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The Clogging Effect in the Process of Protein Separation by Ultrafiltration

GHEORGHE BATRINESCU[1#], ROXANA-ELENA SCUTARIU[1,2#], NICOLAE-IONUT CRISTEA[1], IOANA-ALEXANDRA IONESCU[1], GHEORGHE NECHIFOR[2*]

[1] National Research and Development Institute for Industrial Ecology-ECOIND, Pollution Control Department, 71-73 Drumul Podu Dambovitei Str., 060652, Bucharest, Romania

[2] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Analytical Chemistry and Environmental Engineering, 1-7 Polizu Str., 011061, Bucharest, Romania

Abstract: *In this study, five ultrafiltration membranes (polysulfone, cellulose acetate and polyethersulfone) were tested in the treatment of aqueous protein solutions similar to wastewater from fermentation industries. The experiments were made in tangential flow filtration. The permeate flux for the five membranes tested at the optimum pressure of 3 bar decreased due to the effect of clogging the pores by the macromolecular protein solutions. Cellulose acetate membranes showed the lowest permeate flux (Ac-Cel1=152.4 L/m^2.h and Ac-Cel2=40.3 L/m^2.h) which doesn't recommend them for the ultrafiltration process of bovine serum albumin. When a polysulfone membrane was used in several cycles of protein-containing wastewater ultrafiltration, the permeate flow decreased progressively from one cycle to another due to the internal clogging of the membrane (501.6 L/m^2.h up to 444.0 L/m^2.h). Regarding the ultrafiltration of protein solutions with a suspended yeast content, the clogging was predominant on the membrane's surface, which results in a decrease of the permeate flux by over 50%.*

Keywords: *bovine serum albumin, membrane clogging, protein, ultrafiltration, yeast*

1. Introduction

Membrane techniques include a group of separation processes in which the characteristics of a membrane (electric charge, porosity, selectivity) are used to separate the components of a liquid and gas feed streams according to size. In these membrane processes, the feed stream is separated into two: the fraction that permeates through the membrane (permeate), and the fraction containing the components that have not been transported through the membrane (concentrate) [1].

Membrane separation processes include reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), microfiltration (MF) and electrodialysis (ED). Membrane technology plays a important role in the broad range of industrial applications. Membrane processes, unconventional processes are mainly used in obtaining drinking water from sea water by reverse osmosis (the largest application), in the branches of the food industry (milk concentration and fractionation [2-3], separation and concentratıon of proteins from whey [4], clarification of blood orange juice [5-6], bovine serum albumin (BSA) [7], environmental protection (wastewater treatment from different industrial area) [8-11].

Ultrafiltration is the most important process in membrane technology and has the ability to remove colloids and different macromolecules with a molecular weight ranging from 1000 to 100000 Da [12-17]. The UF process is performed by applying a specific pressure in order to separate the particles according to their sizes [11] and molecular weight. Ultrafiltration presents a series of advantages, like low energetic consumption, low operating temperature, and the absence of phase transition [18].

The ultrafiltration performance is based on a number of factors, including membrane selectivity and flux, good mechanical, chemical and thermal stability of the membrane material, minimal fouling during operation, and good compatibility with the feed solution [19-20].

**email: doru.nechifor@yahoo.com* *#Authors with equal contribution*

The pollution effect induced on the environment due to the wastewater resulted from the fermentation industry is primarily due to the very high organic load caused by proteins and yeast residues as reported by several authors in scientific literature [21-23]. Thus, for the treatment of these wastewaters at the source (in order to reduce the impact on the operation of the treatment plant) two main phases were used: water purification by a conventional process (flocculation and/or classical filtration) followed by microfiltration and/or ultrafiltration.

Proteins are common pollutants found in the wastewater, and many authors have demonstrated that proteins cause severe ultrafiltration membrane fouling [24-27]. Previous studies have demonstrated the fouling characteristics of UF membranes by polysaccharides and proteins, when they are present alone in the solution, wastewater or water to be treated [24-25]. Also, a significant amount of research has been performed using dead-end ultrafiltration. Regarding the interaction of BSA and yeast with membranes, most of the reported work has been performed with cellulose acetate membranes [26-28]. Because of this, the organic compounds (BSA and yeast) have also been used in the present study.

The aim of the paper is to highlight the properties of new membranes obtained from known materials such as polysulfone, cellulose acetate and polyethersulfone in separation processes of organic impurities such as proteins from liquid media. For this, the problem of membrane clogging was studied, a defining phenomenon for the operating parameters in ultrafiltration. The studied membranes belong to the group of those presented in the literature [29-32] as being intended for the processes of removing proteins from liquid media. The novelty of the paper consists in the study of clogging of new membranes whose characteristics differ depending on the composition of the coagulation solution [33].

The effect of the clogging phenomenon was studied both after a single ultrafiltration step and after several stages between which a clogging phase was interlaced by washing the membranes with water. This last aspect also has a novelty character, not being currently reported in the specialized literature. The thermal stability of membranes was investigated by thermal gravimetric analysis (TGA). The effect of proteins on the morphology of the membranes was studied by scanning electron microscopy (SEM). This study attempts to elaborate an integrated system of ultrafiltration for the treatment of wastewater in the fermentation industry.

