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Treatment by Nanofiltration of the Bleed Stream of a Continuous Alcohol Fermentor

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ABSTRACT

In continuous ethanol fermentation processes, which include product removal and recovery steps, to limit the concentration of secondary products, a bleed stream may be used. This stream contains several small molecular weight organic fermentation products and substrate sugars. To recover sugars and to minimize the amount of waste, the bleed stream is treated by nanofiltration. Three types of membranes were used in these experiments: 500 and 200 mwco (molecular weight cut off) polyamide and a thin-film composite membrane with 99.2% NaCl rejection. Separation was achieved in two stages. First, separation of sugars was studied with 500 and 200 mwco membranes, and 84% total sugar

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rejection was obtained. Second, by-products, which constitute the high total organic carbon (TOC) of the wastewater, were separated from the bleed stream by using the thin-film composite membrane. The 95.1% of organics leading to TOC could be removed from the bleed by this method. Based on these results, a waste process, consisting of two nanofiltration units with a 500 mwco membrane and a thin-film composite membrane, were suggested for the recovery of sugars and water.

Key Words: Bleed stream; Nanofiltration; Ethanol fermentor; Water recovery; Sugar rejection; Membrane rejection.

INTRODUCTION

Waste treatment and waste minimization have become important issues in the last decade due to their implication for the protection of the environment. Membrane processes started to be exploited for this purpose, in addition to their wide use in separations.[1–3] In this study, it is proposed to use nanofiltration for the treatment of a real bleed stream of a continuous ethyl alcohol fermentation process. Ethyl alcohol can be produced commercially in continuous fermentation systems using *Saccharomyces cerevisiae*. In the early studies, it has been shown that fermentation products may become toxic to the yeast and show inhibitory effects. To increase the productivity, as well as to prevent the inhibition of yeast, the fermentor is coupled with a separation unit in many continuous fermentation processes. Such applications may involve a stripping column,[4,5] a prevaporation unit,[6] or a membrane–bioreactor unit[7] to remove the product ethanol.[8] During fermentation, in addition to ethyl alcohol, some by-products also are produced.[9,10] Most of these by-products have inhibitory effects on the microorganisms as well.[10] The volatile by-products can be removed simultaneously from the broth during the stripping of ethyl alcohol. However, the utilization of a stripping column or pervaporation unit alone will lead to accumulation of the nonvolatile secondary products (high molecular weight) to the point where they may become toxic to the yeast. This can be readily overcome by continuous withdrawal of a bleed stream from the fermented beer. This bleed is considered as a waste of the process.

The effluent of a continuous ethanol fermentor will normally have ethanol in the range 20–63 g/L, depending on the operating conditions and productivity of the process.[4–7] When the fermentor is coupled with a separation unit, some proportion of the volatile substances of the broth stream will be removed with the product. As a result, the concentration of ethanol within the bleed is expected to be lower. However, some portion of ethanol and other low molecular weight secondary products, which cannot be completely separated with the stripping column, as well as higher molecular weight substances such as glycerol, will still be a problem if the bleed is to be recycled to the fermentor. The average composition of a bleed stream exiting a real ethyl alcohol fermentation process is given in Table 1.[9,10] This stream exhibits high levels of chemical oxygen demand (COD) and total organic carbon (TOC). According to water quality standards for discharging from fermentation processes of the Turkish Ministry of Environment-2002, the COD of the waste stream should not exceed 150 g/L. Therefore, such streams cannot be directly discharged to the environment, and they require treatment.[11] On the other hand, treatment of a bleed with conventional methods is very difficult. In ethyl alcohol production by conventional fermentation processes, approximately 10 L of bleed is discharged to obtain 1 L of 98% ethyl alcohol.[10] The focus of this study, is therefore, to minimize the amount of waste by recovering water and sugar from the bleed. Additionally, minimization of the sterilization expenses also is a goal.

