Separation of Lactic Acid from Cheese Whey Fermentation Broth Using Cross-Flow Ultrafiltration and Nanofiltration Membrane System

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Abstract

Cheese whey is one by-product that can be used to produce lactic acid. The processes of lactic acid production include two key stages which are (a) fermentation and (b) product recovery. In this project, lactic acid was produced from cheese whey using B. longum. After 48 hours of fermentation, nearly 100% of the lactose was converted and the lactic acid yield reached 0.81 g/g lactose utilized without any nutrient addition.

Ultrafiltration membrane was used to separate cells and protein from the above fermentation broth. About 94% of the proteins were retained by the ultrafiltration membrane with MWCO of 5,000 and 20,000 Daltons. Nanofiltration membrane was used to further separate lactic acid from lactose in the ultrafiltration permeate, 99-100% of lactose can be retained in the concentrate and 40-60% of lactic acid can be recovered in the permeate using a nanofiltration membrane of DS-5DK. Higher initial lactic acid caused significant higher permeate flux, lower lactose retention, and higher lactic acid recovery. Increased transmembrane pressure caused significant higher permeate flux, higher lactose retention, and lower lactic acid recovery.

Introduction

Manufacturing of cheese produces large volumes of whey as a by-product. The United States generates nearly 1.2 billion tons of cheese whey per year (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. 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 *Lactobacillus 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). To 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 three moles of acetate and two moles 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 (a) fermentation and (b) 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 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.

Ultrafiltration can remove dissolved macromolecules with MWCO between1000 and 100,000 Dalton (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 molecular weight cutoff (MWCO) around 20,000 Dalton (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 200-1,000 Dalton (16). Nanofiltration membrane with MWCO around 400 Dalton 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 process for lactic acid recovery. The protein and cells have been separated by ultrafiltration unit in the previous study. The objectives of this study were two-fold: (1) to evaluate membranes that could be used to retain high levels of lactose and ultimately high recovery ratios of lactic acid in the 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, USA) into a liter of deionized (DI) water and stirring for 5 minutes at ambient temperature. The composition of the deproteinized cheese whey powder was as follows: crude protein (total nitrogen $\times$ 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 minutes.

MICROORGANISM AND CULTURE MEDIA

Bifidobacteria 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 to 24 h under anaerobic conditions. This was used as a pre-culture 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 5.0-liter bench top fermentor. The pH of the broth was maintained at 5.5 by neutralizing the acid with 5N ammonium hydroxide during fermentation. The agitation speed of the fermentor was maintained at 150 rpm, while the temperature was maintained at 37°C. Samples were withdrawn every 2 h during the first 8 hours and every 12 h during the remaining fermentation process. The fermentation was lasted for 48 h.

MEMBRANE SEPARATION

The ultrafiltration membrane system consisted of a recirculation pump, cross flow ultrafiltration module (OPTISEP, North Carolina SRT, Inc., Cary, NC), and an online permeate weighting unit (Figure 1). The media was fed from the fermentor at constant velocities via the recirculation pump. The concentrate was recycled to the fermentor while permeate was collected in a reservoir placed on an electronic balance. The balance was interfaced via RS232 to a computer that continually recorded time and permeate weight at 30 s intervals. Two types of membranes (PES5 and PES20, Nadir Filtration GmbH, Wiesbaden, Germany) with MWCO of 5,000 and 20,000 Dalton were used in the ultrafiltration experiments. The membrane polymer consisted of permanently hydrophilic polyethersulfone and polysulfone.

