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Demineralization of Protein Hydrolysates from Enzymatic Hydrolysis of Leather Shavings Using Membrane Diafiltration

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ABSTRACT

Pressure-driven membrane separations operated in the diafiltration mode appear promising as an efficient demineralization method of waste protein hydrolysates from leather production. The conclusion drawn from our experimental results is that nanofiltration membranes should be used rather then ultrafiltration ones as they show complete passage of the salts with high retention of the protein hydrolysates. In addition to the classical membrane separation operating parameters, which are crossflow fluid velocity, transmembrane pressure difference, and temperature, the required residual salt concentration as well as acceptable protein losses affect the effectiveness of the process.

INTRODUCTION

The leather manufacturing industry is a large producer of wastes. The waste which is the most difficult to minimize is from the tanning operation. This effluent contains hazardous chromium compounds as well as conventional pollutants, i.e., biological materials (mainly proteins) and suspended solids.

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Nevertheless, these chrome leather shavings offer potential for the production of some valuable substances. Recently, it has been shown in the literature (1–3) that chrome leather shavings can successfully be treated with enzymes to recover a soluble protein. After separation of the reaction mixture using a pressure filter, a solid chromium cake and filtrate (protein hydrolysates) are obtained. The chromium cake can be recycled or used in other industrial applications while the protein hydrolysates have potential for use in cosmetics (moisturizing creams, lotions, hair sprays), biomedical products (burn dressings, implant coverings), and animal feed products. In order for the protein hydrolysates to be used, however, requires high purity of the product to prevent an allergic response when used on or in the body. In this context the problem arises because enzymatic protein hydrolysates from leather shavings usually contain a relatively large quantity of common salts as a result of hydrolysis and neutralization processes used in the original enzymatic processing. Thus, demineralization of the protein product is needed prior to evaporation and drying. Potential processes for removing salts from macromolecular compounds include ion exchange, electrodialysis, and pressure-driven membrane separations (ultrafiltration and nanofiltration) operated in the diafiltration mode. Of these known techniques, ion exchange requires short cycle times due to the high salt content in the processed protein hydrolysate. Thus, large amounts of chemical regenerates and water are needed. Electrodialysis, on the other hand, does not achieve complete demineralization due to a rapid reduction in conductivity, and thus electrodialysis must usually be followed by ion exchange to achieve the required degree of demineralization. Potential protein destabilization may occur in both mentioned processes. Therefore, the major target of the work presented in this paper was to carry out some pressure-driven membrane separation experiments with commercial protein hydrolysate products to find both the membrane type and optimal parameters for the demineralization process.

EXPERIMENTAL

The goal of the experimental program was primarily to find a suitable separation membrane and consequently to determine the operational conditions under which the best combination of protein demineralization and recovery as well as of capital and operating costs is achieved.

The enzymatic protein hydrolysate (EPH) used was supplied by AERRSC, Philadelphia (USA). It was acquired by enzymatic hydrolysis of shavings (chrome-containing waste obtained during shaving of chromium-containing leather) under the working conditions according to a US patent (3). The composition of the powdered product was 72% wt of protein hydrolysates, 17.5% wt of mineral salts, 10.5% wt of water, and <20 ppm of chromium. The

protein hydrolysate contains a relatively large number of individual amino acids in a proportion similar to that of hydrolyzed collagen. The mean relative molecular weight was determined to be 10 kDa by using gel column chromatography. The powdered product was dissolved in deionized water at 50°C to prepare feeds.

Nine types of nano- and ultrafiltration membranes were tested in the experimental loop. The membranes were made of many different materials including aromatic polyamides, polysulfones, polyethersulfones, and various modifications of them. Characteristics of the membranes are summarized in Table 1.

The experiments were carried out in a crossflow filtration unit equipped with a flat crossflow module (TZA 944, Amafilter, The Netherlands) with a membrane area of 44 cm^2. The solution was pumped from a thermostated reservoir by a positive displacement diaphragm pump (Hydra-Cell, PMA 279). The velocity and pressure in the retentate loop were varied independently by means of a pump controller and an appropriate needle valve. The