Separation of by-products from a real bleed stream is particularly difficult, since it contains many types of compounds with different physico-chemical properties. Moreover, many products are heat labile and can be adversely affected by evaporation. Thus, membrane processes prove to be suitable alternatives for separation of products from a real fermentation broth.

Microfiltration and ultrafiltration are establishing themselves as viable technologies in many primary downstream treatments in biotechnology.[1,2] Reverse osmosis and nanofiltration on the other hand, should be useful in recovering microsolutes.[13] There are several studies in which nanofiltration has been used to treat fermentation broths.[13–16] In the study by Toledo,[13] reverse osmosis was used to separate ethyl alcohol from fermentation broth to increase productivity. In that process, glucose was separated simultaneously with ethyl alcohol and was recycled to the fermentor. As a result, productivity

Table 1. Composition of the real bleed stream of a continuous ethanol fermentation process that is integrated with a separation unit of ethanol.

Substance	Concentration (g/L)
Sugar	0.16–0.26
Acetic acid	2.65–3.94
Ethyl alcohol	8.2–14.96
Glycerol	6.68–9.08
TOC	14,700–16,000
COD	47,000–49,000

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of the process was increased through the use of reverse osmosis. In another study, separation of lactic acid from fermentation broth by reverse osmosis was performed.[14] A cellulose acetate reverse osmosis membrane was attached to the fermentor and operated to remove lactic acid as it was formed, while recycling the sugars and other components. A high ratio of rejections was obtained in spite of the low operating pressure.[14] Separation of butanol via reverse osmosis was studied by Garcia et al.[15] Both synthetic mixtures and real fermentation broths were investigated, and it was found that permeate fluxes obtained with the real stream is about one-third of those obtained with a synthetic feed. In addition to those examples in which nanofiltration is primarily used to separate fermentation products, there are a few applications in which waste minimization is achieved by nanofiltration.[16] All of these studies indicate that to determine the process parameters for a wastewater treatment plant, real solutions should be used.

In this work, the possibility of stepwise treatments of the bleed stream using nanofiltration was studied. First, unused sugars are to be separated and recycled to the fermentor. Second, the by-products are to be separated. Remaining water is to be recovered and recycled to the fermentor. By doing so, the amount of waste is to be minimized; valuable feed materials are to be recovered, and the plant is to be operated at the highest capacity.

EXPERIMENTAL

Materials and Methods

Three types of membranes have been studied for the treatment of the bleed stream: asymmetric polyamide membranes with 500 and 200 mwco (Berghof Filtration, Germany, BM5 and BM2, respectively), and thin film composite membranes with 99.2% NaCl rejection (Amafilter, Holland, ROM3). Cellulose acetate membranes (0.2 μm) (Strautorius, Germany) were used for prefiltration purposes.

In the nanofiltration experiments, Berghof's high-pressure filtration units was used.[17,18] The system is equipped with a high-pressure pump of three plungers capable of maintaining a liquid system pressure up to 60 bar. Pulsation in the pressure was prevented by using a 30 bar pulsation damper. The system contains a chamber that holds an element of a flat membrane with 7.6 cm diameter. Flow geometry of this chamber is designed to obtain cross flow to minimize concentration polarization and membrane fouling. The flow rate and the operating pressure were controlled independently by stainless-steel needle valves located at the outlet of the module. Temperature was controlled with a countercurrent double-pipe heat exchanger.

