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Lactic Acid Recovery From Cheese Whey Fermentation Broth Using Combined Ultrafiltration and Nanofiltration Membranes

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Abstract

The separation of lactic acid from lactose in the ultrafiltration permeate of cheese whey broth was studied using a cross-flow nanofiltration membrane unit. Experiments to test lactic acid recovery were conducted at three levels of pressure (1.4, 2.1, and 2.8 MPa), two levels of initial lactic acid concentration (18.6 and 27 g/L), and two types of nanofiltration membranes (DS-5DK and DS-5HL). Higher pressure caused significantly higher permeate flux and higher lactose and lactic acid retention ($p < 0.0001$). Higher initial lactic acid concentrations also caused significantly higher permeate flux, but significantly lower lactose and lactic acid retention ($p < 0.0001$). The two tested membranes demonstrated significant differences on the permeate flux and lactose and lactic acid retention. Membrane DS-5DK was found to retain 100% of lactose at an initial lactic acid concentration of 18.6 g/L for all the tested pressures, and had a retention level of 99.5% of lactose at initial lactic acid concentration of 27 g/L when the pressure reached 2.8 MPa. For all the tests when lactose retention reached 99–100%, as much as 64% of the lactic acid could be recovered in the permeate.

Index Entries: Cheese whey; fermentation; lactic acid; membrane; lactose; nanofiltration.

Introduction

Manufacturing of cheese produces large volumes of whey as a byproduct. The United States generates nearly 1.2×10^9 t of cheese whey/yr *(1)*. It is estimated that as much as 40–50% of the whey produced is disposed of as sewage or as fertilizer applied to agricultural lands with the rest being used primarily as animal feed. Cheese whey contains about 4.5–5% lactose, 0.6–0.8% soluble proteins, 0.4–0.5% w/v lipids, and varying concentrations of mineral salts *(2)*. Therefore, there is an interest to utilize lactose from cheese whey in the production of value-added products. Lactic acid is one such value-added product that is produced from processing cheese whey.

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Lactic acid is a natural organic acid and has many applications in the pharmaceutical, food, and chemical industries. It is used as an acidulant and as a preservative, and also as a substrate in the production of biodegradable plastics and other organic acids (1,3).

Lactic acid can be produced by fermentation of sugar-containing substrates such as cheese whey using *Lactobacillus helveticus* (3,4), and *L. casei* (5,6). *L. helveticus* is a thermophilic and acidophilic bacterium that can grow under conditions inhibitory for most contaminant microorganisms (3). *Bifidobacterium longum* is a bacteria that can both convert lactose into lactic acid and also produce an anti-bacterial compound, which can boost the immune system in its host. *Bifidobacterium* spp. produces high yield of L-(+) lactic acid compared with D-(–) lactic acid (7).

Most previous studies examining lactic acid production have concentrated on increasing *B. longum* cell production by cell immobilization and optimized pH (8,9). Till date, there has been no report on using *B. longum* to produce lactic acid from cheese whey. By the *Bifidum* pathway, the fermentation of two moles of hexose results in 3 mol of acetate and 2 mol of lactate (7), but there has been no report on the metabolic pathway of *B. longum* to convert lactose to lactic acid.

The processes of lactic acid production include two key stages which are fermentation and product recovery. The biggest challenge in lactic acid production lies in the recovery and not in the fermentation step (10). A successful lactic acid recovery approach is that of continuous fermentation in a recycled reactor in which the cells, protein, and lactose are separated by a filtration unit and returned to the fermentor while the lactic acid is removed in the permeate.

Ultrafiltration can remove dissolved macromolecules with molecular weight cutoff (MWCO) between 1000 and 100,000 Da (11). An important hurdle in the application of membrane technology in whey processing is the decline in permeate flux during the operation. The permeate flux decline during ultrafiltration of cheese whey is attributed to concentration polarization and membrane fouling (12). Cells and proteins can be successfully separated from the cheese whey fermentation broth using ultrafiltration membrane with MWCO around 20,000 Da (13,14).