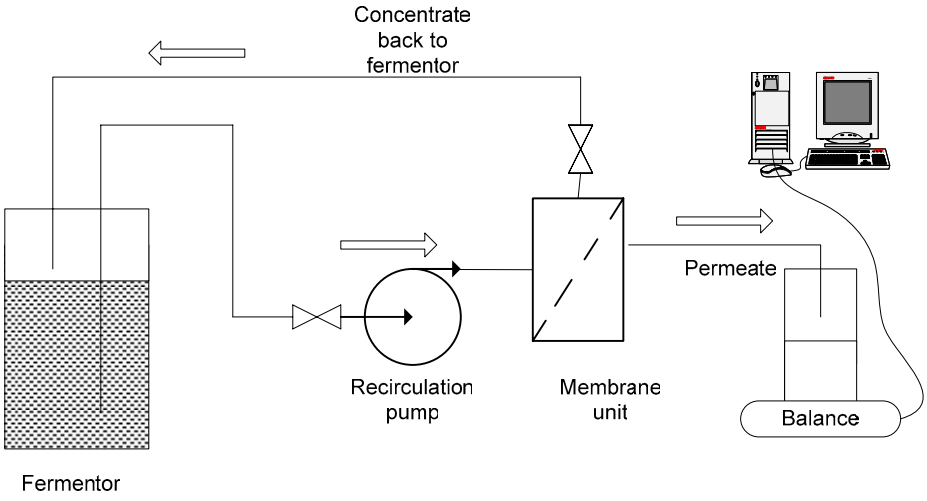


Figure 1. Schematic diagram of the membrane separation system

In the nanofiltration system, the pump and ultrafiltration unit in the ultrafiltration system was replaced with a high pressure pump (M03-S, Hydra-cell, Minneapolis, MN, USA) and nanofiltration membrane unit (SEPA CF II, Osmonics, Minneapolis, MN, USA). The two tested nano membranes (DS-5DK and DS-5HL, Osmonics, Minneapolis, MN, USA) in this study

could retain 98% of $MgSO_4$ but had different levels of permeate flux. No MWCO information was provided by the manufacturer.

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

ANALYSES

Lactose, lactic acid, and acetic acid were measured 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_3PO4 solution at a flow-rate of 1ml/min. The temperatures of the detector and of the column were maintained at 35°C and 60°C respectively.

The total nitrogen was analyzed using the macro-Kjeldahl method. Samples were digested using a block digestion (FOSS Tecator, Sweden) and analyzed for nitrogen on a Tecator Kjeltec auto 2400 analyzer (FOSS Tecator, Sweden). When the protein nitrogen was determined, the samples were precipitated using a trichloroacetic (TCA) solution before nitrogen analysis (11). The digestion and analysis procedure for crude protein was the same as that for total nitrogen analysis.

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

$$Conversion\ ratio(\%) = \frac{initial\ lactose\ conc. - residual\ lactose\ conc.}{initial\ lactose\ conc.} \times 100\% \quad (1)$$

The lactic acid yield was expressed as grams of lactic acid produced per gram of lactose used.

$$Lactic\ acid\ \ yield(g/g) = \frac{lactic\ acid\ \ produced}{lactose\ used} \quad (2)$$

Lactic acid production efficiency was expressed as:

$$Lactic\ acid\ \ production\ efficiency(\%) = \frac{actual\ lactic\ acid\ \ yield}{stoichiometric\ lactic\ acid\ \ yield} \times 100\%$$

Stoichiometric lactic acid yield=0.95 (3)

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.

$$\textit{Permeate flux},\ J = \frac{permeate\ volume}{membrane\ area \times time}\ (l\,m^{-2}h^{-1}) \quad (3)$$

The component retention (%) was defined as:

$$Retention = \left(1 - \frac{C_{LP}}{C_{L0}}\right) \bullet 100 \quad (4)$$

C_{L0} = concentration of component in feed stream, C_{LP}= concentration of component in permeate.

