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Separate and Concentrate Lactic Acid Using Combination of Nanofiltration and Reverse Osmosis Membranes

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Abstract The processes of lactic acid production include two key stages, which are (a) fermentation and (b) product recovery. In this study, free cell of *Bifidobacterium longum* was used to produce lactic acid from cheese whey. The produced lactic acid was then separated and purified from the fermentation broth using combination of nanofiltration and reverse osmosis membranes. Nanofiltration membrane with a molecular weight cutoff of 100–400 Da was used to separate lactic acid from lactose and cells in the cheese whey fermentation broth in the first step. The obtained permeate from the above nanofiltration is mainly composed of lactic acid and water, which was then concentrated with a reverse osmosis membrane in the second step. Among the tested nanofiltration membranes, HL membrane from GE Osmonics has the highest lactose retention (97±1%). In the reverse osmosis process, the ADF membrane could retain 100% of lactic acid to obtain permeate with water only. The effect of membrane and pressure on permeate flux and retention of lactose/lactic acid was also reported in this paper.

Keywords Cheese whey · Lactic acid · Membrane · Nanofiltration · Reverse osmosis

Introduction

Cheese whey is an important by-product from the cheese manufacturing industry. Typically, 100 g of milk yields 10 g of cheese and 90 g of liquid whey. 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 [1]. Cheese whey disposal has long been a problem for the dairy industry. Most medium and small cheese producers still dispose of their whey or whey

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permeate directly on farmland, which can pose an environmental risk. In response, regulations for land spreading of cheese whey are tightening and looking to ban land spreading of cheese whey. Ultrafiltration/diafiltration has been used to separate whey protein from lactose sugar and other components in the whey. Whey protein has found a good market as a food additive or protein supplement. The permeate stream after ultrafiltration/diafiltration is mainly composed of lactose, salts, and a lot of water, which can be dried to produce whey permeate powder (deproteinized whey). Drying of whey permeate need to remove large amount of water, which is an energy-intensive process. The lactose sugar fraction in cheese whey can be used to produce value-added products such as lactic acid, ethyl alcohol, and methane gas or to grow cells for an antibacterial compound, but this is not currently in full-scale production.

Lactic acid is a natural organic acid and has many applications in the pharmaceutical, food, and chemical industries. It is used as acidulant and preservative, and recently, its potential as substrate for the production of biodegradable plastic has been actively pursued. Approximately half of the world's supply of lactate is produced by fermentation process. Lactic acid has been produced by fermentation of sugar-containing substrates including cheese whey using *Lactobacillus helveticus* [2, 3] and *Lactobacillus casei* [4, 5] in most of the previous studies. *Bifidobacterium longum* was used to produce lactic acid in our previous research [6]. *B. longum* is a bacterium that can convert lactose into lactic acid and also produce an antibacterial compound, which can boost the immune system in its host [7].

The processes of lactic acid production include two key stages, which are (a) fermentation and (b) product recovery. The biggest challenge in lactic acid production lies in the recovery and not in the fermentation step [8]. A successful lactic acid recovery approach is that of continuous fermentation in a recycled reactor where 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.

Several different approaches and combinations have been applied for lactic acid recovery from fermentation broth. Neutralization with a base followed by filtration, concentration, and acidification is a traditional process for recovery of lactic acid; this process yields calcium salt as a by-product, causing high chemical cost and waste generation [9]. Other alternative lactic acid recovery processes such as solvent extraction, adsorption, direct distillation, nanofiltration, and electrodialysis have also been studied. The solvent extraction process is limited by unfavorable distribution coefficients and environmental problem because of the use of hazardous solvents. Adsorption process requires regeneration of ion exchange resin and also has the disadvantages such as short lifetime of adsorbents, low capacity, and additional filtration [10]. Direct distillation is an energy-intensive process, and it will causes product transformation, such as the polymerization of lactic acid [11]. Membrane technologies have been proving their advances in the fields of separation and purification. There has been a shift toward membrane separation processes because they are often more capital and energy efficient when compared with chemical separation processes [12]. Membrane processes have several advantages over many of the traditional separation techniques such as distillation, extraction, ion exchange, and adsorption. No energy-intensive phase changes or potentially expensive solvents or adsorbents are needed for membrane separations, and simultaneous separation and concentration of both inorganic and organic compounds is possible. Nanofiltration membrane with a molecular weight cutoff (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 [13]. In our previous research, ultrafiltration was used to separate cells and proteins from cheese whey fermentation broth. The obtained permeate was then passed through the nanofiltration unit to separate lactic acid from lactose [14].

