Processing of Soybean Soaking Water with a NF−RO Membrane System and Lactic Acid Fermentation of Retained Solutes

Yuan-Kuang Guu,* Chiu-Hsia Chiu, and Jing-Kun Young

Department of Food Science and Technology, National Pingtung University of Science and Technology, Neipu, Pingtung 912, Taiwan

Recovery of water and soluble materials of soybean soaking water was done using a membrane system consisting of nanofiltration (NF) and reverse osmosis (RO). Retentates from NF and RO were collected when the weight concentration ratio (WCR) had reached 7 and 6, respectively, which was equivalent to a total WCR of 3.5 for the combined NF−RO system. The retentates were inoculated with the probiotic cultures of *Lactobacillus acidophilus* CCRC 10695 and *Bifidobacterium longum* CCRC 11847. Under the fermentation condition (pH 5.5, 37 °C), up to 7 g/L organic acids, mostly lactic acid, could be harvested after 24-h fermentation. The fermentation broth was centrifuged to remove cells, pasteurized, and then formulated to a test drink, which was well accepted as compared with market samples. The membrane-permeated water was nearly neutral in pH with a Hunter *L** value above 90, which could possibly be reused in the plant site for cleaning and soaking purpose.

Keywords: *Soybean soaking water; probiotic cultures; lactic acid fermentation; water recovery; nanofiltration; reverse osmosis*

INTRODUCTION

Soybean products, such as tofu (soybean curd), tokan (pressed hard soybean curd), tohua (soybean jello), and soybean milk are widely consumed in Taiwan and other Asian countries (Snyder and Kwon, 1987; Fukushima, 1991; Shuy, 1991). A large amount of water, about 12−15 times the volume of soybean, is used to clean and soak the beans before further processing. It takes about 5−7 h to complete the traditional soaking process at room temperature. With fermentable solubles and in the open air, the soaking water is very susceptible to the growth of microorganisms (Chen, 1993). Recently, an improved soaking process has been adopted by a leading soybean processing company for automatically replacing and discharging soaking water by a programable time controller (Chen, 1993; Guu and Chiu, 1994). However, this new process has not been able to reduce the amount of water required for soaking. The discharged soaking water is forwarded to the wastewater treatment plant without any recovery, causing a great waste of water resources. This soaking water contains about 0.08% (w/w) crude proteins and 0.2% (w/w) carbohydrates, and the COD is frequently as high as 10 000 ppm, which makes the soaking water not reusable if not properly treated (Guu and Chiu, 1994). Most of the crude proteins and carbohydrates in the soaking water are soluble peptides, glucose, fructose, and trace amounts of oligosaccharides such as raffinose (Guu and Chiu, 1994). These soluble materials could be easily separated by nanofiltration (NF) or reverse osmosis (RO) membranes to recover water (Guu and Zall, 1992; Young and Guu, 1995; Guu, 1996). The membrane-recovered retentates may be further treated by fermentation for recovery of the soluble materials or discharged with a remarkable reduction in volume.

Certain microorganisms can utilize mono-, di-, and oligosaccharides in the medium and generate lactic acid during fermentation. Typical strains include *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*, etc. (Mital and Steinkraus, 1979; Vickroy, 1985; Desjardins et al., 1990; Huang, 1992; Montelongo et al., 1993). Some of these probiotics have been reported to produce pleasant organics to mask beany flavor in the soy milk fermentation (Huang, 1992) or to produce lactic acid in the soybean cooked syrup (Matsuda and Ueda, 1995).

According to statistics, about half of the lactic acid produced globally has been the fermentation products of lactic acid bacteria capable of utilizing various kinds of carbohydrates (Vickroy, 1985). In this study, two available probiotic cultures, *L. acidophilus* CCRC 10695 and *B. longum* CCRC 11847, were inoculated into the NF−RO retentates and incubated at 37 °C to investigate the feasibility of fermenting soluble materials in the retentate to produce lactic acid.

MATERIALS AND METHODS

Materials and Probiotic Cultures. Soybean soaking water (Hen-Yi Foods Co., Ltd., Kaohsiung, Taiwan) was first sedimented and centrifuged to remove dirt and debris and then fed to the membrane system. Composition of the pretreated soaking water is shown in Table 1.

Lyophilized probiotic strains, *Lactobacillus acidophilus* CCRC 10695 and *Bifidobacterium longum* CCRC 11847, were from the Culture Collection and Research Center at the Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan. These strains were first activated by CCRC method (FIRDI, 1992). Cultivations of CCRC 10695 and 11847 were at 37 °C for 48 h using the original and modified (by adding into 0.5 g of cystein) MRS (Man−Rogosa−Sharpe) broth (Difco Lab., Detroit, MI), respectively, and saved as stock cultures. Total microbial counts in the stock cultures were in the range of 10^8 CFU/mL.

