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Evaluation of cleaning efficiency of ultrafiltration membranes fouled by BSA using FTIR-ATR as a tool

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ABSTRACT

The goal of this paper was to study the cleaning of two polyethersulfone (PES) membranes of different molecular weight and fouled with BSA solution. Ultrafiltration (UF) membranes were tested in a flat sheet module. Fouling experiments were carried out at a transmembrane pressure of 2 bar and cross flow velocity of 2 m/s during 2 hours. Cleaning experiments were performed at 1 bar and 2.2 m/s. To compare the efficiency of different cleaning solutions (NaOH and P3-Ultrasil 115), quantification of residual proteins on the membrane was carried out by FTIR-ATR. To have a better understanding of the cleaning process, characteristics of the feed solution and of the membranes were considered and contact angle of the membranes before and after the cleaning was measured. Membrane resistances were also calculated at the different stages. Results from resistances showed that reversible fouling prevail over irreversible fouling for both membranes. P3-Ultrasil 115 was a better cleaning agent than NaOH

solution since cleaning efficiencies (CE) of 100% for both membranes were achieved for P3-Ultrasil 115 solution. Residual proteins on the membrane after the cleaning were measured both by FTIR-ATR and Pierce-BCA method. Results showed that 100% of permeability recovery did not imply the complete BSA removal from the membrane. However, these measurements corroborated that P3-Ultrasil 115 had removed a higher amount of proteins than NaOH solution.

Keywords: Ultrafiltration; Bovine Serum Albumin; FTIR-ATR; fouling; membrane cleaning.

1. Introduction

Membrane processes are considered as excellent technologies for many industrial applications (Delaunay et al., 2008). Particularly, in the dairy industry, ultrafiltration (UF) is widely used in the processing of whey and milk products for the standardization of the protein content (Diagne et al., 2013, Muthukumaran et al., 2004, Karasu et al., 2009).

UF membranes are an excellent alternative that reduce the separation cost and at the same time increase the product yield (Levitsky et al., 2012). UF process offers many advantages such as low-energy requirement and high permeate flux at low transmembrane pressure. It has entailed that UF processes are often used in the dairy industry. However, the bottleneck of UF processes is the membrane fouling during the production step (Diagne et al., 2013) and the sub-consequent permeate flux decline. Thus, an effective cleaning procedure will be necessary.

Membrane fouling is due to the deposition on the membrane surface and inside the pores of the organic and inorganic compounds in feed solutions (Muthukumaran et al. 2005b). These compounds cause cake and gel layer, adsorption and pore clogging (Argüello et al., 2003; Juang and Lin, 2004; Muthukumaran et al., 2005a and Kyllönen et al., 2006). Membrane fouling, both reversible (removed in the water rinsing step) and irreversible (removed in the chemical cleaning) (Cheryan 1998 and Muthukumaran et al., 2005b) had to be frequently eliminated in order to restore the membrane initial permeability.

In dairy industries, membrane fouling is mainly produced because of proteins and ions by adsorption or deposition (Zhu and Nyström 1998) onto the membrane surface and to internal pore blockage (Argüello et al., 2003 and Muthukumaran et al., 2005a). The main protein that milk contains is casein. These compounds form micelles in milk which consist of sub-micelles linked together by means of calcium and phosphorous bridges (Hausmann et al., 2013). Thus, these ions play a critical role in membrane fouling (Merin and Cheryan 1980 and Gésan et al., 1995).

Bovine serum albumin (BSA) has been used as model protein to describe the behavior of UF and MF membranes related to membrane fouling by proteins due to its low cost and high availability (Kelly and Zydney 1995). However, BSA fouling modeling is complex since BSA molecules form aggregates and particle size distribution will be of paramount importance to describe fouling mechanisms (Palacio et al., 2003). This explains that contributions on this topic are still being published in the bibliography (Hwang and Sz 2011).

It seems clear that a better understanding of the membrane cleanig step would contribute to the optimisation of the overall process efficiency (Rabiller-Baudry et al.,

2002). The cleaning procedure should be as economically viable as possible. In addition, membranes used in food industries are cleaned also to satisfy hygienics standards (Popovic et al., 2010).

Membrane cleaning methods are divided into chemical and physical. However, chemical processes are the most often used. Chemical cleaning agents include: alkalis, acids, metal chelating agents, surfactants, oxidizing agents and enzymes (Al-Amoudi and Lovitt 2007). It is important to note that a group of specific surfactants and alkaline solutions are commonly used to remove organic fouling. Sodium hydroxide solution and P3 Ultrasil 115 (Ecolab, Spain) were employed in this study. The cleaning solution must spread into the fouling layer and inside the pores acting by dissolution and then solubilization to remove the fouling layer from the membrane (Zhu and Nyström 1998; Levitsky et al., 2012 and Naim et al., 2012).

A key factor in the study of the membrane cleaning is the quantification of the residual proteins on the membrane under several cleaning conditions, as they are the main components of the membrane fouling (Bégoin et al., 2006a; Bégoin et al., 2006b and Rabillet-Baudry et al., 2012). These works quantified the residual proteins on the membranes by FTIR-ATR. However, no studies comparing FTIR-ATR data with other methodologies like Pierce-BCA method after extraction have been found in the bibliography. Besides, a different procedure for determining the calibration line for quantifying proteins by FTIR-ATR is proposed.

The main objective of this work was to study the influence of two cleaning reagents (P3-Ultrasil 115 and NaOH) on the cleaning efficiency in terms of permeability recovery, residual proteins on the membrane and membrane characteristics (contact angle). For it, membrane resistances in series approach has been applied to permeate

fluxes data. Besides, estimation of the deposited proteins on the membrane by FTIR-ATR and by Pierce-BCA method has been carried out.

2. Materials and methods

2.1. Ultrafiltration pilot plant.

UF laboratory plant was equipped with a Rayflow flat sheet module from ORELIS (France) which allows to work with two membranes of 100 cm^2 each one, working in series and operating by cross-flow filtration mode. The tank for the feed and cleaning solution has a capacity of 15 liters.

2.2. Membranes

The UF membranes tested in this study were from Microdyn Nadir. One of them was a polyethersulfone (PES) membrane with a molecular weight cut-off of 5 kDa (membrane UP005) and the other one was a hydrophilic polyethersulfone (PESH) membrane with a molecular weight cut-off of 30 kDa (membrane UH030). The membranes can be used at operating conditions of pH and temperature in the range of 0-14 and until 95ºC respectively.

