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Optimization of lactic bio-acid separation from actual post-fermentation broth using nanofiltration process and fouling analysis

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ABSTRACT

In this study, lactic bio-acid was separated from the actual post-fermentation (initially pre-treated) broth using pilot-scale nanofiltration (NF) setup. The experiments were carried out using two nanofiltration modules equipped with ceramic tubular membranes made of TiO_2 with cut-off equal to 200 or 450 Da. In addition, the effect of transmembrane pressure (1.20 and 1.60 MPa) on the purification process was also under evaluation. The collected data were used to analyze the fouling that occurred in the NF process of multicomponent pre-treated actual post-fermentation broth. Comprehensive analysis of membrane blocking was made by the resistance-in-series method and flux decline study. The desirability function methodology was used to optimize the NF process. The optimal conditions of separation of assumed targets and constraints were calculated on the basis of the determined empirical model. It was shown that the best results can be obtained using the NF system with 360 Da membrane and at TMP = 1.60 MPa. However, such an NF process is impossible to conduct due to the unavailability of the module with 360 Da membrane and thus, we proposed the alternative optimal condition, that is, a 450 Da membrane and TMP = 1.48 MPa. Nevertheless, it was concluded that the use of both membranes (200 and 450 Da) has some advantages, which inspired us to further study. We investigated the performance of the two-stage NF system and compared the results with those obtained in the one-stage approach. It turned out that the proposed concept could be beneficial considering final retention ratios and the overall duration of separation.

Keywords: Lactic bio-acid; Post-fermentation broth; Nanofiltration; Optimization

1. Introduction

Striving for continuous technological development, while simultaneously taking care of the environment, forces the search for alternative sources of valuable substrates. Lactic acid, a precursor of biocompatible and biodegradable polymer – polylactide [1,2], belongs to the group of compounds of high technological significance. The importance of lactic acid stems from its wide application in the textile, food, and pharmaceutical industries [2,3]. Lactic acid can be produced by chemical synthesis [4] or using microorganisms in the process of waste biomass fermentation [2,5–7]. Besides its eco-friendly nature, lactic acid biosynthesis has many advantages, for example, offers a high yield of the target product. Moreover, the fermentation-based lactic acid may be produced as the preferred L(+) optical stereoisomer, the one that can be metabolized by the human body [3], which is a great advantage in favor of bioconversion.

However, due to the complex composition, the actual post-fermentation broth needs the application of a

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multi-stage purification process to provide effective separation of the desired product [8–10]. For this purpose, it is possible to employ, both pressure-driven and electrically-driven membrane techniques [11–16], which are characterized by low energy demand and belong to the group of low-carbon processes. Laube et al. [12] have separated lactic acid from the post-fermentation broth using a spiral-wound membrane module for the cross-flow nanofiltration. Dey and Pal [17] have investigated the use of a two-stage membrane purification (microfiltration combined with nanofiltration) of lactic acid from the actual post-fermentation broth in the membrane-integrated fermenter. A similar study has been conducted by Sikder et al. [18] who additionally proposed a techno-economic analysis of such a separation process.

However, purification using selective membranes has also drawbacks. The major disadvantage, especially in the case of pressure-driven membrane processes used for purification of multi-component mixtures, is a drop in separation efficiency during the process due to the deposition of particles on the membrane surface [9,19,20]. Such a phenomenon reduces the membrane permeability and may eventually cause a complete blockage of the flow. The unfavorable effect of membrane fouling can be reduced by conducting the membrane process under appropriate conditions determined by optimization methods. Considering numerous possible approaches, it is worthwhile to use the design of experiment methodology [21,22], which allows conducting a statistical analysis of the influence of selected process parameters on experimental results and permits building a mathematical model of the process. Aydoğan et al. [23] have reported using response surface methodology to optimize the extraction of lactic acid from aqueous solution. Also, Kumar et al. [24] have used statistical optimization in order to find the best performance conditions of lactic acid extraction using the green emulsion ionic liquid membrane. So far, the nanofiltration (NF) of lactic acid from actual post-fermentation broth has not been optimized in order to increase the effectiveness of the separation process and enhance the content of the lactic acid in the permeate flux. Our paper is the first to describe the employment of a combination of factorial design and desirability function methodology to intensify membrane separation of organic acid from the post-fermentation broth.

The aim of this study is the optimization of lactic acid separation from the pre-treated actual post-fermentation broth by NF and analysis of membrane fouling in the process of separation. Experiments were planned to employ full factorial design, with the transmembrane pressure and cut-off of ceramic NF membrane as the input variables, whereas the lactic acid (LA) retention, acetic acid (AA) retention, L-pyroglutamic acid (L-PGA) retention, calcium ions retention, magnesium ions retention, and permeate flux were the output variables. Nanofiltration was optimized using the desirability function method based on the multi-dimensional linear regression model fitted to the experimental data. Such an approach allows optimization of many dependent variables combined into one function. Moreover, the flux decline and membrane fouling were evaluated in order to determine the mechanisms leading to a drop of the separation efficiency and selectivity. Additionally, the performance of a two-stage NF system was investigated and compared with that of a one-stage approach.

2. Materials and methods

2.1. Materials

The actual post-fermentation broth after bioconversion to lactic acid (other main components are shown in Table 1) was delivered by the Poznan University of Life Sciences (Poland). For the purpose of removal of unreacted substrates, biomass, and by-products of the fermentation process, the broth was subjected to pre-treatment using centrifugation, microfiltration (cut-off 50 kDa), and ultrafiltration (cut-off 15 kDa). The average pH of the solution was equal to 4.8 ± 0.1.