TABLE 1
Selected Characteristics of Membranes Used

Code	Manufacturer/ trademark	Material	Separation characteristics		Maximum operating conditions		
			Nominal	EPH retention[c] (%)	pH	Temperature (°C)	Pressure (MPa)
M1	Dow(DDS)/HC-50	Thin-film composite	40–50[a]	95	2–10	60	6
M2	Liko/X-50-60-25	Cellulose acetate	10[b]	63	3–8	40	2.5
M3	Hoechst/UF-PES-4/PP60	Polyethersulfone	4[b]	81	1–14	90	4
M4	Dow(Film Tec)/NF-40	Thin-film composite	45[a]	96	2–11	45	4
M5	Nitto/NTR-7410	Polymer composite	5–10[a]	87	2–11	40	3
M6	Nitto/NTR-7250	Polyvinyl alcohol	50–60[a]	95[d]	2–8	40	3
M7	Toray/SU-610	Ultra-thin-film composite	50–60[a]	93	2–11	35	1.5
M8	Celfa/DRA-40	Polyethersulfone/ polyamide	40[a]	99	2–11	45	4
M9	Hoechst/NF-PES-5	Polyethersulfone	3–8[a]	99 (fouling)	1–14	90	5

[a] NaCl retention (%) reported by producer.
[b] MWCO (kDa) reported by producer.
[c] Maximum value (pressure, velocity, and concentration dependent).
[d] Sulfate retention in the presence of EPH: $R_{sulfate}$ = 12% (in contrast to negligible retention of all other membranes).

resulting feed velocities and average transmembrane pressures reached up to 4.44 $m \cdot s^{-1}$ and 3.5 MPa, respectively. Two pressure transducers were placed upstream and downstream of the membrane module in the retentate loop. Because of atmospheric pressure on the permeate side, the transmembrane pressure difference was then taken as the average of both measured values. The retentate flow rate was monitored by using a flowmeter.

After the membrane was placed in the membrane module, distilled water was circulated in the test loop at the maximum operating pressure for about 8 hours. During this time a compaction of the membrane was observed, and this led to a stable water permeability. After each set of experiments with protein hydrolysates the circuit and membrane were rinsed with water and the pure water flux was measured again under the same conditions until steady state was obtained. Differences in the steady-state pure water flux were taken as a measure of the fouling tendency of the membrane. Measurements with the test solutions were performed in the following modes: 1) at constant feed concentration, i.e., the permeate and retentate were returned to the feed tank; 2) with increasing retentate concentration, which means that the retentate was returned to the feed tank and the permeate was collected separately; and 3) diafiltration, which, in contrast to the previous mode continuously added pure water to the feed to prevent any loss of volume. Flux was recorded as a function of time, and permeate and retentate samples were both analyzed for proteins and sulfate anions by refractometry and conductometric titration, respectively. To prevent protein gelation, the measurements were performed at 40 ± 2°C. Duplicate experiments for selected conditions showed good reproducibility of the data measured.

RESULTS AND DISCUSSION

Choice of the Membrane

Initially the experiments were performed as a series of runs with various types of membranes. Each run was carried out in the same manner, with a constant protein hydrolysate concentration of 4% wt, a feed velocity of 3 $m \cdot s^{-1}$, and the transmembrane pressure being changed stepwise in the range from 0.5 to 3.5 MPa. Observed membrane retention coefficients R_{obs} for both sulfate anions and protein hydrolysates (EPH) were determined from experimental data by using the equation

$$R_{obs} = 1 - (C_P/C_R)$$

where C_R and C_P denote retentate and permeate solute concentrations, respectively. The data obtained were plotted in graphs, as illustrated in Fig. 1, to show the separation and performance characteristics of the membrane. The conclusion that can be drawn from the experimental results summarized in