The lactic acid recovery (%) was defined as:

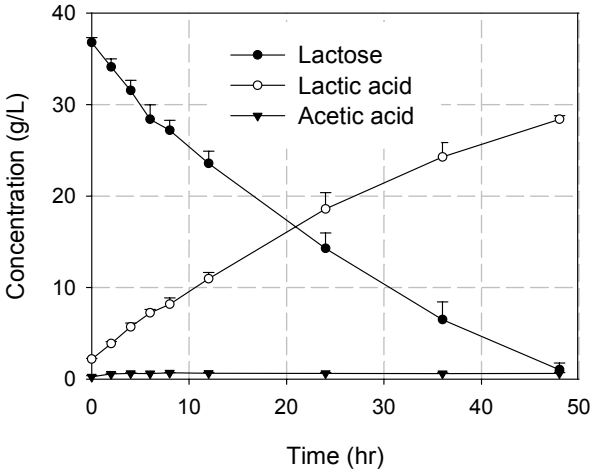
$$Lactic\ acid\ recovery = 1 - lactic\ acid\ retention\ ratio \quad (5)$$

RESULTS AND CONCLUSIONS

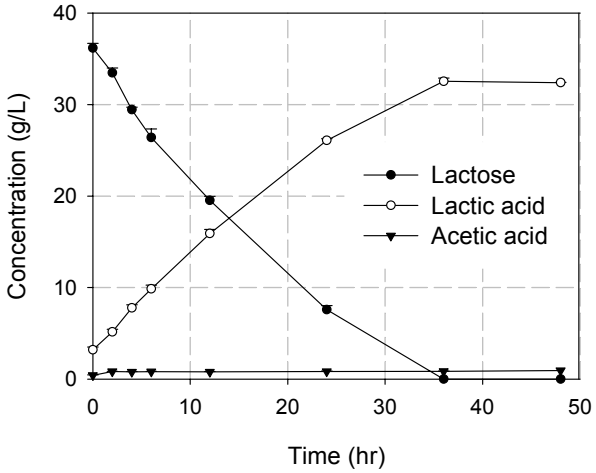
FERMENTATION

The lactose, lactic acid, and acetic acid concentrations obtained during the 48 hours of fermentation with free cell *B. longum* are shown in Figure 2. The results show that the lactose utilization efficiency increased from 97% to100% and the lactic acid production efficiency increased from 77% to 85% when the cell density was increased from 2.6 x 10^8 to 5.2 x10^8 cfu/ml. The lactic acid yield was 0.73 and 0.81g/g at cell density of 2.6 x 10^8 and 5.2 x10^8 respectively.The production of acetic acid was negligible in comparison to that of lactic acid production.

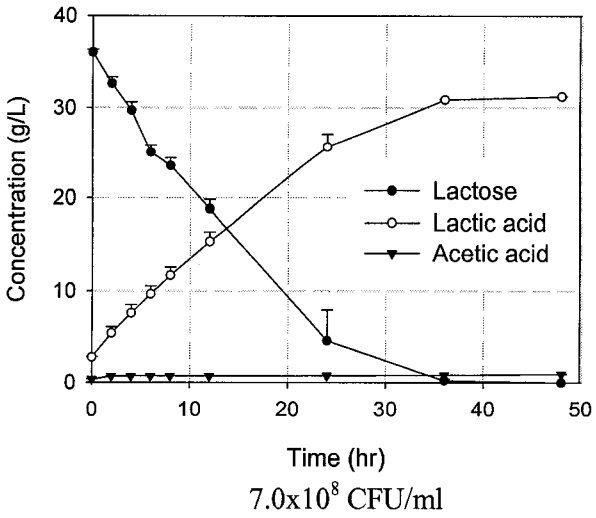
The lactose conversion ratio and lactic acid yield are similar to results of other lactic acid producing bacteria such as *L. helveticus.* Tango and Ghaly (3) obtained a lactose utilization value of 92-95% and a lactic acid yield of 0.86 g lactic acid/g lactose when using immobilized *L. helveticus* with nutrient supplement at 36 h of fermentation. Most of the previous works were focused on obtaining high lactose conversion ratios and lactic acid yields. These experiments were carried out with immobilized cells and nutrient supplementation. In this study, free cells of *B. longum* were grown with no nutrient supplements, which would significantly reduce the cost of lactic acid production and be more compatible with the current fermentation facilities.



