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Enzymatic Cleaning of Inorganic Ultrafiltration Membranes Fouled by Whey Proteins

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The aim of this work was to study the cleaning of inorganic membranes fouled with whey protein solutions using the enzymatic formulation Alcalase (Novo Nordisk A/S). Hydraulic and chemical methods were considered to characterize the cleanliness of the membranes. Cleaning efficiency was observed to be a function of the operating conditions. The operating conditions tested were the following: recycling versus non-recycling of permeate, pH of the cleaning solution, addition of alkali to regulate the pH, enzymatic agent concentration, and cleaning time. The best conditions to perform the cleaning were related to the best conditions to hydrolyze whey proteins in a discontinuous reactor using the same enzyme preparations. Very high cleaning efficiencies (>90%) were achieved in short operating times (20 min). However, residual matter was observed on the membrane surface.

KEYWORDS: Inorganic membranes; enzymatic cleaning; whey protein solutions; cleaning efficiency

INTRODUCTION

The application of ultrafiltration (UF) and microfiltration (MF) in the food industry and particularly in the dairy industry has attracted increasing interest in recent years. These techniques are widely used for the concentration and separation of proteins from whey (*1*, *2*). However, the main problem of membrane techniques is the reduction of permeate flux with time due to membrane fouling, which also produces changes in the selectivity and decreases the overall process productivity. To maintain the membrane's performance, it is necessary to periodically stop the process to clean the membrane. Cleaning consumes time, energy, chemicals, and water, thus increasing production costs. The optimization of cleaning parameters can save money and increase the membrane life. The cleaning step has to be effective, easy, and fast, with no risks for the membrane and the rest of the installation (*3*).

Untill recently, very little attention had been paid to membrane cleaning. Also, cleaning sequences were almost identical for different types of membranes and feed solutions. In recent a few studies about the cleaning of membranes were published (*4*−*6*). Most of the protocols consisted of series of acid−alkaline cleaning cycles, although a few authors considered the possibility of using enzymatic formulations to clean organic membranes (*7*, *8*). The utilization of enzymatic detergents presents several advantages over conventional ones such as the easier neutralization of cleaning effluents and their biodegradability (*9*).

Flux reduction during filtration is mainly due to two types of phenomena: concentration polarization and fouling. Concentration polarization is considered to be a reversible phenomenon, whereas fouling effects are characterized by an "irreversible" decline in flux. Membrane fouling is due to the deposition and accumulation of particles on the membrane surface and/or the crystallization and precipitation of small molecules on the surface and within the membrane pores. The nature and extent of fouling depend on the characteristics of the solute and solute−membrane interactions (*2*). When dairy solutions are filtered, one of the main contributions to fouling is the adsorption of proteins on the membrane surface and into the membrane pores (*10*, *11*). Therefore, a cleaning formulation able to degrade the proteins that constitute the deposits could be used for membrane cleaning after whey ultrafiltration.

In a previous work by the authors (*12*) the protein hydrolysis ability of different commercial detergents was studied using a discontinuous reactor. Two enzymatic (P3-Ultrasil 62 and P3-Ultrasil 53) and two nonenzymatic (P3-Ultrasil 13 and P3-Ultrasil 10A) formulations supplied by Henkel Ibérica, S.A. (Barcelona, Spain), were tested. It was concluded that only the enzymatic detergents were able to produce a significant hydrolysis of whey proteins. The best results were achieved with P3-Ultrasil 62 (Henkel Ibérica) at temperatures between 48 and 52 °C. Higher temperatures resulted in strong enzyme denaturation, thus causing a dramatic decrease of the enzymatic activity. The maximum hydrolysis degree (∼20%) was reached in 20 min. pH was observed to decrease during the process due to the proteins hydrolysis (*13*). The optimum initial pH was within the range of 10.3−10.8, which led to an average pH of 9.5−10.0.

In this work the utilization of the enzymatic formulation Alcalase to clean inorganic membranes used in the fractionation

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Table 1. Composition of the Whey Protein Concentrate

compound	WPC Eurial (g/kg)	compound	WPC Eurial (g/kg)
β-lactoglobulin	525	total main proteins	749
α-lactalbumin	165		
BSA	19	fat	<10
immunoglobulin G	40	salts	<20

of whey proteins was studied. Whey protein concentrate (WPC) solutions were used to foul the membranes, as these solutions are used as feed streams for whey protein fractionation. The influence of operating conditions (pH, time, concentration, etc.) on the cleaning efficiency was tested, and the results were compared to those obtained in the discontinuous reactor.

MATERIALS AND METHODS

Feed Solutions. Membranes were fouled with solutions prepared from Eurial WPC, which was kindly supplied by Eurial-Européen d'Industries Alimentaires (La Roche, France). This is a powdered WPC produced from sweet rennet whey, pretreated by thermocalcic aggregation and microfiltration, and concentrated by successive ultrafiltration (UF) and diafiltration (DF) operations. Its composition is indicated in **Table 1**. The rest of the solids are mainly other proteins and peptides, such as caseinomacropeptide (CMP), and lactose. The amount of low molecular weight compounds, such as salts, in this WPC was very low due to the intensive UF and DF steps. The preparation of the solutions from this WPC was carried out in filtered tap water up to a final concentration of 10−50 g/L, with further adjustment of pH and ionic strength (0.1 M NaCl).

