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Separation of post-fermentation glycerol solution by nanofiltration membrane distillation system

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ABSTRACT

The fermentation process generates a broth containing the metabolites, residual substrates and mineral salts. Various unit operations are required for the separation, purification and concentration of organic compounds from the fermentation broth. In this work, nanofiltration (NF) and membrane distillation (MD) were proposed as a method for the preparation of preliminary separated concentrates for a further separation by other processes, such as rectification. The study was carried out using the real broths obtained from the fermentation of glycerol solutions with the *Leuconostoc mesenteroides* and *Citrobacter freundii* bacteria. The obtained broths were subjected to the pretreatment by microfiltration, followed by a further separation with NF. The NF270 membrane was used for NF process, and the rejection of citric acid and multivalent ions close to 100% was achieved. The permeate and retentate from NF process were subsequently concentrated about four to five times using membrane distillation. The membrane fouling during the NF and MD processes was observed.

Keywords: Glycerol; Fermentation; 1,3-Propanediol; Membrane distillation; Nanofiltration

1. Introduction

A biotechnological synthesis of organic compounds, such as 1,3-propanediol (1,3-PD) and carboxylic acids from crude glycerol, appears to be an attractive alternative route for chemical synthesis [1–3]. The post-fermentation broths contain the dissociated and non-dissociated forms of the organic products and residual mineral salts; therefore, different unit operations are required for the separation of these solutions [3].

With regard to the complex composition of broths, the major cost of carboxylic acids and other bioproducts synthesis is due to its downstream separation and purification stages [4]. The traditional separation and purification involve a series of unit processes such as filtration, acidification, neutralization, crystallization, carbon adsorption, evaporation, distillation and ion exchange [3–5]. The separation of even one major product of fermentation requires several processes. The methods for the separation of 1,3-propanediol from fermentation broth using the following steps, removal of biomass, removal of proteins, concentration of broth and the separation of 1,3-propanediol by silica gel chromatography, were presented [6].

The investigations on the separation and purification of carboxylic acids and polyols from

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fermentation broths by membrane processes have been carried out by several researchers [3–9]. Membrane-based processes can produce high purity carboxylic acid bypassing many of the steps and chemicals of the conventional processes [5,7–9]. Nanofiltration (NF) in a downstream separation can replace the multiple purification stages by a single process. In this case, the separation of microbial cells has to be performed prior to a microfiltration (MF) step [5].

NF is an effective method of selective removal of the ionic species. In the case of uncharged solutes such as sucrose and non-dissociated compounds, the solution transport mainly occurs through diffusion and the convection mechanism, whereas the transport of charged solutes such as lactate is mainly governed by the Donnan exclusion effect [5,7–12]. Thus, the ionic rejection is mainly affected by three parameters (effective pore radius, ratio of membrane porosity to membrane thickness and effective charge density of membrane) [8,9]. A membrane charge density depends on the solution pH, solute concentration, chemical nature of ions and the membrane composition [12]. The membrane charge originates from the dissociation of ionisable groups at the membrane surface as well as those within the membrane pore structure. The dissociation of the surface groups is strongly depended on the pH of contacting solution and because the membrane surface chemistry is amphoteric in nature, the membrane may exhibit an isoelectric point at a specific pH. The isoelectric point for NF270 membrane was determined to be in the pH range of 3.25–4.1 [12].

The applications of NF process for the separation, purification and concentration of products from streams have been emerging in various fields, such as fermentation product separation, sugar fractionation and sugar concentration [7–9]. The microbiological processes are associated with the generation of the considerable amounts of diluted effluents from the bioreactors. In the integrated separation processes, the NF can be used with economic advantages not only to pre-concentrate these solutions before the next evaporation step, but also to recycle the process water [7].

Distillation and rectification are the most widely used separation techniques; however, energy consumption is very high. The post-fermentation solutions often contain 80–90% of water. Therefore, replacing distillation partially or completely by other separation technique may result in considerable savings in energy [13]. In the case of separation of high-boiling components, a preliminary removal of solvent (water) significantly facilitates their rectification [14].

It was found that the operational costs for xylose concentration by NF were one-fourth of those for the conventional evaporation process [10]. However, an effective pressure-driving force for NF differs significantly from applied transmembrane pressure. It is decreased by osmotic pressure, which increases with the increase of solutes concentration. Therefore, it is difficult to prepare the concentrated solutions in the NF process, even when high transmembrane pressures are used. The solutions can be concentrated up to the saturation state in a process of membrane distillation (MD). This process is useful for the separation of the volatile compounds and for the concentration of non-volatile solutes [15].

MD is a process involving the evaporation of water through the non-wetted pores of a hydrophobic macroporous membrane. The driving force is the vapour partial pressure difference between the two sides of the membrane [15,16]. The liquid/vapour interface is located at the membrane pore inlet, and the pores are filled only by the gas phase. As a result, the osmotic pressure does not affect the driving force in MD, and this process can be applied for the separation of highly saline solutions. Therefore, the MD process is proposed for the production of drinking water from brines and is considered as a complementary process to RO and NF to perform a further concentration of brines in order to increase the global water recovery of the desalination process [15–22].

The evaporators are used for the removal of a part of water from the feed before the rectification [16]. In the case of installation with the productivity smaller than 45,000 kg/h, the application of MD is more cheaper than a three-stage evaporation system [23,24]. Moreover, the MD process is carried out below the boiling point, what enables the application of low-temperature heat source. The waste glycerol is formed during the biodiesel production in the petrochemical installations. The petrochemical industries often have substantial amounts of waste heat. If this waste heat can be utilized, the additional energy requirement of MD will be significantly reduce and the process will become economically viable [25].