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Analyses of by-products were done by high-performance liquid chromatography (Shimadzu CR4A Chromotopac, Japan), by using a differential refractive index detector and an organic acid column (HPX-Aminex, Biorad, California, USA). Concentration of total sugar was determined by the sulfuric acid–phenol method.[19] Concentration of glucose was determined by a *D*-glucose analyzer (YSI 1500 sidekick, Ohio, USA). Ethyl alcohol concentration was measured by using a gas chromatograph (Shimadzu GC-14, Japan) with a poropak-Q column. Total organic carbon of the bleed was measured by a carbon analyzer (ION X 1555B, Colorado, USA), and COD was determined by the open reflux method.[18]

A real bleed stream of a continuous ethanol fermentation process, in which *S. cerevisiae* was used in a fluidized bed reactor, is studied.[10] Previously, the importance of using a real stream rather than a synthetic stream has been discussed. Prior to starting the nanofiltration experiments, bleed streams of different fermentation runs were accumulated to have sufficient volume. An accumulated bleed stream provided a solution with a time-averaged composition. Concentrations of ethanol, glycerol, acetic acid, and total sugars, as well as COD and TOC of this stream, are reported in Table 1. Prior to membrane separations, free cells were removed from the bleed stream by centrifugation. Following the separation of the free cells, hydrogen peroxide was added to prevent microbial growth of any kind. In the final stage of preparation of the bleed stream, the solution was prefiltered through a 0.2 μm cellulose acetate membrane to remove high molecular weight substances.

Studies on sucrose–water and glucose–water separations, using 500 and 200 mwco membranes, and a sucrose–glucose–water system with 500 mwco membranes have been reported in our earlier publication.[17] Our previous study has shown that feed flow rates >18.9 mL/sec should be used to reduce concentration polarization.[17] In this study, 29.7 mL/sec was used as the feed flow rate, which is the highest rate possible with our experimental setup. The second parameter, which affects the performance of the separation, is pressure. The operating pressure has been chosen based on previous details permeate flux studies.[17] In summary, experiments were carried out at the pressure of 30 atm and a feed flow rate of 29.7 mL/sec.

For each nanofiltration experiment, 2-L bleed streams were used. During the experiments, permeation rate was measured, and its variation with time was monitored. Experiments were continued until a steady state was reached. After measuring the same permeate flux at least three times, the operation was terminated. Samples were taken at these steady-state conditions, and the concentrations of sugars, ethyl alcohol, glycerol, acetic acid, and acetaldehyde in the effluent were measured. Sugars are the substrates that are to be recycled; thus, their concentrations were monitored in the feed and permeate streams. Since the permeate stream is planned to be recycled to the fermentor, the concentration of inhibitory products is monitored. Those monitored inhibitory products are ethanol (the main product), acetic acid (representative of a low molecular weight by-product), and glycerol (a high molecular weight by-product).

RESULTS AND DISCUSSION

Experimental results obtained with four different membranes using bleed streams with average compositions are given in Table 1 are reported in Table 2. The variations in the composition of the real bleed stream, which is used as a feed in these experiments, is minor, thus its effect is negligible. Reported rejection results are the averages of three steady-state measurements. Since the measured rejection values at three different times did not vary significantly, the assumption of steady-state behavior of the permeate flux was validated. In the first two columns of Table 2, results for 200 and 500 mwco membranes are reported. In the last two columns of Table 2, results of the thin film composite membrane units (used in series) are reported. Additionally, in this table, water and real-stream permeability values, and rejections of the selected constituents

Table 2. Results of bleed stream experiments (30 atm operation pressure, 29.7 mL/sec feed flow rate).

	500 (BM5)	200 (BM2)	Thin-film composite (ROM3) 1st unit	Thin-film composite (ROM3) 2nd unit
Distilled water flux (kg/m^2hr)	126.00	16.92	40.68	41.04
Permeate flux (kg/m^2hr)	46.80	4.68	15.48	28.87
Total sugar (% at feed)	0.26	0.19	0.16	9.6×10^{-3}
Rejection (%), total sugar	84.00	94.70	98.40	84.00
TOC (% at feed)	16,148	18,765	14,700	2,000
Rejection (%), TOC	27.60	91.40	95.10	75.00
COD (mg/L feed)	47,600	59,600	49,000	4,000
Rejection (%), COD	18.48	92.45	97.96	71.00
Glycerol (g/L feed)	9.08	6.87	6.68	0.15
Rejection (%), glycerol	42.4	95.00	97.00	100.00
Acetic acid, (g/L feed)	3.94	2.98	2.65	0.45
Rejection (%), acetic acid	37.80	80.00	85.00	99.00
Ethyl alcohol, (g/L of feed)	14.96	9.95	8.20	1.00
Rejection (%), ethyl alcohol	6.69	67.00	84.40	99.00

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are reported. Detailed evaluations of these results are given in the following paragraphs.