Cleaning Solutions. As feed solutions are mainly composed of proteins, a commercial proteolytic enzyme was selected as cleaning agent. The proteolytic enzyme considered was Alcalase 0.6 L (Novo Nordisk A/S), which is produced from the microorganism *Bacillus licheniformis*, and the main enzymatic component is Subtilisin Calsberg. The molecular weight of this enzyme is ~27.3 kg/mol, and the reported optimal conditions ranged between 50 and 70 °C and pH of 6−10. In some experiments NaOH was added to the cleaning solutions to adjust the pH.

Experimental Setup. Experiments were carried out in a standard ultrafiltration device. The inorganic membrane Carbosep M6, supplied by Orelis, S.A., was selected as it is being used for the fractionation of whey proteins. It consisted of a 6 mm internal diameter tube with a ZrO_2 filtering layer on a carbon support. Membranes of 25, 60, and 120 cm length were used. Carbosep M6 membranes are reported to have a molecular weight cutoff of 340 kDa.

Operating Conditions. The fouling−cleaning experiments consisted of several steps: membrane conditioning−disinfection, rinsing, water flux measurement, membrane fouling with WPC solutions, rinsing, water flux measurement, cleaning, rinsing, and water flux measurement. The membrane conditioning was performed before the first use of the membrane. After this step, the initial water flux, J_{wi}, was recorded to calculate the intrinsic hydraulic resistance, R_m, which served as a reference for the cleaning procedure efficiency. All of the cleaning cycles were performed at 50 °C as the enzymes showed the highest activity at this temperature (*12*). The values of pressure and feed flow were selected so that the shear stress was not too high in order to avoid enzyme denaturation. However, the flow rate should not be low as it could induce a large boundary layer. Higher flow rates increase the sweeping of substances deposited on the membrane surface. Some authors recommend using the same pressure and the same or higher feed flow as the ones used to foul the membrane (*4*). Tap water filtered through a series of 10, 5, 2, 1, and 0.2 μm microfilters (fouling index < 3) was used to rinse the membranes and prepare the solutions. Operating conditions are summarized in **Table 2**. The ratio of cleaning solution volume to membrane area was 44 L/m^2 for all of the runs. Most of the experiments were repeated several times to check the reproducibility of the results, which was observed to be very high. Average values are presented.

Table 2. Operating Conditions for the Fouling–Cleaning Cycles

feed stream	v (m/s)	ΔP (bar)	t (min)	T (°C)	operation
NaOCl, pH 11	2	0.24–1	15–30	50	conditioning
water	2	0.24–1	10	50	rinsing
WPC Eurial, 10–50 g/L, 0.1 M NaCl, VCR 1	2–6	1.4–2.6	85–130	50	fouling
water	2	1	10	50	rinsing
Alcalase	2	1	30–180	50	cleaning
water	2	1	10	50	rinsing
NaOCl, pH 11	2	0.24–1	15–30	50	disinfection
water	2	0.24–1	10	50	rinsing

Table 3. Chromatographic Conditions for Protein and Peptide Analyses

parameter	SE-HPLC	RP-HPLC
column	TSK-GEL 2000SW, 300 × 7.5 mm	PLRP-S 300 A, 8 mm, 150 × 4.6 mm
supplier	Tosoh Corp.	Polymer Laboratories Ltd.
solvent	pH 6, 50 mM phosphate buffer, 15 mM in sodium sulfate	A: Milli-Q water/ 0.1% TFA B: [(20%) Milli-Q water/ (80%) ACN]/0.1% TFA
elution	isocratic	gradient (see below)
run time	15 min	30 min
injected vol	20 mL	20 mL
flow rate	1 mL/min	1 mL/min
temp	25 °C	40 °C
UV detection	214 nm	210 nm
column washing	Milli-Q water, 60 min, 1 min/mL; 10% ACN, 180 min, 0.2 mL/min; once a week	ACN: Milli-Q water (7:1), 40 °C, 60 min, 1 mL/min, after 20 samples

elution gradient for RP-HPLC

minute	0	1	8	16	22	22.5	23	23.5	30
% B	40	40	45	53	58	100	100	40	40

Analytical Methods. Protein hydrolysis degree (HD) was defined as the relationship between the number of peptidic linkages hydrolyzed per unit of substrate mass (n) and the total number of peptidic linkages per unit of substrate mass (n_{tot}):

$$HD = (n/n_{tot}) \times 100 \quad (1)$$

The hydrolysis degree was determined by means of the *o*-phthaldialdehyde method (*14*) as described by Arguello et al. (*12*), using a PU 8700 UV−vis spectrophotometer (Philips Scientific).

In this work an arbitrary unit (u) was defined to characterize the activity of the enzyme. This activity unit was considered as the amount of product (in milliliters) necessary to achieve 10% hydrolysis degree in 10 min at a pH of 9.5 and a temperature of 50 °C and for a concentration of WPC solution of 1 g/L. The activity was determined in a discontinuous reactor as described in ref *12*. The same type (Eurial WPC) and concentration (1 g/L) of WPC and the same operating conditions (50 °C and pH 8.1) were used for all of the measurements. The activity experimentally measured for the enzyme formulation selected in this work was 1.40 units/mL.

The amount of whey proteins and the molecular weight of the peptidic fragments formed during the hydrolysis were determined by chromatography using a reversed phase (RP) PLRP-S 300 A column of 150 × 7.5 mm (Polymer Laboratories, Inc.) and a size exclusion (SE) TSK-GEL 2000 SW column of 300 × 7.5 mm (Tosoh Corp.), respectively, and a Hewlett-Packard HP 1050 HPLC chromatograph, following the procedure described in Argüello et al. (*12*). The chromatographic conditions used are shown in **Table 3**. Reversed phase analyses were carried out using a modification of Resmini's method.