2.3. Fouling solution

Bovine serum albumin (BSA, purity>98%, Sigma-Aldrich, Germany) solution was employed as organic foulant. Solutions with a concentration of 1% w/w and pH 7.5 were prepared for the fouling step. According to the manufacturer, the molecular weight of the BSA is about 66 kDa. Mixing of the stock solution was performed for a period of 24 h in a glass beaker with magnetic stirring to guarantee the complete BSA dissolution and then the stock solution was stored at 4ºC.

2.4. Contact angle measurements

The contacts angle of the UH030 and UP005 membranes (virgin, fouled and cleaned) were measured on Dataphysics OCA instrument (Data Physics Instruments GmbH, Filderstadt, Germany). To measure the contact angle of each membrane a surface of 1500 mm^2 in pieces of 60 mm of length and 25 mm of width was analyzed. The contact angle was determined by measuring the average contact angle (right and left) of 10 extra pure water drops on the membrane surface **(10 different locations for each membrane).** The time for the water droplet to reach the equilibrium with the membrane surface is important because if there is mobility of the polymer chains, the functional groups in contact with the drop of water can change and interfere with the results. In this case, the time was about 10-20 seconds until the equilibrium was confirmed.

2.5. Quantification of residual proteins

2.5.1. Quantification of residual proteins by FTIR-ATR

The quantification of the small amount of proteins on the flat membrane was performed by the Fourier Transform Infrared- Attenuated Total Reflectance (FTIR-ATR) analysis. FTIR tests were carried out by using a Bruker infrared spectrometer (Bruker, Germany). Membrane samples were cut in pieces of 5 cm^2. The crystal material was diamond and the infrared beam enters the ATR crystal at an angle of 45°. ATR spectra were obtained in the 4000-600 cm^{-1} region, using 32 scans and 4 cm^{-1} resolution to background spectra recorded in the air.

In this experiment, UH030 and UP005 membranes were analyzed using FTIR-ATR technique under different experimental conditions: membrane before first use (virgin), after being fouled with BSA and after the cleaning with the tested solutions (NaOH and P3 Ultrasil 115).

Although there are three bands useful to reflect the secondary structure of proteins, the Amide I band is the most commonly used in secondary structure analysis because it is the most sensitive to structural changes (Glassford et al., 2013). Amide I band is located close to 1656 cm^1 and it is due to C=O vibration (Delaunay et al., 2008). The most representative band of the PES (membrane material) is located between 1200 cm^{-1} and 1275 cm^{-1} and it is due to the vibration of the ether bond (C-O-C). Thus, quantification of deposited proteins in the range 0.5-350 $\mu g/cm^2$ is based on the ratio A_{amidaI}/A_{PES} instead of heights ratio, what is proposed by other authors (Bégoin 2004 and Bégoin et

al., 2006a, Paugam et al., 2013). A_{amidaI} is the area under the curve due to the 1656 cm^{-1} band and A_{PES} is the area under the curve due to the 1240 cm^{-1} band.

To obtain the calibration line the following sequence of experimental steps was carried out:

1. Rinsing of the membranes with distilled water during 2 hours, drying at room temperature during 24 hours and storing in a desiccator at least 30 minutes. Weighting of the membranes pieces (6 pieces of 900 mm^2) (P_1).

2. Submersion of the 6 membranes pieces in solutions of different BSA concentrations (5, 10, 15, 20, 25, 30 $g \cdot l^{-1}$) during 24 hours.

3. Drying at room temperature during 24 hours and storing in a desiccator at least 30 minutes and weighting again of the membranes pieces (P_2). At this moment the amount of proteins per membrane area ($\mu g/cm^2$) can be obtained from the P_1 and P_2 values.

4. Analysis with FTIR-ATR of the different membrane pieces including the virgin membrane that was used as control membrane. Fouling membrane is represented only by Amide I band since in this case the fouling solution only consisted BSA molecules.

5. Determination of the calibration line from the obtained data of amount of proteins ($\mu g/cm^2$) and ratio $A_{amidaI}/A_{PES.}$

In Eq. 1 and Eq. 2 the calibration line (UH030 and UP005 membrane respectively) relating areas and protein mass on the membrane can be observed:

$$A_{1656}/A_{1240} = 0.003 \cdot [\text{proteins}, \mu g/cm^2] + 0.3487 \qquad r^2 = 0.970 \qquad (1)$$

$$A_{1656}/A_{1240} = 0.0036 \cdot [\text{proteins}, \mu g/cm^2] + 0.3133 \qquad r^2 = 0.974 \qquad (2)$$

187 2.5.2. Quantification of residual proteins by Pierce-BCA method after soxhlet extraction

188

189 The extraction of BSA from the membranes was carried out using a method developed
190 by Puro et al., 2002. The protein extractions from membrane samples after being fouled
191 with BSA and after the cleaning with the tested solutions (NaOH and P3 Ultrasil 115)
192 were carried out in a Soxhlet extractor. 350 ml of acetone-water solution (9:1) was used
193 as a solvent and the total time for each extraction was 2 h. After that, acetone was
194 separated by distillation with a rotary evaporator equipment. Finally, the amount of
195 proteins in each sample was measured by Pierce BCA test. Experimental procedure of
196 Pierce BCA test was: 1 ml of BCA working reagent was added to 1 ml of each sample,
197 then samples were incubated at 60ºC for 1 hour and after cooling at room temperature,
198 the absorbance samples were measured with the spectrophotometer at 562 nm.

199

200

201 **2.6. Experiments and measurements**

202

203 2.6.1. Membrane water flux

204 The membrane water flux was measured at the beginning of the experiment and after
205 the first and second rinsing. Distilled water was used as feeding solution and
206 measurements were performed at 25ºC and at transmembrane pressure of 2 bar.

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2.6.2. Membrane fouling

The fouling tests were carried out with BSA at transmembrane pressure of 2 bar, at 25ºC and during 2 hours. The cross-flow velocity was maintained at 2.0 m/s. To keep the BSA concentration constant both the permeate and the retentate streams were continuously recirculated to the feed tank. The flux was measured each 3 min using a balance KB-800-2 (Kern, Germany) with an accuracy of ± 0.01 g. The permeate flux was calculated from mass data, changing to volume data considering the permeate density as the water density at the operating temperature. Thus, flux was calculated dividing the difference between permeate volumes by the time between measurements and the membrane area.