Nanofiltration is a pressure-driven membrane process with a MWCO situated between reverse osmosis and ultrafiltration. The nanofiltration membrane has already been used in the demineralization of salted, acid, and sweet cheese whey (15). The process could separate monovalent salts and organics in the molecular weight range 200–1000 Da (16). Nanofiltration membrane with MWCO around 400 Da was demonstrated to retain about 97% of lactose and 12–35% of lactate at pH 3.3 in a nanofiltration membrane reactor (17). Nanofiltration of cheese whey has been evaluated based on the permeate flux to improve the demineralization rate by Alkhatim et al. (18). In their research, they found that lower pH was observed to have higher permeability of sodium and potassium.

Jeantet et al. *(17)* also found out that decreasing pH resulted in decreased retention of lactic acid.

This study was the second step of a three-step membrane separation (ultrafiltration, nanofiltration, and reverse osmosis separation) process for lactic acid recovery. The protein and cells have been separated by ultrafiltration unit in the previous study *(14)*. The objectives of this study were twofold: (1) to evaluate membranes that could be used to retain lactose as concentrate while recover lactic acid in permeate; and (2) to study the effects of transmembrane pressure and initial lactic acid concentration of feed stream on the permeate flux, lactose retention, and lactic acid recovery using nanofiltration.

Materials and Methods

Cheese Whey Media

Cheese whey media was prepared by dissolving 50 g of deproteinized cheese whey powder (Davisco Foods International, Inc., Eden Prairie, MN) into a liter of deionized (DI) water and stirring for 5 min at ambient temperature. The composition of the deproteinized cheese whey powder was as follows: crude protein (total nitrogen × 6.38) 6.8%, crude fat 0.8%, lactose 78.6%, ash 9.4%, and moisture 4.4%. The solutions were autoclaved at 103°C for 10 min.

Microorganism and Culture Media

B. longum was obtained from the National Collection of Food Bacteria (NCFB 2259). Stock culture of this strain was maintained in 50% glycerol and Man Rogosa Sharpe (MRS) broth media at –80°C. Active cultures were propagated in 10 mL MRS broth at a temperature of 37°C for 18–24 h under anaerobic conditions. This was used as a preculture to initiate cell production of higher volume with a 1% inoculation into 100 mL fresh MRS broth, incubated at 37°C for 24 h.

Fermentation

Fermentation was conducted in a stirred 5-L bench top fermentor. The pH of the broth was maintained at 5.5 by neutralizing the acid with 5 *N* ammonium hydroxide during fermentation. The agitation speed of the fermentor was maintained at 150 rpm and the temperature was maintained at 37°C. Samples were withdrawn every 2 h during the first 8 h and every 12 h during the remaining fermentation process. Each fermentation test lasted for 48 h.

Ultrafiltration Separation

The fermentation broth was first filtrated through an ultrafiltration membrane unit. Two types of membranes (PES5 and PES20, Nadir Filtration

GmbH, Wiesbaden, Germany) with MWCO of 5000 and 20,000 Da were used in the ultrafiltration experiment. The membrane polymer consisted of hydrophilic polyethersulfone and polysulfone. The permeate of the ultrafiltration process was collected for this nanofiltration study. The average true protein concentration in the ultrafiltration permeate was less than 0.02% (*14*). As most of the lactose was converted after 48 h of fermentation, the lactose concentration of permeate was adjusted to 22 g/L by the following two methods. The first method involved adding 22 g of lactose in 1 L of permeate which produced a solution with lactose and lactic acid concentration of 22 and 27 g/L. The second method used the permeate obtained by ultrafiltration of the unfermented cheese whey to adjust the lactose concentration. The lactose and lactic acid concentrations obtained by the second method were about 22 and 18.6 g/L, respectively.