In this work, the membrane processes were used for a preliminary separation of post-fermentation glycerol solutions before their further separation. The MF was applied for biomass removal and feed clarification. The NF was used for feed desalting and a preliminary separation of bioproducts. The MD process was used for the concentration of solutions obtained from the NF process.

2. Experimental

2.1. Glycerol fermentation

The post-fermentation broth contains, besides the bioconversion products, many other constituents such

as proteins used as nutrients or generated by bacteria e.g. extracellular polysaccharides. These compounds may cause the membrane fouling; therefore, the studies were carried out with the real solutions. Two types of bacteria strains were used for the fermentation: generating extracellular polysaccharides and those that did not generate these polysaccharides.

The glycerol fermentation was carried out using *Leuconostoc mesenteroides* and *Citrobacter freundii* bacteria (isolated and characterized in the Department of Biotechnology and Food Microbiology, Poznań University of Life Science, Poland). *L. mesenteroides* is facultative anaerobic Gram-positive non-pathogenic bacteria spherical to lenticular in shape. The genus *Leuconostoc* synthesizes extracellular polysaccharide dextran on a medium with sugars (e.g. glucose and fructose). This genus generated a kind of slime around bacteria cells. *C. freundii* are facultative anaerobic Gram-negative bacilli of Enterobacteriaceae family. *C. freundii* cells do not contain a thick cell wall made up of peptidoglycan and the outer membrane does not contain dextran [26,27].

A composition of prepared fermenting solutions was presented in Table 1. After sterilization, a medium was inoculated with *C. freundii* bacteria in a lag phase (10% v/v) and the batch fermentation was performed using a 2 L bioreactor (LiFlusGX, Biotron Inc, Korea) at 300 K for 3 days.

In the case of *L. mesenteroides*, the bacterial culture was revived (24 h) on MRS medium (BTL, Poland) at a temperature of 300 K. The used MRS medium contained the following per liter: yeast extract–4 g, meat extract–8 g, peptone K–10 g, $K_2HPO_4{\cdot}3H_2O$–3.4 g, CH_3COONa–5 g, ammonium citrate–2 g, $MnSO_4{\cdot}4H_2O$–0.05 g, $MgSO_4{\cdot}7H_2O$–0.2 g and glucose–20 g. After sterilization, the fermentation solution was inoculated with *L. mesenteroides* bacteria in a lag phase (10% v/v) and incubated (309 K) in the LiFlusGX bioreactor for 4 days. The stirrer velocity was 300 rpm for both kinds of bacterial cultures and the pH stabilization was performed. As a result, the pH decreases during the performed fermentations, and the final values equal to 4.4–4.8 and 3.5–3.8 pH were obtained for *C. freundii* and *L. mesenteroides* bacteria, respectively.

Table 1
The initial composition of fermentation broths

Broth ingredients (g/L)	*C. freundii*	*L. mesenteroides*
Glycerol	20	20
Yeast extract	2	5
Meat extract	1.5	0.8
Peptone K	2.5	20
Ammonium citrate	–	0.3
Glucose	–	2
$K_2HPO_4{\cdot}3H_2O$	3.4	0.6
KH_2PO_4	1.3	–
$MgSO_4{\cdot}7H_2O$	0.4	0.1
$(NH_4)_2SO_4$	2	–
$CaCl_2{\cdot}2H_2O$	0.1	–
$CoCl_2{\cdot}6H_2O$	0.004	–
CH_3COONa	–	0.5
$MnSO_4{\cdot}4H_2O$	–	0.05

2.2. Nanofiltration

The SEPA GE membrane cell (GE Osmonics, USA) with NF 270 membrane (Filmtec^{TM} Membranes, USA) was used for NF study. An active membrane area was 150 cm^2. Prior to the first use, the membrane was compacted at a transmembrane pressure of 1.0 MPa for 5 h at 298 K, using deionized water as a feed. The TMP was increased from 0 to 1.0 MPa applying a step of 0.05 MPa every 15 min.

The NF permeate flux and solute rejections as a function of the transmembrane pressure were measured, in the range between 0.3 and 1.0 MPa. The initial volume of feed was minimum 3 L. All the NF experiments were performed at a constant flow rate of the feed equal to 10 L/min and at a temperature of 298 ± 1 K.

After fermentation, the obtained broths were pre-treated using a dead-end MF (membrane filter 0.45 μm, Millipore), and the obtained filtrate was used as a NF feed. The volume of bioreactor was equal to 2 L (the initial NF feed volume 3–5 L), therefore, the fermentation was several times repeated and the MF filtrate was stored in the frozen state. The NF process was also performed with single-component aqueous solutions (2 g/L) of glycerol, 1,3-PD, citric acid and acetic acid in order to determine the separation properties of NF270 membranes, which are not presented in the membrane element specification. The permeate was recycled to the feed tank to sustain a constant solute concentration during the test. The continuous feed concentration studies were also performed. The membrane was flushed only with deionized water between NF experiments carried out with different solutes. During a shutdown period, the NF module was filled with distilled water. In order to limit the biofouling intensity, the NF installation (module disconnect from the installation) was several times rinsed using a 0.5 wt.% NaOH and H_3PO_4 solution, followed by a final rinsing of installation with distilled water.