Nitrogen compounds for the RP- and SE-HPLC standards were protein hydrolysate P-0431, lactoferrin from bovine colostrum L-4765, α-lactalbumin L-6010, albumin bovine A-0281, β-lactoglobulin L-6879, γ-globulins G-500, L-methionine M-9625, L-cysteine C-1276, L-asparagine A-0884, L-leucine L-1512, and pepstatin p-4265 from Sigma. Solvents and salts for HPLC were acetonitrile (ACN) of HPLC grade from Aldrich, reference no. 27,071-7, and from Panreac, reference no. 263093, trifluoroacetic acid (TFA) anhyrous T-1647 from Sigma, Milli-Q water, dipotasium hydrogen phosphate 131512, sodium phosphate anhydrous 131716, and potasium dihydrogen phosphate 131509 from Panreac.

Permeate flux was measured gravimetrically, and the kinematic viscosity (ν) of each cleaning product was determined using a Cannon−Fenske capillary viscosimeter (Afora). Dynamic viscosity (μ) was calculated from density (ρ) at 50 °C, which was measured using an Abbe densimeter (Sibuya Optical). An automatic titrator Titroprocesor 686, equipped with a Dosimat 665 Metrohm (Roncaire, Velizy, France), was used to maintain the feed tank pH in the pH-stat experiments.

The evaluation of the chemical cleanliness of the membrane was performed by means of infrared spectroscopy (IR) and X-ray photoelectron spectroscopy (XPS) as described in Labbe et al. (*15*). Measurements were performed after and before each cleaning cycle, and results were compared with those of the new membrane.

IR spectra were registered on Perkin-Elmer spectrometers, dispersive (783) and Fourier transform (1710) models. Different types of evaluations were carried out: (a) upper layers, 1−10 μm from the upper part of the deposits were taken; and (b) lower layers, the material closest to the membrane support was considered.

XPS supplies information from very thin surface layers (no more than 10 nm thickness). Measurements were taken through a VSW HA 100 hemisphere analyzer fixed on an ultrahigh vacuum bell. The surface under study was exposed to unmonochromatized X-rays from a thin Mg/Al source (Mg Kα line, 1250 eV; Al Kα line, 1487 eV). The relative atomic composition was recorded, and results were expressed by means of the relationships C/Zr, Ca/Zr, N/Zr, and C/N, which represent the ratio between the relative atomic composition of the corresponding atoms.

CLEANING EFFICIENCY

Hydraulic and/or chemical methods can be considered to characterize the cleaning efficiency (*6*).

Hydraulic Characterization. Permeate flux data was used to evaluate the membrane hydraulic resistance (R), according to Darcy's law

$$R = \Delta P/\mu J \tag{2}$$

and where ΔP is the transmembrane pressure, J is the permeate

$$R_{uf} = R_m + R_f = R_m + R_{if} + R_r \tag{3}$$

flux, and R_{uf}, R_m, and R_f, are, respectively, the total resistance of the fouled membrane, the intrinsic hydraulic resistance of the membrane, and the resistance due to membrane fouling, which combines reversible (R_r) and irreversible (R_{if}) phenomena. The error in this evaluation was estimated by summing the relative errors as described in Daufin et al. (*3*) and were found to be <6.7% in this work.

Several authors (*3*, *11*) proposed the comparison between the hydraulic resistance of the cleaned membrane, R_{cw}, and the intrinsic hydraulic resistance of the membrane to evaluate the cleaning efficiency. Thus, the membrane can be considered to be clean when $R_{cw} - R_m/R_m \leq 0.067$. The difficulties in cleaning the membrane depend on its initial degree of fouling. To take into account this effect, the cleaning efficiency (E_{RW}) was evaluated in this work as the relationship between the hydraulic resistance removed during the cleaning ($R_{if} - R_{cw}$) and the hydraulic resistance that should have been removed to reach 100% efficiency ($R_{if} - R_m$):

$$E_{RW} = \frac{R_{if} - R_{cw}}{R_{if} - R_m} \times 100 \tag{4}$$

Table 4. Summary of the Results Obtained in the Discontinuous Reactor Using Different Enzyme Formulations

enzyme preparation	T_{opt} (°C)	initial pH_{opt}	av pH_{opt}	max HD[a] (%)	hydrolysis time (min)
P3-Ultrasil 62	48–52	10.3–10.8	9.5–10.0	20	20
Alcalase	48–52	7.2–9.7	6.2–8.4	20	20

[a] Hydrolysis degree.

Chemical Characterization. From the chemical point of view, the membrane is considered to be clean when there are no substances attached to its surface that do not belong to the membrane material itself. IR and XPS measurements provide information on the composition of the membrane surface. New and fouled−cleaned membranes were characterized, and the results were compared to check the presence of any residual matter after the cleaning cycle.

RESULTS AND DISCUSSION

The ability of the enzyme preparation to hydrolyze whey proteins was tested using a discontinuous reactor as described in Argüello et al. (*12*). The results obtained are summarized in **Table 4** and compared to those obtained in a previous work with P3-Ultrasil 62. The enzyme formulations were able to reach a maximum hydrolysis degree of 20% in 20 min. Alcalase was observed to be much less affected by pH than P3-Ultrasil 62, while it kept high activity at a much broader pH interval. The enzyme preparation manufacturer reported optimal conditions for the utilization of the enzyme of 50−70 °C and pH of 6−10. However, it was observed that the activity of the enzyme significantly decreased when the temperature was raised to >52 °C, and the optimal pH interval was narrower (6.6−9.7).