2. Materials and methods

2.1. Chemicals and membranes

The membranes were obtained using the following materials:

- polysulfone (Psf), Mw = 35000 g/mol, (Aldrich, USA);
- cellulose acetate (Ac-Cel), Mn = 50000 g/mol, (Sigma-Aldrich, USA);
- N-methyl-2-pyrrolidone (NMP), (Merck, Germany);
- polyvinylpyrrolidone (PVP) K30, M=40000 g/mol, (Fluka, USA);
- polyethylene glycol (PEG) 4000, M = 3500-4500 g / mol, (Scharlau Chemie SA, Spain);
- ultrapure deionized water, (Milli-Q Integral 15, Merck, Millipore, France);
- ethyl alcohol, 96%, (Chimreactiv SRL, Bucharest);
- glycerin, 99.5%, (Chempur, Poland).
- polyethersulfone membrane (coded PES), 50 kDa MWCO, Sartorius Stedim Biotech GmbH (Germany).

The synthetic solutions used in the experiments were obtained using the following standard materials:

- protein - bovine serum albumin (BSA) having the following characteristics: Bovine Serum Albumin Fraction V, Mw = 68 kDa, Sigma Aldrich, Germany;
- food baking yeast (Saccharomyces Cerevisiae) with a dry matter (d.m.) of 35% and a protein content of 14%.

The initial concentrations of protein and yeast in the synthetic samples were established so as to reach values close to the CCO-Cr indicator of those of the real systems (CCO-Cr>1000 mg O_2/L).

2.2. Methods

Five membranes were used in experiments. Four membranes were made in the laboratory from polysulfone (two from polysulfone coded Psf1 and Psf2) and cellulose acetate polymer (two from cellulose acetate coded Ac-Cel1 and Ac-Cel2). The polymeric membranes used in the experiments were made using the phase inversion process and the immersion-precipitation technique as described in previous work [33]. Thus, the working mode and the main phases of the process are presented schematically in Figure 1.

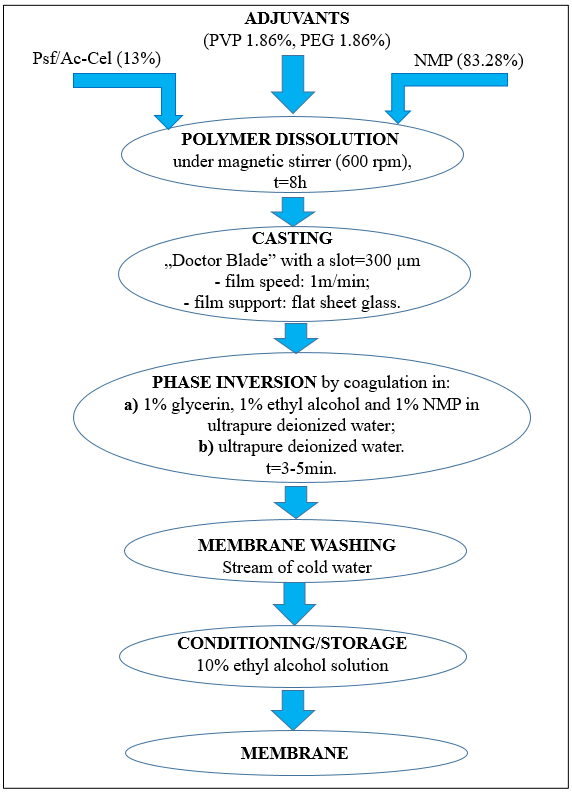

Figure 1. Preparation method of the membranes

The fifth membrane used in experiments was a commercial polyethersulfone membrane (coded PES), with a molecular weight cut-off of 50 kDa from Sartorius Stedim Biotech GmbH (Germany).

Protein solutions were analyzed both in the initial form and after the ultrafiltration process (permeate and concentrate) by determining the *pH*, *electrical conductivity*, *turbidity*, *chemical oxygen consumption* (CCO-Cr) and *total nitrogen content*.

The following methods and equipment's were used:

- pH: SR EN ISO 10523: 2012; CONSORT C932 multiparameter;
- electrical conductivity (EC): SR EN 27888:1997; CONSORT C932 multiparameter;
- turbidity: SR EN ISO 7027:2001; SPECORD 205;
- CCO-Cr: SR ISO 6060:1996;
- total nitrogen: SR ISO 6060:1996, Shimadzu analyzer, TOC-L CPH/CPN model.

The obtained membranes were characterized both in terms of morphological characteristics by SEM and TGA as well as in terms of flux characteristics.

The membranes were characterized by point of structural view through scanning electron microscopy (SEM) using a SEM Quanta FEG 250 equipment, Thermo Fischer Scientific and by thermogravimetric analysis, using a STA 409 PC equipment, produced by Netzsch (Germany). The hydrodynamic characteristics of the membranes were determined in tangential flux regime, using a LABCELL-CF1 Koch Membrane System, described in other study [13]. The installation was equipped in each experiment with a circular membrane of 76 mm in diameter and an effective active area of 28 cm^2. The

experiments were performed at a working pressure of 3 bar, established as the optimal value in the other experiments [33].

2.3. Calculations

For the hydrodynamic characterization, the ultrapure deionized water flux was determined for each membrane in the UF experiments using equation 1:

$$J = \frac{V}{t \times S} \quad (1)$$

where:

- J - flux of liquid through the membrane (L/m^2□h);
- V - volume of liquid (L) passing through the membrane during time (t);
- t - time (h) in which the volume V of liquid is collected;
- S - membrane area (m^2).

The efficiency of the membrane in the ultrafiltration process was analyzed by determining the degree of rejection, according to formula 2:

$$R = \frac{\left(C_f - C_p\right)}{C_p} \times 100 \quad (2)$$

where:

- R - protein removal rate (%);
- C_f and C_p - protein concentrations in the feed solution and permeate solution (mg/L), respectively.