2.6x10^8 CFU/ml



5.2x10^8 CFU/ml



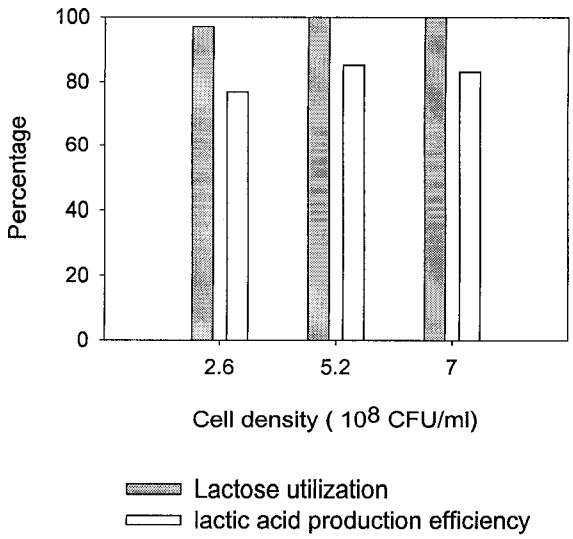
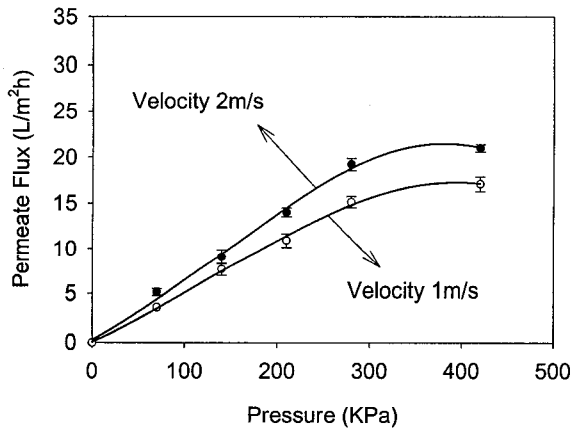
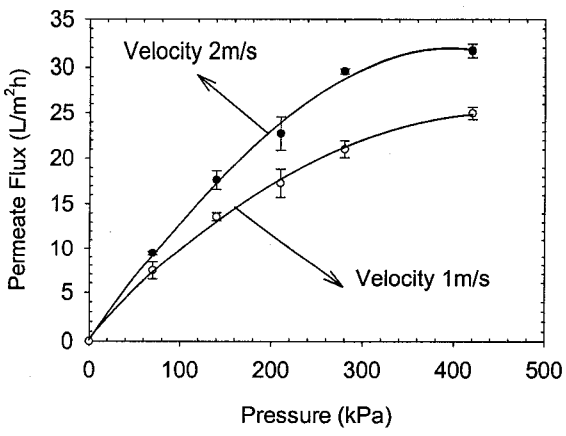


Figure 2. Effect of cell density on the lactic acid production using free cell *B. longum*