Nanofiltration

The nanofiltration system consisted of a recirculation pump, nanofiltration unit (SEPA CF II, Osmonics, Minneapolis, MN), and an online permeate weighting unit. The diagram of nanofiltration membrane system is shown in Fig. 1. The ultra filtered permeate was circulated from the fermentor to the membrane unit at a constant velocity via a positive pump (M03-S, Hydracell, Minneapolis, MN) which can run at pressure up to 8.4 MPa. Two Nano membranes (DS-5DK and DS-5HL, Osmonics, Minneapolis, MN) were used in the experiments. Both the membranes could retain 98% of $MgSO_4$ but had different levels of permeate flux. No MWCO information was provided by the manufacturer. The surface area of the membrane is 140 cm^2. The hold up volume of the membrane unit is 70 mL. The fermentor was used to maintain the ultra filtered permeate at constant temperature and pH. The concentrate was recycled to the fermentor via the pump while permeate was collected in a container placed on an electronic balance. The reading of the balance was continually recorded at 30-s intervals by a computer. The transmembrane pressure and cross-flow feed velocity were adjusted by a manual valve and pump controller. The pressure was measured by a standard pressure gauge. The cross-flow feed velocity was calculated based on the flow rate and section area of the membrane channel. The cross flow velocity used in the experiments was 0.5 m/s. The transmembrane pressure levels used were 1.4, 2.1, and 2.8 MPa. The pH of the ultra filtrated permeate was kept at 5.5 and the ultra filtrated permeate was agitated at 200 rpm in the fermentor. The nanofiltration process lasted for 1 h. Duplicate samples of initial feed stream, of permeate and of concentrate were collected at the end of nanofiltration for analysis to determine the lactose and lactic acid concentrations. An alkali–acid treatment method was applied to the membrane system in the following steps:

1. Fully open the recirculation and permeate valves.
2. Flush with tap water for 5 min.

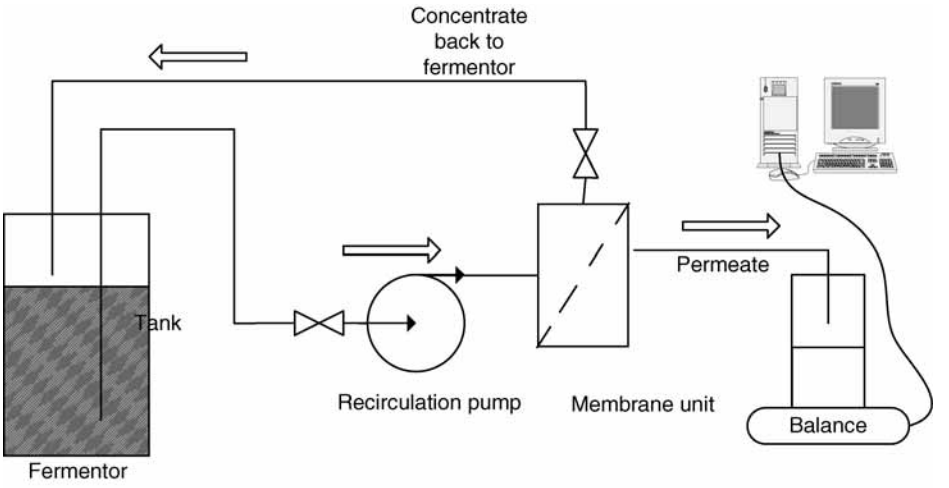

Fig. 1. Schematic diagram of the nanofiltration membrane separation system.

3. Circulate 2 L of 4% phosphoric acid for 10 min.
4. Rinse with tap water for 5 min.
5. Circulate 2 L of 0.1 *N* NaOH solution for 10 min.
6. Rinse with 10 L of DI water for 5 min.

Analyses

Lactose, lactic acid, and acetic acid were determined by high-performance liquid chromatography (Waters, Milford, MA) with a KC-811 ion exclusion column and a Waters 410 differential refractometer detector. The mobile phase was 0.1% H_3PO_4 solution at a flow-rate of 1 mL/min. The temperatures of the detector and of the column were maintained at 35°C and 60°C, respectively.