For the determination of protein concentrations equations 3 and 4 were used, based on CCO-Cr indicators and total nitrogen content:

$$R_C = \frac{\left(CCOCr_i - CCOCr_p\right)}{CCOCr_i} \times 100 \quad (3)$$

$$R_N = \frac{\left(N_i - N_p\right)}{N_i} \times 100 \quad (4)$$

where:

- CCO-Cr_i - chemical oxygen consumption of the initial solution (mg O_2/L);
- CCO-Cr_p - chemical oxygen consumption of permeate (mg O_2/L);
- N_i - total nitrogen content of the initial solution (mg/L);
- N_p - total nitrogen content of the permeate (mg/L).

The efficiency of the membranes in the ultrafiltration process was deducted from the degree of reduction of the permeate flux (DRPF), compared to the initial flux of ultrapure deionized water, calculated according to formula 5:

$$DRPF = \frac{\left(J_i - J_p\right)}{J_i} \times 100 \quad (5)$$

where:

- J_i - ultrapure water flux of the membrane before the ultrafiltration process (L/m^{2}h);
- J_p - membrane permeate flux in the ultrafiltration process (L/m^{2}h).

Highlighting the phenomenon of membrane clogging during the ultrafiltration process was made possible by calculating the degree of reduction of the ultrapure water flux (DRWF) according to formula 6:

$$DRWP = \frac{\left(J_i - J_a\right)}{J_i} \times 100 \quad (6)$$

where:

- J_i - ultrapure water flux of the membrane before the ultrafiltration process (L/m^{2}h);
- J_a - ultrapure water flux of the membrane after the ultrafiltration process (L/m^{2}h).

3. Results and discussions

The initial flux characteristics of the tested membranes are presented in the form of deionized water flux (Table 1). From the point of view of the morphological characteristics, SEM images and TGA were selected for the relevant Psf2 membrane before being used in the process: Figure 5 (a) for SEM and Figure 6 (a) for TGA.

3.1. Separation of proteins from liquid media by ultrafiltration

In this set of experiments, we aimed to establish the operational parameters in the process of ultrafiltration of a liquid media with protein content. To perform the experiments, a standard protein solution (BSA) with a concentration of 1 g/L was used. The solution was obtained by dissolving the protein in ultrapure deionized water, under magnetic stirring (500 rpm), at room temperature (20°C) for 1 h.

Subsequently, the LABCELL-CF1 Koch Membrane System laboratory installation was equipped with the corresponding membranes. After that, the feed tank was supplied with a content of 500 mL of the protein solution. The process was initialized and the working pressure was adjusted to 3 bar. The time needed to collect a final volume of 250 mL permeate was noted. The process was performed so as to obtain a concentration ratio of 1/1 (volume of permeate/volume of concentrate).

The results obtained for the permeate fluxes in the case of the five membranes studied in the ultrafiltration process of BSA protein solution 1g/L, at a constant pressure of 3 bar, were graphically represented in Figure 2 (variation of fluxes depending on the permeate volume).

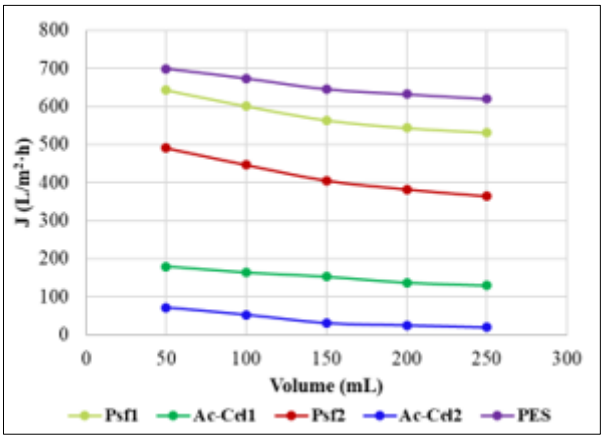

Figure 2. Variation of membrane fluxes in the UF process
of 1g/L BSA solution, at a pressure of 3 bar and a concentration ratio of 2/1

The data presented in Figure 2 highlights the fact that with the obtained increase in the permeate volume (from 50 to 250 mL), the membrane flux decreases for all the studied membranes, as an effect of the clogging of their pores with the protein macromolecules. The flux variation was lower in the case of Ac-Cel1 and Ac-Cel2 membranes than in the case of the other three membranes (Psf1, Psf2 and PES), which have a comparable decrease. This does not lead to the conclusion that the Ac-Cel1 and Ac-Cel2 membranes, respectively, would be the most suitable for the UF process of the BSA solution. It was found that the two membranes have the lowest fluxes of all, which do not recommend them for such a process in technologically and economically efficient conditions. The small variation in flux from the first volume of permeate to the last fraction obtained was due to the fact that the clogging from the

beginning of the process become inefficient further during it.

Process-acceptable fluxes were obtained from all three other membranes (Psf1, Psf2 and PES). For a better selection of the most suitable membranes, the degree of reduction in the permeate flux (DRPF) compared to the essential characteristic of the membranes must be taken into account (the flux of distilled water). It's calculated using formula (5) and using the water average fluxes. The results were presented in Table 1.

Table 1. Degree of permeate flux reduction (DRPF) of membranes in the UF process of BSA solution 1g/L

Membrane type	Average flux water ($L/m^2 \cdot h$)	Average flux permeate ($L/m^2 \cdot h$)	DRPF (%)
Psf1	2432.8	576.5	**76.3**
Ac-Cel1	799.5	152.4	**80.9**
Psf2	1037.1	417.7	**59.7**
Ac-Cel2	674.4	40.3	**94.0**
PES	1537.1	653.6	**57.5**

It was observed that Ac-Cel1 and Ac-Cel2 membranes have the highest values for DRPF, which do not make them suitable for the ultrafiltration process under efficient conditions. The conclusion was also valid for the Psf1 membrane, which at a DRPF value of 76.3% was also unsuitable for this process.