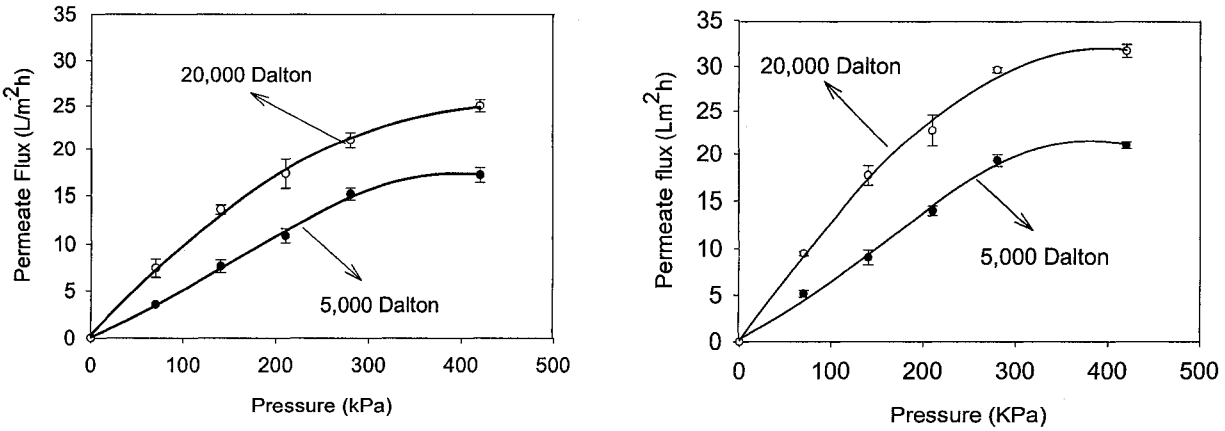
MEMBRANE SEPARATION

Ultrafiltration Figure 3 shows the effects of transmembrane pressure, cross flow velocity and MWCO on the permeate flux at 21°C. The fermentation broth was obtained by fermentation for 48 h. Each separation test lasted 2 h and the permeate flux was calculated based on the permeate volume collected in the 2h test. The permeate flux values in Figure 3 are the average of two replicate tests. It can be discerned that increased transmembrane pressure caused an increase of the permeate flux. Beyond a certain pressure, the increase in permeate flux with pressure was negligible which indicates that there is an optimum pressure to obtain the maximum permeate flux. Similar results were also reported by Vigneswran and Kiat (13) who obtained the optimum pressure for maximum permeate flux during the ultrafiltration of polyvinyl alcohol solution at different concentrations.





(a) MWCO: 5,000 Dalton (b) MWCO: 20,000 Dalton



(c) Cross flow velocity: 1 m/s (d) Cross flow velocity: 2 m/s

Figure 3. Effect of transmembrane pressure, cross flow velocity, and membrane cutoff on permeate flux

Results in Figure 3 also indicate that higher cross flow velocity caused higher permeate flux for the membrane with MWCO of both 5,000 and 20,000 Dalton. At the same cross flow velocity, the membrane with MWCO of 20,000 Dalton had a higher permeate flux than that with MWCO of 5,000 Dalton. The analysis of variance performed on the permeate flux data using a statistical package from the SAS System (SAS Institute, Cary, NC) showed that pressure and cross flow velocity had significant ($P < 0.0001$) effects on the permeate flux. Most of the interactions between the parameters were not significant.

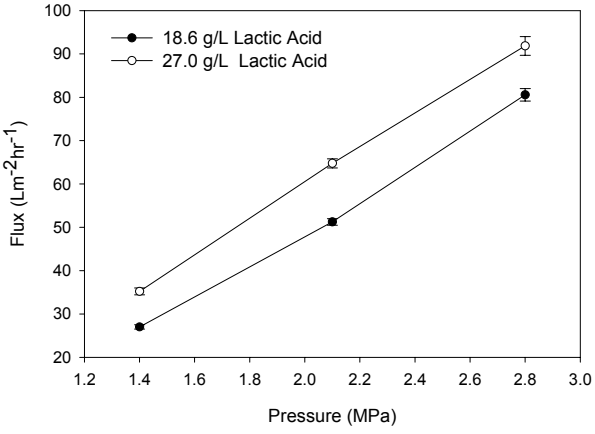
The average crude protein (total nitrogen) retention ratios for membranes with MWCO of 5,000 Dalton and 20,000 Dalton were 72.0 and 53.9%, respectively. The average protein retention ratio was 94.0% for both of the two membranes with MWCO of 5,000 Dalton and 20,000 Dalton. It can be concluded that most of the protein is retained by the ultrafiltration membranes with both MWCO of 5,000 and 20,000 Dalton. We conclude that most of the detected raw protein in permeate is non-protein nitrogen, which has smaller MWCO than protein.

Nanofiltration

Figures 4a, and 4b show that permeate flux increased with the increase of transmembrane pressure. Higher permeate flux could be obtained at higher initial lactic acid concentrations. When the initial lactic acid concentration was increased from 18.6 g/L to 27.0 g/L, the permeate flux increased about 30%, 26%, and 14% at pressure 1.4, 2.1 ad 2.8 MPa, respectively for membrane of DS-5DK. Among the two tested membrane of DS-5DK and DS-5HL, higher permeate flux levels were obtained with membrane of DS-5HL (Figure 5b). The analysis of variance performed on the permeate flux data showed that membrane, pressure and initial lactic acid concentration has significant ($P < 0.0001$) effects on the permeate flux. The interaction between these parameters were not significant ($P=0.045$ and 0.11, respectively).