2.6.3. Cleaning experiments

The cleaning procedure depended on the aim of the test, since partial cleanings were required according to the goals of this work. Table 1 details the three different procedures carried out in concordance with the above mentioned objectives. The general procedure included a first rinsing step with distilled water, a chemical cleaning step and a final rinsing step (in continuous feed mode) until distilled water characteristics were reached.

The first rinsing step was carried out at 25ºC, at transmembrane pressure of 1 bar, at cross flow velocity of 2.2 m/s during 30 minutes (only during the first 5 minutes, this step was carried out in continuous feed mode). Flux was measured every 5 minutes.

The chemical cleaning step (with NaOH or P3 Ultrasil 115 solution) was carried out at a transmembrane pressure of 1 bar and at a cross flow velocity of 2.2 m/s during 30 minutes and in total recycle mode. The optimum conditions of temperature and concentration of the cleaning solutions were chosen according to previous studies (Luján-Facundo et al., 2013). Thus, the tested temperature and concentration were 45ºC and $0.4 \cdot 10^{-3}$ g/l (pH 9) for NaOH solution and 35ºC and 0.7% v/v (pH 12.73) for Ultrasil solution.

Each experiment was repeated at least twice, but if the results differed significantly, the experiment was repeated three times and the mean values were reported. Results were reproducible.

Table 1: Type of procedure and operating conditions for the experiments.

Type of procedure	**1**	**2**	**3**
Stage	Initial flux	Initial flux	Initial flux
	BSA fouling	BSA fouling	BSA fouling
	-	First rinsing	First rinsing
	-	Flux after rinsing	Flux after rinsing
	-	NaOH (45ºC, pH 9)	Ultrasil (35ºC, 0.7%)
	-	Second rinsing	Second rinsing
	-	Final flux	Final flux

2.6.4. Evaluation of cleaning efficiency (CE) and resistances

Cleaning efficiency was calculated according to Eq. 3 defined by (Blanpain-Avet et al., 2009). This parameter was used to evaluate the rinsing process.

$$CE = \frac{R_t - R_c}{R_t - R_m} \cdot 100 \quad (3)$$

Where, R_m is the initial membrane resistance, calculated from Darcy's law equation (Eq.4) using the initial water flux (J_w) measured before each fouling experiment; R_c is the cleaning resistance, calculated using Eq. 4 replacing J_w by the cleaned membrane flux (J_{wc}), which was measured after the second rinsing water; R_t is the membrane resistance after the fouling step calculated using Eq. 4, replacing J_w by membrane flux after the fouling step (J_t).

$$R_m = \frac{\Delta P}{\mu \cdot J_w} \quad (4)$$

R_{irrev} was calculated applying Eq.5, where J_{wr1} is the membrane flux after the first water rinsing and R_{rev} was calculated according to Eq. 6.

$$R_{irrev} = \frac{\Delta P}{\mu \cdot J_{wr1}} - R_m \quad (5)$$

$$R_t = R_m + R_{rev} + R_{irrev} \quad (6)$$

Finally, it is important to define the membrane residual resistance (R_{res}) that refers to the membrane resistance remaining after the cleaning step in comparison with the initial membrane resistance. It is defined by Eq. 7:

$$R_{res} = R_c - R_m \quad (7)$$

2.6.5. Statistical analysis

A statistical analysis was carried out with STATGRAPHICS Centurion XVI. In this way, a multiple variable analysis was studied and p-values between CE's, contact angle and the amount of residual proteins were calculated.

3. Results and discussion

3.1. BSA feed solution and membranes characteristics: zeta potential and contact angle

BSA concentration in the feed solution was 10 g/l. BSA solution and membrane zeta potential values vary with the pH. The point at which BSA solution or membrane changes its charge and the zeta potential becomes zero is called isoelectric point (pI). In a previous study (Luján-Facundo et al., 2013), it was shown that the pI of the BSA solution was 4.57. In addition, the zeta potential of the BSA at pH 7.5 (feed solution pH in the fouling experiments) was around -55 mV. Thus, the organic foulant was charged negatively in the fouling tests (Kuzmenko et al., 2005; Porcelli and Judd 2010 and Jun et al., 2011). According to a previous study (Luján-Facundo et al., 2013), UH030 and UP005 membranes showed a negative zeta potential (-6.33 and -8.07 for membrane UH030 and UP005 respectively, values shown in Table 2). Taking into account that zeta potential of BSA at pH 7.5 was extremely negative (-55 mV), it can be affirmed that

membrane charge was not a significant factor on the different fouling behavior of both membranes.

Contact angle measurements were also evaluated to complete the characterization of the membrane surface. Contact angle is directly related with the degree of hidrophilicity of the membrane and it depends on the membrane material and the membrane porosity (Susanto and Ulbricht 2007). It is considered that membranes are hydrophilic if contact angle is not higher than 90º (Muthu et al., 2014). As it can be observed in Table 2, both membranes are considered hydrophilic and contact angle values were similar and in concordance with the literature (Metsämuuronen and Nyström 2009 and Balcıoğlu and Gönder 2014). However, UH030 shows slightly higher contact angle than UP005 membrane. This fact can be attributed to UH030 membrane is rougher than UP005 as reported by other authors (Li et al., 2013 and Hou et al., 2014).

Table 2: Membrane characteristics obtained experimentally.

	UH030	**UP005**
Molecular weight cut-off *	30 kDa	5 kDa
Membrane resistance (m^{-1} (R_m))	$\approx 3 \cdot 10^{12} \pm 1.39 \cdot 10^{12}$	$\approx 1 \cdot 10^{13} \pm 0.03 \cdot 10^{13}$
Zeta potential (mV)	-6.33 ± 0.004	-8.07 ± 0.065
Contact angle (º)	65.54 ± 3.21	54.27 ± 3.17
Roughness (nm)	12.12 ± 3.16	1.59 ± 0.20

*value supplied by the manufacturer

3.2. Membrane fouling

Fig. 1 depicts the evolution of flux (J_{BSA}) during BSA ultrafiltration as a function of time at constant transmembrane pressure of 2 bar. The trend of flux for both tested membranes was similar and the steady flux was reached after about the first 36 minutes.