The lactic acid productivity was evaluated by, (a) lactic acid yield and (b) conversion ratio. The conversion ratio was expressed as follows:

$$\text{Conversion ratio (\%)} = \frac{\text{Initial lactose concentration - Residual lactose concentration}}{\text{Initial lactose concentration}} \times 100\%$$

The lactic acid yield was expressed as grams of lactic acid produced/g lactose used:

$$\text{Lactic acid yield (g/g)} = \frac{\text{Lactic acid produced}}{\text{Lactose used}}$$

The performance of membrane separation was evaluated by using three criteria: (a) permeate flux, (b) lactose retention, and (c) lactic acid recovery. The permeate flux was calculated by measuring the quantity

of permeate collected during a certain time and dividing it by the effective membrane area for filtration.

$$\text{Permeate flux} = \frac{\text{Permeate volume}}{\text{Membrane area} \times \text{Time}} (\text{L/m}^2\text{h})$$

The lactose retention (%) was defined as:

$$\text{Retention} = \left(1 - \frac{\text{Concentration of lactose in the permeate}}{\text{Concentration of lactose in the feed stream}}\right) \times 100$$

C_{L0} = concentration of component in the feed stream;
C_{LP} = concentration of component in the permeate.
The lactic acid recovery (%) was defined as:

$$\text{Recovery} = \frac{\text{Concentration of lactic acid in the permeate}}{\text{Concentration of lactic acid in the feed stream}} \times 100$$

Analysis of variance was performed using a statistical package from the SAS System (SAS Institute, Cary, NC).

Results and Discussion

Fermentation

The lactose, lactic acid, and acetic acid concentrations obtained during the 48 h of fermentation are shown in Table 1. The values in the table represent the averaged values of two runs. The results show that about 97.2% of the lactose was converted and that 0.73 g lactic acid was produced from 1 g of lactose used. The production of acetic acid was trivial in comparison to that of lactic acid. The production of acetic acid could be caused by the nonstrict anaerobic fermentation conditions and contamination of broth. The lactose conversion ratio and lactic acid yield are similar to results of other lactic acid producing bacteria such as *L. helveticus.* Tango and Ghaly *(3)* obtained a lactose conversion ratio of 92–95% and a lactic acid yield of 0.86 g lactic acid/g lactose when using immobilized *L. helveticus* with nutrient supplement at 36 h of fermentation. Shahbazi et al. *(1)* obtained a lactose conversion ratio of 79% and lactic acid yield of 0.84 g lactic acid/g lactose using immobilized *L. helveticus* in bioreactor. Most of the previous work targeted at obtaining high lactose conversion ratios and lactic acid yields have used immobilized cells and nutrient supplementation. In the current experiments, free cells of *B. longum* were grown with no nutrient supplementation, which would significantly reduce the cost of lactic acid production and be compatible with current fermentation facilities.

Nanofiltration

The testing results of permeate flux, lactose retention, and lactic acid recovery are shown in Table 2. The values in the table are the average

Table 1
The Lactose Conversion Ratio and Lactic Acid Yield of Cheese Whey Fermentation Using Immobilized *B. longerm*

Time (h)	Lactose concentration (g/L)	Lactic acid concentration (g/L)	Acetic acid concentration (g/L)	Conversion ratio (%)	Yield (g lactic acid/ g lactose)
0	36.8	2.2	0.2		
2	34.1	3.9	0.6	7.3	0.63
4	31.5	5.7	0.6	14.3	0.67
6	28.4	7.2	0.6	22.8	0.6
8	27.2	8.2	0.7	26.1	0.62
12	23.6	11	0.6	35.9	0.66
24	14.3	18.6	0.6	61.2	0.73
36	6.5	24.3	0.6	82.3	0.73
48	1	28.4	0.6	97.2	0.73

Table 2
Permeate Flux, Lactose Retention, and Lactic Acid Recovery During Nanofiltration

Membrane type	Initial lactic acid concentration (g/L)	Pressure (MPa)	Flux (L[/m^2]h[/r])	Lactose retention (%)	Lactic acid retention (%)	Lactic acid recovery (%)
DS-5DK	18.6	1.4	27	100	45.7	54.4
		2.1	51.3	100	56.2	43.9
		2.8	80.6	100	63.4	36.6
	27	1.4	35.2	94.7	23.2	76.9
		2.1	64.8	96.8	30.8	69.3
		2.8	91.9	99.5	36.5	63.5
DS-5HL	18.6	1.4	56.5	82.2	33.7	66.4
		2.1	87.1	87.3	45.5	54.5
		2.8	126.2	90.7	49.6	50.4
	27	2.1	102.2	73.4	24.8	75.2

of two runs. Figure 2 shows the decreasing of permeate flux with time during nanofiltration at different transmembrane pressures. The permeate flux kept nearly constant when the transmembrane pressure was 1.4 MPa. Increased transmembrane pressure caused more decreasing of permeate flux during nanofiltration process.