The retention of solute (R) was calculated from Eq. (1) taking into account the concentration values of particular components in the feed and permeate streams:

$$R = [(C_F - C_P)/C_F] \times 100\% \quad (1)$$

where C_F and C_P are the solute concentrations in the feed and permeate, respectively.

2.3. Membrane distillation

The studies on the concentration of NF permeate and retentate were carried out using a variant of direct contact MD (DCMD). The MD installation consisted of two thermostatic loops (feed and distillate), which were connected to a capillary membrane module. The polypropylene membranes Accurel PP S6/2 (Membrana GmbH, Germany) were used. The applied capillary membranes had the pore sizes with the nominal diameter of 0.2 μm and the porosity of 73% (the manufacturer's data), and the outside/inside diameters equal to 2.6/1.8 mm. The MD module (shell diameter 18 mm) was equipped with 18 membranes S6/2 with a length of 230 mm. The membrane area (lumen side) was 234 cm^2. During the MD studies, the inlet temperatures of feed and distillate streams were kept at 353 and 293 K, respectively. Peristaltic pumps were used to circulate the feed and distillate, and the flow rate of feed and distillate stream was equal to 14 ± 0.5 ml/s.

2.4. Analytical methods

The compositions of separated solutions were determined using a high-performance liquid chromatograph HPLC UlitiMate 3,000 (Dionex, USA) with a refractometer detector RI-101 (Shodex) and a column HyperREZ XP H (Thermo Scientific, USA) through which a H_2SO_4 solution (0.005 M) was flowing (0.6 ml/min). The pH values and electrical conductivity were measured with a 6P Ultrameter (Myron L Company). The feed and permeate turbidity was measured using a Hach 2100AN IS Turbiditimeter (USA) instrument.

The anion and cation concentrations were determined using an ion chromatography method with a conductivity detector (850 Professional IC, Herisau Metrohm-Switherland). The separation of anions was achieved on a 1.7 × 3.5 mm Metrosep RP guard column in series with a 250 × 4.0 mm Metrohm A Supp5-250 analytical column. An analytical column 150 × 4.0 mm Metrosep C2-150 was used for the separation of cations.

In order to identify the functional groups on the membrane surfaces, the attenuated total reflection Fourier transform infrared (ATR-FT-IR) analyses were performed using a Nicolet 380 FT-IR spectrophotometer connected with Smart Orbit diamond ATR accessory (Thermo Electron Corp., USA). At least, four ATR-FT-IR spectra for each membrane sample were recorded in the region 400–4,000 cm^{-1} at resolution 0.482 cm^{-1} and with 50 scans for sample.

The morphology of deposit layer formed on the membrane surface was studied using a scanning electron microscopy (SEM).

3. Results and discussion

3.1. Post-fermentation solutions

The initial concentrations of nutrients (salts and protein) in the glycerol solutions and the type of used bacteria (Table 1) affected the content of post-fermentation solutions. The average composition of obtained solutions was presented in Table 2. The broth with *L. mesenteroides* bacteria contained, besides carboxylic acids, the unfermented glycerol (5.4 g/L). In the case of broths with *C. freundii* bacteria, the concentration of residual glycerol was lower (2.94 g/L) and the presence of 1,3 propanediol was also detected. The post-fermented broths were pre-filtered (0.45 μm) and the obtained filtrates with turbidity below 0.2 NTU were applied for NF studies. The NF installation was rinsed with distilled water, and about 0.6–0.8 L of water remained in the pipes after water drain from the NF installation. This operation caused that the broth was diluted by a residual distilled water; therefore, the initial feed concentration during the NF study was slightly lower than that presented in Table 2.

3.2. Characteristic of NF270 membrane

The NF process is usually proposed to remove the divalent ions from water. Due to the charge

Table 2
The composition of post-fermentation solutions

Component (g/L)	*L. mesenteroides*	*C. freundii*
Glycerol	5.4	2.94
Lactic acid	12.2	3.17
Citric acid	1.2	0.68
Acetic acid	1.5	0.48
Formic acid	0.07	0.02
Succinic acid	0.034	–
1,3-PD	–	3.5
Cl^-	0.04	0.34
PO_4^{3-}	0.35	2.42
SO_4^{2-}	0.145	1.47
NH_4^+	0.13	0.57
K^+	0.32	1.72
Na^+	0.52	0.38
Ca^{2+}	0.011	0.027
Mg^{2+}	0.01	0.038

interactions, the negatively charged ions are rejected more effectively. For that reason, the divalent co-ions usually exhibit higher rejection than monovalent ones. In this study, the $MgSO_4$ rejection was over 95%, and $MgCl_2$ was equal to 53% (Fig. 1), and these results were close to those presented in a membrane element specification. The rejection of organic compounds is affected in a significant way by their molecular weights. The nominal molecular weight cut-off (MWCO) of used NF270 membrane is at a level of 150 Da [28]. The NF study of model solutions confirmed that the retention coefficients decreased with the molecular mass of separated solutes and increased with increasing transmembrane pressure to 1.0 MPa (Fig. 1). The retention amounted to 36% for 1,3-PD (76.09 g/mol) was obtained. The lowest retention was observed for acetic acid (60.05 g/mol), which amounted to only a few per cent. The retention of citric acid (192.13 g/mol) and glycerol (92.09 g/mol) was at a level of 85 and 50%, respectively. The similar results were obtained in a previous work [12], where the glycerol rejection increases over the pressure range from 46.9 to 65.7%, and stabilizes for TMP higher than 1.0 MPa.