The mechanisms for BSA fouling have been studied for many years ago. Thus, (Kelly and Zydney 1995) reported that protein fouling is produced by two different mechanisms: deposition of BSA aggregates on the membrane surface and chemical attachment to the previously deposited proteins.

UP005 membrane showed lower fouling degree since the flux-decline profile was less prominent than membrane UH030. This fact is mostly related with three parameters that could control its fouling (Vatanpour et al 2014): hydrophilicity (evaluated from contact angle), surface charge and surface roughness. Besides, the different pore size of the two membranes can affect the membrane fouling in spite of the high molecular weight of BSA.

In addition, there were two reasons to corroborate that UH030 was more prone to fouling than UP005 membrane. On one hand, permeate flux during BSA ultrafiltration decreased at a higher extent for UH030 membrane (11 $l/m^2 \cdot h$) than for UP005 membrane (5 $l/m^2 \cdot h$). On the other hand, taking into account the normalized values ($J_{BSA}/J_{water\ before\ fouling}$), UH030 membrane had higher flux decline than UP005 membrane. These two reasons corroborate that UH030 was more prone to fouling than UP005 membrane.

According to Table 2, UP005 showed lower contact angle than UH030. In agreement with Rahimpour and Madaeni 2010, the higher the hydrophilicity of the membrane surface is, the better the antifouling properties are. Besides, UP005 has a lower roughness than UH030 as it was showed in a previous work (Luján-Facundo et al., 2010). This low roughness value implies low trapping of the BSA on the peak and valleys of the membrane surface (Vatanpour et al., 2014). All of these characteristics explain the lower flux diminution for UP005 in comparison with UH030 membrane.

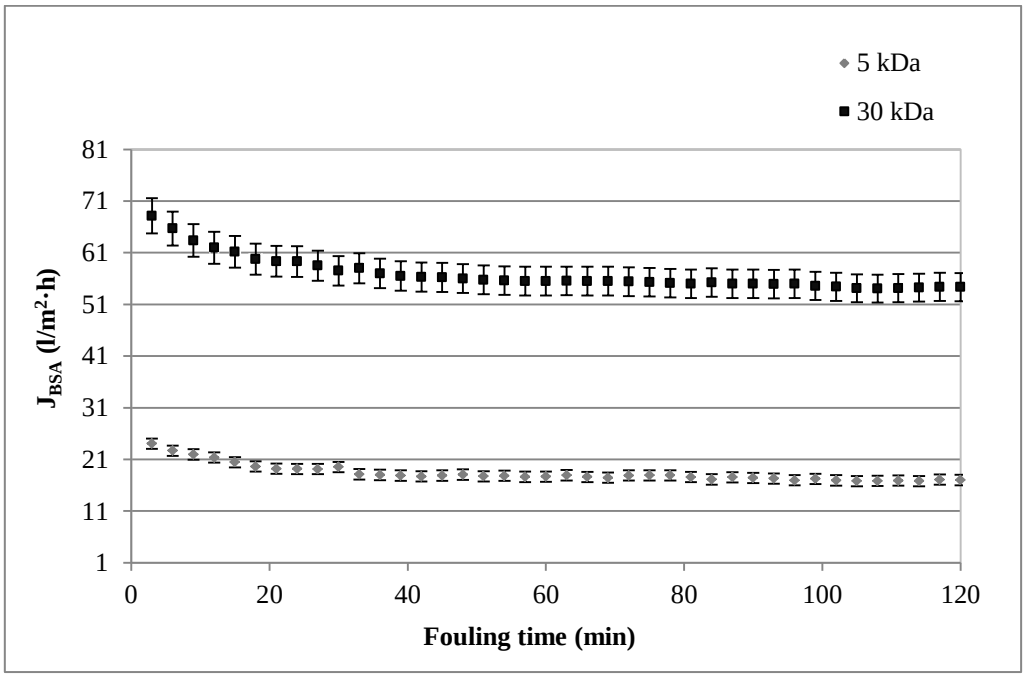


Figure 1: Evolution of flux during BSA ultrafiltration with time.

3.3. Cleaning efficiency and membrane resistances

3.3.1. Effect of chemical cleaning on cleaning efficiency

The highest value for cleaning efficiency for both membranes was achieved when P3 Ultrasil 115 was used, with a value of 100%, what means a total flux recovery. With NaOH solution, the CE values were 88.05% and 83.10% for UH030 and UP005, respectively. These results are consistent with previous studies (Rabiller-Baudry et al., 2002; Popović et al., 2009; Levitsky et al., 2012 and Diagne et al., 2013) which also showed for P3 Ultrasil solution better results than for NaOH solution. Comparing both membranes, CE values were very similar and there were not significant differences. P3 Ultrasil cleans mainly by breaking the bonds between the foulant and the membrane surface, what prevents the BSA from re-deposition. On the contrary, NaOH reacts with BSA and as a consequence the hydrolysis or swelling of BSA can occur. This could hinder the complete removal of proteins (Popović et al., 2010). Thus, hydrolysis can drive to amino-acids penetration into the membrane pores and swelling of the proteins in the pores would make very difficult their removal.

As it can be observed in Table 3, contact angle measurements after the fouling experiments showed an increase in the value of this parameter due to BSA deposition on the membranes, what implied a diminution in hydrophilicity. Although, BSA is a hydrophilic protein, the membrane properties after fouling become more hydrophobic in the same way that other authors have reported (Razali et al., 2014).

By contrast, membranes after cleaning showed lower contact angle than fouled membranes. These values were even a few lower than the values measured in the virgin

membranes. Specifically, results from membrane cleaned with NaOH were lower than membranes cleaned with P3 Ultrasil 115. These results suggested that membranes became more hydrophilic due to surface modification by NaOH and because of the presence of hydrophilic functional groups (i.e. –OH). This is in concordance with the results reported by other authors (Levitsky et al., 2011; Baek et al., 2012; Levitsky et al., 2012; Naim et al., 2012 and Li et al., 2013).

Table 3: Contact angles values for membrane UH030 and UP005 fouled and cleaned.

