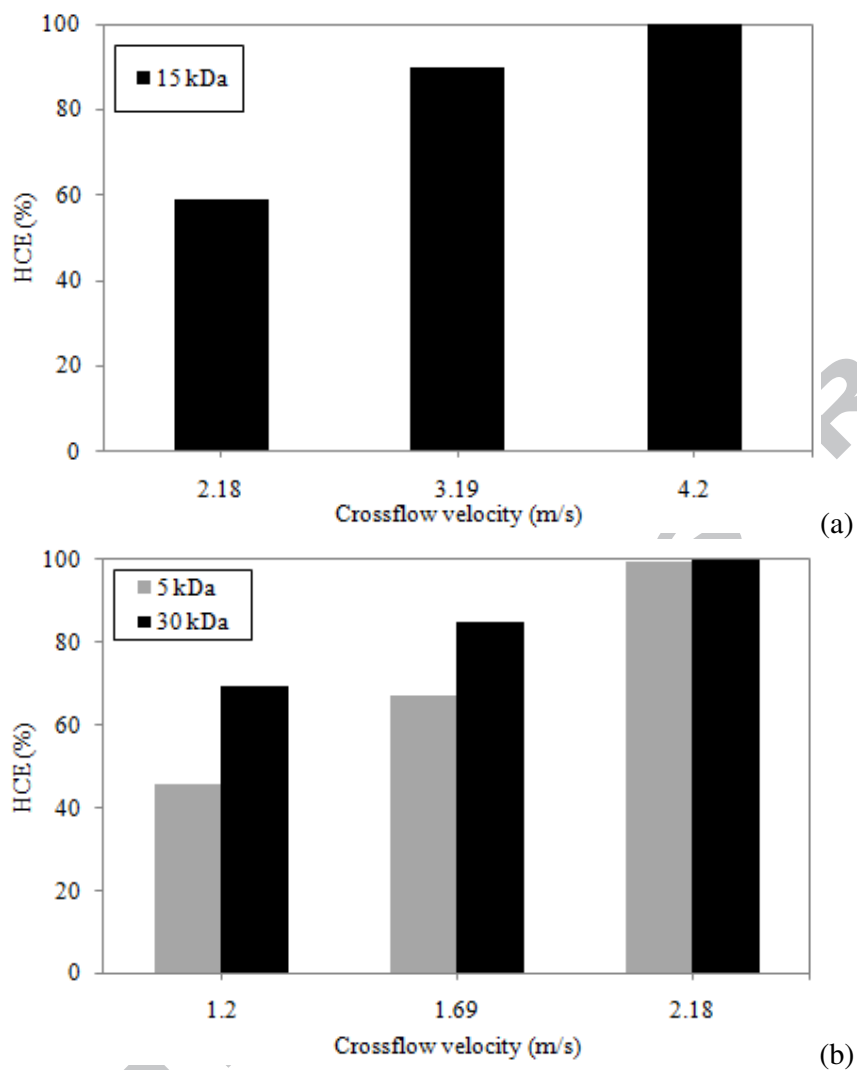
Fig. 10. Influence of crossflow velocity on the values of HCE for the membranes of 15 kDa (a) and 5 and 30 kDa (b), when temperature is 50 ºC and NaCl concentration is 2.5 mM for the 15 kDa membrane and 5 mM for the 5 and 30 kDa membranes.
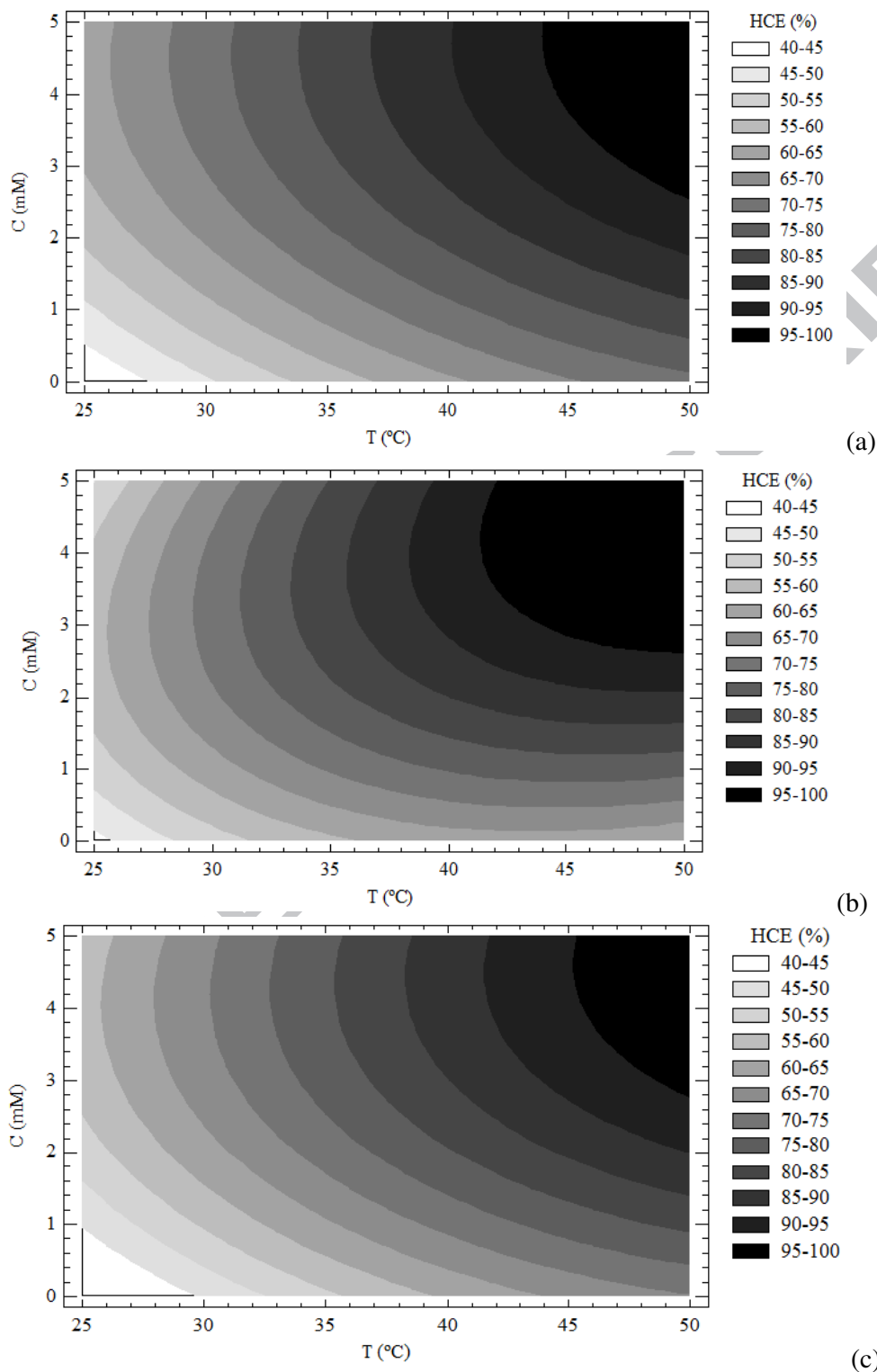
Fig. 11. Contour plot for HCE as a function of temperature and NaCl concentration for the membranes of 5 kDa (a), 15 kDa (b) and 30 kDa (c) at a crossflow velocity of 2.18 m·s^{-1} for the 5 and 30 kDa membranes and 4.2 m·s^{-1} for the 15 kDa membrane.