The influence of several factors on the membranes' cleaning efficiency was studied. These factors were the following.

Permeate Recycling during Cleaning. Different approaches can be considered to carry out the cleaning process: (a) recycling of both retentate and permeate to the feed tank; (b) retentate recycling to the feed tank while permeate is removed from the system; and (c) removal of both permeate and retentate from the system. The last approach involves the consumption of very large amounts of cleaning solutions so that it is not very often employed and was not considered in this research.

The transmission of enzymes through UF and MF membranes is usually high (*16*). The molecular weight of the enzyme used (27.3 kg/mol) is much lower than the molecular weight cutoff of the membranes considered, so that a high transmission is expected. If permeate is not recirculated, the amount of enzymes available for the cleaning can decrease at the same time as the volume of cleaning solution. Its concentration in the feed tank would increase unless its transmission through the membrane is 100%. As a result of the degradation of the deposits by the enzymes, the permeate can contain species that can foul the membrane, so that the recycling of the permeate could produce a reduction of the cleaning efficiency.

To study the influence of permeate recycling on the cleaning efficiency, a set of experiments was carried out with Carbosep M6 membranes. **Figure 1** shows the evolution of the hydraulic resistance (R_c) with time during the cleaning process with and

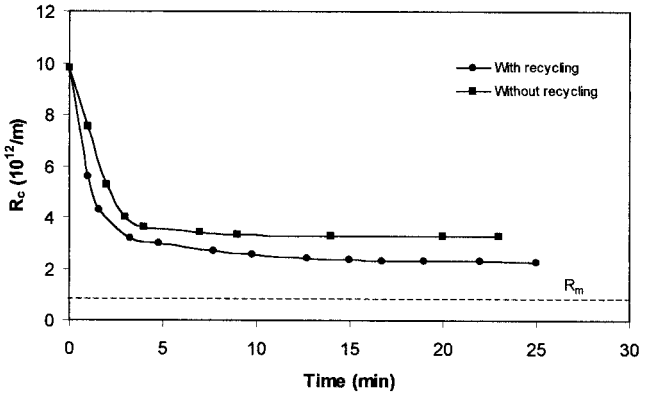

Figure 1. Evolution of the hydraulic resistance during the cleaning of Carbosep M6 membranes with Alcalase (1.40 units/L, 8.7 initial pH).

without permeate recirculation. It can be observed that the evolution of R_c with time does not present a minimum, but it reaches a stationary value. Therefore, it can be considered that there is no important redeposition of protein fragments on the membrane or, if there is, it does not affect the hydraulic resistance.

It can be observed that R_c decreases more rapidly when permeate is recirculated, until a steady state is reached. The steady value of R_c is lower when permeate is recycled, thus obtaining higher cleaning efficiency. From these results it can be suggested that there is high transmission of enzymatic activity to the permeate. This was demonstrated using the permeate to catalyze the hydrolysis of small amounts of WPC solutions in a discontinuous reactor following the procedure described by Argüello et al. (*12*). Moreover, the enzymatic activity of the retentate was measured at the beginning and at the end of the experiments with and without permeate recycling, using 100 mL of Eurial WPC solution in a discontinuous reactor. It was first observed that the activity had decreased during the membrane cleaning in both cases. It was also noted that the enzymatic activity was higher when permeate had not been removed, thus explaining the higher cleaning efficiency. As permeate is being removed, the cleaning solution volume decreases. Therefore, the volume is higher when permeate is recycled, so that the activity loss suffered by several enzymes can be compensated by the higher amount of enzymes available for the cleaning. The presence of protein fragments in the permeate was also detected and was the result of the enzymatic hydrolysis. As the efficiency is higher when permeate is recirculated, it can be considered that these fragments do not significantly foul the membrane.

Permeate recirculation is also more advantageous from the economical point of view, as lower amounts of cleaning solutions are required. Taking into account these results, the permeate recycling operating mode was selected to carry out the rest of the experiments.

The relative amount and size of the protein fragments present in the permeate and retentate during the cleaning process are depicted in **Figure 2**. It can be noted that the amount of protein fragments both in the retentate and permeate increases with time as a result of their incorporation to the solution due to proteins hydrolysis. The amount of protein fragments in the permeate is higher, and it was observed that there was a constant increase of the species with molecular weight under 3 kg/mol. However, the evolution of the concentration of species with molecular weight between 3 and 20 kg/mol shows a maximum at a cleaning time of 13 min. Hydrolysis takes place on the proteins deposited on the membrane and also on the protein fragments

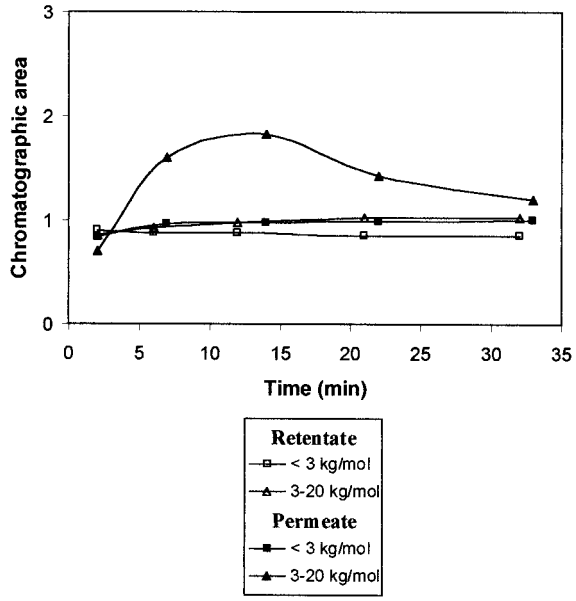

Figure 2. Evolution of the chromatographic area with time for the protein fragments present in the permeate and retentate during the cleaning of Carbosep M6 membranes with Alcalase (1.40 units/L) at pH 8.1.