2.2. Nanofiltration setup

The separation processes were carried out using a pilot-scale NF setup (Intermarsz, Poland) equipped with two one-channel tubular ceramic membranes (Inopor, Germany) with cut-off equal to 200 and 450 Da (Fig. 1). Each membrane was made of TiO_2 and had an effective surface area equal to 0.0125 m^2. Detailed characterization of membranes is available in Table 2. The process of LA separation from the pre-treated post-fermentation broth was carried out using the NF unit operated at two different TMP (1.20 and 1.60 MPa) and T = 25°C ± 2°C. During each NF process, the retentate was circulated through the NF membrane module in a closed-loop, while the permeate was collected. The initial volume of the feed solution was equal to 6 L and was pumped at the volume flow rate of 700–750 L/h. Each of the separation processes was proceeded until obtaining the permeate volume equal to 3 L.

Table 1
Composition of pre-treated actual post-fermentation broth

Compound	Quantity (g/L)	pKa	Molecular weight (g/mol)
Lactic acid (LA)	113.70	3.86	90.08
Acetic acid (AA)	6.24	4.756	60.05
L-pyroglutamic acid (L-PGA)	4.39	–1.76	129.12
		3.48	
		12.78	
Ca^{2+}	0.0051	–	40.08
Mg^{2+}	0.0954	–	24.31

The NF processes were repeated twice, each time using a new portion of the feed solution. The samples of both the retentate and the permeate were taken for further analysis after collecting 100; 250; 500; 1,000; 1,500; 2,000; 2,500; and 3,000 mL of permeate.

2.3. Two-stage NF setup

The two-stage NF separation was conducted using the equipment and parameters described in section 2.2 (Nanofiltration setup). The first stage was NF_I of initially pre-treated post-fermentation broth on the module equipped with Inopor 450 Da membrane and at optimal TMP. The separation process NF_I was proceeded until obtaining the permeate volume equal to 6 L. The second stage was NF_{II} of the permeate obtained in the NF_I process on the module equipped with Inopor 200 Da membrane and at TMP = 1.60 MPa. The NF_{II} process was proceeded until obtaining the permeate volume equal to 3 L.

2.4. Analytical methods

The contents of LA, AA, and L-PGA in the permeate and feed solutions obtained in NF processes were determined using high-performance liquid chromatography HP Agilent 1100 Series (Germany), equipped with an autosampler, interface (HP 35,900), RI detector (HP 1047A), pump (HP1050), and a separating column rezex ROA-organic acid H + (8%), phenomenex1. The eluent of 2.5 mM H_2SO_4 solution was constantly supplied at the rate of 0.6 mL/min. The column temperature and that at the input to the detector was 50°C, P = 0.2 MPa.

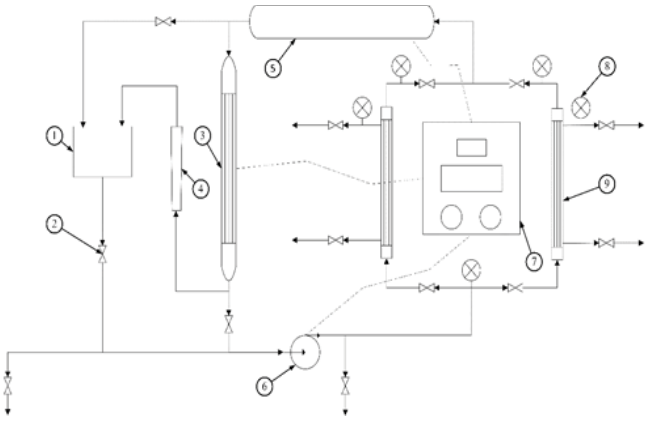

Fig. 1. Scheme of the pilot-scale NF setup: (1) feed vessel, (2) valve, (3) heat exchanger (cooling), (4) flow meter, (5) heat exchanger (heating), (6) pump, (7) control panel, (8) pressure gauge, and (9) membrane module.

The collected samples were diluted with deionized water and analyzed for Mg^{2+} and Ca^{2+} concentration with an atomic absorption spectrometer contrAA 300 (Analytik Jena AG, Germany) at 285.21 and 422.67 nm, respectively. Each measurement was replicated three times to determine the average concentration. The standard solutions were prepared by dissolution of the standard stock solutions of Mg^{2+} and Ca^{2+} (Sigma-Aldrich, Poland) in deionized water in the concentration range from 0.05 to 0.4 ppm and 0.25 to 2 ppm, respectively.

2.5. Calculation

The flux of permeate was calculated from a linear regression equation fitted to the experimental data of a volume change in time. The retentions of individual components of the pre-treated post-fermentation broth were calculated using the Eq. (1):

$$R = \left(1 - \frac{C_{p,i}}{C_{f,i}}\right) \times 100\% \tag{1}$$

where R is the retention ratio, %; $C_{p,i}$ is the concentration of a given component in permeate solution, g/L; $C_{f,i}$ is the concentration of a given component in the feed solution, g/L.