Table 1

Experimental data for the statistical analysis.

MWCO (kDa)	T_c (ºC)	C (mM)	v (m·s^{-1})	HCE (%)
5	25	0	2.18	41.10
	25	2.5	2.18	59.20
	25	5	2.18	60.17
	37.5	0	2.18	57.49
	37.5	2.5	2.18	80.21
	37.5	5	2.18	89.59
	50	0	2.18	75.49
	50	2.5	2.18	94.36
	50	5	2.18	99.58
	50	5	1.69	67.00
	50	5	1.20	45.74
15	25	0	4.20	45.77
	25	2.5	4.20	56.49
	25	5	4.20	49.03
	37.5	0	4.20	59.99
	37.5	2.5	4.20	80.68
	37.5	5	4.20	91.49
	50	0	4.20	58.99
	50	2.5	4.20	100.00
	50	5	4.20	100.00
	50	2.5	3.19	90.07
	50	2.5	2.18	58.93
30	25	0	2.18	35.94
	25	2.5	2.18	58.31
	25	5	2.18	53.32
	37.5	0	2.18	54.24
	37.5	2.5	2.18	77.71
	37.5	5	2.18	87.55
	50	0	2.18	72.67
	50	2.5	2.18	91.23
	50	5	2.18	100.00
	50	5	1.69	85.11
	50	5	1.20	69.27

Table 2

ANOVA results for the model equations that relate the hydraulic cleaning efficiency with the design variables.

MWCO (kDa)	Parameter	Coefficient	Estimated value	p-value
5	Constant	a (%)	-77.179	0.0002
	T_c	b ($^\circ$C^{-1})	1.448	0.0000
	C	c (mM^{-1})	10.922	0.0024
	C^2	d (mM^{-2})	-1.186	0.0292
	v^2	e (m$^{-2}\cdot$s^2)	17.024	0.0000
15	Constant	a (%)	-40.939	0.1015
	$T_c\cdot C$	f ($^\circ$C$^{-1}\cdot$mM^{-1})	0.590	0.0001
	C^2	d (mM^{-2})	-3.207	0.0006
	v	g (m$^{-1}\cdot$s)	22.468	0.0026
30	Constant	a (%)	-46.222	0.0090
	T_c	b ($^\circ$C^{-1})	1.556	0.0001
	C	c (mM^{-1})	11.948	0.0058
	C^2	d (mM^{-2})	-1.345	0.0493
	v^2	e (m$^{-2}\cdot$s^2)	8.873	0.0022

Table 3

Optimal values of the design variables obtained with a pattern-search optimization method.

MWCO (kDa)	T_c ($^\circ$C)	C (mM)	v (m$\cdot$s^{-1})
5	50	4.61	2.18
15	50	4.56	4.20
30	50	4.44	2.18

The efficiency of several saline solutions to clean the UF membranes was tested.

The efficiency was related to the operating conditions and the membrane MWCO.

Saline solutions were able to clean the low MWCO membranes.

Optimal operating conditions to perform the cleaning were selected.