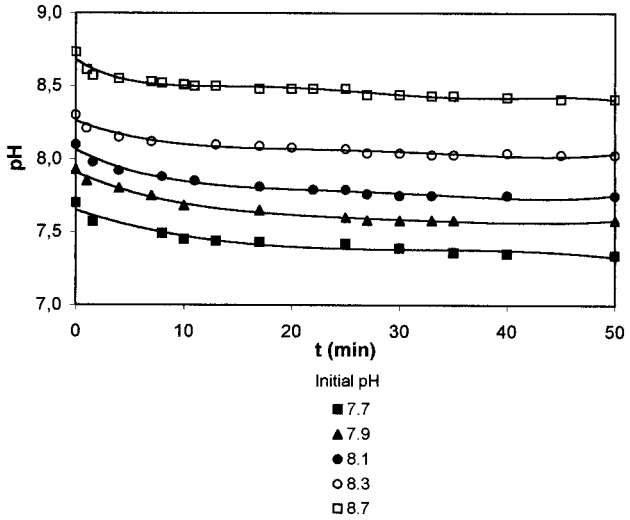

Figure 3. Variation of pH during the cleaning of Carbosep M6 membranes with Alcalase (1.40 units/L).

that are being incorporated to the solution. At the beginning of the cleaning process the amount of fragments incorporated into the solution increases, but afterward they are hydrolyzed so that the concentration of species with molecular weights of 3−20 kg/mol decreases and the concentration of species with the lowest molecular weight increases.

Cleaning Solution pH. To test the influence of the solution pH on the cleaning efficiency, the pH of the original cleaning solution was modified by adding small amounts of 3 N NaOH solution to the desired value.

In the experiments carried out in the discontinuous reactor it was established that the optimum pH for protein hydrolysis using Alcalase was within the range of 7.2−9.7. pH decreased during the process due to proteins hydrolysis (*13*), the average value being 6.2−8.4. Similar variation (depicted in **Figure 3**) was observed during membrane cleaning. However, the reduction of pH with time was smaller than the one observed in the discontinuous reactor, probably due to a lower amount of substrate.

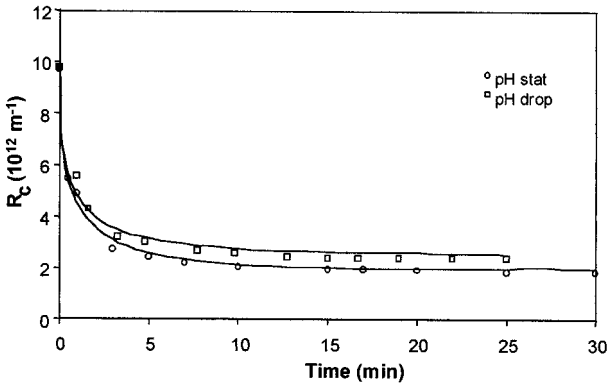

Figure 4. Evolution of the hydraulic resistance with time during the cleaning of Carbosep M6 membranes with Alcalase for pH-drop and pH-stat operating modes (initial pH 8.1, 1.40 units/L).

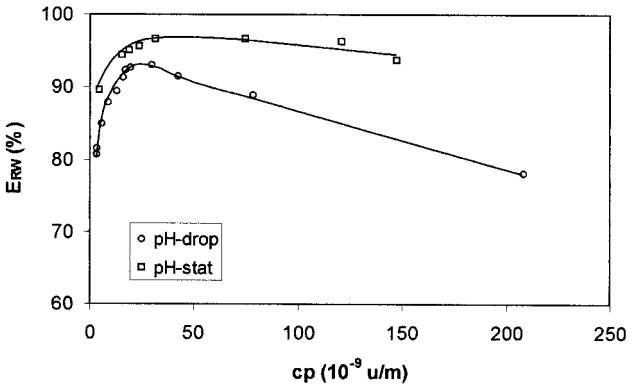

Figure 5. Evolution of the cleaning efficiency with the cleaning parameter (cp) for pH-drop and pH-stat operating modes.

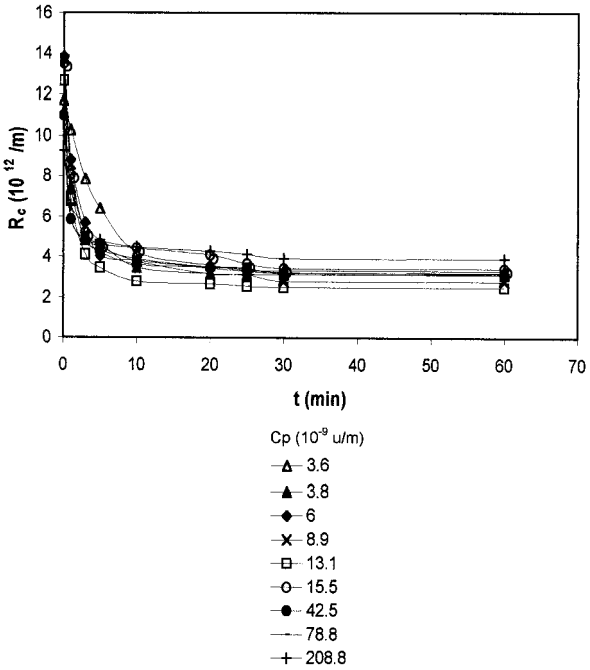

Figure 6. Evolution of the hydraulic resistance with time for different values of the cleaning parameter (cp).