2.6. Fouling analysis

In this study, the fouling phenomena of the ceramic membranes were evaluated assuming the resistance-in series model based on the Darcy general equation [25]:

$$J = \frac{\Delta P}{\mu R_T} = \frac{\Delta P}{\mu\left(R_m + R_c + R_b\right)} \tag{2}$$

Table 2
Specification of nanofiltration membranes

Membrane parameters	200 Da membrane	450 Da membrane
Material	TiO_2	TiO_2
Type	Tubular	Tubular
Number of channels	1	1
External diameter, mm	10	10
Hydraulic diameter, mm	7	7
Active area, m^2	0.0125	0.0125
Mean pore size, nm	<0.9	0.9
Cut-off, Da	200	450
Membrane permeability for deionized water (determined experimentally), L/(m^2h MPa)	164.5	347.7

where J is the permeate flux, L/(m²h); μ is the dynamic viscosity, Pa·s; ΔP is the transmembrane pressure (TMP), Pa; R_T is the total filtration membrane resistance, m^{-1}; R_m is the internal membrane resistance, m^{-1}; R_c is the reversible resistance (cake layer resistance), m^{-1}; R_b is the irreversible resistance (pore blocking resistance), m^{-1}. Particular resistances were determined using the Eqs. (3)–(6):

$$R_m = \frac{\Delta P}{\mu \cdot J_0} \tag{3}$$

$$R_T = \frac{\Delta P}{\mu \cdot J_b} \tag{4}$$

$$R_b = \frac{\Delta P}{\mu \cdot J_1} - \frac{\Delta P}{\mu \cdot J_0} \tag{5}$$

$$R_c = R_T - R_m - R_b \tag{6}$$

where J_0 is the flux of permeating deionized water before the NF process; J_b is the flux of the permeate at the end of the NF process; J_1 is the flux of permeating deionized water after hydraulic washing. Besides, the relative flux (RF) was defined as:

$$\mathrm{RF} = \left(\frac{J_b}{J_0}\right) \cdot 100\% \tag{7}$$

while the flux recovery was calculated from the Eq. (8):

$$\mathrm{FR} = \left(\frac{J_1}{J_0}\right) \cdot 100\%. \tag{8}$$

Moreover, flux decline during the filtration process was determined from the Eq. (9) and was interpreted as a drop of flux due to irreversible fouling:

$$\mathrm{FD_{irr}} = 100 - \mathrm{RF} \tag{9}$$

On the other hand, Eq. (10) stands for the reversible flux decline caused by concentration polarization:

$$\mathrm{FD_{rev}} = \mathrm{FR} - \mathrm{RF} \tag{10}$$

2.7. Cleaning procedure

After each NF process, the unit was subjected to a cleaning procedure in order to recover the initial permeate flux value and permeability of the fouled membrane. The clean-up process consisted of the following steps:

- *Hydraulic washing*: (a) step $\mathrm{I}_{h,\mathrm{w}}$ is at the volume flow rate of 700–750 L/h using deionized water, t = 15 min, T = 30°C ± 2°C; (b) step $\mathrm{II}_{h,w}$ is at the volume flow rate of 700–750 L/h using deionized water, t = 15 min, T = 30°C ± 2°C;
- *Chemical cleaning*: (a) step $I_{\mathrm{ch},c}$ is using 2% sodium hydroxide solution, t = 30 min, T = 35°C ± 2°C; (b) step $\mathrm{II}_{\mathrm{ch},c}$ is using 2% nitric acid solution, t = 30 min, T = 35°C ± 2°C;
- Restoration of the initial conditions of the unit using deionized water (pH ≈ 6 and δ < 5 μS/cm).

2.8. Experimental design

Two-level full factorial design was employed to study the effects of two independent variables (factors), that is, transmembrane pressure (*A*) and the membrane cut-off (*B*). Each factor was considered at two levels which were determined according to the capability of the pilot-scale unit. Both coded and non-coded values of independent variables are presented in Table 3.

Six dependent variables were taken into account to ensure a comprehensive analysis of the NF process. The outputs were LA retention, AA retention, L-PGA retention, calcium ions retention, magnesium ions retention, and the final flux of permeate. The first-order linear regression model was fitted to each response, but it contained only statistically significant factors and interactions. Retention values presented in this paper are the averages of samples retention taken after collecting 100; 250; 500; 1,000; 1,500; 2,000; 2,500; and 3,000 mL of permeate.

2.9. Optimization setup

Simultaneous optimization of the obtained equations was performed using the desirability function method proposed by Derringer [26,27]. Each experimental response value was transformed to a desirability value d_i, where $0 \le d_i \le 1$. The value of d_i increases as the "desirability" of the corresponding response increases [26]. The higher the value of the permeate flux and the solutes retention ratio (except LA), the more efficient the process. The lower the value of LA retention, the higher the desirability. Moreover, each response was assigned to a weight which corresponded to its importance (Table 4). The value of weight w_i for each NF process response was determined in order to minimize the content of by-products of bioconversion and inorganic ions (Table 1) in the permeate, while maximizing the permeability of the membrane. Moreover, the weighted overall desirability D of the considered system of equations was calculated as follows [27]:

$$D = \left(d_1^{w_1} \cdot d_2^{w_2} \ldots d_n^{w_n}\right)^{\frac{1}{\sum w_i}} \tag{11}$$

It was necessary to impose constraints on both dependent and independent variables to avoid physically inconsistent

Table 3
Levels of independent process variables used in the experimental design

	Factor	
Name	TMP	Cut-off
Unit	MPa	Da
Coded name	*A*	*B*
Low level (–1)	1.20	200
High level (+1)	1.60	450

results of optimization, for example, a negative value of the flux. All variables were considered between limits set by the two-level two-factor full factorial design and maxima and minima of the responses. Moreover, the retention ratio of AA was excluded from optimization due to its negative value and high variance in each separation process.

