



Separation and purification of benzylpenicillin produced by fermentation using coupled ultrafiltration and nanofiltration technologies

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Abstract

The purpose of this study was to evaluate the capacity of using coupled ultrafiltration—nanofiltration technologies for separation and purification of benzylpenicillin (BP). More specifically, we verified the efficiency of three ultrafiltration (UF) membranes (cut-off of 5000, 30,000 and 100,000 Da) to remove impurities that cause stable emulsion during the chemical extraction of the antibiotic. We also tested the effectiveness of a nanofiltration (NF) membrane (cut-off of 300 Da) to concentrate the benzylpenicillin recovered from permeates and to decrease the osmotic pressure by reducing the ionic charge of the broth. Results have shown that high recovery (89.0–91.0%) can be obtained in permeate generated by the 30,000 and 100,000 UF membranes, but a slight emulsion will be formed during phase separation. With the 5000 UF membrane, lower recovery is obtained (81.0%) but no emulsion is produced, leading to a high solvent extraction yield (94.6%). The nanofiltration of 30,000 and 100,000 UF permeates leads to very high recovery (98.0%), but stable emulsions are formed, reducing the chemical extraction yield (80.0–82.6%). For the nanofiltration of 5000 UF permeate, excellent recovery of the antibiotic is noted (97.4%) leading to high extraction yield (92.4%) with no emulsion formed. Diafiltration step should be applied during UF procedure in order to increase the antibiotic recovery in the generated permeates.

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1. Introduction

One of the major problems regarding the antibiotic's production by fermentation resides in the extrac-

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tion and purification technologies of these molecules. According to Gossele et al. (1989), the purification costs can vary from 20 to 50% of the total production cost. The conventional isolation processes of benzylpenicillin (BP) from fermented broth have been extensively described in the literature (Hersbach et al., 1984; Gossele et al., 1989; Nabais and Cardoso, 1995, 1999, 2000). The important steps are the separation of mycelial cells, the chemical extraction of the active molecule, and the purification and crystallization of the antibiotic. The most important concern is at the chemical extraction level, where stable emulsions can be frequently formed. These emulsions are generally a mixture of protein materials and cannot be eliminated with conventional techniques of gravitation or centrifugation (Nabais and Cardoso, 1999). This phenomenon leads to contamination of the final product, low extraction yield, high solvent losses in the extracted broth and clogging of the pumping or separation equipment (Nabais and Cardoso, 1999, 2000). One of the most promising solutions regarding the emulsion problem is to use the ultrafiltration (UF) technology in order to remove emulsifying agents (Nabais and Cardoso, 1995, 1999, 2000). It eliminates the use of anti-emulsion products or flocculants, and a substantial volume of fermented broth can be filtered. Some authors have used microfiltration membranes for the clarification of fermentation broth and cell harvesting (Hooper et al., 1998; Adikane et al., 1999; Davies et al., 2000), but their ability to remove protein solids are restricted since their molecular weight cut-offs (MWCO) are too high to retain most of protein materials. This could be problematic since stable emulsion can be formed at the chemical extraction level. The UF process can be combined with reverse osmosis (RO) to concentrate antibiotic ultrafiltered broths, leading to low losses of BP in the permeate and a high antibiotic recovery for high volumetric concentration factors (Datta et al., 1977; Nabais and Cardoso, 2000).

Few studies have proposed the use of nanofiltration (NF) in order to concentrate antibiotic ultrafiltered broths. This membrane technology can provide many advantages compared to the concentration of organic molecules by RO. First, the operating pressures used are significantly lower in the case of NF, which represent an economical benefit. Second, the NF membrane porosity is slightly higher than RO membrane, inducing enhanced permeate flux and hence, reducing the

operating time. Considering that several antibiotics like penicillin are very sensitive to hydrolysis and that filtration time is one of the most critical parameter to control during separation phase, the NF technology can represent a potential alternative. Finally, the NF process can increase the purity level of organic molecules retained in the concentrate, by removing a significant charge of inorganic ions (Peeters et al., 1998; Van der Bruggen et al., 1999). In fact, most of NF membranes are partially permeable to cationic and anionic elements, reducing the concentrate conductivity. This might contribute to a reduction of osmotic pressure and promote the use of low operating pressures comparatively to RO technology.

The purpose of this study is to demonstrate that UF and NF technologies can be combined to improve separation and purification of BP produced by fermentation. More specifically, we compared UF membranes with different molecular weight cut-offs in order to evaluate their removing potential of protein materials and their impact on the chemical extraction performance. We also tested a NF membrane to verify its capacity to recover and concentrate the penicillin from UF permeates, to reduce substantially the ionic charge of the broth and to increase the chemical extraction performance by reducing the solvent volume used.