Permeate Flux

Figure 3 shows that permeate flux increased with the increase of transmembrane pressure. When the transmembrane pressure increased

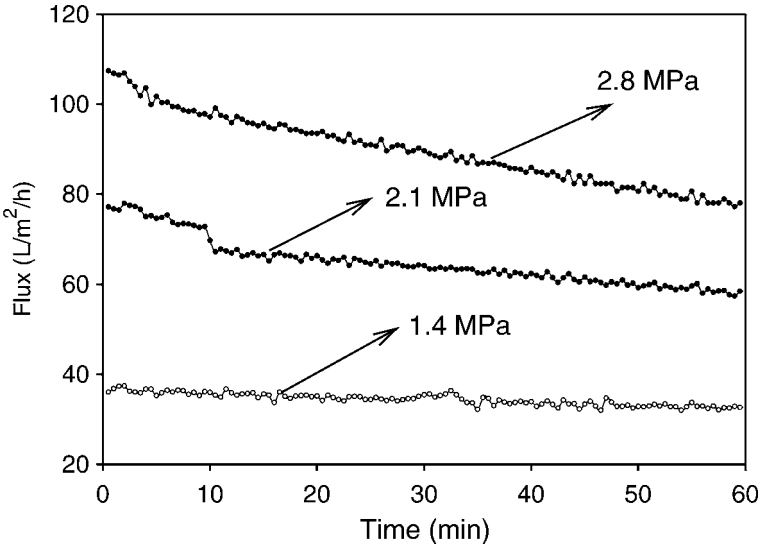

Fig. 2. Permeate flux profiles during nanofiltration (membrane: DS-5DK, initial lactic acid concentration: 27 g/L).

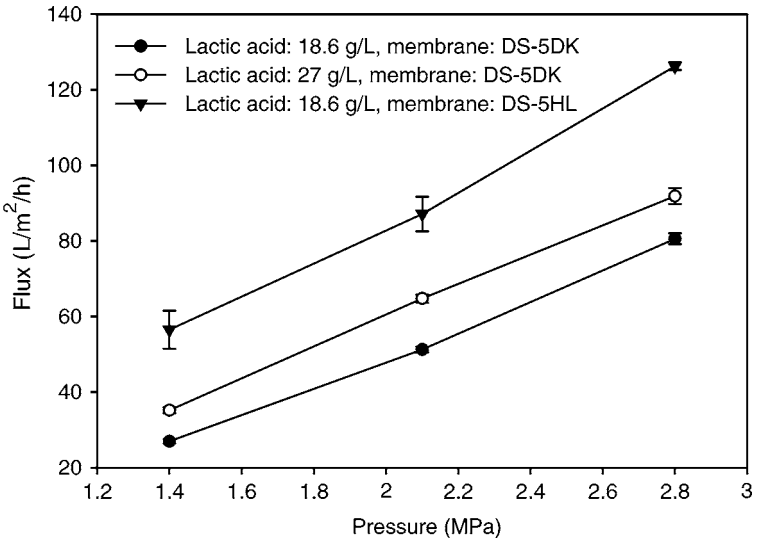

Fig. 3. Effects of pressure, membrane, and initial lactic acid concentration of feed stream on nanofiltration permeate flux.

from 1.4 to 2.8 MPa, the permeate flux increased 2–3 times for all of the tested membranes.

Figure 3 shows that higher permeate flux could be obtained at higher initial lactic acid concentration. When the initial lactic acid concentration increased from 18.6 to 27 g/L, the permeate flux increased about 30, 26, and 14% at pressure 1.4, 2.1, and 2.8 MPa, respectively, for membrane of DS-5DK

(Table 2). It can be concluded that accumulation of lactic acid during fermentation process could speed up the nanofiltration process.