3. Results and discussion

3.1. Influence of TMP and membrane cut-off on the efficiency of the NF process

The influence of TMP and cut-off of the NF membrane on the duration of NF process and its effectiveness were evaluated. Four processes of LA separation were conducted to perform a comparative analysis of the process. The results of the experiments are presented in Table 5 and the collected permeates are compared in Fig. 2. As expected, both low value of TMP (1.20 MPa) and small cut-off (200 Da) extended the duration of separation. Nevertheless, the time of the NF separation process using the module equipped with Inopor 450 Da membrane increased almost twice with the change of TMP, while using the module equipped with Inopor 200 Da membrane, the separation lasted about 24 min longer. Such a phenomenon was probably caused by the difference in the contribution of the concentration polarization to the overall blockage of transport through the membrane.

The retention of the main components of the broth was greater in the case of Inopor 200 Da membrane. According to Sanches et al. [28], the permeate flux is affected by the rise of the osmotic pressure difference between the retentate and permeate streams, which is mostly caused by the presence of charged solutes. Moreover, a fouling mechanism for salt-rejecting membrane in colloidal solutions, based on the osmotic pressure phenomenon, was proposed [29]. Besides, the coordination complexes of calcium and magnesium cations with natural organic matter (NOM) are capable of bonding with the membrane surface, which accelerates the concentration polarization [30–33].

For the membrane with the cut-off equal to 450 Da, the rise of TMP could lead to higher permeation of organic compounds than for the other membrane (200 Da). Besides the composition presented in Table 1, the broth could contain other organic matter such as non-ionic saccharides (i.e., lactose, sucrose, and raffinose [9,38]), which had remained in the broth after the bioconversion process. What is more, molecular weight of the above-mentioned compounds is greater than the molecular weight cut-off of one of the used membrane but lower than that of the other one. High-molecular-weight uncharged solutes take part in building up the cake layer, which leads to pore size reduction and contributes to a drop of permeate flux. The mentioned effect has also been observed by Goulas et al. [34] in the NF separation of sugars model solutions on the module equipped with a flat sheet asymmetric cellulose acetate membrane.

Table 4
Optimization details

Response	Target	Weight (1–10)
Lactic acid retention (%)	Minimize	3
L-pyroglutamic acid retention (%)	Maximize	1
Calcium ions retention (%)	Maximize	2
Magnesium ions retention (%)	Maximize	2
Average permeate flux (L/(m²·h))	Maximize	4

Fig. 2. Comparison of the initial post-fermentation broth (a) and permeates obtained in NF process: (b) 450 Da membrane and TMP = 1.60 MPa, (c) 450 Da membrane and TMP = 1.20 MPa, (d) 200 Da membrane and TMP = 1.60 MPa, and (e) 200 Da membrane and TMP = 1.20 MPa.

Table 5
Results of experiments

Output	NF setup (Da/MPa)			
	450/1.60	450/1.20	200/1.60	200/1.20
Duration of the separation (min)	64.25	128.00	373.78	397.22
Final flux (L/(m²·h))	226.42	113.88	39.54	36.62
LA retention (%)	7.01	5.47	11.60	11.11
AA retention (%)	–12.97	–25.91	–33.20	–23.89
L-PGA retention (%)	7.31	4.52	25.52	26.62
Mg^{2+} retention (%)	29.66	25.51	46.69	38.44
Ca^{2+} retention (%)	28.63	31.25	34.72	33.44

In the system with Inopor 450 Da membrane, there was a great rise of the permeate flux with increasing TMP, whereas for the system with Inopor 200 Da membrane, the flux difference was relatively small (Table 5). The concentration of molecules with molecular weight larger than the value of cut-off of the used membrane, rise rapidly near the membrane surface. Such a phenomenon leads to an increase in concentration polarization and the creation of the gel layer, which contributes to the flux drop [30]. Moreover, the process was conducted in the semi-closed loop (i.e., the retentate was returned to the vessel with the feed), which strengthened the above-mentioned effect, due to continuous increase in the compounds concentration in the feed solution. The proposed effect was well seen for the system with Inopor 450 Da, whereas in the case of separation on the other membrane, the gel layer did not cause a relevant difference in the flux drop and blockage of the membrane surface. Such an observation could indicate that there was a similar gel layer generated for both separation processes conducted on the NF module equipped with Inopor 200 Da membrane and the effect of TMP on this membrane was smaller than in the system with Inopor 450 Da membrane.

Another crucial experimental output to be considered as the influence of these parameters (i.e., membrane cut-off and TMP) on the retention of organic acids. The values of retention determined using Eq. (1) are presented in Table 5 and in Fig. 3.

The pKa value of LA is lower than the initial pH of pre-treated actual post-fermentation broth, which indicates the presence of LA in the ionized form in the feed solution (Fig. S1). In addition, the zeta potential of the used TiO_2 ceramic membranes is positive in acidic pH, due to protonation of hydroxy groups to $–OH_2^+$ [35,36]. This means that the surface of this type of membrane reveals a tendency to adsorb organic acid anions and retain them in the feed solution [36]. The increase in TMP led to increasing LA retention ratio. A similar effect has been noted by González et al. [37]. On the other hand, the effect of TMP was small in comparison with the effect of the increase in the membrane cut-off. Although the LA molecular weight was lower than the molecular weight cut-off of both used membranes, its retention took place. However, the bigger the sieve effect (i.e., the smaller the pores' diameters), the greater the retention of LA. Furthermore, the degree of LA dissociation was greater than 50% (Fig. S1) which could play a key role in its retention in the NF process [38]. A similar observation was made for L-PGA, but the retention of L-PGA was much higher in the NF system with Inopor 200 Da membrane in comparison to that with Inopor 450 Da membrane. The mechanism of the L-PGA removal was the same as that of LA, but the ratio of its retention was greater than that of LA. The observed effect could be explained by the higher molecular weight of L-PGA and its multivalence. In contrast to the organic acids mentioned above, AA was not excluded in any NF process considered. Moreover, the retention ratio of AA has a negative value, which implies a higher concentration of AA in the permeate than in the retentate. Kang and Chang [39] have pointed out the negative retention ratio of monovalent anions (e.g., acetic acid) in the NF process of the simulated fermentation broth containing succinate and proposed the pumping and Donnan effects as the explanation of such an observation. What is more, the small size of AA particles and ions, which indicates their high mobility, contributes to easy permeation through the membrane pores.