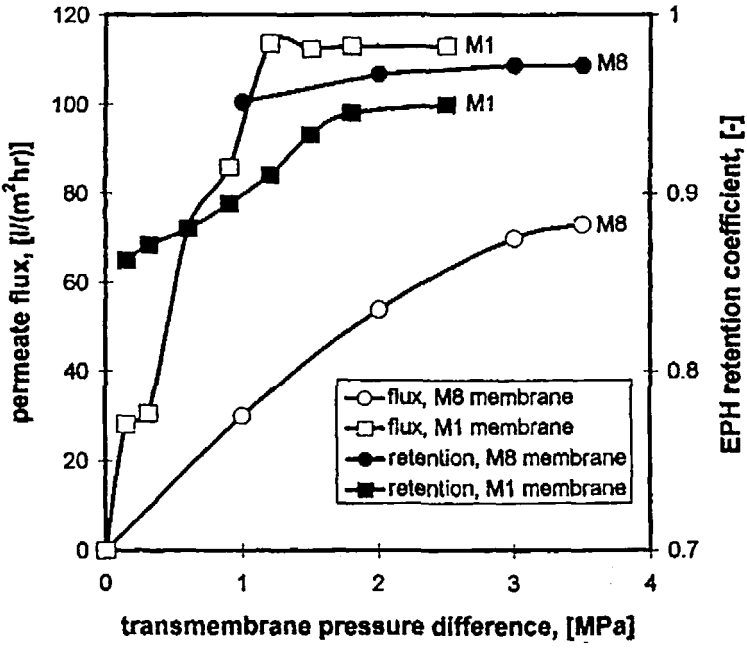

FIG. 1 Variation of the permeate flux and EPH retention coefficient with transmembrane pressure difference for a retentate velocity of 3 m/s.

Table 1 is that nanofiltration membranes should be used rather then ultrafiltration ones as they show complete passage of sulfate anions with higher retention of protein hydrolysates. The considerable loss of protein hydrolysates into the permeate for ultrafiltration membranes with a nominal cutoff of 4 and 10 kDa is probably due to the polydispersity of the protein hydrolysates, and thus the presence of a considerable portion of hydrolysates of very low molecular weight in the feed. Moreover, the flux was often higher for nanofiltration membranes despite the fact that pure water permeability for these membranes was smaller. Another conclusion is that the salt (NaCl) retention coefficient given by membrane manufacturers is only an indicative property of the nanofiltration membranes used and in some cases is even misleading. Membranes characterized by the same retention coefficient but prepared by different manufacturers had quiet different separation properties for the system investigated, even in the case of membranes of the same polymeric material.

Two nanofiltration membranes with the code names M1 and M8 were found to be the best ones. Therefore, all subsequent experiments were con-

ducted solely with these nanofiltration membranes. As reported by the membrane manufacturers, the M1 membrane (DDS/HC-50) is more resistant to temperature compared to the M8 one (60 vs 45°C). On the other hand, the M8 membrane (Celfa/DRA-40) has a higher pH upper limit (11). The retention of test salt (NaCl) solutions is reported to be about 40% for both membranes. Our experiments with sulfate anions without proteins in the testing solution indicate that the retention of Na_2SO_4 is higher than 85% for both membranes. Surprisingly, in comparison with these membrane characteristics, the observed retention of divalent sulfate anions in the presence of proteins was negligible. Such a result deserves further investigation and probably relates to the fact that sulfate ions were rejected to the extent that there are balanced electroneutrality requirements in the vicinity of the membrane. In this connection it is necessary to note that protein hydrolysates are also negatively charged at the pH value used. A similar phenomenon has also been reported by authors (8, 9) for feeds consisting of high salt concentrations mixed with low concentrations of organics. They even observed negative salt rejections for some feed compositions. This illustrates that the presence of electrolytes in feeds of organics, and vice versa, permits realization of the Donnan effect: sulfate anions are forced into the permeate with increasing protein hydrolysates concentration. Thus each application of this type must be carefully tested and monitored to be sure that the desired results are being obtained.

Effects of Transmembrane Pressure Difference and Crossflow Velocity

The retentate crossflow velocity and transmembrane pressure difference were found to be important factors influencing both the permeate flux and separation efficiency. In Fig. 1 the steady-state flux is plotted versus the transmembrane pressure difference for both membranes tested. The well-known limiting flux behavior is observed for the M1 membrane, which suggests that there is little advantage to be gained from operating at a higher pressure differences than 1.2 MPa. On the other hand, no constant value of permeate flux is reached for the M8 membrane even at the 3.5 MPa pressure difference. The reason for this behavior is that the M8 membrane which consists of a polyethersulfon active layer on a polyamide support, exhibits considerably higher hydrodynamic resistance even in the case of pure water flow. Nevertheless, this disadvantage is balanced by the higher retention of enzymatic protein hydrolysates (EPH). Since the total transmission of sulfate anions was observed for both membranes, the retention of EPH is the dominating factor affecting the selectivity of the demineralization process. The EPH retention data for both membranes tested are presented in Fig. 1 as a function