The optimum pH for membrane cleaning can be affected by several factors, not just by the optimum pH for proteins hydrolysis. One factor that can be important is the ionic exchanger character of ZrO_2 membranes, which can exchange cations or anions with the solution depending on the value of the pH (*17*). Another factor can be the contribution to the cleaning of the NaOH added to adjust the pH. The optimum pH value was considered to be the one that produces the highest cleaning efficiency at the same operating conditions (temperature, flow rate, transmembrane pressure, enzyme concentration, time, and hydraulic resistance at the beginning of the cleaning process).

When Alcalase was used to clean Carbosep M6 membranes, it was observed that the cleaning efficiency was not very much affected by the initial pH. The cleaning efficiency for an enzymatic activity of 1.40 units/L was ~93% within the pH range studied (6.6−10.0). In fact, it had been observed from the experiments performed in the discontinuous reactor that Alcalase maintains high activity in a very broad pH interval (6.6−9.7). This variable is expected to be much more important for those enzymes more affected by pH as is the case of P3-Ultrasil 62 (**Table 4**). The optimum pH range is very similar to that obtained in the discontinuous reactor, thus indicating that enzymatic hydrolysis of proteins is the main factor that produces membrane cleaning. To check the contribution of the NaOH to the cleaning, several membranes were cleaned with diluted NaOH solutions at the optimum pH. The efficiency was observed to be <10%, thus demonstrating that the high cleaning efficiency achieved is mainly due to the enzymatic hydrolysis of the deposited proteins.

The reduction of pH with time that was observed during the cleaning process could affect the enzymatic activity. To avoid pH variation, the continuous addition of alkali (pH-stat method) was considered.

Figure 4 compares the removal of hydraulic resistance when a Carbosep M6 membrane fouled with WPC Eurial was cleaned with Alcalase + NaOH at variable (pH drop) and constant (pH stat) pH. In both cases R_c decreases very quickly, reaching a steady state in 15−20 min, but cleaning efficiency was observed to be higher when the pH was kept constant. This enzyme preparation is very little affected by pH and shows maximum activity at a very broad pH range (6.2−9.7). Therefore, the improvement in cleaning efficiency in pH-sat operating mode could be explained by the synergic effects between the enzymatic and the chemical cleaning caused by the NaOH added to the solution.

To study the effect of the added NaOH, additional cleaning experiments were performed in the absence of enzyme in both pH-drop and (initial pH value of 8.1) and pH-stat (constant pH value of 8.1) operating modes. Small hydraulic efficiencies were achieved in both cases: 5.0% in pH-drop and 10.0% in pH-stat. The difference between both values was very similar to that obtained for pH-drop (~93% efficiency) and pH-stat (~97% efficiency) enzymatic cleaning. Therefore, it can be suggested that the difference between the two cleaning modes can be due to the contribution of the added NaOH.

However, most of the enzymes are active only in a very narrow pH range, and they show an optimum pH value for enzymatic activity, as can be observed for P3-Ultrasil 62 and P3-Ultrasil 53 in Argüello et al. (*12*). In this case, keeping the pH constant can be much more important. The slow diffusion of product away from the fouling layer can create a pH profile in the boundary layer. This would mean that the pH at the membrane surface would be lower than that of the bulk solution, which would decrease protease activity. To better maintain surface pH, a buffer could also be added to the cleaning solution.

Enzymatic Agent Concentration. From the economic point of view the determination of the optimum amount of enzyme required to clean the membrane is very important. Lower amounts of enzyme can result in low cleaning efficiencies or

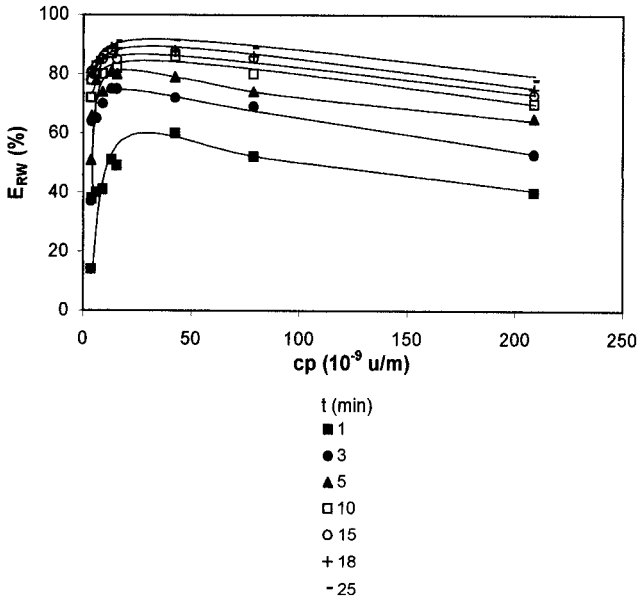

Figure 7. Evolution of the cleaning efficiency with the cleaning parameter (cp) for different cleaning times.