Due to the complex composition of the pre-treated actual post-fermentation broth, the NF separation is merely a stage needed to isolate and purify LA. Different membrane processes, such as a conventional electrodialysis or an electrodialysis with a bipolar membrane, are sensitive to the presence of Ca^{2+} and Mg^{2+} ions, which may form a scaling layer [40,41]. It was found that the retention ratio of divalent inorganic cations in the NF process was several times higher in comparison with the %*R* of the considered organic acids (Table 5 and Fig. 4). Significant differences in the retention of inorganic ions and organic acids were observed especially in the system with NF membrane of 450 Da cut-off. The retention was mainly caused by the electrical repulsion of $–OH_2^+$ groups present on the surface of the used NF ceramic membranes in acidic pH. In this situation, there was a possibility of organic anions sorption onto the membrane surface which may have changed the membrane characteristics [42,43] and weakened the electrical repulsion effects. Nevertheless, the sieve effect also played an important role due to the higher stokes radii of the considered inorganic cations than those of organic acids molecules [42]. The highest retention ratio of both divalent ions (Ca^{2+} and Mg^{2+}) was observed for NF separation on the module equipped with Inopor 200 Da membrane using TMP equal to 1.60 MPa. However, the results of the Ca^{2+} retention were burdened with uncertainty due to the high variance of experimental data. Such a problem was probably caused by the low concentration of this cation and the possible presence of various calcium forms (i.e., cation, coordination complex with the lactate [31], insoluble salt).

The value of two considered nanofiltration parameters (i.e., the membrane cut-off and TMP) have also influenced the color of the permeate obtained (Fig. 2). In the module equipped with Inopor 200 Da membrane, the collected permeates were noticeably discolored (which indicates the higher retention of colored compounds) in comparison with the color of the permeates in the system with the membrane with the cut-off equal to 450 Da. Even the used

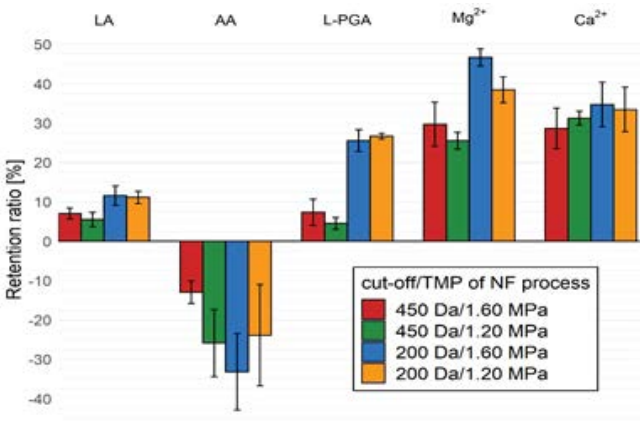

Fig. 3. Comparison of the retention ratio of main broth components in the NF process.

TMP caused a noticeable visual difference. The higher the TMP, the weaker the color of the permeate. The color compounds also were partly retained in the retentate solution, similarly as has been reported by Chidambaram et al. [44] as far as the NF process of the textile dye bath wastewater is concerned. This effect could be caused by compacted cake layer which played a role of an additional physical filter and enhanced the retention of color compounds as has been pointed out by Liu et al. [25].

3.2. Fouling analysis

The essential part of the nanofiltration process analysis is evaluation of the fouling process. The contribution of different types of fouling to flux decline is shown in Table 6 and was calculated according to Eqs. (7)–(10). As pointed out in section 3.1 (Influence of TMP and membrane cut-off on the efficiency of the NF process), there was a profound difference in the impact of TMP on the flux decline between the two investigated membranes. As for the system with Inopor 200 Da membrane, the relative values of permeate flux were relatively close for both applied values of TMP, while the change in the TMP value for 450 Da membrane generated a relevant difference. Only a closer insight into different types of fouling reveals that the larger flux decline for the membrane with a cut-off equal to 450 Da and TMP = 1.20 MPa was caused by the larger percentage of reversible fouling (i.e., concentration polarization, which enhances backward diffusion and gel layer formation). A similar observation has been made in our previous paper [38], when analysing the NF separation of the succinic acid from the post-fermentation broth. Such a phenomenon was not observed for the processes run using the Inopor 200 Da membrane, therefore the values of specific fouling types were similar regardless of the applied TMP value.

Despite the dissimilarities in the fouling process of both considered membranes, the proposed hydraulic cleaning procedure (section 2.7 (Cleaning procedure)) provided a comparable flux recovery for both membranes, regardless of the applied TMP. However, the FR values were higher for the separation process on Inopor 450 Da membrane than for that with 200 Da membrane, which was caused by a greater percentage contribution of the irreversible fouling (described in details in section 3.2 (Fouling analysis)). Importantly, the used chemical bath allowed recovering the initial membrane efficiency after each separation process. Other researchers have also suggested sufficient cleaning procedures to get rid of the post-NF fouling [10,45–47].

3.3. Resistance-in-series

Based on the Darcy law (Eq. (2)), which is a phenomenological description of the flow through a porous medium, the presence of different types of resistance was studied. The total resistance R_T against the permeate flux was divided into its specific types R_m, R_b, R_c, and treated as their sum. The values determined using Eqs. (3)–(6) are presented in Fig. 4.