2. Material and methods

2.1. Sample preparation

The fermentation broth used was produced in a previous study, and consisted of fermented cheese whey liquor containing mycelial cells of *Penicillium chrysogenum* and the BP. The collected broths were filtered on a filter bag (10 μm porosity) under gravitational conditions, in order to remove a large part of the aggregated mycelium. Filtration was carried out at 4 °C since penicillin is very sensitive to hydrolysis at temperature higher than 15 °C (Hersbach et al., 1984; Nabais and Cardoso, 1999). An acceptable loss of only 0.9% of penicillin was measured during this step.

2.2. Filtration unit configuration

A process flow diagram for operating the cross-flow filtration unit is shown in Fig. 1. The system includes pressure gauges (immediately adjacent to the

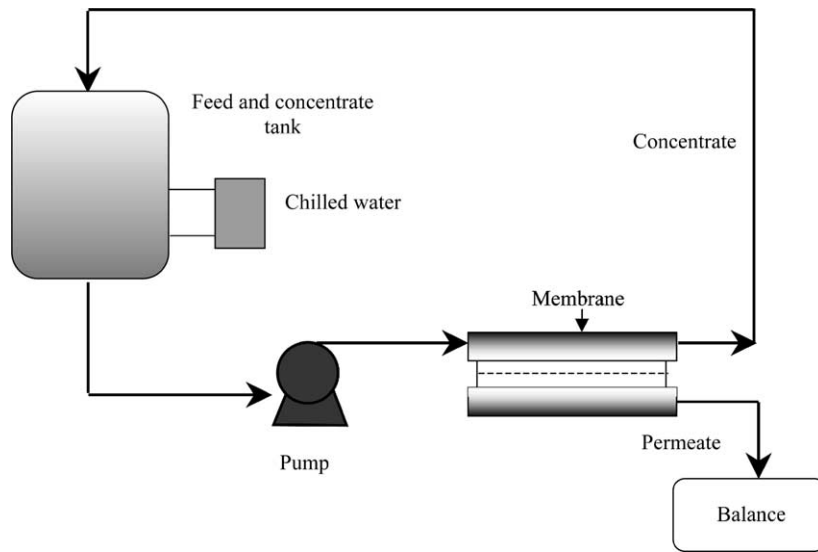


Fig. 1. Process flow diagram for membrane filtration.

membrane module) on the feed and concentrate lines. Flat sheet membranes (155 cm²) were used. All experiments were run in a batch concentration mode, with the concentrate recycled to the feed tank (6 L capacity) and the permeate collected in another container chilled with ice (reducing penicillin degradation), on an electronic balance (and Electronic Balance, model EP-40 kg, ± 0.5 g). Water at 4 °C was circulated around the reservoir and membrane module. The permeate temperature was maintained between 4 and 12 °C. At the beginning of each run, the system was disinfected with sodium hypochlorite (300 ppm). The filtration unit was chemically sterilized (without membrane) by recycling the chlorinated water during 1 h at room temperature, after which, the system was rinsed with distilled water. The distilled water permeability of each membrane used in this study was measured before and after each experiment in the same experimental conditions (temperature, operating pressure, cross-flow velocity) that were used for the UF and NF tests. All filtration runs were conducted at constant pressure. Hence, the permeate flux decline was related to fouling problems instead of pressure variation.

2.3. Ultrafiltration tests

Three flat sheet organic membranes of different MWCO were selected for this study: UE-50 (TRISEP,

USA, 100,000 Da), HFM-100 (LCI-KOCH, USA, 30,000 Da), HFK-328 (LCI-KOCH, USA, 5000 Da). The operating pressures applied for each membrane was 15, 25 and 80 psig, respectively. They were in the range used at industrial scale, which is from 7 to 70 psig (Aptel and Buckley, 1996). The tests were realized at constant pressure, consequently the membrane's fouling will induce a decrease of permeate flux. The tangential cross-flow velocity (CFV) was fixed to 0.50 m/s for UE-50 and HFM-100 membranes, and to 0.65 m/s for HFK-328. A higher CFV would have generated a more elevated pressure on the membrane inducing a rapid fouling and a decrease of the permeation flux. The inlet and outlet pressures and the permeate flux were recorded at a specific interval. Temperature, pH and conductivity were measured in the feed tank, the concentrate and the permeate lines. The operating filtration time for each tested membrane varied from 5.0 to 6.5 h. The original feeding volumes were 2 or 3 L.