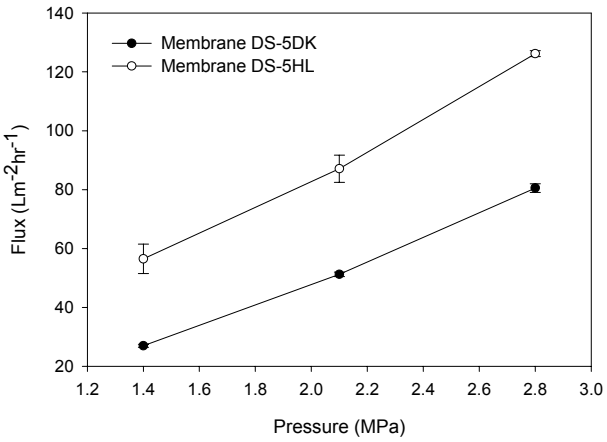
Figure 4c and 4d show that lactose retention increased with the increase of transmembrane pressure. Lower lactose retention was obtained at higher initial lactic acid concentration. When the DS-5DK membrane was used, 100% retention of lactose was obtained at initial lactic acid concentration of 18.6 g/L for all tested transmembrane pressures. When the initial lactic acid concentration was increased to 27.0 g/l, lactose retention rates of 94.7, 96.8, and 99.5% were obtained at pressure levels of 1.4, 2.1, and 2.8 MPa, respectively. This indicates that at higher initial lactic acid concentrations, higher lactose retention can be obtained by increasing transmembrane pressure. When the DS-5HL membrane was used to separate media with initial lactic acid concentration of 18.6 g/L, with the same pressure levels as for the DS-5DK membrane, lactose retention rates were 82.2, 87.3, and 90.7%, respectively. At most of the test conditions, 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.

Increased retention of lactic acid corresponded positively with increased lactose retention (Figure 4e and 4f). Increases of transmembrane pressure were associated with lower levels of lactic acid recovery in permeate. Higher lactic acid recovery was obtained at higher initial lactic acid concentration. When the initial lactic acid concentration increased from 18.6 g/L to 27.0 g/l, the lactic acid recovery increased from 54.4, 43.9, and 36.6 % to 76.9, 69.3 and 63.5 at pressure of 1.4, 2.1 and 2.8 MPa, respectively for membrane of DS-5DK. Considering the effects of increased initial lactic acid concentration on the permeate flux and lactose concentration, both of the increased permeate flux and lactic acid retention are desired while the decreasing of lactose retention need to be compensated by optimized parameter such as increased pressure.



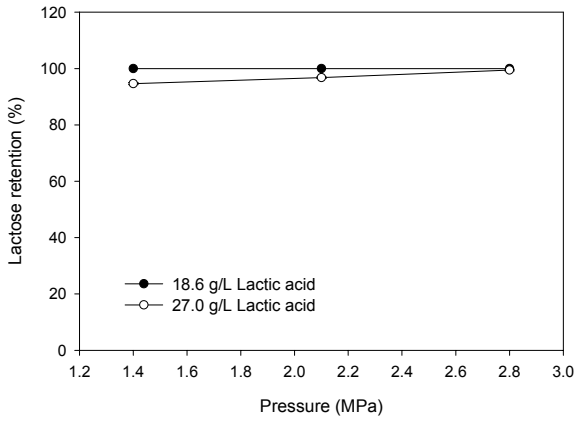
a.