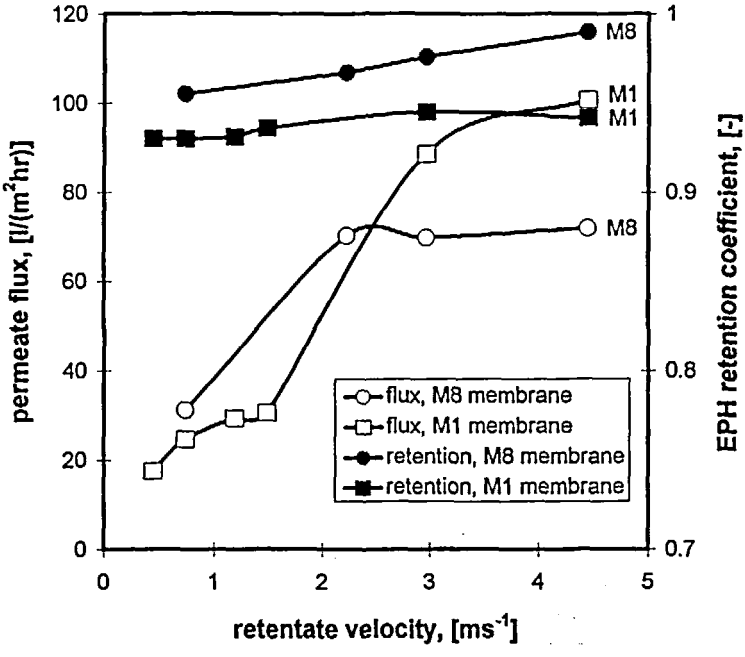

FIG. 2 Variation of the permeate flux and EPH retention coefficient with retentate velocity for constant transmembrane pressure differences of 1 MPa (M1 membrane) and 3 MPa (M8 membrane).

of transmembrane pressure difference and in Fig. 2 as a function of retentate velocity. It can be seen that the EPH retention varied across the whole range of operating conditions from 0.86 to 0.99%. At first sight the retention changes do not seem to be very important. However, the EPH concentration in the permeate becomes 14 times larger when the EPH retention decreases from 0.99 to 0.86%. The increase in EPH rejection with increasing transmembrane pressure difference suggests a mechanism for the transport of protein hydrolysates and solvent across the membrane based on the idea that protein flux is almost invariant with pressure, whereas the solvent (mineralized water) flux increases with an increasing pressure difference. The net result is that a higher solvent flux dilutes the EPH concentration in the permeate, resulting in a higher calculated EPH retention. Of course, this is a simplified explanation because the transport can be further complicated by the concentration polarization (4, 5). This phenomenon is characterized by an increase of the EPH concentration at the surface of the membrane above that of the bulk solution as a result of both convective transport to the membrane and the back-diffusive

transport. For protein composed feeds, the increase in permeate flux usually increases the protein concentration at the membrane surface to a limiting concentration. Under these conditions, proteins begin to form a gel at the membrane surface. Once a gel layer is formed, it is often the limiting resistance to flow, simultaneously affecting the retention of the system. For example, as it can be seen in Fig. 2, a higher retentate velocity results in an increased permeate flux as the gel layer thickness is reduced. Assuming again the above-mentioned mechanism for transport of water and protein hydrolysates across the membrane, a higher permeate flux can be attributed to the increasing the portion of solvent pore flow. Thus, the net result is that higher EPH retention is observed for higher feed velocities.

The key point derived from these measurements is that there is an obvious advantage in terms of EPH retention for the conditions of a high transmembrane pressure difference as well as of a high retentate crossflow velocity. Nevertheless, the cost factors, both capital and operating, must be weighed against EPH recovery when considering these favourable conditions.

Effect of EPH Concentration

Experiments were carried out at various feed concentrations of enzymatic protein hydrolysate ranging from 2.3 to 15% wt. The results are plotted in Fig. 3. It can be seen that the permeate flux follows fairly well the law of gel model of concentration polarization (7). According to this model, plotting steady-state permeate flux versus ln ($C_R - C_P$) should give a straight line with ln($C_{GEL} - C_P$) as the intercept. Using this procedure, the gel-like concentration C_{GEL} was found to lie between 35 and 40% wt for the systems investigated. In fact, the value of the gel concentration is usually much higher for gelatin proteins which can display liquid behavior at concentrations as high as 60% (5, 6). Thus, it is often recommended that C_{GEL} be used only as a model fitting parameter rather than as a result of the theoretical soundness of the gel model (7). Nevertheless, recently Élysée-Collen et al. (6) studied the effect of added $(NH_4)_2SO_4$ on the morphology of the concentration polarization layer during membrane separation of gelatin, and their results indicated that in the presence of salt a gel can form at a wide range of protein concentrations. The data of Fig. 3 can be also used to find the optimal EPH concentration prior to the demineralization process under which diafiltration is performed as fast as possible with the smallest membrane area. It can easily be derived that for total rejection of EPH, the optimal initial EPH concentration is the gel concentration multiplied by 0.37 (5). For the system investigated, the optimal EPH concentration equals 14 ± 1% wt.