The calculated R_T value was different for each conducted NF process. As for the separation processes on Inopor 450 Da membrane, the R_T increased with decreasing TMP. Such an effect was caused by the slower fluid cross-flow during the separation process, which was unable to reduce the formation of the cake layer (i.e., its thickness increased). Thus, the reversible resistance R_c increased as well as the overall resistance, whereas the intrinsic membrane resistance R_m and irreversible resistance R_b associated with pore blockage were almost constant, regardless of the applied TMP.

The opposite and much more complex phenomenon was observed in the system with the membrane of 200 Da cut-off. The highest value of R_T was observed for the NF process with TMP = 1.60 MPa. Moreover, both R_b and R_c brought almost the same contributions to the overall resistance. Nevertheless, the occurrence of the reversible fouling could be accelerated by the pore blockage. As mentioned in section 3.2 (Fouling analysis), the pre-treated actual post-fermentation broth possibly contained saccharides which can depose on the membrane surface. For example, the raffinose is able to permeate through the Inopor 450 Da membrane, but is retained by Inopor 200 Da membrane (due to the sieve effect regardless of TMP). Perhaps the adsorption of particles in pores (i.e., irreversible fouling) and deposition on the membrane surface strengthens the cake layer build-up, which resulted in the increase in its thickness and viscosity. As Liu et al. [25] have claimed, high TMP compacts the cake layer, which acts as an additional physical filter and improves the retention of small organic compounds.

The dominant role of R_c has been observed by Park et al. [48] for the NF process of model solutions containing high-molecular-weight organic foulants (i.e., humic acid, sodium alginate, and bovine serum albumin) and salts. On the basis of literature [29], Park et al. [48] have suggested that the colloidal deposit layers hinder the back diffusion of salts ions, which induces the osmotic pressure. Moreover, as Thuy and Boontawan [19] have highlighted, the flux decline during the NF process of pre-treated actual post-fermentation broth containing succinic acid is mostly caused by the concentration polarization and the cake layer formation.

Table 6
Analysis of flux decline during NF process

Output	NF setup (Da/MPa)			
	450/1.60	450/1.20	200/1.60	200/1.20
Relative flux (RF, (%))	40.70	27.29	15.02	18.55
Flux recovery (FR, (%))	64.48	63.12	47.96	50.67
Total flux decline (FD, (%))	59.30	72.71	84.98	81.45
Irreversible flux decline (FD_{irr}, (%))	35.52	36.88	52.04	49.33
Reversible flux decline (FD_{rev}, (%))	23.78	35.83	32.94	32.12

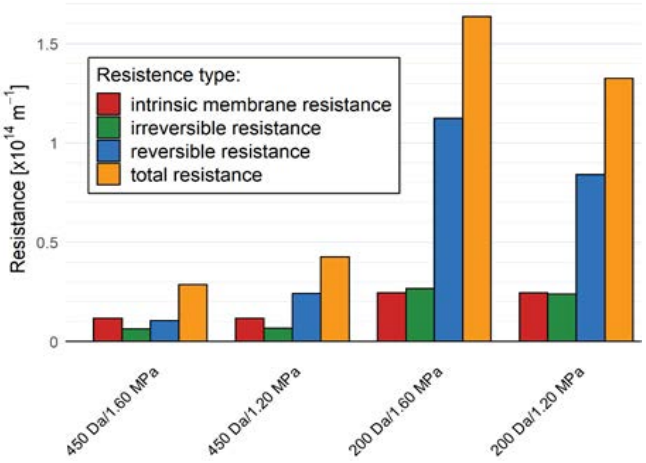

Fig. 4. Membrane fouling during NF of the LA from pre-treated actual post-fermentation broth at different NF setups.

3.4. NF optimization

The experimental data were analyzed using ANOVA in order to determine statistically significant (α = 0.05) factors in the phenomenological model of LA separation from the pre-treated actual post-fermentation broth using a tubular ceramic NF membrane. As mentioned in section 2.8 (Experimental design), the input parameters considered were TMP and cut-off of the membrane, but also the interaction of these factors was taken into consideration. The linear regression equations in the non-coded form are presented as Eqs. (12)–(16):

$$\text{Flux} = 229.1 - 1.006 \cdot A - 211.94 \cdot B + 1.096 \cdot AB \quad (12)$$

$$\text{RLA} = 16.66 - 0.0351 \cdot A - 0.865 \cdot B + 0.01045 \cdot AB \quad (13)$$

$$\text{RPGA} = 56.86 - 0.13486 \cdot A - 10.475 \cdot B + 0.03875 \cdot AB \quad (14)$$

$$\text{RMg} = 14.13 - 0.00234 \cdot A + 28.88 \cdot B - 0.04115 \cdot AB \quad (15)$$

$$\text{RCa} = 21.98 + 0.0381 \cdot A + 11.01 \cdot B - 0.03905 \cdot AB \quad (16)$$

The coefficients of determination R^2 of all equations (Eqs. (12)–(16)) are higher than 0.99, which indicates a satisfactory description of the experiment by the proposed system of equations.