high cleaning times, and higher amounts of enzyme can increase costs and even result in further membrane fouling. The optimum amount of cleaning agent depends on a number of factors, such as the degree of membrane fouling, operating conditions, volume of cleaning solution (V), and membrane area (A_m). To take into account some of these factors, the cleaning parameter (cp) was defined as the relationship between the initial enzymatic activity (a), the hydraulic resistance that should be removed during the cleaning ($R_{if} - R_m$), and membrane area:

$$\text{cp} = a/(R_{if} - R_m)A_m \tag{5}$$

The optimum concentration of enzymatic agent was considered to be the one that produces the highest cleaning efficiency for a certain membrane area and membrane fouling ($R_{if} - R_m$). **Figure 5** shows the cleaning efficiency for different values of cp. It can be observed that the maximum efficiency that can be reached is a function of the operating mode (pH-stat vs pH-drop). The optimum value of cp was $\sim 32 \times 10^{-9}$ units/m for both operating modes. It can also be observed that higher amounts of enzyme than the optimum result in a decrease of cleaning efficiency, probably due to membrane fouling caused by the cleaning agent and/or by redeposition of solutes on the membrane surface. This effect was more important in the pH-drop operating mode, probably due to the contribution to the cleaning of the NaOH added in the pH-stat operating mode.

Cleaning Time. Cleaning time was defined as the time required to reach a steady value of the hydraulic resistance, and

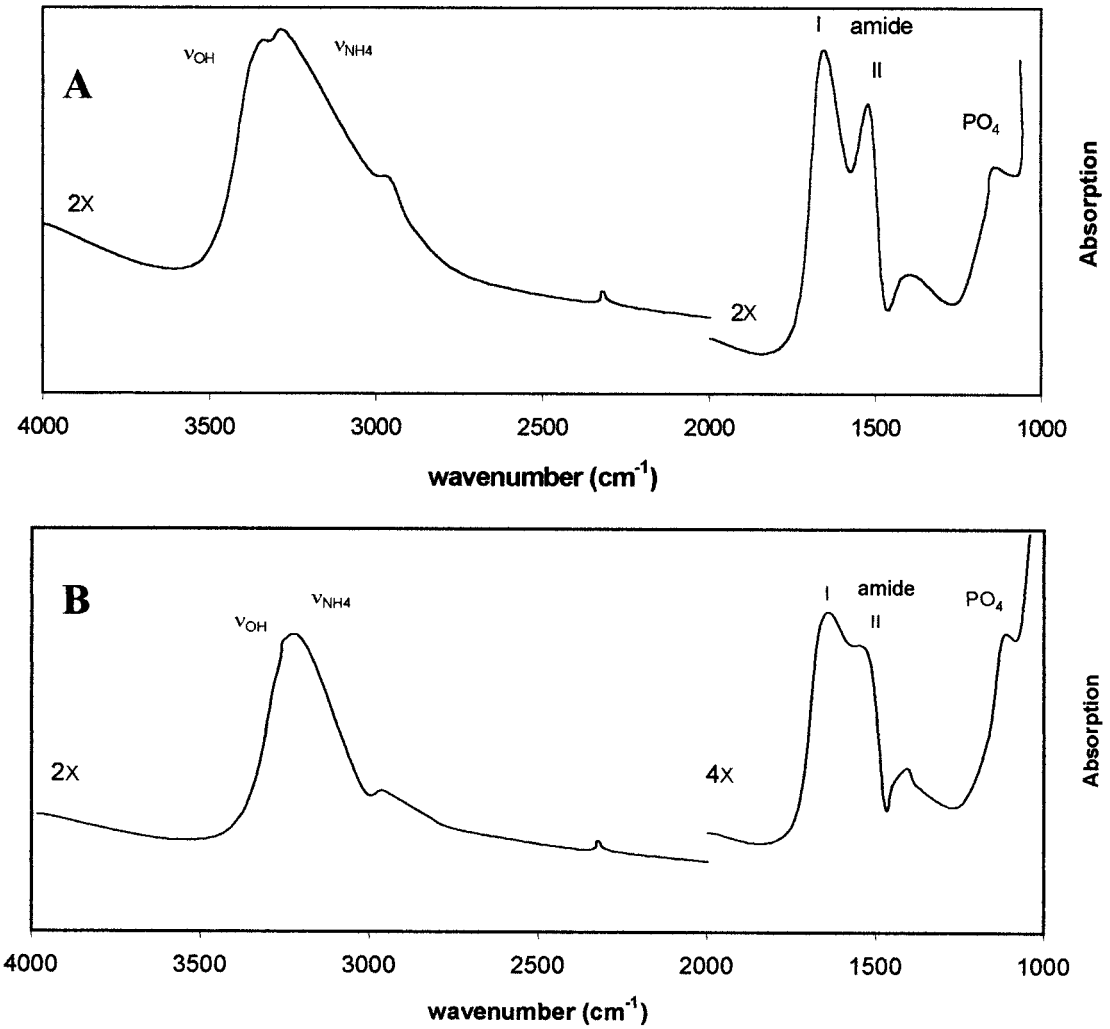

Figure 8. IR spectra of (A) Carbosep M6 membrane after a fouling–rinsing cycle and (B) Carbosep M6 membrane after a fouling–rinsing–cleaning–rinsing cycle.

it was observed to be ~20 min, independently of the value of cp, as can be observed in **Figure 6**. According to the results obtained in the discontinuous reactor (**Table 4**), the maximum hydrolysis degree was achieved in 20 min. Therefore, the cleaning time can be related to the time required to hydrolyze whey proteins. Some authors (*4*) reported much higher cleaning times when organic membranes were cleaned with enzymatic detergents.