The effectiveness of separation in the NF process is governed by two mechanisms: sieving effect and charge interactions. As glycerol is neutral solute, its retention is only due to the sieving effects, according to which the molecules with sizes larger than the pore size are rejected by the membrane. Thus, the retention of neutral solutes depends on the membrane pore radius and hydrodynamic radius [8]. The optimum effective pore size for NF270 membrane was calculated as 0.43 nm [12]. The Stokes diameter of acetic acid is 0.38 nm [9,10]; hence, the size exclusion alone was unable to account for the high retention of acetic acid. On the other hand, the retention of ions by a negatively charged NF270 membrane may be greater due to the Donnan effect. The pKa values of citric acid are 3.13, 4.76, and 6.40, and that for acetic acid pKa is 4.75 [28]. The pH of NF feed (Fig. 1) was equal to 3.5, thus the acetic acid was undissociated and only citric acid (partially dissociated) retention was additionally enhanced by the influence of the negative charge of the membrane. In the studied case, the broths also contained lactic acid (90.08 g/mol). The pKa of this acid is equal to 3.86 at 298 K, and at 313 K, the pKa was estimated to be 3.67 [29]. Thus, at the pH values below 3.8 pH, a low retention of lactic acid by NF270 membrane can be expected.

Such large differences in the rejection of particular compounds create the possibility of the application of NF process for a preliminary separation of broths from glycerol fermentation.

3.3. NF separation of post-fermentation solutions

The separation of post-fermentation solutions by NF process confirmed the results obtained for single-component solutions presented in Fig. 1. In each studied case, the rejection of multivalent ions was high, which is characteristic for NF membranes, and the obtained results were similar to those presented in Fig. 2. These data indicated that the majority of salts present in the post-fermentation solutions were rejected by NF270 membrane and they were retained in the NF retentate.

The results presented in Fig. 3 demonstrated that the separation of organic solutes contained in the post-fermentation solutions could be divided into two groups: with rejection below 40% and above 80%. The rejection of lactic acid was higher for solution obtained from fermentation with *C. freundii*, mainly

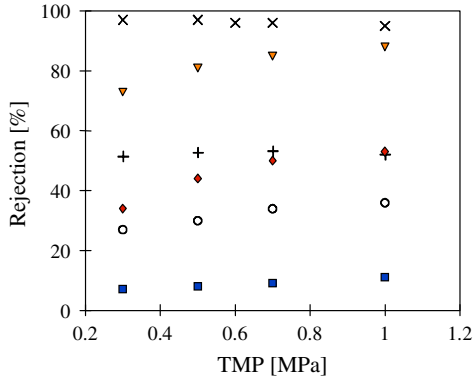

Fig. 1. The influence of transmembrane pressure on the rejection during NF process of different single-component solutions (2 g/L): (x) $MgSO_4$, (▼) citric acid, (+) $MgCl_2$, (◆) glycerol, (○) 1,3-propanediol and (■) acetic acid.

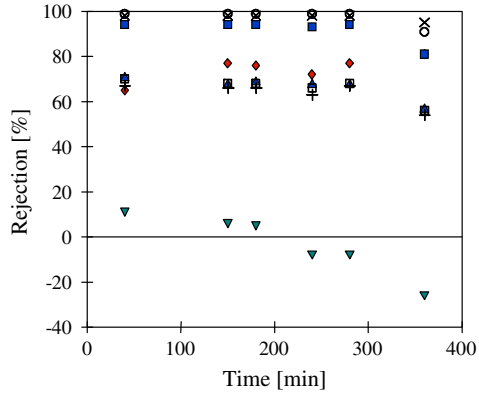

Fig. 2. The rejection of salts during the NF process of post-fermentation solution. Fermentation with *C. freundii*. TMP = 1.0 MPa. Ions: (x) SO_4^{2-}, (■) PO_4^{3-}, (○) Mg^{2+}, (◆) Ca^{2+}, (+) Na^+, (□) NH_4^+, (▲) K^+ and (▼) Cl^-.

due to higher pH (4.4–4.9), which was above the pK values of lactic acid (pK = 3.8). After 200 min of process duration, the feed concentration was started. As a result of a continuous concentration, the salts rejection was decreased. Finally, when the feed was concentrated about threefold, the rejection was close to that obtained for broth with *L. mesenteroides* (Fig. 4), which had a significantly higher initial concentration (Table 2). This indicates that not only a degree of acid dissociation, but also the feed concentration affected the rejection level (screening effect).

To study the effect of solute concentration on the permeate flux decline, one series was performed with threefold diluted feed (Fig. 4, series S1). Although the different level of acetic acid rejection was observed, the dilution of solution fermented with *L. mesenteroides* has an insignificant influence on the retention of lactic and citric acids. It was probably associated with a low value of the pH (3.5) of diluted feed. In this case, the citric acid was dissociated (pK = 3.13), but lactic acid (pK = 3.8) was in the undissocited form, and only the sieving effect affected the rejection of the lactic acid, and the separation results were similar to those for more concentrated solutions.

The negative retention of chloride ions (up to −20%) and acetic acid (up to −120%) was observed (Figs. 3 and 4). Moreover, the negative rejection of chloride was also observed for the separation of citrate and chloride solutions [30]. The negative retention of acetic acid was found when NF was used for the separation of xylose and acetic acids [9]. The observed results suggested that the intermolecular interactions, based on the Donnan exclusion, play an important role in the solutes separation during the NF process. The rejection of solutes depends not only on their size, but also on the differences in the mobility of the ions.