Permeate fluxes measured with 500 and 200 mwco membranes using 1% synthetic sucrose solution and the real bleed stream (see Table 2) are compared in Fig. 1. The permeate fluxes measured in these experiments, when the 500 mwco membrane is used, indicate that permeate flux obtained with the real bleed containing 0.3% total sugar is much lower ($\sim$16 kg m^{-2} h^{-1}) than the permeate flux obtained with the 1% synthetic sucrose solution ($\sim$90 kg m^{-2} h^{-1}). This may be due to the presence of several organics in the real bleed and that their presence interferes with the transport mechanism.[14,15] Additionally, steric effects, as well as acidity and polarity, may be factors of importance on the permeation behavior.[19,20] Similar observations have been reported earlier for a system using real fermentation broths.[15] Moreover, the so called "gel layer" may become more pronounced in the case of the real bleed stream, as it may contain high molecular weight substances. Similar behavior is observed with the 200 mwco membrane when the real bleed stream is used. The dependence of permeate flux on feed composition was reported previously and was also supported from a comparison of the results of 3rd and 4th columns of Table 2. The results indicate that for the thin film composite membranes with the same pure water fluxes, permeate flux is nearly doubled when more dilute feed is used (Fig. 2). Thus, real bleed streams should be used rather than the synthetic streams for a realistic assessment of the process.

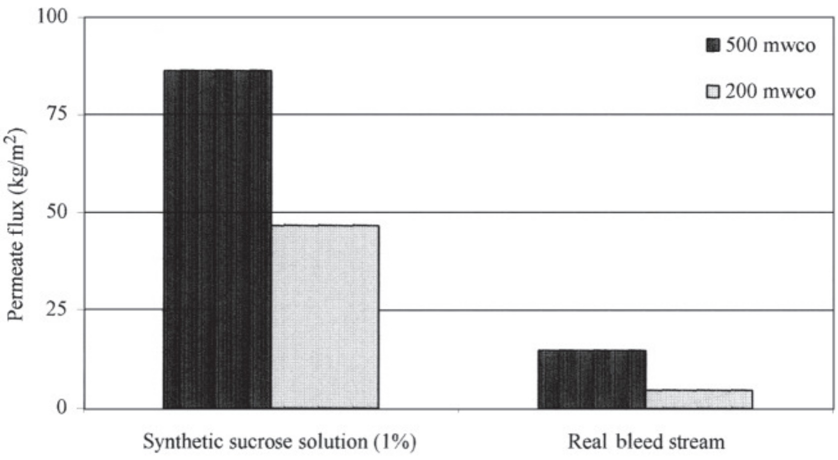

Figure 1. Permeate flux of 1% sucrose solution and bleed stream (30 atm operating pressure, 29.7 mL/sec feed flow rate).