Between the two tested membranes DS-5DK and DS-5HL, higher permeate flux was obtained with membrane of DS-5HL (Fig. 3). When the initial lactic acid concentration of 18.6 g/L, the permeate flux values obtained with membrane of DS-5HL were about 100, 70, and 57% higher than that of the membrane DS-5DL at pressures of 1.4, 2.1, and 2.8 MPa, respectively (Table 2). These results are in agreement with manufacturer provided permeate flux data (the permeate flux of membrane DS-5HL is 77% higher than that of the membrane DS-5DK at 140 KPa). Membranes with higher permeate flux have the potential to increase the capacity of the nanofiltration membrane unit.

The membrane, pressure, and initial lactic acid concentration showed significant ($p < 0.0001$) effects on the permeate flux. The interaction between these parameters were not significant ($p = 0.045$ and 0.11, respectively).

Retention of Lactose

Lactose retention increased with the increase of transmembrane pressure (Fig. 4). Figure 4 shows that lower lactose retention was obtained at higher initial lactic acid concentration. When the DS-5DK membrane was used, 100% retention of lactose was obtained at initial lactic acid concentration of 18.6 g/L for all tested transmembrane pressures. When the initial lactic acid concentration was increased to 27 g/L, lactose retention of 94.7, 96.8, and 99.5% were obtained at pressure levels of 1.4, 2.1, and 2.8 MPa, respectively (Table 2). This indicates, using this membrane, that at higher initial lactic acid concentrations, nearly 100% lactose retention can be obtained by increasing transmembrane pressure.

When the DS-5HL membrane was used to separate ultra filtered permeate with initial lactic acid concentration of 18.6 g/L, with the same pressure levels as for the DS-5DK membrane, lactose retention were 82.2, 87.3, and 90.7%, respectively. A lactose retention of 73.4% was obtained at initial lactic acid concentration of 27 g/L and transmembrane pressure of 2.1 MPa (Table 2). At most of the test conditions, the lactose retention of DS-5HL was lower than 91%, while the lactose retention for membrane of DS-5DK reached about 99–100%. These results indicate that, in comparison with the DS-5HL membrane, the DS-5DK membrane should be used for separating lactose from lactic acid in nanofiltration process. The transmembrane pressure, lactic acid concentration and membrane showed significant effects on the lactose retention ($p < 0.0001$, 0.0005, and 0.01, respectively).

Jeantet et al. *(17)* reported that lactose retention was kept constant at 97% ± 2% with transmembrane pressure for 400 Dalton Millipore nanofiltration membrane (spiral wound module). In our study, transmembrane pressure caused increase of lactose retention. At initial lactic acid concentration of 18.6 g/L, lactose retention was kept at 100% at all the studied transmembrane pressures.

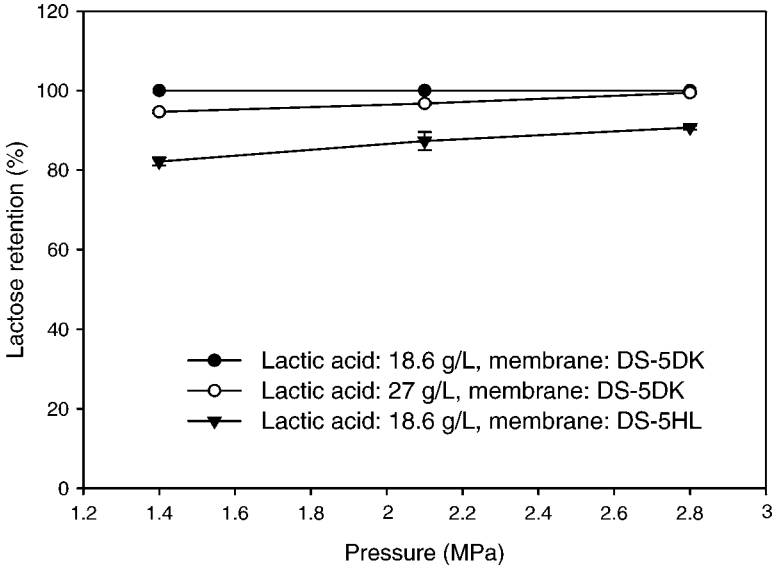

Fig. 4. Effects of pressure, membrane, and initial lactic acid concentration of feed stream on lactose retention during nanofiltration.