An important role in selecting the best membranes for the ultrafiltration process of the protein solution is played by the parameter degree of rejection (protein removal rate) - R, determined with formulas 3 and 4, based on physical-chemical analysis.

The results obtained from the physical-chemical analysis of the samples taken during the five experiments performed and the degree of rejection (R) are included in Table 2.

Table 2. The results of the physical-chemical analysis of the samples resulting from the UF experiments of the BSA solution 1g/L and the degrees of rejection of the membranes

Sample	pH	EC (µS/cm)	T (NTU)	CCO-Cr (mgO_2/L)	N (mg/L)	R_C (%)	R_N (%)
I*	6.7	29	2	1320	151.2	-	-
Psf1, P**	6.8	20	1.5	841	96.95	36.3	35.9
Psf1, C***	6.1	48	23	1760	195.8		
Ac-Cel1, P	6.6	18	1.6	880	102.3	33.3	32.3
Ac-Cel1, C	5.8	26	42	1692	193.6		
Psf2, P	6.8	12	1.1	61.6	6.5	95.3	95.7
Psf2, C	5.7	56	144.9	2420	285.9		
Ac-Cel2, P	6.1	14	1.3	202.4	25.8	84.7	82.9
Ac-Cel2, C	5.9	43	116	2304	268.5		
PES, P	6.4	14	1.2	48.3	7.7	96.3	94.9
PES, C	5.9	34	150.4	2492	284.7		

I* - initial solution of BSA 1g/L (used in all experiments);
P**- permeate resulting from UF with Psf1 membrane;
C***- concentrate resulting from UF with Psf1 membrane.

Variations in pH and electrical conductivity were generated by the distribution of protein between the 2 fractions; permeate and concentrate. These two parameters were influenced by the free amino and carboxylic groups in the structure of amino acids that enter the macromolecular chain of BSA. Turbidity also correlates with the protein content of each fraction. It was found that it decreases in all permeate fractions, regardless of the type of membrane and increases in concentrate due to the increase in concentration correlated with the degree of rejection.

The values corresponding to the CCO-Cr indicator show that only the Psf2, Ac-Cel2 and PES membranes ensure a permeate quality that allows it to be discharged into the sewerage network (maximum value 500 mg/L according to NTPA 002/2005). In the case of Psf2 and PES membranes, the CCO-Cr values for the permeate were lower than those normed by NTPA 001/2005 of 125 mg/L, being feasible for the direct discharge in natural emissions.

The rejection degrees calculated both according to the CCO-Cr values and to the nitrogen content are adequate for the UF process (over 90%) in the cases of Psf2 and PES membranes. For the Ac-Cel2 membrane the value of approx. 84% is close to the threshold that would recommend its use in the studied process. However, the very low flux value mentioned above for this membrane does not make it possible for use in feasible technical-economic conditions. It should be mentioned that the experimental results obtained, regarding the content of the nitrogen element in the BSA macromolecule, are in accordance with the data from literature [29]. These data indicate a nitrogen content in the BSA structure used of about 16%. At a nitrogen content of 151.2 mg/L determined for the initial BSA solution containing 1 g/L the protein, results from a calculation a value of 15.1% N. The value obtained justifies the use of this indicator in the calculation of rejection, in correspondence with the one calculated using CCO-Cr values. The data presented in Table 2 highlights a good correlation between the values of the degree of rejection calculated with the two methods.

3.2. The effect of membrane's clogging in the UF process of liquid protein

To highlight how membrane clogging influences the performance of the process of ultrafiltration of proteins from liquid media, two sets of experiments were performed using Psf2 and PES membranes.

✓ In the first set aimed at the repeated use of a Psf2 type membrane, in 3 cycles of UF, with its washing with deionized water (between cycles) in order to unclog.

✓ In the second set aimed the influence of the protein concentration in the feed solution on the clogging of Psf2 and PES membranes.

All experiments were performed at a constant pressure of 3 bar, established as the optimal value in previous studies. In both experimental sets the clogging effect was highlighted both by processing the data obtained by physical-chemical analysis and by structural/morphological analysis of the membranes using SEM and TGA methods.

3.2.1. Membrane clogging after their use in repetitive cycles

A new membrane sample of Psf2 type was used in this experiment (a round membrane with a diameter of 76 mm cut from lot 2 of the three obtained membranes).

The ultrapure deionized water flux of the membrane was determined in the first phase; continued with the ultrafiltration of 500 mL of 1 g/L BSA solution, at a pressure of 3 bar, up to a concentration ratio of 4/1 (permeate volume/concentrate volume). After the first UF cycle, the ultrapure water flow of the membrane was determined again. In this experiment, there is practically a clearing of the membrane produced by the polarization of the protein concentration, deposited on its surface (washing of the membrane surface in a tangential regime). The UF process and unclogging operations were repeated in the next two cycles.

Figure 3 and Figure 4 present the flux variations depending on the permeate volume for the two situations: deionized water flux and respectively permeate flux for the 3 UF cycles.