	Contact angles membranes (º)	
	UH030	**UP005**
Virgin	65.54 ± 3.16	54.27 ± 3.48
Fouled	75.37 ± 4.25	76.2 ± 4.31
NaOH	57.17 ± 4.39	53.33 ± 4.02
P3- Ultrasil 115	60.87 ± 5.88	73.25 ± 5.21

3.3.2. Membrane resistances

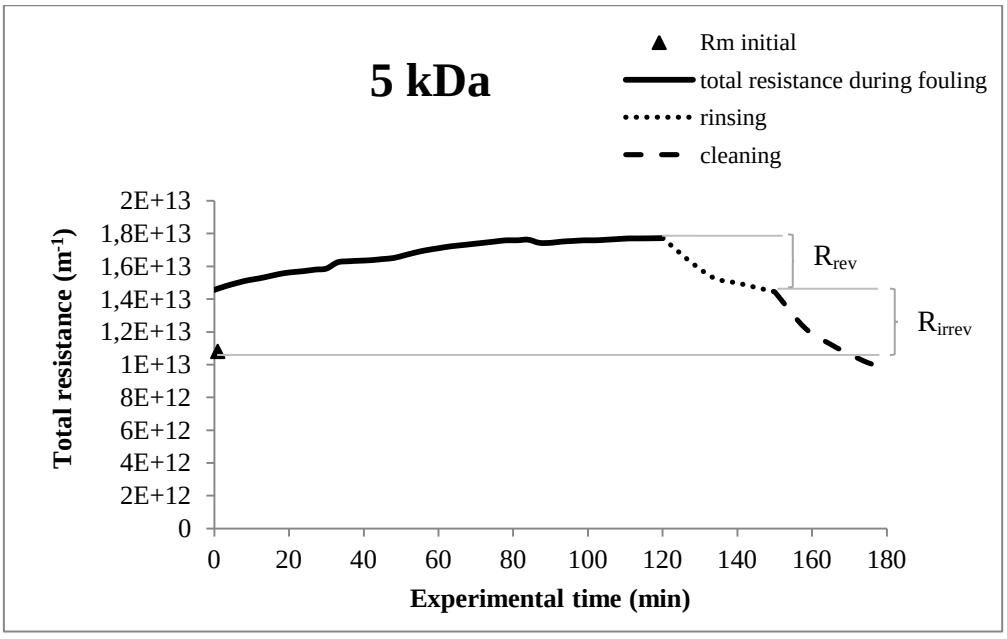
Membrane resistances R_m (initial and final), $R_{rev,}$ R_{irrev} and R_{res} were calculated as explained in Section 2.6.4 for a deeper comparison between the fouling and the cleaning of the two membranes and the efficiency of both tested cleaning agents (Huyskens et al., 2008 and Minehara et al., 2014).

According to Fig.2 and Fig.3, it seems clear that in general terms the reversible fouling (after rinsing) was more pronounced than irreversible fouling (after chemical cleaning). It indicates that strongly attached to the membrane BSA molecules provided the lowest contribution to the membrane fouling. In spite of it, it is very important to focus on the

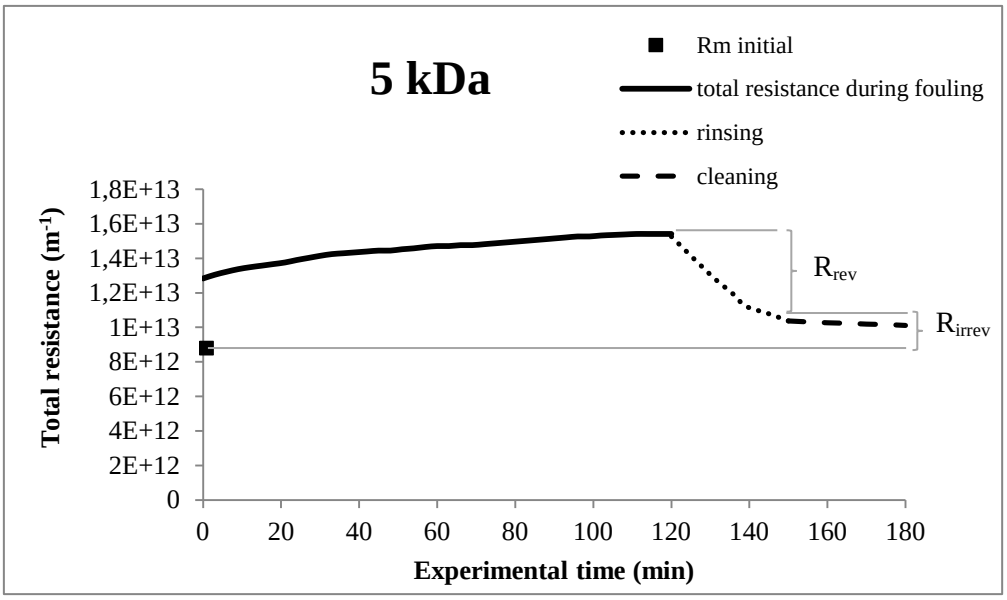
chemical cleaning step in order to maximize flux recovery, which is the main objective of this study.

In addition, the lowest resistances were obtained for membrane UH030. This fact was related with the higher pore size of the membrane UH030 comparing with UP005. There was no residual resistance when Ultrasil was used for both membranes, what implies that the permeability value was recovered totally after cleaning in comparison with the initial permeability value before fouling. By contrast, when NaOH solution was used, a residual membrane resistance remained.

a)