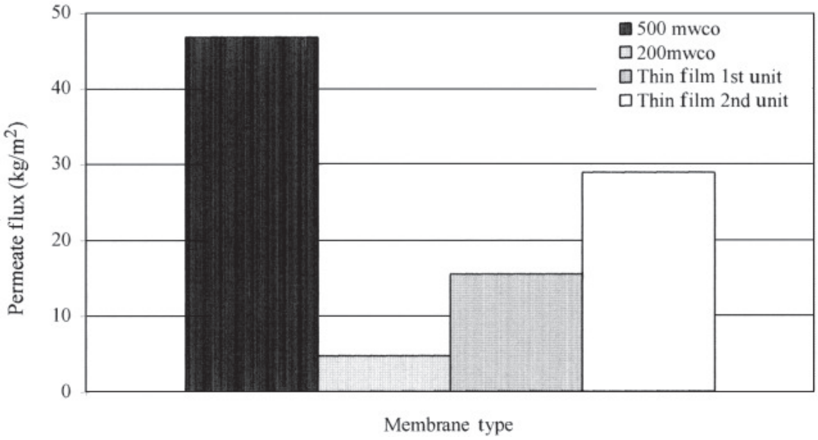

Figure 2. Permeate fluxes of the thin-film composite membranes with real bleed stream (30 atm operating pressure, 29.7 mL/sec feed flow rate).

The performance of the substrate recovery process, rejections of total sugar and glycerol (inhibitory product with the highest molecular weight) are examined and are shown in Fig. 3. The 500 mwco membrane rejects a significant amount of total sugars. However, the 200 mwco membrane and the thin-film composite membranes have a higher rejection of total sugars. The difference of the rejections of glycerol by the 500 and 200 mwco membranes is

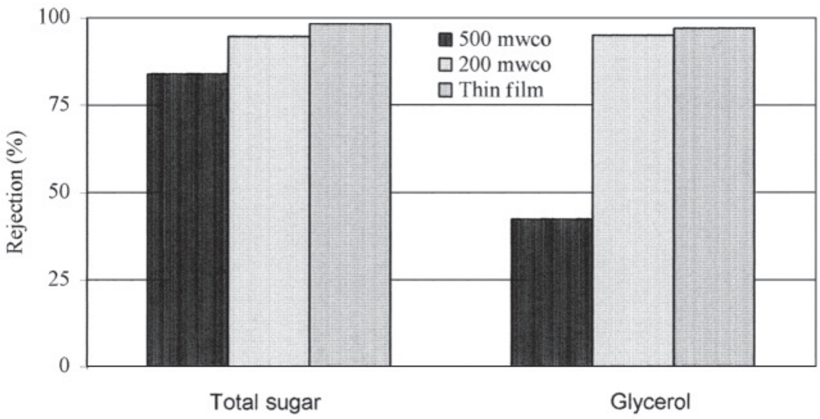

Figure 3. Rejection of total sugars and glycerol (30 atm operating pressure, 29.7 mL/sec feed flow rate).

more pronounced. It is observed that the 200 mwco and the thin-film composite membranes reject most of the glycerol as well as sugars. Since sugars are aimed to be recycled to the fermentor, the concentration of glycerol must be minimized in the retentate. As a result, the 500 mwco membrane is to be preferred for sugar recovery due to its lower glycerol rejection. For a specific application, a trade-off between substrate loss and inhibition due to recycled high molecular weight secondary products should be considered in membrane selection.

One of the purposes of this study is to minimize the amount of the waste. To evaluate the properties of the waste, its TOC and COD values are measured. From Table 1, it can be seen that TOC and COD of the feed bleed stream are very high, and it is impossible to discharge this stream directly into the environment.[13] Figure 4 indicates the rejection of the TOC and the COD by the three membranes used in this study. It can be seen from Fig. 4 that filtration with the 500 mwco membrane results in only a small decrease in the TOC and the COD. The main reason for this reduction is the removal of sugars. On the other hand, other membranes (the 200 mwco and the thin-film composite) remove most of the TOC and the COD. This shows that the 200 mwco and the thin-film composite membranes reject, besides sugars, most of the organics that may have inhibitory effects on the microorganisms. The thin-film composite membrane shows slightly high rejections compare with the 200 mwco membrane. Unfortunately, the TOC and COD values of the permeate stream are still high and not suitable for discharge to the environment. The possibilities are to recycle this permeate stream to the fermentor or to use a second membrane unit in series to decrease the solute concentrations.