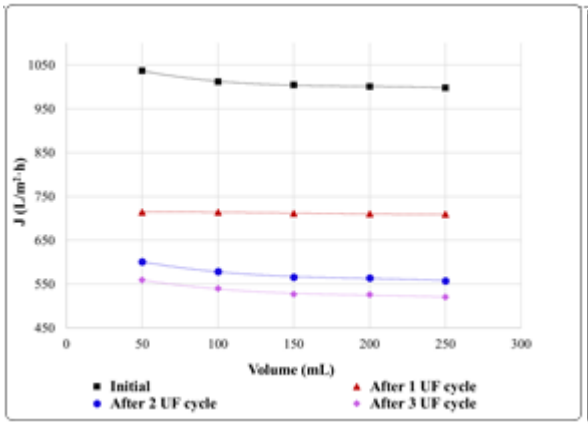

Figure 3. Variation of deionized water fluxes before and after UF 1g/L BSA solutions

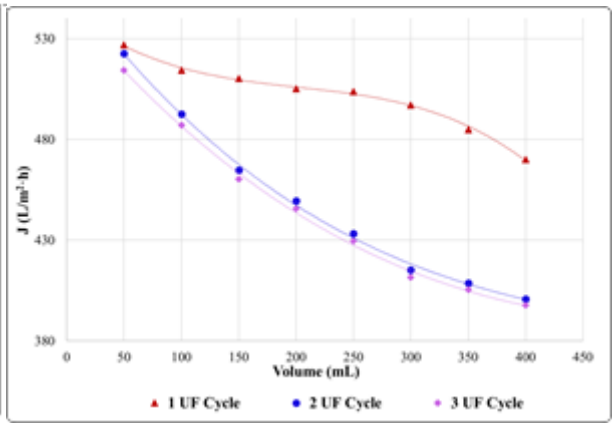

Figure 4. Variation of permeat fluxes in the 3 UF cycles

The data presented in Figure 3 and Figure 4 highlight the aspects related to the clogging of the membranes during the UF process of the protein solutions. Thus, the deionized water fluxes of the membrane decrease after each ultrafiltration cycle; the most pronounced decrease was recorded after the first cycle of UF and in comparison, to the initial value of the flux it is 29.54%. This denotes the effect of internal clogging (penetration of associated protein macromolecules into the membrane microstructure). The deionized water flux after the second cycle of UF compared to the water flux determined after the first cycle is 19.45% lower and the one after cycle 3 compared to the one from cycle 2 is 6.81% lower. This behavior is the consequence of the increase in the internal clogging as a higher number of UF cycles were performed. There is a tendency to saturate the microscopic structure of the membrane with protein macromolecules. Practically after 3 cycles of UF the internal clogging is almost complete. The decrease of the deionized water flux at this stage (internal saturation clogging) compared to the initial one was 47.11%.

The effect of membrane clogging is also highlighted by the way in which the permeate flux varies in the three UF cycles of the 1g/L BSA solution. In the first cycle of UF there is a decrease of 50.37% in the permeate flux (average value) compared to the average deionized water flux. In the second cycle the decrease is 55.62% and in the third 56.07%. The decrease of the flux in the first cycle of UF by about 50% is the consequence of both the clogging of the membrane's surface generated by the concentration polarization layer and the internal clogging of the membrane.

The effect of clogging the surface, according to the obtained results, was diminished by washing the membrane's surface between cycles, in a tangential flux regime. However, the internal clogging is cumulative up to a point, aspect demonstrated by the tendency of the flattening in the decrease of the permeate fluxes in cycles 2 and 3 towards a value of about 56%. The cumulative effect of internal clogging was very well highlighted in Figure 4, in which for cycles 2 and 3 it is observed that as the volume of permeate collected increases the flux decreases to a much greater extent than in the case of the first cycle. The fact that the allure of the two curves corresponding to cycles 2 and 3 was the same, denotes the tendency of saturation of the internal clogging of the membrane.

In Figure 5 SEM images of the surfaces of the Psf2 membrane were presented, after the first cycle of UF (analysis of sample no. 1 from the Psf2 membrane) and after the washing of the membrane at the end of cycle 3 of UF (analysis of sample no. 2 from the Psf2 membrane).

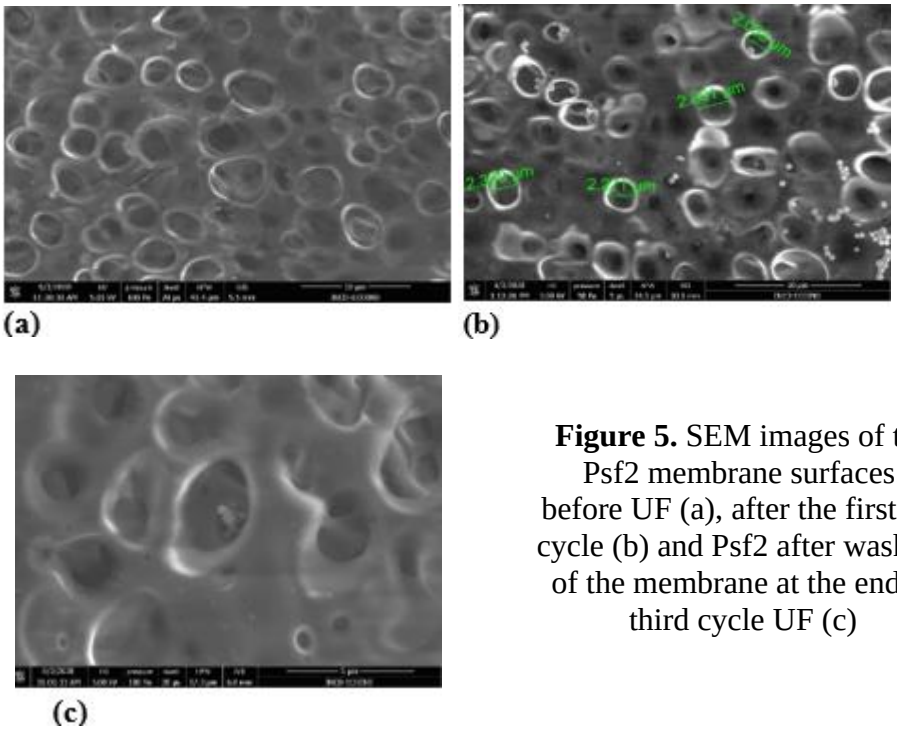

Figure 5. SEM images of the Psf2 membrane surfaces: before UF (a), after the first UF cycle (b) and Psf2 after washing of the membrane at the end of third cycle UF (c)