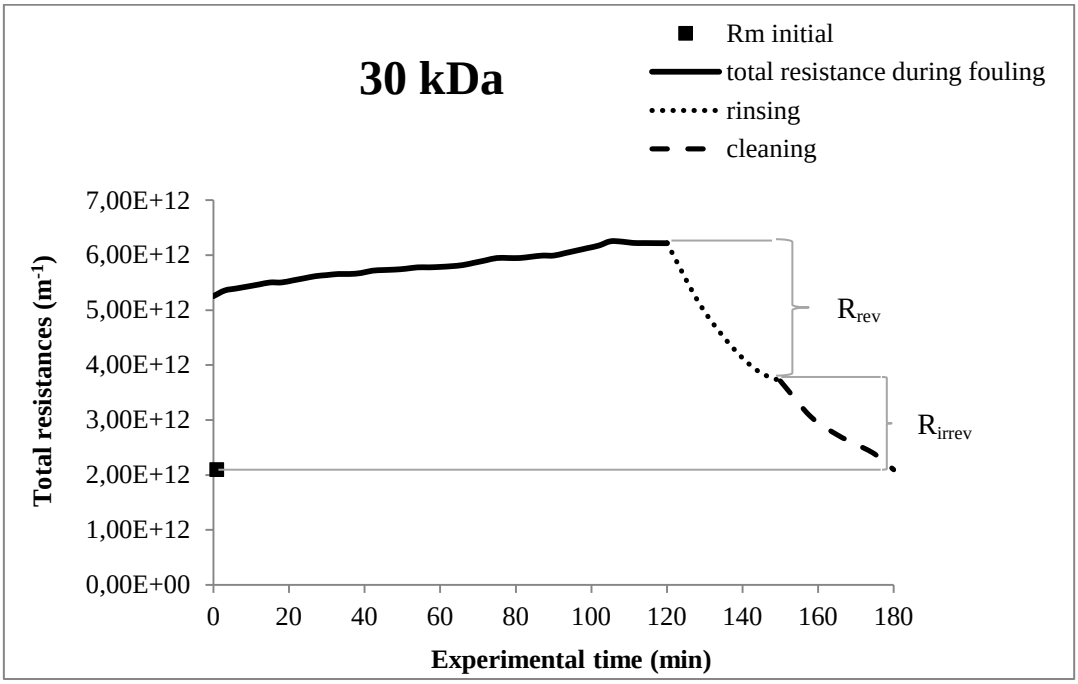
403 b)



404
405 **Figure 2: Evolution of UP005 membrane resistance during the experiment using:**
406 **(a) Ultrasil solution. (b) NaOH solution.**
407

408

409 a)



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b)

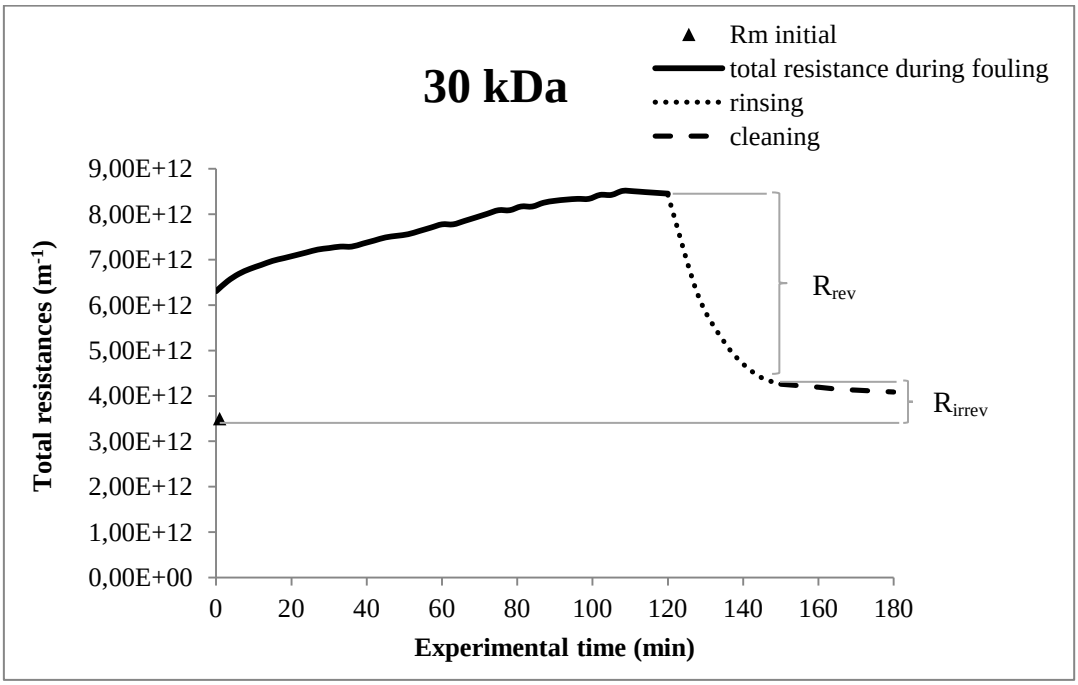

Figure 3: Evolution of UH030 membrane resistance during the experiment using: (a) Ultrasil solution. (b) NaOH solution.

3.4. Identification and quantification of organic fouling

Two methods have been employed to quantify the amount of residual protein on the membrane: FTIR-ATR analysis and Pierce-BCA after solid-liquid extraction. In this study, quantification analysis was considered as an optimal tool to corroborate and compare results in terms of permeability recovery and remaining BSA.

Virgin FTIR-ATR spectra of the membranes fouled by BSA, membranes cleaned by NaOH solution and membranes cleaned by P3 Ultrasil 115 solution were shown in Fig. 4 and Fig. 5. The most representative bands were provided in Table 4. It is important to highlight on the Fig. 4 and Fig. 5 that the Amide I band at 1655 cm^{-1} that it is the BSA fingerprint on the membrane surface and the ether band at 1240 cm^{-1} that is related to

the membrane material (PES). Both peaks areas were used for quantifying (Table 5) the amount of residual protein remaining on the membrane following Eq. 1 and Eq. 2 (Section 2.5.1).

Table 5 shows the amount of residual proteins that remained on the membrane after fouling with BSA and cleaning with NaOH and P3 Ultrasil 115 solutions. As expected, after the evaluation of flux decay in fouling tests, membranes without cleaning had higher values of residual proteins on the UH030 membrane than in UP005 independently of the quantification method used. This result was in concordance with Section 3.2 about membrane fouling in which it was showed that UH030 was more prone to fouling than UP005. However, the chemical cleaning did vary the membrane surface characteristics (table 3 shows the variation in the membrane contact angle). Thus, UH030 became more hydrophilic than UP005 after the cleaning with Ultrasil what drove to a lower amount of residual proteins in the membrane. In the case of NaOH, results of residual proteins after the cleaning from FTIR-ATR analyses were very similar for both membranes what coincide with similar contact angle values of both membranes.

A small amount of BSA remained on the membrane after cleaning. However, this fact did not impede good results in terms of CE as it can be observed in Section 3.3.1. Nevertheless, it may cause higher fouling in the following tests due to BSA attachment to BSA molecules previously adsorbed to the membrane (residual BSA). Both FTIR and Pierce-BCA results corroborated the CE values previously reported (Section 3.3.1) from a qualitative point of view; thereby it can be concluded that Ultrasil solution was a better cleaning agent than NaOH solution.