The evolution of antibiotic separation was estimated by collecting samples of the concentrate and permeate every 500 mL of generated permeate (called «instantaneous samples»). These samples were collected immediately after the membrane unit in the concentrate return pipe and in the permeate exit pipe. Since these samples are not collected in the feeding or permeate tanks, they are used only to follow the BP instantaneous separation in both fractions at a time t . Samples

were also taken in the final volume of permeate and concentrate (called «composite samples») in order to evaluate the recovery rates of penicillin. Before each test, a sample of the fermented broth was collected. All samples were stored at -20°C until chemical analysis. Several parameters were calculated in order to evaluate the performance of BP recovery (in the permeate) for each tested membrane. The variables used are defined as follow:

The BP percentage of recovery by mass balance to permeate

$$\% \text{ of recovery} = \left(\frac{C_{\text{permeate}} \times V_{\text{permeate}}}{C_{\text{feed}} \times V_{\text{feed}}} \right) \times 100 \quad (1)$$

The BP percentage of degradation or loss during UF process

% of degradation

$$= \left[1 - \left(\frac{C_{\text{concentrate}} \times V_{\text{concentrate}} + C_{\text{permeate}} \times V_{\text{permeate}}}{C_{\text{feed}} \times V_{\text{feed}}} \right) \right] \times 100 \quad (2)$$

The instantaneous BP percentage of permeation through the UF membrane:

$$\% \text{ of permeation} = \left(\frac{C_{\text{permeate}(t)}}{C_{\text{concentrate}(t)}} \right) \times 100 \quad (3)$$

The volumetric concentration factor (C_f) at time t

$$C_{f(t)} = \frac{V_{\text{feed}}}{V_{\text{concentrate}(t)}} \quad (4)$$

where t is the time of filtration (min); C_{permeate} is the benzylpenicillin concentration in the generated permeate (g/L); C_{feed} is the benzylpenicillin concentration in the initial feed volume (g/L); $C_{\text{concentrate}}$ is the benzylpenicillin concentration in the generated concentrate (g/L); $C_{\text{concentrate}(t)}$ is the benzylpenicillin concentration in the instantaneous concentrate collected in the return pipe at time t (g/L); $C_{\text{permeate}(t)}$ is the benzylpenicillin concentration in the instantaneous permeate collected in the exit pipe at time t (g/L); V_{permeate} is the permeate volume obtained (L); V_{feed} is the initial feeding volume (L); $V_{\text{concentrate}}$ is the concentrate volume obtained (L); $V_{\text{concentrate}(t)}$ is the concentrate volume at time t .

2.4. Nanofiltration tests

The flat sheet organic membrane selected for the NF tests was a NF-270 (Filmtec, 300 Da MWCO). The assays were realized in the same conditions used for UF tests, except that the operating pressure was fixed at 200 psig. The tangential cross-flow velocity was adjusted to 0.65 m/s. A volume of 1 L of each UF permeate generated previously was used for these tests. Each filtration run was conducted until the feed volume was concentrated up to 200 mL, which represent the dead volume of the filtration unit. The sampling sequence and the permeate/concentrate characterization were the same as described for UF tests. Parameters were calculated in order to evaluate the performance of BP recovery (in concentrate) for each tested membrane. The variables used are defined as follow:

The BP percentage of recovery by mass balance to concentrate

$$\% \text{ of recovery} = \left(\frac{C_{\text{concentrate}} \times V_{\text{concentrate}}}{C_{\text{feed}} \times V_{\text{feed}}} \right) \times 100 \quad (5)$$

The BP percentage of loss to permeate

$$\% \text{ of loss} = \left(\frac{C_{\text{permeate}} \times V_{\text{permeate}}}{C_{\text{feed}} \times V_{\text{feed}}} \right) \times 100 \quad (6)$$

The signification of each variable is the same as described previously (Eqs. (1) and (2)). The degradation percentage of BP (according to the mass balance) and the volumetric concentration factor were established as described for UF calculations (Eqs. (2) and (4)).

2.5. Chemical extraction procedures

To estimate the UF and NF performances on the subsequent steps of penicillin purification, we proceeded to conventional chemical extraction with butyl acetate (Hersbach et al., 1984; Brunner, 1985; Yang et al., 1994; Nabais and Cardoso, 1995, 1999). First, the UF permeates and the NF concentrates were acidified at pH 2.0 with H_2SO_4 (6N) to transform the benzylpenicillin into penicilloic acid, a form that is more soluble into the solvent (the $\text{p}K_a$ value for BP is 2.5). A volume of 100 mL of acidified broth was mixed with 25 mL of butyl acetate (volumetric ratio of 4:1 as proposed by Hersbach et al., 1984), and the two phases system vigorously agitated for 5 min in ice bath. Afterwards separation of phases was achieved by gravity at

4 °C. The two volumes were separated and measured. The BP concentration was immediately measured in both phases. To facilitate the interpretation of extraction yield, four parameters were calculated as follow:

The BP extraction yield in butyl acetate

$$\% \text{ of extraction} = \left(\frac{C_{\text{solvent}} \times V_{\text{solvent}}}{C_{\text{ML}} \times V_{\text{ML}}} \right) \times 100 \quad (8)$$