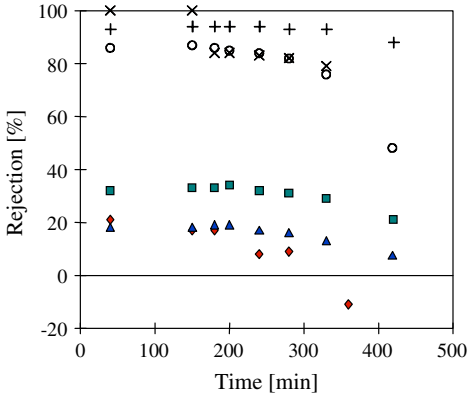

Fig. 3. The rejection of broth components during the NF process. Fermentation with *Citrobacter freundii*. TMP = 1.0 MPa. (x) succinic acid, (+) citric acid, (○) lactic acid, (◆) glycerol, (▲) 1,3-propanediol and (◆) acetic acid.

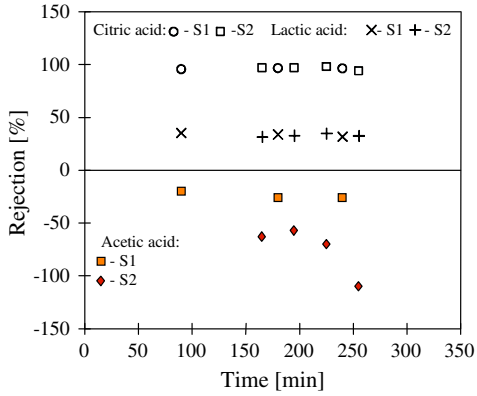

Fig. 4. The rejection of broth components during the NF process. Fermentation with *L. mesenteroides* bacteria. TMP = 1.0 MPa. S1 and S2–series with broth 1 (diluted) and broth 2, respectively.

The negative retention of acetic acid was strongly dependent on the feed concentration. However, this dependence was less pronounce when the feed concentration was three times lower, during the first measurement series (Fig. 4, series S1). The change of negative rejection from −60 to −120% was observed during the second series (Fig. 4 after 200 min of NF process), when a continuous concentration of the feed was applied. The rejection of acetic acid for the solution fermented with *C. freundii* was initially at a level of 20%. In this case, the pH of feed was equal to 4.8. This value was close to the pKa of acetic acid (4.75 pH) and was above the isoelectric points of NF270 membrane (3.25–4.1 pH). Under these conditions, the rejection level was increased due to the charge interactions among the ions of a partially dissociated acid and the NF membrane surface. However, the rejection decreases during the feed concentration (after 200 min of NF), and the negative rejection was also observed in this case.

3.4. Membrane fouling

The changes of permeate flux during the NF process used for the separation of post-fermented solutions were presented in Figs. 5 and 6. In both cases, an initial decrease of the permeate flux and then its stabilization was observed for NF studies with the constant feed concentration. Subsequently, a continuous concentration of the feed (after 200 min of NF process duration) was carried out and the permeate flux decline was again observed.

The feed concentration has usually a significant influence on fouling, which is the major cause for the flux decline. In order to investigate the fouling

intensity, the NF studies were carried out with both diluted and concentrated feeds. However, the obtained results (Figs. 5 and 6) indicate that the changes of feed concentration have an insignificant impact on the membrane fouling in the concentrations range examined. During the initial period of process, in both cases it was found that the obtained permeate flux rapidly decreased to a level which is two to three times lower than that determined for distilled water (122 L/m^{2}h). Such a large decline in the efficiency during the broths separation was also observed in other works [5,8,11]. After the initial changes, the permeate flux was at a relatively constant level for the consecutive 100–150 min. Over this period, the permeate was recycled to the feed tank in order to maintain the constant concentration of solutes.

After 200 min of NF process duration (Figs. 5 and 6), a continuous feed concentration was initiated, and finally more than a twofold reduction of feed volume was obtained. Due to an increase in the feed concentration, a rapid decline of the permeate flux was observed, which was higher for solutions fermented with *L. mesenteroides* bacteria. However, the maximum permeate flux determined after completing the studies was only lower by 10–15% than the initial flux, for both kinds of used post-fermenting solutions. The results obtained for the solutions fermented with *L. mesenteroides* were presented in Fig. 7. It was found that such a large decline of the permeate flux during the NF process mainly resulted from the increase of solutes concentration (osmotic pressure increase).

A decline of maximum permeates flux that was determined after completing the broths separation was found to be only slightly larger than that for a diluted broth (Fig. 7). This indicates for a limited intensity of fouling, which was probably mainly caused by the adsorption of organic compounds on the membrane surface. In a previous work with NF270 and NF90 membranes [31], it has been demonstrated that the organic fouling has a level characteristic for a given membrane material during the separation of post-fermented glycerol solutions (*Lactobacillus casei*).