From Figure 5 (b) it can be seen that the deposits of protein conglomerates on the surface of the membrane are visible, compared with the initial condition - Figure 5 (a). The majority of those that have penetrated inside the pores, highlighting the clogging generated by the concentration polarization. After washing the membrane, seen in Figure 5 (c) these conglomerates are removed from the surface, being found only inside the pores, highlighting the internal clogging.

Figure 6 presents the thermograms of the initial Psf2 membrane (a), the Psf2 membrane after the first UF cycle, noted Psf2UF (b) and Psf2 after washing at the end of UF cycle 3, noted Psf2C (c).

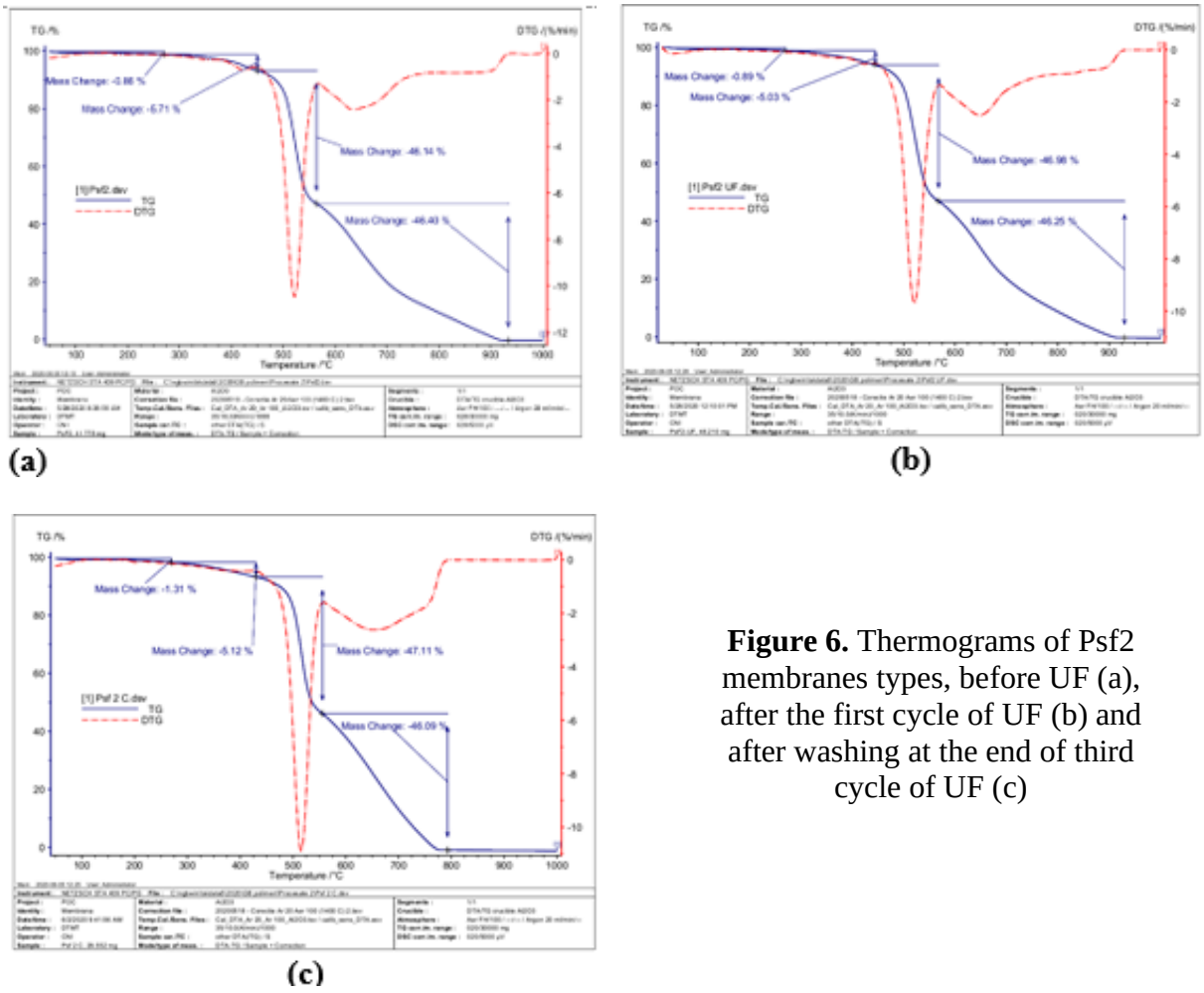

Figure 6. Thermograms of Psf2 membranes types, before UF (a), after the first cycle of UF (b) and after washing at the end of third cycle of UF (c)

The three thermograms are similar in terms of geometry and loss of mass in temperature areas above 400°C. A careful analysis of the temperature zone of 275°C, shows that the mass loss for the Psf2UF membrane is higher than that of the initial membrane (Psf2) with a percentage of 3.5%, while for the Psf2C membrane the loss is higher than that of the initial membrane with a percentage of 52.3%.