The BP percentage of loss into the aqueous phase

$$\% \text{ of loss} = \left(\frac{C_{\text{aqueous}} \times V_{\text{aqueous}}}{C_{\text{ML}} \times V_{\text{ML}}} \right) \times 100 \quad (9)$$

The BP percentage of degradation during extraction

% of degradation

$$= \left[1 - \left(\frac{C_{\text{solvent}} \times V_{\text{solvent}} + C_{\text{aqueous}} \times V_{\text{aqueous}}}{C_{\text{ML}} \times V_{\text{ML}}} \right) \right] \times 100 \quad (10)$$

The solvent loss

% of solvent loss

$$= \left(\frac{V_{\text{solvent added}} - V_{\text{solvent obtained}}}{V_{\text{solvent added}}} \right) \times 100 \quad (11)$$

where C_{solvent} is the benzylpenicillin concentration in the solvent (g/L); C_{ML} is the benzylpenicillin concentration in the mother liquor (extracted liquor) (g/L); C_{aqueous} is the benzylpenicillin concentration in the aqueous phase (g/L); V_{solvent} is the volume of the solvent (organic phase) obtained (L); V_{ML} is the volume of the original mother liquor (L); V_{aqueous} is the volume of aqueous phase obtained (L); $V_{\text{solvent added}}$ is the volume of solvent used at the beginning of extraction (L); V_{obtained} is the volume of solvent measured after separation phase (L).

2.6. Assay of penicillin

Samples were analyzed for BP by HPLC (Waters Co.) using a Supelco C₁₈ Nucleosil column (Sigma-Aldrich, 250 mm × 4.6 mm; 5 μm particle size) at 40 °C. The injection volume was 20 μL. The mobile phase consisted of 80% (v/v) 0.05 M KH₂PO₄, pH 5.5, and 20% (v/v) acetonitrile (Fisher, HPLC grade). The flow rate of the mobile phase was 1 mL/min. The eluted

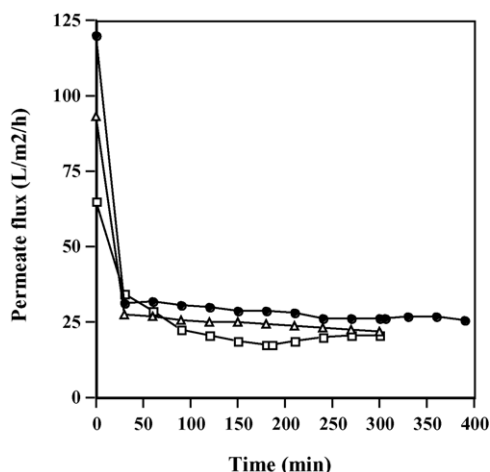


Fig. 2. Variation of permeate flux for the three UF runs. UE-50 membrane, operating pressure: 15 psig, initial volume: 3 L, final C_f : 15.0 (●), HFM-100 membrane, operating pressure: 25 psig, initial volume: 2 L, final C_f : 10.0 (▲), HFK-328 membrane, operating pressure: 80 psig, initial volume: 2.1 L, final C_f : 10.5 (□). The first data at time = 0 represents the permeate flux of distilled water.

peaks were detected at 205 nm in a UV spectrophotometer (Varian, model 9050) and the peak's area was determined by the Turbochrom software (V. 6.1.2) from Waters Co.

3. Results

3.1. Ultrafiltration tests

The results of UF tests are presented in Fig. 2 (Tables 1 and 2). The initial BP concentration in the feed varied from 0.8 to 1.0 g/L. The pH and conductivity values are similar in both concentrate and permeate and temperature was slightly higher in permeate (Table 1). Permeate flux with distilled water at the beginning of UF tests ($t=0$) are fairly high (Fig. 2) with values of 120, 93 and 65 L/m²/h for UE-50, HFM-100 and HFK-328 membranes, respectively. Significant fouling of the membranes are noticed early since these values decrease rapidly after 30 min of filtration (31 L/m²/h for UE-50 and HFM-100, 34 L/m²/h for HFK-328), which represent a reduction of 74% (UE-50), 67% (HFM-100) and 48% (HFK-328) compared to original permeate flux. However, the flow stabilizes after 30 min and declines very slowly until the end of

Table 1

Physico-chemical characterization (average value) of the concentrate and permeate generated for each ultrafiltration run