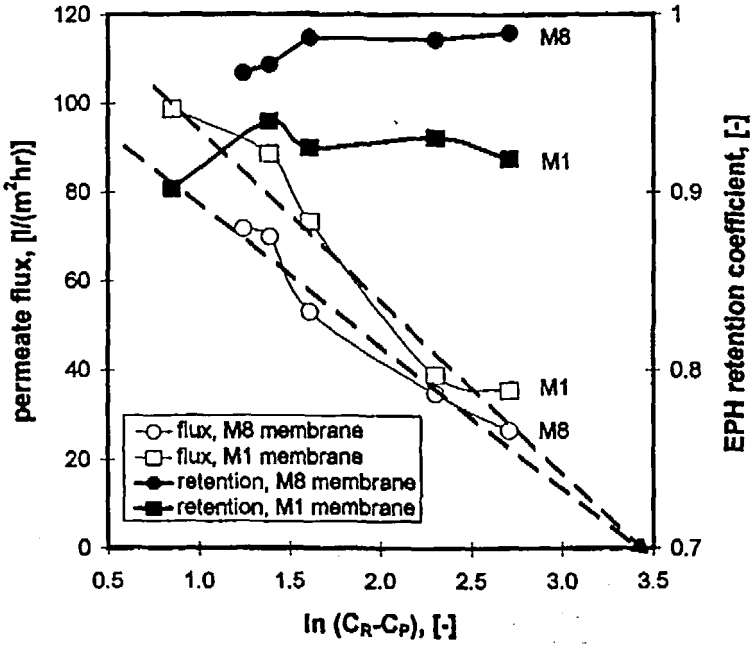

FIG. 3 Semilogarithmic variation of the permeate flux and EPH retention with EPH concentration.

Design Considerations

The design of a diafiltration system should balance the desired selectivity and the price to pay for it in terms of time, membrane area, and volume of washing solvent. To illustrate these key design considerations a simple batch demineralization system was proposed and analyzed on the basis of laboratory experiments. The design was for a 4 × 40″ spiral-wound nanofiltration module (5 m^2 of active membrane area) equipped with the M8 membrane. The operating conditions were chosen with a transmembrane pressure difference of 3 MPa, a feed crossflow velocity of 3 $m \cdot s^{-1}$, a protein concentration before diafiltration of 15% wt, and a diafiltration dilution (volume of added water: original concentrate volume) of 5.3 : 1. By using a simple mathematical model combining mass balance for the system with the equation which quantifies the flux (see Ref. 4; the parameters have been determined from the experiments mentioned above), the overall performance of the demineralization system was predicted. This achieved 90% protein recovery and a 200 times reduction

of salt concentration at an average permeate flux of 15 $L \cdot m^{-2} \cdot h^{-1}$. The amount of the demineralized protein hydrolysate was found to be 6.9 kg of the pure demineralised protein product during a 5 hour production cycle. In the Czech Republic the total specific cost of this demineralization process has been evaluated to be approximately 53 Kč*/kg of the pure demineralized protein product, which is rather high. The cost estimates also show that the cost of membrane replacement and related capital costs are dominated by the operating costs, mainly the power consumed for pumping the recycle stream and the use of relatively expensive pure water for diafiltration and membrane cleaning. However, the number of variables is so great that changes in the system size, the desired degree of demineralization, as well as in the prices of energy and pure water can cause considerably lower specific costs.

CONCLUSIONS

Nanofiltration operated in the diafiltration mode is a very promising method for demineralization of waste enzymatic protein hydrolysates from leather production. The retentate cross flow velocity, transmembrane pressure difference, temperature, and retentate concentration were found to be important factors influencing both the permeate flux and separation efficiency. The best results were achieved using a nanofiltration membrane with the code name M8 (Celfa/DRA 40) operated at 40°C with a moderate crossflow velocity (3 $m \cdot s^{-1}$) and a relatively high transmembrane pressure difference (3 MPa). Under these conditions the protein rejection was higher than 98% whereas the rejection of sulfates was negligible (total transmission). Cost estimates show that for this system the cost of membrane replacement and capital-related costs are dominated by the operating costs, mainly power consumption for pumping the recycle stream and the use of relatively expensive pure water for membrane cleaning and diafiltration.

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* 100 Kč = US$3.47 (March 1997).

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