During shutdown periods (between the experiments), the NF installation was partially filled with deionized water. After several days of shutdown, the growth of micro-organism on the surface of PCV tubes was observed. The feed tank in the used installation was open to the atmosphere, what enables a microbiological contamination. A biofouling was most probably a reason of a further decrease in the permeate flux, determined after 3 days of installation

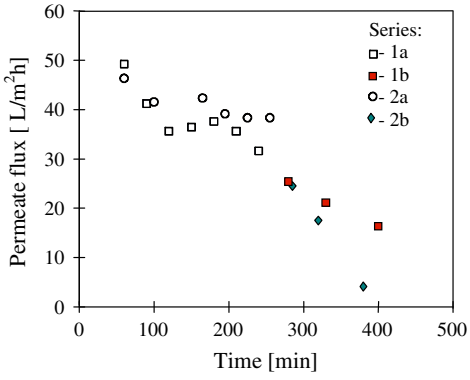

Fig. 5. The NF process of glycerol solution fermented with *L. mesenteroides* bacteria. TMP = 1.0 MPa. Series 1a and 1b–a threefold dilution of broth sample, and 1b and 2b–feed continuously concentrated.

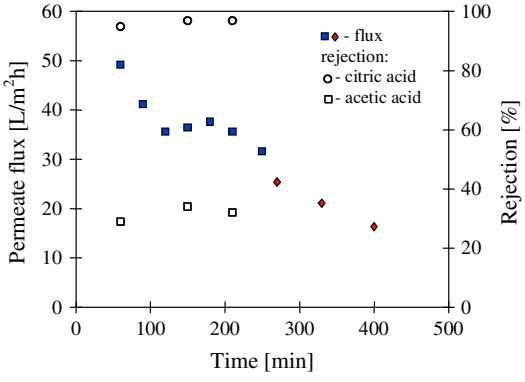

Fig. 6. The NF process of glycerol solution fermented with *C. freundii* bacteria. TMP = 1.0 MPa. The feed was continuously concentrated after 200 min of the process duration.

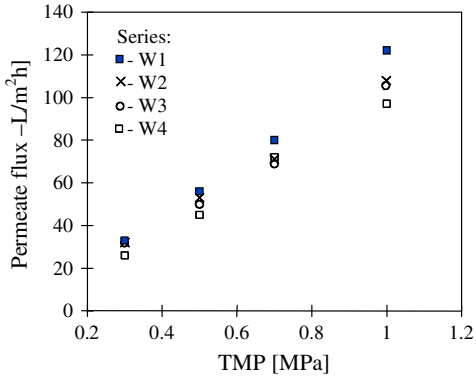

Fig. 7. The influence of TMP on the value of maximum permeate flux (feed-distilled water) determined after successive stages of studies. W1–new membrane, W2–after concentration of diluted broth, W3–after broth separation (series S1 and S2 from Fig. 4, respectively) and W4–three days after W3.

shutdown (Fig. 7, series W4). In order to limit the biofouling intensity, the NF installation (module without membranes) was several times rinsed using 0.5 wt.% solutions of NaOH and H_3PO_4, between the measurements, followed by washing the installation with distilled water. Such a procedure, along with a more accurate drain of water from the NF installation, allowed to practically eliminating the problems with biofouling during a further study. The good results of chemical disinfection indicate that, in a closed MF/NF industrial system, the problems associated with the membrane biofouling can be overcome.

An autopsy of the membrane taken from the module after completing the NF studies also indicated for a slight fouling. It was found that the membrane altered colour from white to brown in certain places. The SEM observation confirmed that the membranes surface was covered by deposit. The SEM images of membrane samples after NF process of solution fermented with *L. mesenteroides* (Fig. 8) were similar to those observed for samples after the filtration of solutions fermented with *C. freundii*.

Larger differences in the membrane fouling were observed between the solutions with *L. mesenteroides* and *C. freundii* bacteria during ATR-FT-IR studies. The IR spectra of the top layer of virgin and fouled NF270 membranes were compared in Fig. 9. Several peaks observed in the IR spectra were characteristic for membrane material examined, especially in the wave number ranging from 500 to 1,800 cm^{-1}. The intensity of peaks was decreased due to the presence of a fouling layer. The differences observed for the absorptions at 3,400, 1,668, 1,550 and 1,100 cm^{-1} can be attributed to the foulants accumulated on the membrane surface. The broad bands in the regions of 1,500–1,800 and 3,000–3,700 cm^{-1} are characteristic for proteins [32,33]. The obtained results indicated that significant differences were observed among the membrane samples fouled by the solutions fermented with *L. mesenteroides*.

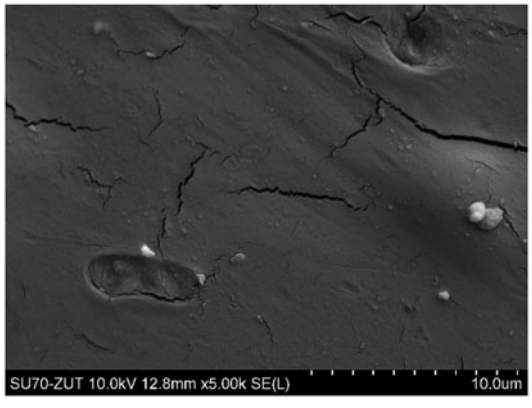

Fig. 8. SEM images of deposit formed on the surface of NF270 membrane after nanofiltration of broth subjected to pre-treatment by MF process. Broth with *L. mesenteroides* bacteria.