Ultrafiltration membrane tested	Temperature in the concentrate (°C)	Temperature in the permeate (°C)	pH in the concentrate	pH in the permeate	Conductivity in the concentrate (mS/cm)	Conductivity in the permeate (mS/cm)	Total filtration time (min)
UE-50 membrane	6.0	12.0	6.0	6.0	22.5	22.5	390 (3.0 ^a)
HFM-100 membrane	7.0	10.0	6.0	6.0	19.5	19.5	300 (2.0 ^a)
HFK-328 membrane	7.6	11.3	5.9	5.9	22.6	22.6	330 (2.1 ^a)

^a Initial feed volume in litres.

filtration time, indicating possibly a surface blocking of the membrane instead of an internal occlusion of the pores. Volumetric concentration factors of 10–15 were obtained in the final composite with the three tested UF membranes (Table 2). The instantaneous BP concentration in the concentrate was almost stable during the filtration runs with the 100,000 UF and 30,000 UF membranes, indicating no significant concentration of the molecule. With the 5000 UF membrane, a slight in-

crease of BP concentration is observed with volumetric concentration of the feed volume. The average BP instantaneous permeation across the membranes was 96.0, 95.0 and 85.0% for the UE-50, HFM-100 and HFK-328 membranes, respectively. The BP recoveries in the UF permeates (final composite) were 91.0, 89.0 and 81.0% for the UE-50, HFM-100 and HFK-328 membranes, respectively (Table 2). These results would have been probably more enhanced with a neg-

Table 2

Results of the UF tests realized with three membranes of different MWCO

C_f	$V_{\text{concentrate}}$ (L)	$C_{\text{concentrate}}$ (g/L)	V_{permeate} (L)	C_{permeate} (g/L)	% of permeation	% of recovery	% of degradation
(a) UE-50 membrane; MWCO of 100,000 Da; initial feed volume: 3 L							
1.0	3.0	1.00*	0.0	–	–		
1.2	2.5	1.00	0.5	0.94	94.0		
1.5	2.0	1.00	1.0	0.97	97.0		
2.0	1.5	1.00	1.5	0.99	99.0		
3.0	1.0	0.99	2.0	0.94	95.0		
Final composite							
15.0	0.2	0.99*	2.8	0.97*	–	91.0	3.0
(b) HFM-100 membrane; MWCO of 30,000 Da; initial feed volume: 2 L							
1.0	2.0	0.80*	0.0	–	–		
1.3	1.5	0.80	0.5	0.77	96.0		
2.0	1.0	0.80	1.0	0.73	91.0		
4.0	0.5	0.82	1.5	0.80	98.0		
Final composite							
10.0	0.2	0.82*	1.8	0.79*	–	89.0	1.0
(c) Membrane HFK-328; MWCO of 5000 Da; initial feed volume: 2.1 L							
1.0	2.1	1.00*	0.0	–	–		
1.3	1.6	1.00	0.5	0.87	87.0		
1.9	1.1	1.05	1.0	0.90	86.0		
3.5	0.6	1.11	1.5	0.90	81.0		
Final composite							
10.5	0.2	1.05*	1.9	0.90*	–	81.0	9.0

Benzylpenicillin concentration at the beginning and at the end of filtration was measured on composite samples (*), whereas during the filtration period, it was measured on instantaneous samples. C_f : volumetric concentration factor; $V_{\text{concentrate}}$: concentrate volume; $C_{\text{concentrate}}$: BP concentration in the concentrate; V_{permeate} : volume of the permeate; C_{permeate} : BP concentration in the permeate; % of permeation: instantaneous percentage of BP moving through the membrane; % of recovery: percentage of BP concentration recovered in the permeate; % of degradation: percentage of BP degradation during UF test.

Table 3

Physico-chemical characterization (average value) of the concentrate and permeate generated for each nanofiltration run

Nanofiltration test	Temperature in the concentrate (°C)	Temperature in the permeate (°C)	pH in the concentrate	pH in the permeate	Conductivity in the concentrate ^a (mS/cm)	Conductivity in the permeate ^a (mS/cm)	Total filtration time (min)
Permeate of UE-50	8.0	6.0	6.0	6.0	22.9/28.5	16.4/22.0	210
Permeate of HFM-100	11.0	10.0	6.0	6.0	20.6/25.2	15.8/20.0	115
Permeate of HFK-328	11.0	10.0	6.0	6.0	22.4/26.1	19.6/24.8	80

^a Conductivity at the beginning/at the end of filtration.

ligible dead volume or a higher feed volume. The percentages of penicillin degradation are low except the one observed with HFK-328 membrane (9.0%).

3.2. Nanofiltration tests

The pH values were stable in both permeate and concentrate (Table 3). The temperature was higher in the concentrate but it did not affect the stability of the molecule since the degradation percentages of BP dur-

ing these experiments were very low (Table 4). We observed that conductivity increased in both fractions during the nanofiltration (Table 3), indicating that partial separation of ions occurred. Therefore, the NF membrane used can provide an additional purification in terms of ionic element separation, and reduces the osmotic pressure of the feed volume. The initial penicillin concentration varied from 0.79 to 0.93 g/L (Table 3).