If high efficiency is not necessary, different combinations of values of cp and time can be considered. In **Figure 7** cleaning efficiency is plotted against cp for different cleaning times. The most favorable pair of values is the one that combines the lowest values of cp and time (cheapest and shortest cleaning cycle) to reach the desired efficiency. Taking into account the shape of the curves, low values of cp require high operating times, so it is necessary to reach a compromise.

From these results it was observed that the enzymatic agent used (Alcalase) was able to clean the inorganic membrane Carbosep M6 fouled with protein-based solutions. High cleaning efficiencies (>90%) were reached in short operating times (20 min). It is expected that similar results can be obtained for other inorganic membranes and/or using other proteolytic enzymes. This point will be tested in a subsequent work.

Chemical Characterization of the Membrane Surface. **Figure 8** shows the IR spectra of a Carbosep M6 membrane after a fouling−rinsing cycle (A) and after a fouling−rinsing−cleaning−rinsing cycle (B). It can be noted that amide I and II bands, which are typical of proteins, present a different profile when panels A and B are compared. This modification can be explained as a result of the difference between high molecular weight polypeptide chains and the low molecular weight polypeptide chains that are formed during the enzymatic cleaning. Amide I and II bands present maximum absorption at 1650 and 1540 cm^{-1}, respectively. Amino groups typically show an absorption band in the region between 3300 and 3500 cm^{-1}, whereas carboxylic groups present absorption bands in the wavenumber regions of 1700−1725 cm^{-1} (as −COOH) and 1550−1630 cm^{-1} (as $-COO^-$). Thus, the increase in the amount of carboxylic groups due to protein hydrolysis can interfere with the resolution of amide I and II bands and can also cause the difference observed panels A and B of **Figure 8**. The increase in the relative amount of COO^- groups was also detected in the XPS measurements. After the membrane had been cleaned with an alkaline nonenzymatic agent, this difference was not observed.

The absorption band in the region between 1000 and 1150 cm^{-1} can be attributed to phosphate. Membranes were heated to 550 °C to destroy the organic matter. The presence of this band after the heating confirms the inorganic nature of phosphate groups.

Table 5 shows the average results obtained from the characterization of Carbosep M6 membranes using IR and XPS spectroscopy. The carbon (C1s) and nitrogen (N1s) structures are typical of organic fouling, whereas the oxygen (O1s) comes from the membrane itself as well as from the fouling. The disappearance of the zirconium ($Zr3p^{3/2}$) structure can be related to the depth of the fouling layer. It can be observed from the table that the relationships C/Zr and N/Zr increase 2 times after the membrane conditioning and the protein content increases 5 times, so that even the conditioning step fouls the membrane from the chemical point of view. However, this fouling does not lead to an increase in the hydraulic resistance. The importance of the rinsing step can be observed when the amounts of matter deposited on the membrane after and before the rinsing are compared. The amount of proteins decreases by ~65% after this step, whereas XPS data show that the fouling layer thickness decreases as the intensity of the Zr peak increases. The amount of matter deposited on the membrane decreases during the cleaning. However, after this cycle residual matter was detected on the membrane surface. It can also be observed that after the cleaning, the amount of proteins on the upper membrane layers is higher than that on the lower layers. It is also possible that a certain amount of enzyme could be part of the residual deposit. This could be advantageous as it could induce a self-cleaning mechanism, as described by Velicangil and Howell (*18*). This effect will be studied in a future paper.

Table 5. Average Results Obtained from the Chemical Characterization of Carbosep M6 Membranes

sample	R (10^{12}/m)	C/Zr[b]	N/Zr[c]	O/Zr[d]	proteins[a] upper layers	proteins[a] lower layers
new	1.11	0.31	0.08	2.80	0.02	0.02
conditioned	1.11 (R_m)	0.63	0.18	3.60	0.10	0.10
fouled	40.0 (R_{uf})	1066.0	206.0	419.0	12.90	11.00
fouled and rinsed	5.57 (R_{cw})	20.0	5.3	8.2	4.60	3.92
cleaned and rinsed	1.41	3.2	0.6	3.9	1.20	0.61

[a] Weight %. [b] Relationship between C1s and $Zr3p^{3/2}$ relative compositions. [c] Relationship between N1s and $Zr3p^{3/2}$ relative compositions. [d] Relationship between O1s and $Zr3p^{3/2}$ relative compositions.

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LIST OF SYMBOLS

a	enzymatic activity
A_m	membrane area
cp	cleaning parameter
E_{Rw}	cleaning efficiency
HD	hydrolysis degree
J	permeate flux
J_{wi}	initial water flux
n	number of peptidic linkages hydrolyzed per unit of substrate mass
n_{tot}	number of peptidic linkages per unit of substrate mass
R	membrane hydraulic resistance
R_c	hydraulic resistance during the cleaning process
R_{cw}	hydraulic resistance of the cleaned membrane
R_f	resistance due to membrane fouling
R_{if}	resistance due to irreversible fouling
R_m	intrinsic hydraulic resistance
R_r	resistance due to reversible fouling
R_{uf}	total resistance of the fouled membrane
V	volume of cleaning solution
ν	kinematic viscosity
ΔP	transmembrane pressure

μ dynamic viscosity
ρ density

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