Permeate flux (membrane: DS-5DK)

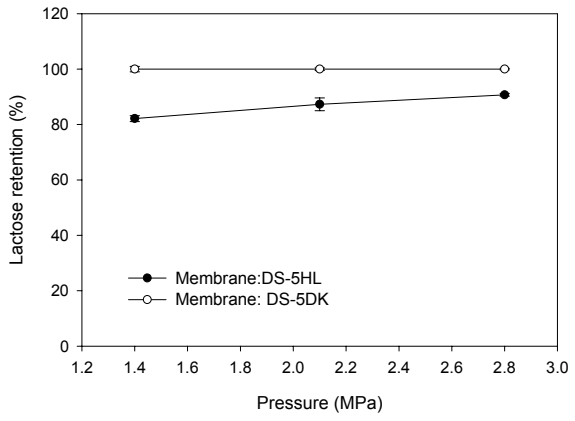


b.

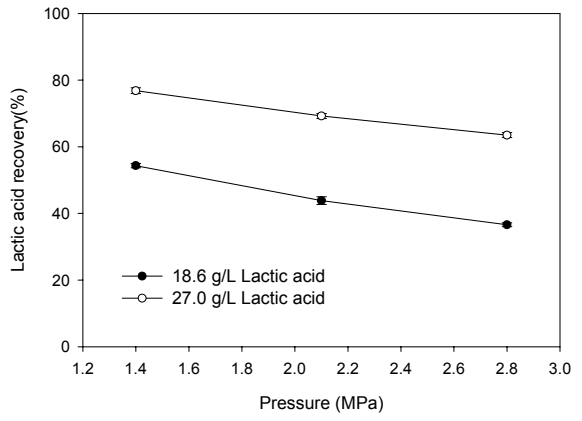
Permeate flux (lactic acid conc. 18.6 g/L)



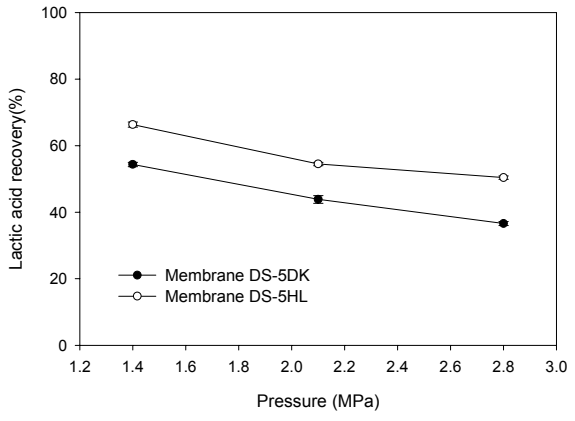
c. Lactose retention (membrane: DS-5DK)



d. Lactose retention (lactic acid conc. 18.6 g/L)



e. Lactic acid recovery (membrane: DS-5DK)



f.Lactic acid recovery (lactic acid conc. 18.6 g/L)

Figure 4 Effects of pressure, membrane, and initial lactic acid concentration on permeate flux, lactose retention, and lactic acid recovery of nanofiltration

CONCLUSIONS

1. *B. longum* has been demonstrated to be promising bacteria for lactic acid production from cheese whey. At pH 5.5, nearly 100% of the lactose was converted and 0.81g lactic acid was produced from one gram of lactose using free cells of *B. longum* and without nutrient supplement. Such high conversion ratios and lactic acid yield could only be obtained with nutrient supplement and immobilized cells for *L. helveticus*.

2. Ultrafiltration can be successfully used to separate protein and bacteria cells from cheese whey fermentation broth. Nearly all cells and proteins were retained by the ultrafiltration membrane with MWCO of 20,000 Daltons. Increased transmembrane pressure and cross flow velocity caused higher permeate flux. Increasing the membrane MWCO from 5,000 Dalton to 20,000 Dalton caused a significantly higher permeate flux and lower crude protein retention ratio.

3. Nanofiltration can be successfully used to separate lactose and lactic acid. 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 g/L and 27.0 g/L. To obtain 100% of lactose retention, transmembrane pressure higher than 2.8 MPa needs to be applied when the initial lactic acid concentration reached 27.0 g/L. For the tests when 99-100% of lactose was retained in the concentrate, the highest lactic acid recovery in the permeate reached 63.5%.

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