The average permeate flux with distilled water (at the beginning of filtration) was 64.9 L/m²/h for the

Table 4

Results of the NF tests realized with the NF-270 membrane (FILMTEC)

C _f	V _{concentrate} (L)	C _{concentrate} (g/L)	V _{permeate} (L)	C _{permeate} (g/L)	% of loss	% of recovery	% of degradation
(a) UF permeate generated by UE-50 membrane							
1.0	1.00	0.93*	0.00	–			
1.3	0.75	1.10	0.25	0.004			
2.0	0.50	1.84	0.50	0.015			
2.9	0.35	2.25	0.65	0.032			
Final composite							
5.0	0.20	4.55*	0.80	0.009*	0.8	98.0	1.2
(b) UF permeate generated by HFM-100 membrane							
1.0	1.00	0.79*	0.00	–			
1.3	0.75	0.98	0.25	<0.001			
2.0	0.50	1.56	0.50	<0.001			
2.9	0.35	2.20	0.65	0.020			
Final composite							
5.0	0.20	3.87*	0.80	0.003*	0.3	98.0	1.7
(c) UF permeate generated by HFK-328 membrane							
1.0	1.00	0.84*	0.00	–			
1.3	0.75	1.07	0.25	0.004			
2.0	0.50	1.63	0.50	0.010			
2.9	0.35	2.03	0.65	0.037			
Final composite							
5.0	0.20	4.09*	0.80	0.011*	1.0	97.4	1.6

Benzylpenicillin concentration at the beginning and at the end of filtration was measured on composite samples (*), whereas during the filtration period, it was measured on instantaneous samples. C_f: volumetric concentration factor; V_{concentrate}: concentrate volume; C_{concentrate}: BP concentration in the concentrate; V_{permeate}: volume of the permeate; C_{permeate}: BP concentration in the permeate; % of loss: percentage of BP loss in the permeate; % of recovery: percentage of BP concentration recovered in the concentrate; % of degradation: percentage of BP degradation during NF test.

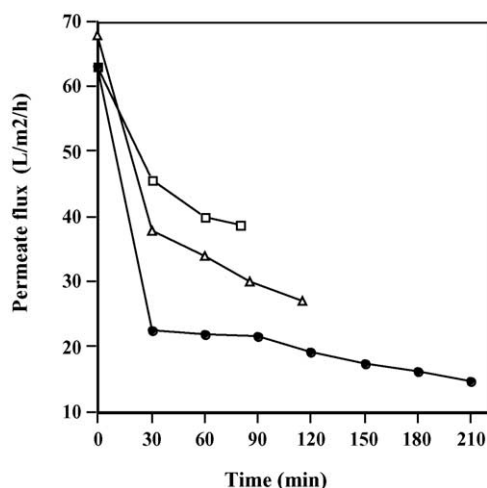


Fig. 3. Variation of permeate flux for the three NF runs. Permeate generated by the UE-50 membrane (●), by the HMF-100 membrane (△), and by the HFK-328 membrane (□). Operating pressure: 200 psig, initial volume: 1 L, final C_f : 5.0. The first data at time = 0 represents the permeate flux of distilled water.

three NF tests conducted. After 30 min of operation, the flows rapidly decreased down to 22.5, 37.9 and 45.4 L/m²/h for UE-50, HFM-100 and HFK-328 permeates respectively, indicating a significant fouling of the NF membrane (Fig. 3). After that, the decrease is slower and more gradual. The flux reduction can be caused by the concentration polarization, by the formation of a cake layer on the membrane surface or by the internal occlusion of the pores. The drop of NF permeate flux is clearly related to the MWCO of the UF membranes used, higher cut-off producing a more

charged permeate according to weak molecule's separation, hence inducing lower NF permeate flow. Also these declines are related to the osmotic pressure of the UF permeates used.

Volumetric concentration factor of 5 was obtained for the three UF permeates tested (which represents the highest C_f that could be reached according to the dead volume of the system and the initial feed volume used). The instantaneous BP concentration in the concentrate increases with the volumetric concentration of the feed volume. The penicillin recovery in the NF concentrates (final composite) was 98.0% for the UE-50 and HFM-100 permeates, and 97.4% for the HFK-328 permeate (Table 4). The antibiotic permeation across the NF membrane is very restricted since the percentages of loss were $\leq 1.0\%$ for all UF membrane tested (Table 4).