The analyzed temperature zone corresponds to the combustion zone of the organic matter. The values obtained confirm the two phenomena: surface clogging in the case of the Psf2UF membrane (after the first ultrafiltration cycle) materialized by the existence of a small amount of protein on the membrane's surface and deep clogging in the case of the Psf2C membrane (after three ultrafiltration cycles) caused by blocking of a larger amount of protein in the pores.

The results obtained from the physical-chemical analysis of the samples taken during the three cycles of UF and the calculated degree of rejection (R) are included in Table 3.

Table 3. The results of the physical-chemical analysis of the samples resulting from the 3 cycles of UF of the BSA solution 1g/L and the degrees of rejection of the membranes

Sample	pH	EC (µS/cm)	T (NTU)	CCO-Cr (mgO_2/L)	N (mg/L)	R_C (%)	R_N (%)
I*	6.7	29	2	1320	151.2	-	-
Psf2a, P**	6.9	16	1.3	100.3	13.2	92.4	91.3
Psf2a, C***	6.5	148	360	5948.8	673.4		
Psf2b, P	6.8	18	1.4	81.7	11.9	93.8	92.1
Psf2b, C	6.4	167	371	6013.2	677.5		
Psf2c, P	6.8	21	1.5	71	9.7	94.6	93.6
Psf2c, C	6.5	183	379	6096.1	689.1		

I*- initial solution of BSA 1g/L (used in 3 cycles experiments);
P** - permeate resulting from UF with Psf2 membrane from UF cycle 1;
C*** - concentrate resulting from UF with Psf1 membrane from cycle 2.

The rejection levels corresponding to a concentration level of 4/1 are lower (in small proportion) than the ones obtained with the same membrane for a concentration level of 2/1. This can be explained by the fact that with the increase in the degree of concentration there is also the increase in the amount of protein that crosses the membrane. As the number of repeated uses (cycles) of the membrane increases, there is a slight increase in the degree of rejection due to the progressive increase in internal clogging. The degree of rejection calculated according to the nitrogen content, has values close to those of the degree of rejection calculated according to the chemical oxygen consumption.

3.2.2. The effect of protein concentration on membranes in the ultrafiltration process

In this set of experiments, the phenomenon of clogging of Psf2 and PES type membranes under the influence of the protein concentration in the feed solution was studied. The conditions for performing the experiments were the same as in the previous set of experiments (in order to make a comparative analysis), namely the working pressure of 3 bar and the concentration ratio of 4/1. In the experiments, a new sample from the Psf2 membrane (sample no. 3) and a PES membrane were used. For the Psf2 membrane, the deionized water flux was determined again in order to verify the reproducibility of its flux characteristics. Then, the actual experiment of ultrafiltration of a protein solution containing 1g/L BSA and 1g (d.m.)/L baking yeast was performed. This solution comes very close to wastewater properties, resulting from washing fermentation vessels in the wine, beer or alcohol industry. The same solution was utilized in the UF experiment using PES membranes. The variation of the permeate flux during the two experiments was shown in Figure 7.

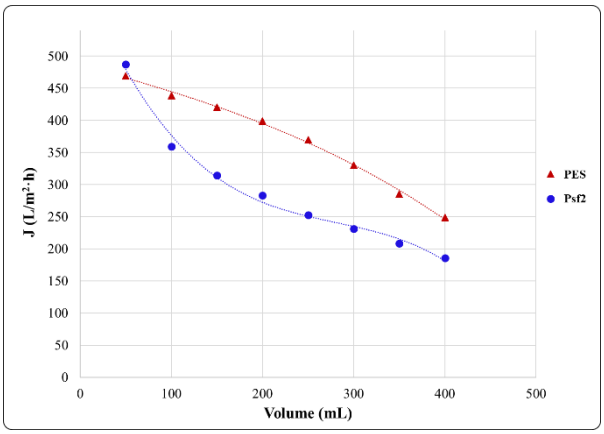

Figure 7. Variation of permeate fluxes of Psf2 and PES membranes in UF of BSA protein solution 1g/L and yeast 1g (d.m.)/L

It was found that in the case of UF of the BSA solution 1g/L, that the permeate flux decreases as its volume increases. The PES membrane (imported) has a relatively constant decrease in the volume range of 50-400 mL, while the Psf2 membrane has a significant decrease in the volume range of 50-200 mL, after which the decrease tends to level its value. The clogging for both membranes is mainly at the surface as a result of the adhesion of yeast fragments, as it had emerged from the SEM images presented in Figure 8.