The optimization was conducted with respect to weights and targets shown in Table 4. The results of the three possible approaches are presented in Table 7 and Fig. 5. The global optimal conditions of the separation were found for TMP = 1.60 MPa and the cut-off of the membrane equal to 360 Da. However, a tubular ceramic membrane with such parameters is unavailable on the market. For this reason, we performed two additional optimization processes to determine which of two used membranes was more suitable for LA separation form the pre-treated actual post-fermentation broth. It turned out that the highest composed desirability was obtained for TMP = 1.48 MPa and the module equipped with Inopor 450 Da membrane. A much higher value of permeate flux and the lower retention ratio of LA were the arguments in favor of such a membrane. The surface plot (Fig. 5) also provides useful information. The higher the value of TMP, the greater the desirability. However, the purification of LA using the NF process performed at extreme values of the cut-off, is less efficient. Both used membranes show advantages and disadvantages. One the one hand, the separation using the Inopor 450 Da membrane was characterized by a high value of a permeate flux, but on the other hand, also by low retention ratio of L-PGA and color compounds. The situation was quite the opposite in the case of the other membrane. These observations correlate fairly well with the results of the fouling process investigation.

The predicted optimal conditions were validated by the additional NF process. Admittedly, the experimental results and the calculated values were not the same, however, the differences were rather inconsiderable (2.1% for permeate flux, 12.5% for LA retention, 7.1% for L-PGA retention, 7.7% for Ca^{2+} retention, and 6.8% for Mg^{2+} retention). Such differences are caused by the specificity of the proposed empirical model, that is, the linearity. Nevertheless, it should be recognized, that the obtained models are good enough to simulate the NF process of LA separation.

3.5. Two-stage NF

As mentioned earlier, both used membranes (200 Da and 450 Da membranes) have advantages and disadvantages. The Inopor 450 Da membrane provided quick separation but the permeate discoloration and L-PGA retention ratio were not satisfactory, while the other membrane showed the opposite behavior. On the basis of the experimental results we decided to check the performance of the two-stage NF system. We assumed that such an approach could enhance the retention of undesired components of the broth and even reduce the duration of the separation. Comparison of the one-stage NF and the two-stage NF results is presented in Fig. 6. The overall duration of the separation using the two-step NF was much shorter (about 70 min) than in the one-step NF separation with the Inopor 200 Da membrane and at TMP = 1.60 MPa. In addition, the final retention ratios of undesired compounds, that is, L-PGA, Ca^{2+}, and Mg^{2+} were greater by 6.43%, 8.91%, and 8.23% respectively. Unfortunately, also the retention ratio of LA raised by 11.79% which is the main disadvantage of such a concept.

4. Conclusion

LA was purified from the initially pre-treated actual post-fermentation broth using a pilot-scale NF setup equipped with the ceramic tubular membrane with a cut-off of 200 or 450 Da. The experiments were conducted at two different values of TMP – 1.20 and 1.60 MPa. The collected data were used to characterize the separation of the broth components as well as the membrane fouling process. Moreover, the NF process was subjected to optimization using the desirability function methodology.

The study of NF process allowed determination of the influence of TMP and the membrane cut-off on the separation efficiency. Each evaluated tubular ceramic membrane

Table 7
Results of optimization: (*A*) overall optimal conditions, (*B*) optimal conditions in the case of Inopor 450 Da membrane, and (*C*) optimal conditions in the case of Inopor 200 Da membrane

		Flux [L/(m²·h)]	LA (%)	L-PGA (%)	Mg^{2+} (%)	Ca^{2+} (%)	Overall desirability	Variable form	Factor	
									Cut-off (Da)	TMP (MPa)
(*A*)	Response	158.93	8.66	13.89	35.81	30.83	0.501	Coded	0.278	1.000
	Desirability	0.170	0.110	0.425	0.236	0.134		Non-coded	360	1.60
(*B*)	Response	193.84	6.56	6.50	28.459	29.39	0.421	Coded	1.000	0.421
	Desirability	0.460	0.518	0.092	0.020	0.019		Non-coded	450	1.48
(*C*)	Response	39.00	11.50	25.72	45.23	34.49	0.106	Coded	–1.000	0.647
	Desirability	4.060e-08	6.890e-05	0.959	0.860	0.905		Non-coded	200	1.53

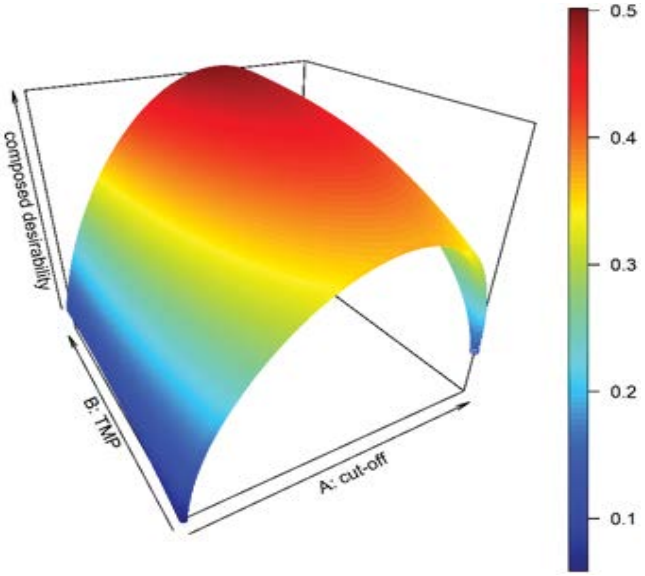

Fig. 5. Surface plot of composed desirability of the NF process.