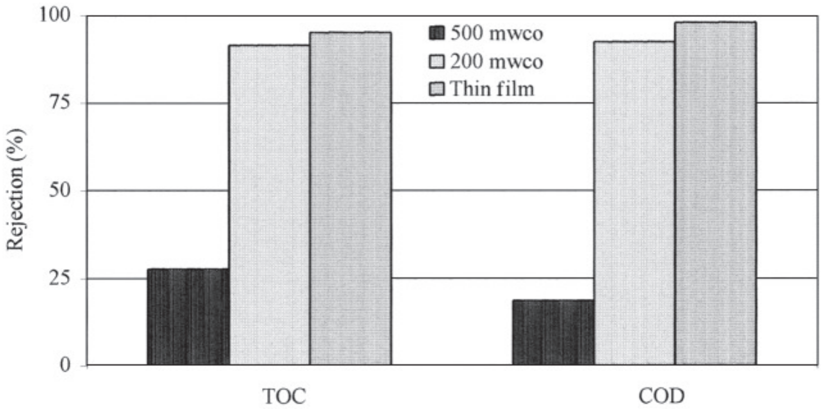

Figure 4. Rejection of TOC and COD (30 atm operating pressure, 29.7 mL/sec feed flow rate).

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For both the recycle case or the discharge case, a low concentration of inhibitory secondary products is desirable. Thus, the effectiveness of the removal of the low molecular weight inhibitory products such as ethyl alcohol and acetic acid need to be monitored. Rejections of these constituents with the three membranes are given in Fig. 5. Both ethyl alcohol and acetic acid permeate through the 500 mwco membrane in substantial amounts. The highest rejection is achieved with the thin-film composite membrane, 84.4% and 85% for ethyl alcohol and acetic acid, respectively. The permeate flux of the thin-film composite membrane unit may be recycled to the fermentor, or it may be further processed in a second thin-film composite membrane unit to decrease the concentration. As can be seen from the last column of Table 2, use of a second thin-film composite membrane unit in series removes the organics almost completely.

These results suggest the possibility of using two or three nanofiltration units in series for waste minimization. Following sugar recovery with 500 or 200 mwco membranes, the permeate flux may be used as a feed to a second unit. In the second unit, thin-film composite membranes, which have the highest TOC and COD rejections among the membranes studied, may prove to be useful for waste minimization. The permeate flux of this second unit can be sent to a third thin-film composite membrane nanofiltration unit, which may be used to decrease the organics concentration in water.

The flow diagram of the proposed waste treatment plant based on the results of the present experimental study is given in Fig. 6. Initially, the bleed stream may be ultrafiltered by using a 0.2 μm cellulose acetate membrane to

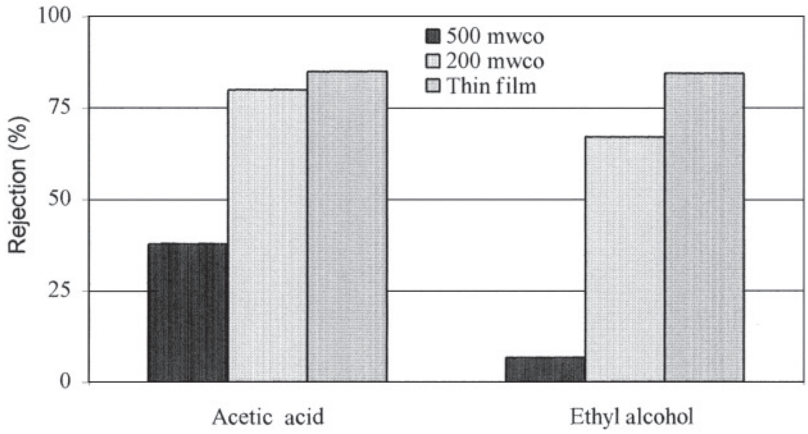

Figure 5. Rejection of ethyl alcohol and acetic acid (30 atm operating pressure, 29.7 mL/sec feed flow rate).