455 The reason why residual proteins in the membrane did not affect the permeate flux was
456 probably due to the fact that the amount of residual proteins was not high enough to
457 block the pores. Argüello et al. (2005) observed the same behaviour using enzymatic
458 cleaning for membranes fouled with whey proteins. They concluded that residual
459 enzymes could lead to a self-cleaning mechanism. Nevertheless, this phenomenon was
460 also observed with acidic cleaning (Paugam et al., 2013). On the contrary, this
461 phenomenon was not observed by the same authors with NaOH using skim milk as
462 foulant.

463 It is important to remark that quantification results from Pierce-BCA method were
464 lower than results from FTIR-ATR analysis. This could be attributed to the fact that
465 methods are completely different and experimental errors in the calibration line (FTIR-
466 ATR, R^2=0.97) due to accuracy in the weight of the membrane pieces with very low
467 protein amount. However, both methods showed similar results in terms of percentage
468 of BSA removal with the cleaning solutions. Thus, it can be concluded that both
469 methods can be valid from a qualitative point of view for determining the efficiency of
470 the cleaning solutions.

471 Taking into account the results showed in Table 6, it can be observed that the cleaning
472 with Ultrasil was the most efficient. The cleaning with NaOH for UH030 membrane led
473 to protein removal efficiencies of 42.7 and 37.34 % for FTIR and Pierce-BCA methods,
474 respectively. For UP005 the values were 10.05 and 12.06% for FTIR and Pierce-BCA
475 methods, respectively.
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Table 4: Assignment of relevant IR absorption bands (Zhu and Nyström 1998; Begoin et al., 2006a and Levitsky et al., 2012).

IR band (cm^{-1})	Range	Functional groups
3307	3190-3550	H-O-H
1655	1600-1700	Amide I: C=0, C-N, N-H
1577	1500-1600	Amide II: C-N, N-H
1487	1475-1600	Benzene rings (alq ar.)
	1460-1550	S-C-S-O_2
1240	1200-1275	R-C-O-C-R
1151	1150-1225	O-H deformation and C-O stretching vibration interaction
1035	About 1030	benzene rings

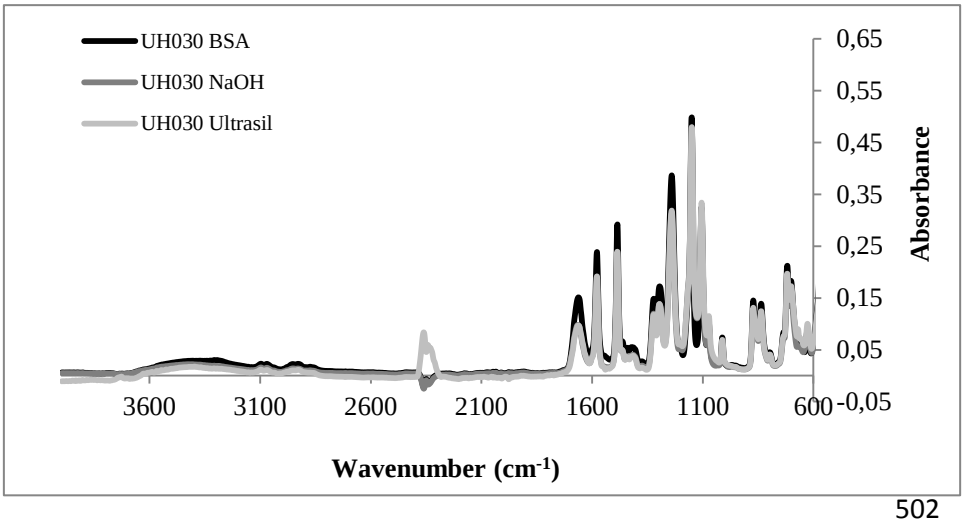
Table 5: Amount of residual proteins that remain on the membrane.

Membrane	UH030 (µg/cm^2)		UP005 (µg/cm^2)	
Method	**FTIR**	**Pierce-BCA**	**FTIR**	**Pierce-BCA**
BSA	114.77 ± 7.85	16.55 ± 3.06	77.42 ± 8.59	8.54 ± 0.009
NaOH	65.77 ± 6.40	10.37 ± 0.04	69.64 ± 5.01	7.51 ± 0.03
P3-Ultrasil 115	44.77 ± 9.66	8.68 ± 0.63	54.64 ± 1.21	5.66 ± 0.0004

Table 6: BSA removal efficiency.

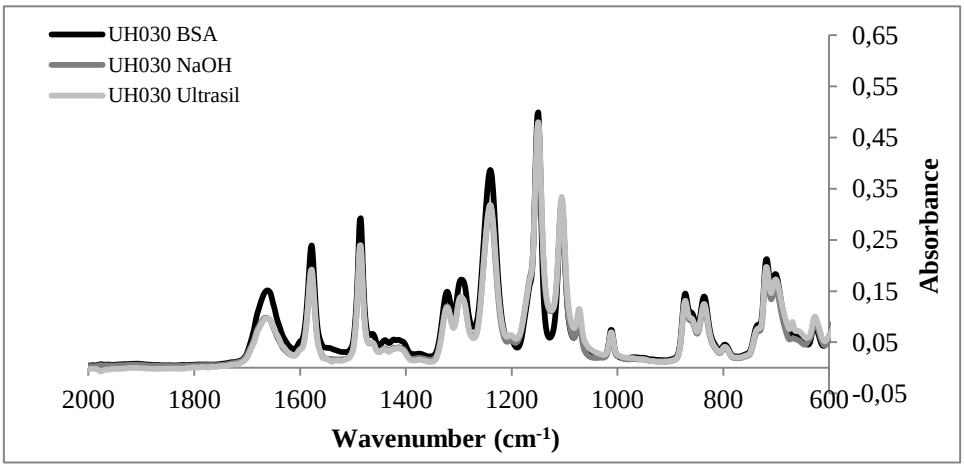
	BSA removal efficiency (%)			
Membrane	**UH030**		**UP005**	
Cleaner	**FTIR**	**Pierce-BCA**	**FTIR**	**Pierce-BCA**
NaOH	42.70	37.34	10.05	12.06
P3-Ultrasil 115	61.00	47.55	29.42	33.72

a)