Electrodialysis might also be an option, but it needs to remove large amounts of salt, which will increase the process cost [15].

The objectives of this study were: (1) to evaluate the performance of nanofiltration filtration membrane for separation of lactose from lactic acid in the fermentation broth and (2) to evaluate the performance of reverse osmosis for lactic acid concentration in the permeate of the nanofiltration. The diagram for the proposed process for lactic acid production and separation was shown in Fig. 1.

Materials and Methods

Fermentation

The whey powder used for the fermentation was obtained from Davisco Foods International (Eden Prairie, MN). The composition of the whey powder in percent w/w is as follows: 6.8% proteins, 78.6% lactose, 0.8% fat, and 4.4% moisture. The whey solution was made by mixing specific amount of whey powder to deionized (DI) water to obtain the designated

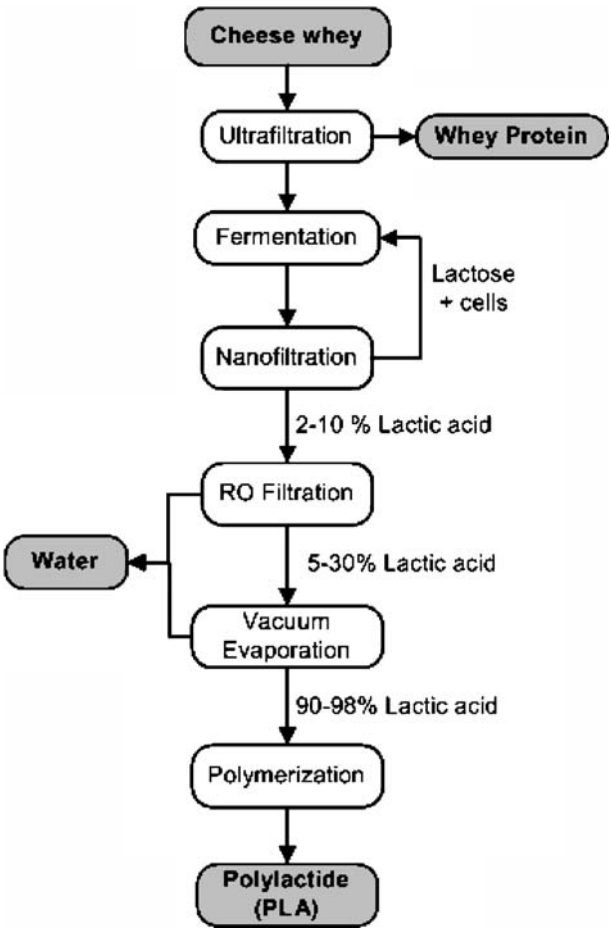

Fig. 1 Flow diagram for lactic acid production and separation from cheese whey

whey concentration. To sterilize the whey solution, it was autoclaved at 110°C for 10 min. The autoclaved cheese whey media was then fermented in a stirred 2.0-L bench-top fermentor with *B. longum*, which was obtained from the National Collection of Food Bacteria (NCFB 2259). The fermentation parameters were as follows: pH 5.5, temperature 37 °C, and agitation 150 rpm. The fermentation broth obtained at different tests was stored in freezer for the membrane separation tests.

Nanofiltration

A cross-flow nanofiltration module (SEPA CF II, GE Osmonics, Minneapolis, MN) was used for this process with a maximum operating pressure of 7.0 MPa. The surface area of the membrane is 140 cm^2. The holdup volume of the membrane unit is 70 mL. The fermentation broth was placed in a 5-L fermentation vessel to control the temperature, agitation, and pH. A bench-top pump (M03-S, Hydra cell, Minneapolis, MN) was used to pump the fermentation broth through the cross-flow membrane separation unit and recycle back to the fermentor (Fig. 2). The permeate was collected on a digital balance attached to a laptop computer with a RS-COM version 2.40 system (A&D, Milpitas, CA) that recorded the amount of permeate collected every 0.5 min. The fermentation broth was kept at constant temperature (37 °C), pH (5.5), and agitation (200 rpm). Transmembrane pressures of 1.4, 2.1, and 2.8 MPa were used in the nanofiltration tests. Each condition was tested twice, and each test lasted for 2 h. Samples of the original broth (before separation), permeate, and retentate were collected for analysis.