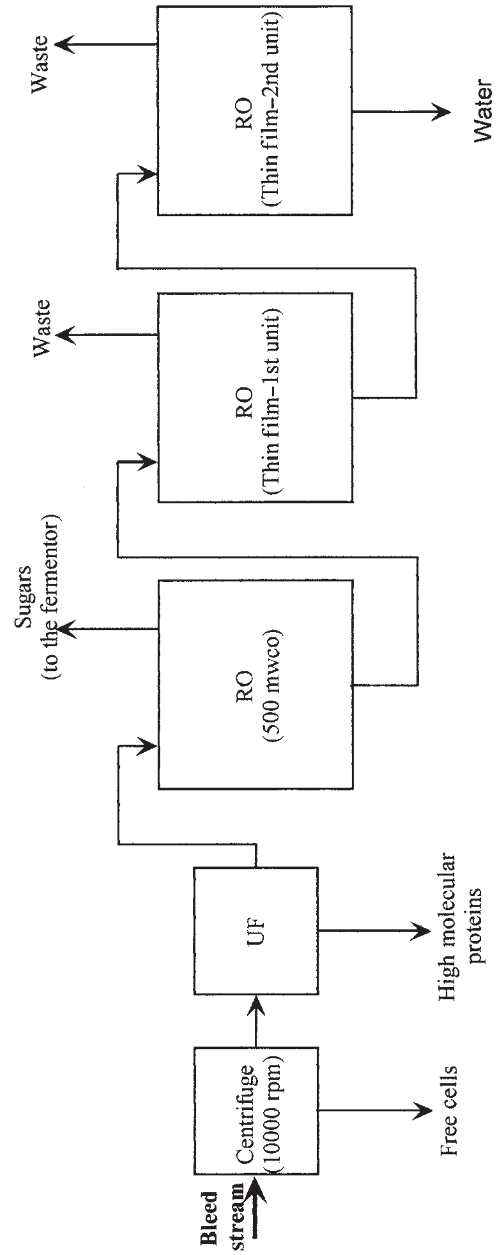

Figure 6. Flow diagram of the proposed waste treatment plant.

remove the free cells and the high molecular weight substances. These high molecular substances may cause fouling at later stages of the process. For that reason, an ultrafiltration unit is recommended, as shown in Fig. 6, although detailed evaluation of its operation is not included in the scope of this study. The sugars are proposed to be separated in a nanofiltration unit that uses a 500 mwco membrane. The retentate of this unit, which contains sugars, can be recycled to the fermentor. The permeate passes on to a second nanofiltration unit. In this unit, a thin-film membrane is proposed to be used for separation of by-products from the bleed. The volume of the retentate, which contains most of the by-products, is reduced dramatically. The permeate stream consists mostly of water, but it also contains small concentrations of organics. Concentration of the secondary products may be further reduced if a third nanofiltration unit is used. Recovered water may be recycled to the fermentor or discharged. The decision to use the third unit should be based on an economical analysis for specific cases.

CONCLUSIONS

In this study, the possibility of using nanofiltration for waste minimization of a continuous ethyl alcohol fermentation process has been introduced. Among the several processes in which separation systems are coupled with fermentation processes, the main focus is the separation or purification of the main product. In this study, on the other hand, simultaneous recovery of sugars and water in combination with waste treatment has been shown to be successful. It has been concluded that using a real stream plays an important role in this evaluation. The permeate flux obtained with a real bleed stream is determined to be smaller that that of the synthetic solution, although its sugar content is lower. All three membranes used in this study reject sugars very well. Because of its low glycerol rejection, 500 mwco membranes are proposed for sugar recovery. Rejections as high as 84% of total sugars are obtained when using this membrane. For the case of water recovery, thin-film composite membranes are used. The use of several membranes in series is shown to increase the performance of the waste minimization process. Since the streams used here are typical, the results of this study should be useful in the design of fermentation system coupled with separation units using nanofiltration.

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