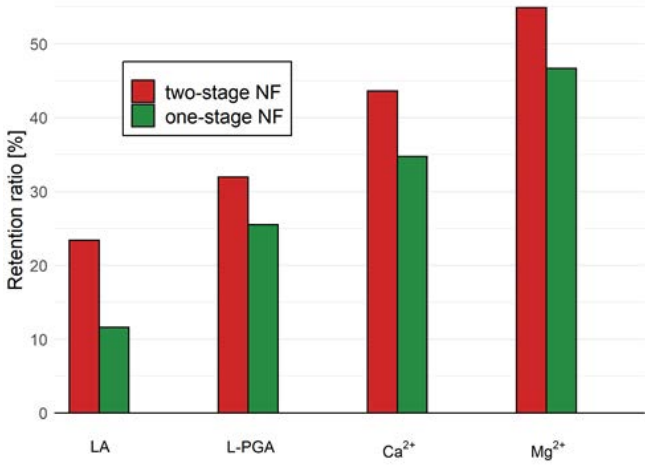

Fig. 6. Comparison of the final retention ratio of the two-stage NF process and the one-stage NF process of initially pretreated post-fermentation broth for (a) LA retention, (b) L-PGA retention, (c) Ca^{2+} retention, and (d) Mg^{2+} retention.

had a different effect on experimental outputs due to the disparity in the size of membrane pores, which affected the membrane blockage phenomena during the separation process. On the one hand, Inopor 450 Da membrane showed a lower flux decline and lower retention of LA than Inopor 200 Da membrane. On the other hand, a higher retention ratio of undesirable broth components was obtained using the module equipped with Inopor 200 Da membrane.

The values of partial resistances determined on the basis of the resistance-in-series model correspond to those obtained from the flux decline analysis. The results of both approaches can be explained by similar effects as have been observed by other researchers in the actual post-fermentation broth separation using pressure-driven membrane techniques. Probably, the transport of particles through the membrane pores was mainly affected by the deposition of bioconversion by-products such as saccharides. What is more, the build-up of a cake layer was accelerated for membranes with smaller pore diameters.

Factorial design employed in combination with desirability function methodology allowed finding the best conditions of NF separation process with respect to the assumed weights and targets. The results of optimization confirmed the previous observation that each used membrane has advantages and disadvantages. The optimal NF process setup was found to be that with the membrane with cut-off equal to 360 Da and TMP = 1.60 MPa. However, it is impossible to carry out such a separation process due to a lack of commercial ceramic tubular membranes with 360 Da cut-off. Thus, we calculated alternative optimal conditions, (i.e., membrane with cut-off equal to 450 Da and TMP = 1.48 MPa, taking into account only commercially available membranes.

The proposed concept of the two-stage NF system combined the features of both used membranes. However, such an approach is not perfect although it has a lot of advantages. On the one hand, the retention ratio of LA is higher than in the case of the one-stage NF separation which limits the content of the main product in the permeate. But on the other hand, in the case of the two-stage NF separation, the retention ratios of undesired compounds are much higher, which allows enhancement of the purity of the solution.

As mentioned earlier, the conducted NF process is only a one step in a multi-stage separation of lactic bio-acid from the actual post-fermentation broth. The collected permeates require further purification in order to obtain LA which could be used as a substrate in the pharmaceutical or polymer industry. As the next steps, the process of discoloration of the solutions should be performed (e.g., using activated carbon) and then, LA should be selectively separated from other acidic compounds, for example, employing electrodialysis combined with reactive liquid–liquid extraction.

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Abbreviations

A	—	Factor *A*
AA	—	Acetic acid
AB	—	Interaction of factors *A* and *B*
ANOVA	—	Analysis of variance
B	—	Factor *B*
FD	—	Flux decline
FR	—	Flux recovery
LA	—	Lactic acid
L-PGA	—	L-pyroglutamic acid
NF	—	Nanofiltration
NOM	—	Natural organic matter
RCa	—	Calcium ion retention
RF	—	Relative flux
RLA	—	Lactic acid retention
RMg	—	Magnesium ion retention
RPGA	—	L-pyroglutamic acid retention

Symbols

C	—	Concentration, g/L
ΔP	—	Pressure difference, MPa
J	—	Permeate flux, L/(m^2 h)
μ	—	Dynamic viscosity, Pa·s
P	—	Pressure, MPa
R	—	Retention ratio, %
R	—	Resistance, m^{-1}
R^2	—	Coefficient of determination, –
T	—	Temperature, °C
t	—	Time, min
TMP	—	Transmembrane pressure, MPa
α	—	Statistical significance, –

Subscripts

0	—	Of deionized water before the separation process
1	—	Of deionized water after hydraulic washing
I	—	First stage of the two-stage NF process
II	—	Second stage of the two-stage NF process
b	—	Pore blocking/of broth
c	—	Cake layer
f	—	Of feed solution
i	—	Of "*i*" component
irr	—	Irreversible
m	—	Of membrane
p	—	Of permeate solution
rev	—	Reversible
T	—	Total

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Supplementary information

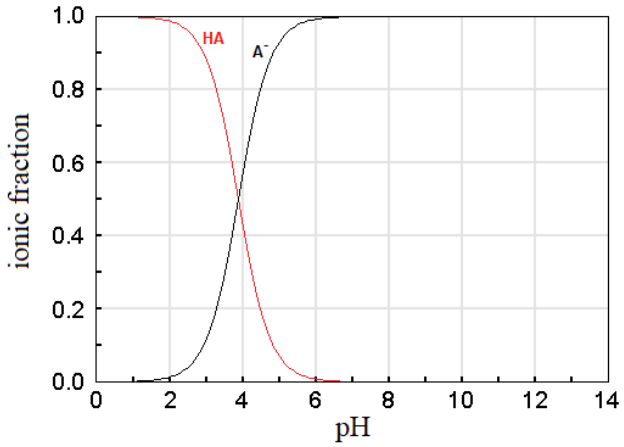

Fig. S1. Degree of dissociation as a function of pH of LA.