3.3. Chemical extraction of benzylpenicillin

The results of chemical extraction are presented in Table 5. A small emulsion was formed during the two-phase separation with the UE-50 and HFM-100 permeates, leading to a noticeable degradation of BP (>6.0%). The extraction yields measured for both permeate were 91.0 and 88.5%, respectively, which are acceptable. Excellent separation was achieved with the HFK-328 permeate with no emulsion produced and a very low BP degradation. Therefore, the highest extraction yield was obtained with this broth (94.6%). The loss of BP in aqueous phase and the loss of added solvent were lower than 5.0% for the three extraction experiments. Regarding the extraction yield ob-

Table 5
Results of the benzylpenicillin chemical extraction from the UF permeates and NF concentrates

Filtration	% of extraction	% of loss	% of degradation	% of solvent loss	Emulsion
Ultrafiltration: UE-50, 100,000 Da	91.0	2.7	6.3	2.0	Light: 1.0% (v/v) (excellent separation)
Ultrafiltration: HFM-10,030,000 Da	88.5	4.8	6.7	4.0	Light: 0.4% (v/v) (excellent separation)
Ultrafiltration: HFK-328, 5000 Da	94.6	3.6	1.8	4.0	None (excellent separation)
Nanofiltration of UE-50 permeate	80.0	4.5	15.5	24.0	High: 12.0% (v/v) (weak separation)
Nanofiltration of HFM-100 permeate	82.6	3.9	13.5	12.0	High: 5.0% (v/v) (weak separation)
Nanofiltration of HFK-328 permeate	92.4	3.3	4.3	4.0	None (excellent separation)

% of extraction: percentage of BP extraction in butyl acetate; % of loss: percentage of BP loss in the aqueous phase; % of degradation: percentage of BP degradation during the chemical extraction.

Table 6
Comparison of the process global yield between solvent extraction of benzylpenicillin from UF permeates and from NF concentrates

UF membranes	Y_{UF} (%)	Y_{NF} (%)
UE-50, 100,000 Da	82.8	71.3
HFM-100, 30,000 Da	78.8	72.0
HFK-328, 5000 Da	76.6	73.0

Y_{UF} : global yield of BP recovery from UF permeate and chemical extraction; Y_{NF} : global yield of BP recovery from UF permeate, NF concentrate and chemical extraction.

tained with the NF concentrates, bad separation was noted with nanofiltered UE-50 and HFM-100 permeates. A sizeable emulsion is formed in both cases, leading to important degradation of the antibiotic (>13.0%) and a significant loss of added solvent (>12.0%). This phenomenon is due to the concentration of emulsifying agents in the concentrate. Consequently, the extraction yields are weak (80.0–82.6%). For the concentrate of nanofiltered HFK-328 permeate, excellent separation was achieved with no emulsion formed and a high extraction yield of 92.4%. Globally, the loss of BP into aqueous phase is not a serious concern since losses were less than 5.0%. According to the results, the volumetric proportion of butyl acetate—filtered broth (1:4) was adequate.

Taking into consideration the global yields obtained using UF permeates followed by direct solvent extraction (Y_{UF}), we found that these values (76.6–82.8%; Table 6) were always better than the global yields obtained when NF was used as an intermediate step between UF and solvent extraction (Y_{NF}) (71.3–73%; Table 6). However, the variation between the Y_{UF} and Y_{NF} values is increasing with an elevation of the MWCO of the UF membranes used.

4. Discussion

The results of this study show that coupled UF/NF technologies can be efficiently used for the separation and the purification of BP produced by fermentation. High extraction yields can be obtained with a good selection of membranes. Also, membrane filtration allows the treatment of great volumes that can be applied at pilot scale and probably at industrial level. The most important problem encountered during membrane filtration is the fouling phenomenon (Ko et al., 1993),

which can be minimized by working with low operating pressure (or low permeate flux). The UF results show a rapid decrease of permeate flow at the beginning, but the flux stabilizes and declines very slowly until the end of the filtration run. As suspected, dissolved and particulate matters were rapidly deposited on the membrane surface, but the permeate flow was related to the MWCO of the UF membrane. We compared our results with those obtained by Nabais and Cardoso (1999) in their study of BP purification with tubular UF membranes characterized by MWCO of 8000 and 20,000 Da. They measured a permeate flux of 24.8 and 28.8 L/m²/h for both membranes respectively, with a final C_f of 2.5. Using the 5000 and 30,000 UF membranes, we obtained a flux of 19 and 22 L/m²/h respectively, resulting in a C_f four to six time higher. Also, they used an operating pressure of 145 psig (10 bars) compared to 25 and 80 psig in our experiment, which lead to a reduction of energy costs. The filtration unit is adequate to maintain stable physico-chemical conditions promoting low penicillin degradation. However, significant loss or degradation was observed with the HFK-328 membrane (MWCO of 5000 Da). Considering that temperature was always kept under 12 °C and that pH remained stable during filtration run, we can assume that a part of BP was retained in the concentrate instead of being degraded. Only 85.0% of BP moves instantaneously across the membrane, which might confirm that a significant amount of penicillin is entrapped in the concentrate compared to the other UF membrane tested. The low porosity of HFK-328 probably increases the entrapment phenomenon. The BP recovery in UF permeates (based on the mass balance) is acceptable considering that feed volumes were concentrated 10–15 times. Nevertheless, in terms of separation, results are less adequate. Generally, yield recovery superior to 90.0% must be obtained for efficient and profitable purification process.