502

503 b)



512 **Figure 4: FTIR-ATR spectra for UH030 membrane:**
513 **a) in the 4000-600 cm^{-1} region b) in the 2000-600 cm^{-1} region.**
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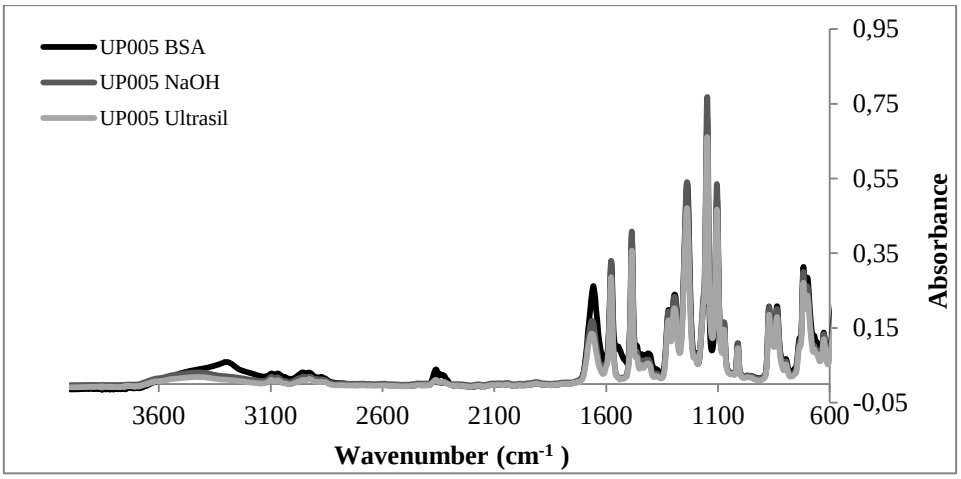
a)



b)

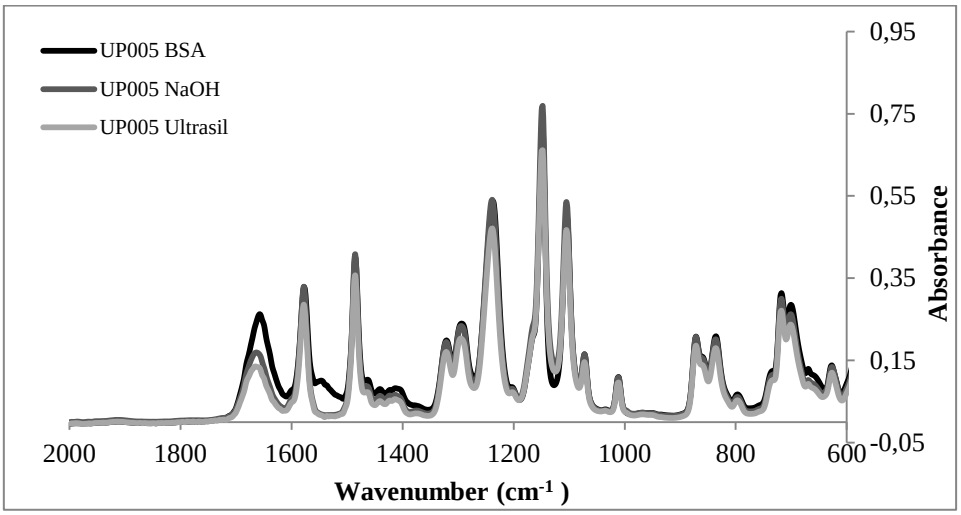


Figure 5: FTIR-ATR spectra for UP005 membrane: a) in the 4000-600 cm^{-1} region b) in the 2000-600 cm^{-1} region.

Table 7 shows the p-values between CE, contact angle measurements and amount of residual proteins obtained from multiple variable analysis. If p-values were lower than 0.05, it was considered that variables were statistically significant at the 95.0% confidence level. As it can be observed in Table 7, there were no statistically significant relation (p-values higher than 0.05) between CE's values and the amount of residual proteins for any case studied, confirming that in spite of the high CE, BSA molecules

remained in the membranes. By contrast, relation between CE's values and contact angle were statistically significant (p-values lower than 0.05) for all cases except for UP005 cleaned with NaOH, what can be explained by the surface modification due to NaOH as explained in section 3.3.1.

Table 7: p-values calculated from multiple variable analysis.

	UH030			
	NaOH/FTIR	NaOH/Pierce	P3 Ultrasil 115/FTIR	P3 Ultrasil 115/Pierce
CE versus contact angle	0.0487	0.0487	0.0247	0.0247
CE versus BSA residual	0.1166	0.7362	0.6952	0.7156
	UP005			
	NaOH/FTIR	NaOH/Pierce	P3 Ultrasil 115/FTIR	P3 Ultrasil 115/Pierce
CE versus contact angle	0.0600	0.0600	0.0109	0.0109
CE versus BSA residual	0.7594	0.7517	0.2302	0.2106

CONCLUSIONS

In this paper the behavior of two UF membranes has been evaluated in terms of fouling after BSA solution filtration and in terms of cleaning with two cleaning solutions (NaOH and P3 Ultrasil 115). It can be concluded that fouling of the UH030 membrane was higher than the UP005, what can be explained both by the different membrane pore size and by characteristics of the membranes such as contact angle, zeta potential and roughness.

Fouling was mainly reversible for both membranes, since membrane resistances calculations showed a higher reversible resistance than the irreversible one. The

562 irreversible resistance was totally eliminated by P3 Ultrasil 115 in the tested conditions;
563 meanwhile CE of the membranes after cleaning with NaOH at a temperature of 45ºC
564 was between 80 and 90%.

565 Almost complete restoration of the initial flux and high values of cleaning efficiency
566 (100%) with the P3 Ultrasil 115 solution did not imply the complete removal of BSA
567 from the membrane as detected by the FTIR-ATR and Pierce-BCA methods after
568 extraction. Thus, this kind of analysis can become an important tool to study membrane
569 cleaning together with the well known study of the membrane in series resistances. In
570 fact, non-eliminated BSA can favour membrane fouling by attachment of BSA of the
571 feed solution on the previously deposited protein molecules.

572

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576

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