In this research, five nanofiltration membranes (CK, DK, DL, GE, and HL) obtained from GE Osmonics were tested. Table 1 shows the product specifications of the membranes.

Reverse Osmosis

The permeate collected from nanofiltration was placed in a 2-L fermentation vessel. Using the same system setup as that for nanofiltration, the lactic acid in the nanofiltration permeate was concentrated with reverse osmosis membrane. However, the pH was not regulated during reverse osmosis separation; only the temperature (37 °C) and agitation

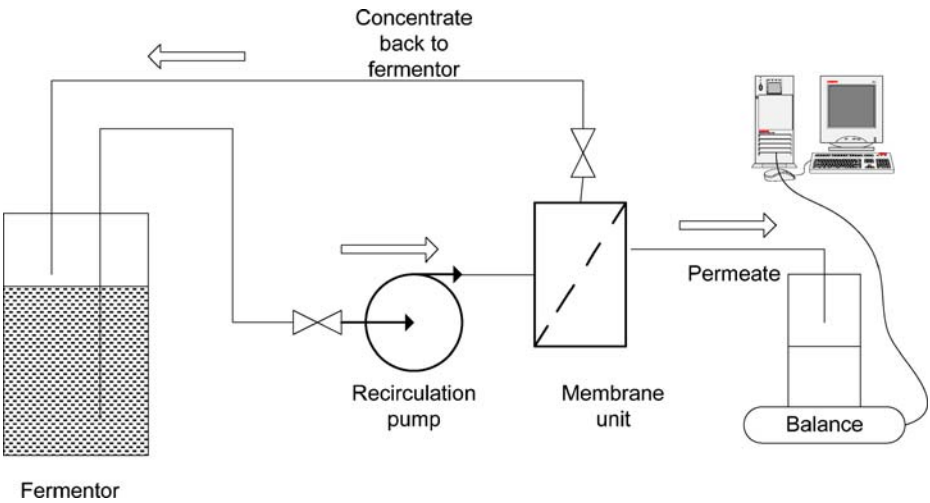

Fig. 2 Schematic diagram of the nanofiltration membrane separation system

Table 1 Product specifications for the five nanofiltration membranes tested.

Class	Polymer	Designation	Rejection size	MWCO (Da)
NF	Cellulose Acetate	CK	92% Na_2SO_4	175–15,000
NF	Thin film composites	DK	98% $MgSO_4$	0–400
NF	Thin film composites	DL	96% $MgSO_4$	0–400
NF	Thin film composites	HL	98% $MgSO_4$	0–400
NF	Composite polyamide	GE	1,000 Da	1,000–200,000

(200 rpm) were controlled. During reverse osmosis separation, two membranes were tested: DS 11 AG and ADF (GE Osmonics) at pressures of 4.1 and 5.5 MPa, respectively. Each test lasted for 75 min. There were two replications for each test. Samples of the original solution, permeate, and retentate were collected for analysis.

Membrane Cleaning

An alkali acid treatment method was applied to the membrane system (both nanofiltration and reverse osmosis separation) in the following steps: (a) fully open the recirculation and permeate valves, (b) flush with tap water for 5 min, (c) circulate 2 L of 4% phosphoric acid for 10 min, (d) rinse with tap water for 5 min, (e) circulate 2 L of 0.1 N NaOH solution for 10 min, and (f) 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 and 60 °C, respectively.

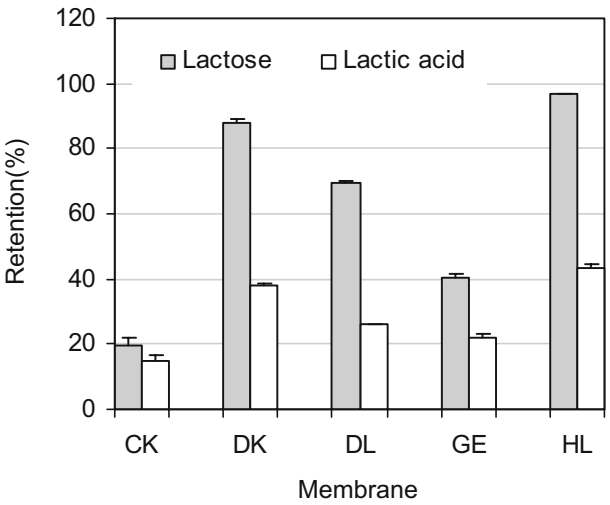

Fig. 3 Effect of membrane on lactose and lactic acid retention (2.1 MPa)