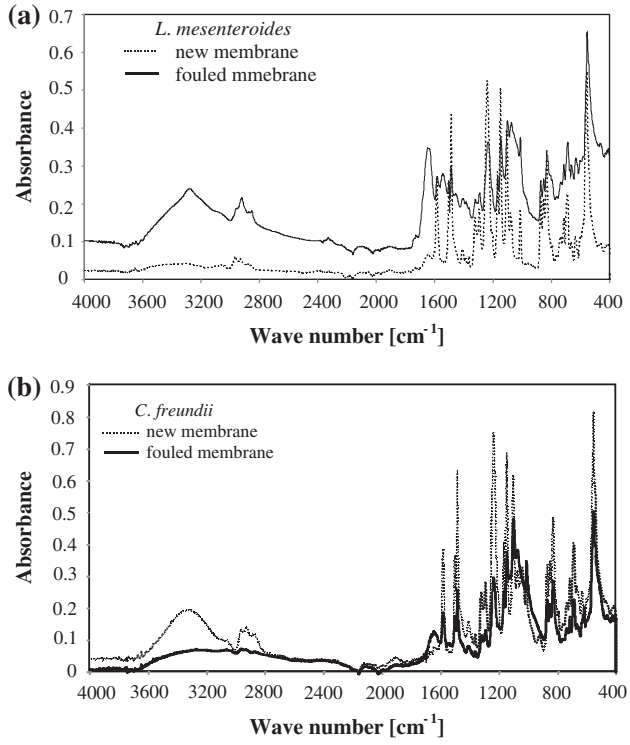

Fig. 9. The IR spectra of the top layer of virgin and fouled NF270 membrane. Glycerol solutions fermented by: (a) *L. mesenteroides*, (b) *C. freundii* bacteria.

3.5. Solution concentration by MD process

The NF permeate mainly contains the compounds (such as 1,3–PD, acetic acid and glycerol) which are rejected by NF270 membrane in a slight degree. For this reason, their concentration was close to that in the post-fermentation solutions. On the contrary, a two-fold to threefold concentration of metabolites, such as citric and lactic acid which were well rejected by NF membrane, was achieved in the obtained retentate. However, a degree of concentration was also restricted in this case by osmotic pressure enhancement. With regard to this, the MD was used for the removal of water from both retentate and permeates.

The courses of the concentration of permeate and retentate from NF by DCMD process was presented in Figs. 10 and 11. A volume of the feed in the MD process was reduced by about fourfold, achieving a high degree of retention of low-volatile compounds,

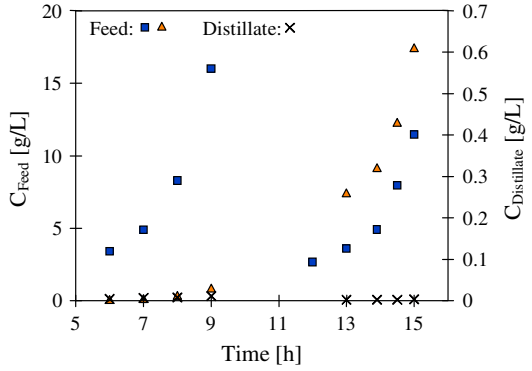

Fig. 10. The concentration of carboxylic acids present in the NF permeate (from 6 to 9 h) and NF retentate (from 12 to 15 h) by MD process. The glycerol solutions fermented by *L. mesenteroides*. (■) *mesenteroides*, (x) lactic acid and (◆) citric acid. $T_F = 353$ K and $T_D = 293$ K.

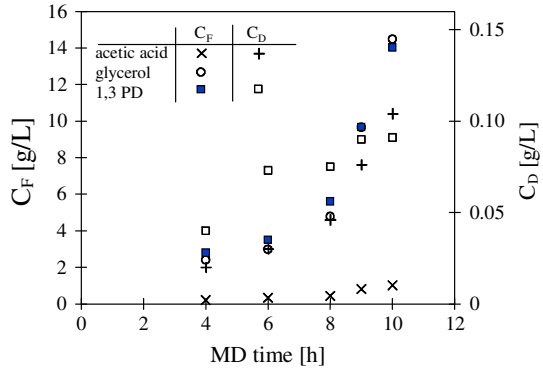

Fig. 12. The changes of the concentrations of acetic acid, 1,3- PD and glycerol in the feed and distillate during MD process of NF permeate obtained from the separation of glycerol solutions fermented by *C. freundii* bacteria. $T_F = 353$ K and $T_D = 293$ K.

such as lactic acid or citric acid (Fig. 10). The obtained distillate contained only small quantities of lactic acid, below 0.003 g/L. In the case of MD process with a solution of compounds having higher volatility, such as acetic and formic acids, they passed through the membrane to the distillate in slightly larger amounts (Fig. 11). In the case under study, the retention of acetic acid amounted to 80%, whereas for formic acid it was about 70%. It can be generally concluded that the majority of metabolites will be retained in the MD retentate, what allows to recycle the obtained distillate into the bioreactor as process water.

The MD process was also used for the concentration of NF permeate obtained from glycerol solutions fermented with *C. freundii* bacteria. In this case, the feed was concentrated by about fivefold (Fig. 12). In spite of a significant increase in the solutes concentration in the feed, only the trace quantities of concentrated compounds were detected in the obtained distillate. For 1,3-PD concentrated to 14.02 g/L, its concentration in the distillate amounted to 0.09 g/L. The distillate containing such small amounts of metabolites can be recycled to the bioreactor as process water.