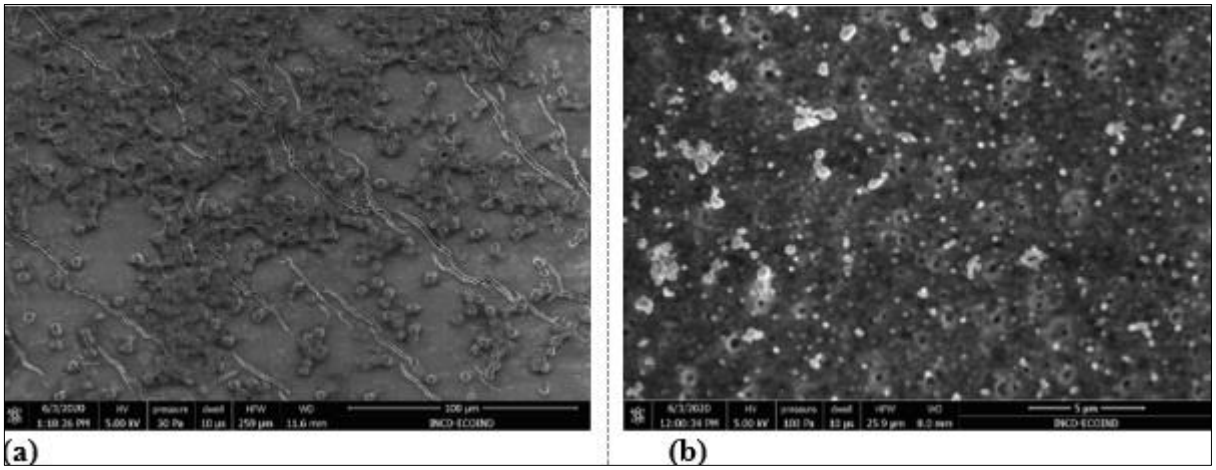

Figure 8. SEM images of the surfaces of the membrane Psf2 (a) and PES (b) after the UF of the protein solution BSA and yeast

This was also confirmed by the thermogravimetric analysis of the two membranes after UF. Figure 9 shows the thermogram of the Psf2 membrane after the UF of the BSA and yeast protein solution, coded Psf2UF A.

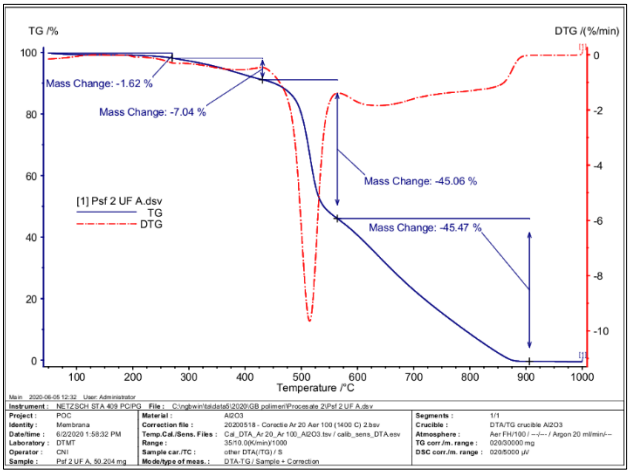

Figure 9. Psf2UF A membrane thermogram

Comparing this thermogram with the one in Figure 6 (b), it was found that the mass loss up at the temperature of 275°C was higher in the case of the membrane through which the UF was the solution of BSA and yeast with 82.02%. The loss of mass retained on the surface of the membranes, which induces the phenomenon of surface clogging was due to the organic matter (yeast and part of BSA).

Both membranes provide acceptable fluxes for an ultrafiltration process. Their use in such processes at industrial level also depends on the degree of rejection. Related to this aspect, Table 4 includes the results obtained from the physical-chemical analysis of the samples related to these experiments.

Table 4. Results of physical-chemical analysis of the samples resulting from the two sets of UF of the solution of BSA 1g/L + yeast 1g (d.m.)/L and the rejection degrees of the membranes

Sample	pH	EC (µS/cm)	T (NTU)	CCO-Cr (mgO_2/L)	N (mg/L)	R_C (%)	R_N (%)
A*	6.0	97	600	3520	295.9	-	-
Psf2A, P**	6.2	25	3.5	123.6	12.34	96.5	95.8
Psf2A, C***	5.6	101	2739	16605	1380.1		
PESA, P	6.3	28	2.6	76.7	8.97	97.8	97.0
PESA, C	5.5	97	1671	16790	1393.6		

A* - initial solution of BSA 1g/l + yeast 1g (d.m.)/L;
Psf2A, P** - permeate resulting from UF solution A with Psf2 membrane;
Psf2A, C*** - concentrate resulting from UF solution A with Psf2 membrane.

In comparison to the results obtained in previous experiments, it is found that both membranes provide the highest degrees of rejection, because of the formation of the protein layer on their surface. As in previous studies, there was a very good correlation of retention values, regardless of the indicator taken as reference (CCO-Cr or total nitrogen content).

4. Conclusions

Ultrafiltration process techniques was employed to removal protein and yeast from synthetic solutions. Five ultrafiltration membranes made from polysulfone, cellulose acetate and polyethersulfone were tested. The optimal conditions were as follows: concentration 1 g/L, pressure 3 bar, temperature 25°C. Under these conditions, the rejection of nitrogen content was determined to be in the range of 32.3% to 95.7%. The rejection for CCO-Cr indicator was in the range of 33.3% to 96.3%.

In the ultrafiltration process of soluble protein solutions, clogging was produced by macromolecules either by clogging the pores on the membrane's surface or by partially blocking the pores inside them. The flux for all tested membranes decreased due to the effect of pores clogging by the protein (BSA and yeast). The cellulose acetate membranes present the lowest flux, compared to the polysulfone membranes and doesn't recommend them for the ultrafiltration process of bovine serum albumin. In the case of using a membrane in several ultrafiltration cycles, the fluxes decrease progressively from one cycle to another because of its internal clogging. In the process of ultrafiltration of concentrated protein solutions and suspended yeast (case similar to that of washing waters of fermenters in the beer, wine or alcohol industry), clogging was predominant on the membrane's surface, as a result of the adhesion of yeast fragments, as it had emerged from the SEM images. The clogging effect is also highlighted by the decrease of permeate fluxes compared to the initially flux of deionized water by over 50%.

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