In order to increase the recovery efficiency, the use of diafiltration combined with UF runs can represent a potential alternative. Nabais and Cardoso (1999) shown that diafiltration of concentrates with several volumes of distilled water can improve the removal of residual penicillin. In our case, since we concentrated the feed volumes up to 10–15, adding one or two diafiltration volumes to the concentrate would conduct most likely to a significant increase of yield recovery in the perme-

ate (for the three tested membranes). The added water could be eventually removed with the nanofiltration process (next step).

The nanofiltration technology is very efficient to accumulate penicillin in the generated concentrate. As seen by the high recovery yields (97.0–98.0%) for the three UF permeate tested. The low percentages of loss and degradation demonstrate that NF filtration unit is suitable for the concentration of ultrafiltered penicillin. We were only able to concentrate the feeding volume up to a C_f value of five, because of the low initial feed volume used and the dead volume of the system. Better results can be anticipated with a high initial feed volume since the permeate flux was still significant at the end of the filtration time with the three permeates tested (Fig. 3). Good results can also be obtained with the reverse osmosis technology (Nabais and Cardoso, 2000), but it implies the use of high operating pressures compared to nanofiltration. Hence, NF represents a more profitable alternative for the concentration and purification of antibiotics. Also, this technology can remove an important ionic charge (reducing the osmotic pressure), which is not negligible. The permeate generated at this level can be used for diafiltration step (UF), improving the process economy by reducing the volumes of distilled water and also by increasing the global yield.

The NF membrane fouling is related to the MWCO of UF membranes utilized at the previous step. Under our experimental conditions (constant pressure), it takes 2.5 less time to nanofiltrate the 5000 UF permeate, which is characterized by low molecular weight material, compared to the 100,000 UF permeate. The low molecular charge reduces significantly the concentration polarization and the cake layer formed on the membrane, and hence promotes a better permeate flux. In fact, the MWCO of UF membranes do not affect the BP concentration, but it plays an important role in the reduction of the fouling problems at NF stage.

The results of chemical extraction showed that UF is adequate for the removal of substances that cause the formation of stable emulsions. However, emulsion was not completely eliminated with the 30,000 and 100,000 UF membranes, which might explain the significant degradation of penicillin (>6.0%) during extraction. The antibiotic is probably trapped in the newly formed emulsion. A better yield was achieved with the

5000 UF permeate (95.0%). Since no emulsion was developed, very low degradation is observed. Similar result was presented by Nabais and Cardoso (1999) where 98.0% of BP was extracted with butyl acetate from the permeate generated by an 8000 UF tubular membrane.

To evaluate the capacity to increase yield extraction and reduce the purification costs, we verified the efficiency of NF to concentrate the penicillin recovered in the UF permeate. According to our results, it is clear that both 30,000 and 100,000 UF membranes cannot reduce sufficiently the emulsifying matters in the filtered broths, since high level of emulsion and penicillin degradation were observed after a five time concentration of the feeding volume. It might also explain the moderate global yields (Y_{NF}) obtained with both membranes. The high solvent losses have contributed to reduce the global yields. Nevertheless, NF can be conducted with the 5000 UF permeate without concerns about emulsion development and giving high penicillin extraction (92.4%). The 5000 MWCO is able to eliminate any residual emulsifying agents, making it the most appropriate choice for the separation and purification of BP produced by fermentation. The use of this membrane also reduces the fouling problem and the loss of permeate flux during the NF step. However, diafiltration step must be combined to the UF run in order to increase the global yield (Y_{NF}). The selected NF membrane represents a relevant option to RO technology with a significant potential to concentrate active biomolecules. Globally the coupling of HFK-328 and NF-270 membranes is the best alternative to improve the separation, purification and concentration of BP. Considering that low operating pressure is required and that decrease of osmotic pressure (due to partial separation of ions) induces a stable permeation, cost energy can be saved compared to RO technology. In order to scaling up this technology to industrial level, more experiments have to be conducted on diafiltration procedure at the UF level. The global yield obtained with both membranes combination and the chemical extraction is not enough high to compete with actual technologies. However, this can be balanced by a cost diminution associated with the reduction of solvent used for extraction. Since the NF procedure is able to concentrate at least five times the initial volume, the volumetric ratio of NF concentrate to solvent can be maximized.

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