An increase of feed concentration, similarly as in other membrane processes, also causes a decline of the permeate flux in the MD process. In the case under study, a fourfold increase of the feed concentration caused that the permeate flux decreased from 450 to 330–337 L/m^{2}day in the case when the permeate from the NF process, as well as the NF retentate, was used as a MD feed (Fig. 13). However, in the latter case, an exchange of concentrated NF retentate into distilled water did not cause a recovery of the initial efficiency of MD process. The observed increase in the

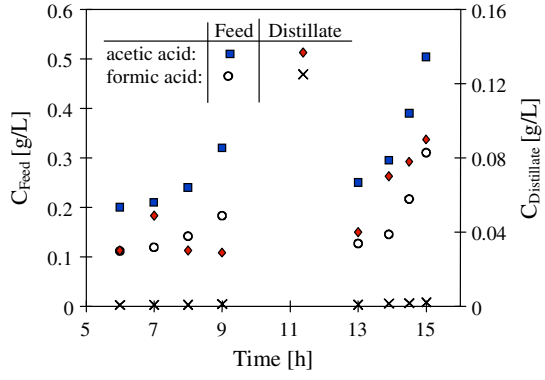

Fig. 11. The changes of the concentrations of acetic and formic acids in the feed and distillate during MD process of NF permeate (6–9 h) and NF retentate (13–15 h). The glycerol solutions fermented by *L. mesenteroides*. $T_F = 353$ K and $T_D = 293$ K.

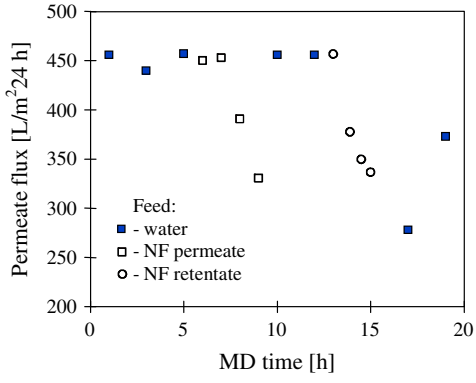

Fig. 13. The changes of MD permeate flux during the concentration of NF permeate and NF retentate obtained during the separation of glycerol solutions fermented by *L. mesenteroides* bacteria.

permeate flux (period from 17 to 20 h) resulted from a fact that the distilled water partly dissolved the foulants from the membrane surface during the MD process. After two-time exchange of distilled water, the process efficiency was obtained at a level of 373 L/m^{2}day, which was lower than the initial 450 L/m^{2}day. This indicates that the MD membranes lose a part of its initial permeability, due to fouling as well as the wettability of a fraction of pores in the membranes. Since the hydrophobic membranes are used in the MD process, the adsorption of organic compounds onto their surface may be facilitated. The concentrated retentate contained the protein substances added to the broth, as was previously demonstrated, which cause a significant membrane fouling in the MD process [34]. Moreover, the electrical conductivity of distillate was increased from initial 5 μS/cm up to 10–13 μS/cm, which indicated that the solutes permeate through a part of pores due to their wettability.

3.6. Separation possibilities

The presented studies confirmed that the post-fermentation solutions cannot be efficiently separated using only one process. With regard to their complex composition, a multi-stage system should be applied for their separation. The number of used processes and their mutual connection depends on the type of product and the conditions of fermentation running. The obtained results demonstrated that the following membrane processes: MF, NF and MD can be utilized to perform a preliminary stage of the separation. However, their application is dependent not only on the expected results of broth separation but also on the method of management of processing effluents.

The biomass retained in the MF process is usually recycled to the bioreactor, which allows to enhance its productivity through the increase of cells concentration in the broth. In the case of polyols production, the prepared 1,3-propanediol permeates to the NF permeate, which is subjected to the separation in the successive processes, such as rectification. Unfermented glycerol partially passed to the permeate, but having the highest boiling point (563 K), it will be concentrated in the evaporator, and after the distillation stage, it can be recycled to the bioreactor. The osmotic pressure limits a degree of the concentration in the NF process, thus, the MD process can be used for the dehydration of NF permeate.

The obtained NF retentate contained remaining glycerol and nutrients; therefore, it can be recycled to the bioreactor. However, the NF270 membrane also rejected several by-products, such as carboxylic acids. With regard to this, a level of inhibitory concentrations for these compounds (which is different and characteristic for each kind of bacteria used) restricts the recirculation of NF retentate to the bioreactor.

The distillation methods also have their limitations because of the formation of azeotropes. One possible method to solve this problem is the application of vacuum distillation. Due to significant differences in the boiling points, a variable pressure distillation can be also used for the separation of NF permeate containing 1,3-PD (boiling point 484–490 K). A preliminary removal of water from treated solutions in the quantities as large as possible facilitates their distillation. A composition of MD distillate was closed to clean water; therefore, it can be reused as process water.

4. Conclusions

The molecular weight of carboxylic acids and the pH of feed (acids dissociation) strongly affect the separation degree in the NF process. Therefore, a different degree of acids rejection, citric acid –97%, and lactic acid –35%, was obtained. Acetic acid separated below these pKa values (3.8) enriched the permeate and a negative retention over 100% was obtained. For higher pH values, equal to 4.5, the rejection of acetic acid at a level of 20% was observed.

The retention of 1,3-PD was increased with increasing transmembrane pressure, and amounted to about 40% for 1.0 MPa.

The used NF270 membrane rejected the majority of salts present in the broth, therefore, they can be partially recycled to the bioreactor (to be used as nutrients) together with obtained retentate. However, this solution is restricted by the inhibition effect of bioproducts, which are also present in the retentate.

The NF permeates and retentate obtained from NF process were concentrated about four to five times in the MD process. The retention of 1,3-PD was over 97%, and was close to 100% for other solutes. As results, the obtained distillate contained a trace quantities of metabolites, which creates the possibility of water reuse.

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