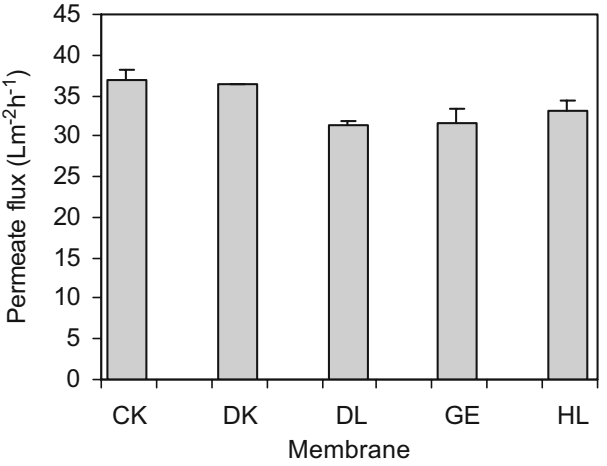

Fig. 4 Effect of membrane on permeate flux (2.1 MPa)

The performance of the membrane was evaluated by using two criteria: (a) permeate flux and (b) component retention. 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.

$$\text{Permeate flux} = \frac{\text{premeat volume}}{\text{membrane area} \times \text{time}} \quad \left(\text{L m}^{-2}\,\text{h}^{-1}\right) \tag{1}$$

The component retention (%) was defined as:

$$\text{Retention} = \left(1 - \frac{\text{concentration of component in the permeate}}{\text{concentration of component in the feed stream}}\right) \times 100 \tag{2}$$

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

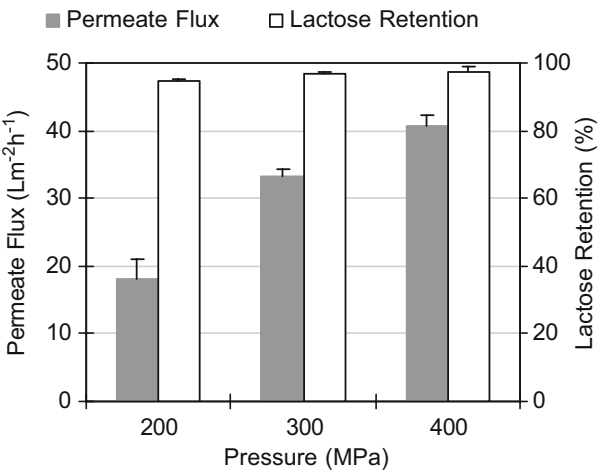

Fig. 5 Effect of pressure on permeate flux and lactose retention of HL membrane

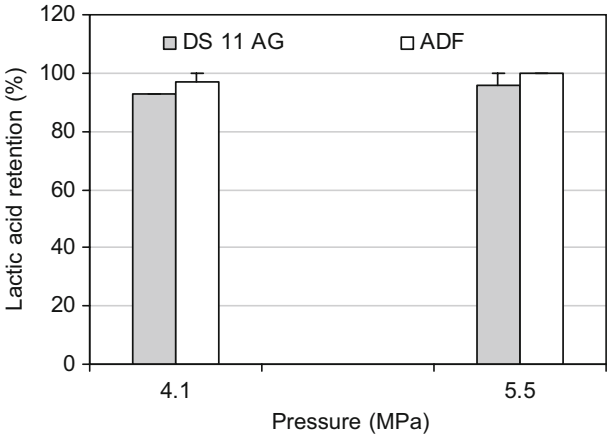

Fig. 6 Effects of pressure and membrane type on lactic acid retention of reverse osmosis separation

Results and Discussion

Nanofiltration

The concentration of lactose and lactic acid in the fermentation both varied with the fermentation conditions such as initial cheese whey concentration, pH, cell density, and fermentation time. The lactose concentration in most of the runs is less than 1%, and the lactic acid concentration is about 3%. The fermentation broth obtained from the fermentation tests was mixed, and the lactic acid and lactose concentration was adjusted to 5 and 2% for nanofiltration tests.