Recovery of Lactic Acid

It can be seen from Table 2 that increased retention of lactic acid corresponded positively with increased lactose retention. Lactic acid recovery in the permeate was used to evaluate the nanofiltration process in this study. Increases of transmembrane pressure were associated with lower levels of lactic acid recovery in the permeate (Fig. 5). Figure 5 shows higher lactic acid recovery was obtained at higher initial lactic acid concentration. At pressures of 1.4, 2.1, and 2.8 MPa, when the initial lactic acid concentration increased from 18.6 to 27 g/L, the lactic acid recovery increased from 54.4, 43.9, and 36.6% to 76.9, 69.3, and 63.5%, respectively (Table 2).

Between the two membranes tested, at the initial lactic acid concentration of 18.6 g/L, higher lactic acid recovery were obtained with membrane of DS-5HL. As the requirement of lactose retention around 99–100% needs to be met firstly, the higher lactic acid recovery of membrane DS-5HL could not change the previous conclusion on the membrane selection based on the lactose retention.

The membrane, pressure, and initial lactic acid concentration showed significant effects ($p < 0.0001$) on the lactic acid recovery. The interactions between these parameters were not significant ($p = 0.19$ and 0.18, respectively).

These results are in agreement with the work of Jeantet et al. *(17)*, who observed that lower pH caused lower lactate retention. In our study, when the pH was kept constant at 5.5, we found out that increasing the initial lactic acid concentration reduces lactic acid retention, i.e., enables higher lactic acid recovery.

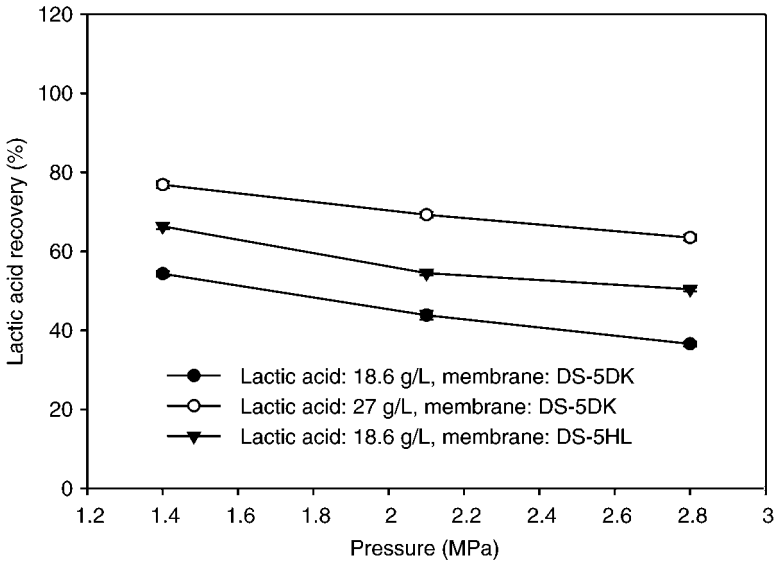

Fig. 5. Effects of pressure, membrane, and initial lactic acid concentration of feed stream on the recovery of lactic acid by nanofiltration.

Conclusions

The tested nanofiltration membrane system has successfully separated lactic acid from lactose in cheese whey fermentation broth. Nearly all the lactose (99–100%) was retained using a DS-5DK membrane at both of the tested initial lactic acid concentration of 18.6 and 27 g/L. Transmembrane pressure higher than 2.8 MPa needed to be applied when the initial lactic acid concentration reached 27 g/L to obtain nearly 100% of lactose retention. Among the tests when 99–100% of lactose was retained in the concentrate, the highest lactic acid recovery in the permeate reached 63.5%. Increasing transmembrane pressure caused higher permeate flux and higher lactose retention, but lower lactic acid recovery. Increased initial lactic acid concentration in the feed stream caused higher permeate flux and higher lactic acid recovery, but lower lactose retention. Further study needs to be conducted to determine the optimized pressure for each initial lactic acid concentration of feed stream to obtain near 100% lactose retention while keeping lactic acid recovery as high as possible.

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