Five nanofiltration membranes, CK, DK, DL, GE, and HL, were tested at the same temperature (37 °C), pH (5.5), and pressure (2.1 MPa). The HL, DK, and DL membranes have the lowest MWCO, therefore allowing little or no solute to pass through the membrane, thereby increasing the lactose retention. The lactose retention of the HL, DK, and DL membrane was 96.8, 88.2, and 69.8%, respectively (Fig. 3). The lactic acid

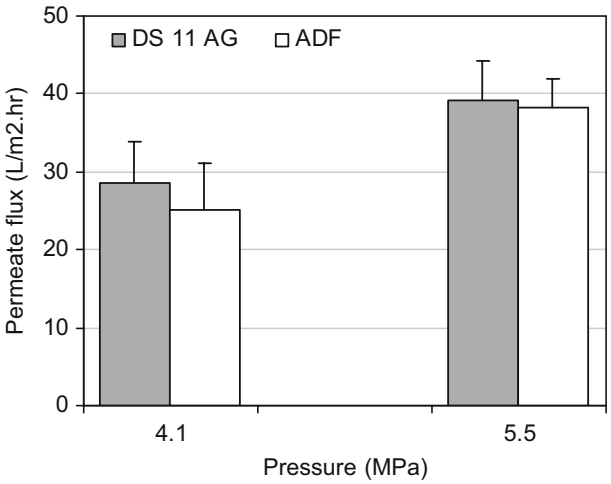

Fig. 7 Effects of pressure and membrane type on permeate flux of reverse osmosis separation

retention of the HL, DK, and DL membrane was 43.7, 38.0, and 26.1%, respectively. The permeate flux was also affected by the membrane type (Fig. 4). The CK membrane has the highest permeate flux of 36.8 L m^{-2} h^{-1}. The lowest permeate flux of 31.3 L m^{-2} h^{-1} was obtained with the DL membrane. The results of these tests show that the HL membrane was most efficient with almost 97% lactose retention and a permeate flux of 33.0 L m^{-2} h^{-1}.

The effects of pressure on lactose retention and permeate flux of the HL membrane were shown in Fig. 5. Permeate fluxes of 18.0, 33.2, and 40.6 L m^{-2} h^{-1} were obtained at pressure of 1.4, 2.1, and 2.8 MPa, respectively. The effect of pressure on lactose retention was not significant ($p>0.3$). The lactose retention kept almost constant at 97±1%. These observations are in agreement with literature data [10].

Reverse Osmosis

Reverse osmosis was applied to concentrate the lactic acid in the permeate of nanofiltration process. The permeate collected from nanofiltration with the HL membrane was used. Two reverse osmosis membranes (DS 11 AG and ADF) were tested at 4.1 and 5.5 MPa.

Slightly higher lactic acid retention was obtained with the ADF membrane compared to that of the DS 11 AG membrane (Fig. 6). The lactic acid retention for the ADF membrane was 96.7 and 100.0% at pressures of 4.1 and 5.5 MPa, respectively, while the lactic acid retention for the DS 11AG membrane was 92.6 and 96% at pressures of 4.1 and 5.5 MPa, respectively. The effect of membrane and pressure on lactic acid retention was not significant ($p>0.1$).

Figure 7 shows the results of permeate flux obtained with DS 11 AG and ADF membranes at pressures of 4.1 and 5.5 MPa. Higher permeate flux was obtained with DS 11AG membrane than that of the ADF membrane, but the effect of membrane on the permeate flux is not significant ($p>0.1$). The results show that permeate flux was significantly affected by the pressure ($p<0.005$). Higher permeate flux was obtained at a higher pressure.

Conclusion

Combined nanofiltration and reverse osmosis membranes could successfully separate and concentrate lactic acid from cheese whey fermentation broth. Nanofiltration membrane could retain about 97% of lactose to obtain permeate mainly containing lactic acid and water. The highest lactose retention of 97% was obtained with the HL membrane. The tested reverse osmosis membranes successfully separated lactic acid from water. Nearly 100% of lactic acid retention was obtained with the ADF membrane.

Further study needs to be conducted on the nanofiltration membrane to obtain 100% lactose retention and lower lactic acid retention. The economic analysis for the lactic acid recovery with membrane separation also needs to be analyzed and compared with that of other downstream processes.

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