Membrane System. A nanofiltration (NF40-2514A) and a reverse osmosis (BW30-2514) membrane, both spiral wound (FilmTec Membranes Co., Minnetonka, MN), were arranged in a NF−RO series (Figure 1). This membrane system was equipped with circulating pumps (CatPump Model 2810,

* Corresponding author.

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Table 1. Compositions of Soybean Soaking Water before and after Membrane Treatments

	total solids (%)	total sugars (%)	crude proteins (%)	ash (%)	COD (ppm)
pretreated soybean soaking water[a]	7.24×10^{-1}	2.00×10^{-1}	7.90×10^{-2}	4.30×10^{-2}	9.92×10^{3}
		Nanofiltration Treatment[b]			
permeate	1.63×10^{-2}	8.30×10^{-3}	nil	1.00×10^{-2}	8.90×10^{2}
retentate	4.96	1.35	5.53×10^{-1}	2.41×10^{-1}	6.39×10^{4}
		Reverse Osmosis Treatment[c]			
permeate	1.80×10^{-4}	nil	nil	nil	2.10×10^{2}
retentate	9.69×10^{-2}	4.94×10^{-2}	nil	5.30×10^{-2}	5.33×10^{3}

[a] Pretreatment with sedimentation and centrifugation (1200*g*, 10 min). [b] Feed was the pretreated soybean soaking water. WCR (weight concentration ratio) of NF was 7. [c] Feed was the permeate from NF. WCR of RO was 6.

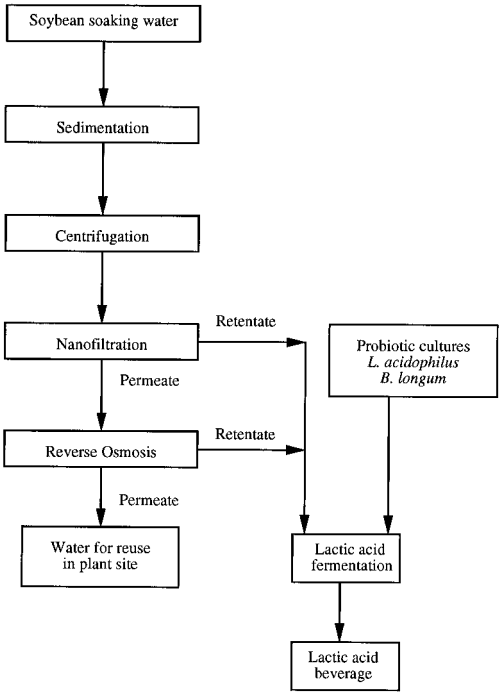

Figure 1. Flow diagram for recovering solubles and water from soybean soaking water.

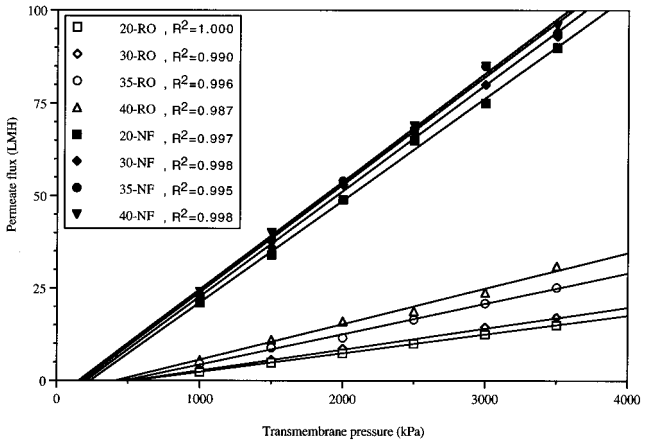

Figure 2. Permeate flux of NF and RO using soybean soaking water as feed under various temperatures and pressures. (LMH, L m^{-2} h^{-1}; 20-RO, RO is operated at 20 °C; 30-NF, NF is operated at 30 °C, etc.)

CatPump Co., Minneapolis, MN), and was operated at 30 °C and a transmembrane pressure of 2500 kPa until a total weight concentration ratio (WCR) of 3.5 was reached.

Fermentation of NF−RO Retentates. The NF−RO concentrated soybean soaking water (250 mL) was inoculated with 5% (v/v) MRS cultivated stock cultures, *L. acidophilus* CCRC 10695 or *B. longum* CCRC 11847, in a 500-mL flask, thus making for initial total microbial counts in the range of 10^6 CFU/mL. When mixed cultures of CCRC 10695 and 11847 were used, the inoculum consisted of equal amounts of these two strains made up to a 5% (v/v) level. Initial fermentation conditions were at pH 5.5 and 37 °C, following CCRC's suggestion (FIRDI, 1992). Final microbial counts in the broth were in the range of 10^9 CFU/mL after 48-h fermentation. Samples were taken from fermentation broth for optical density (OD), pH, titratable acids, and total residual sugars determinations during a 48-h fermentation period. Fermentation broth was centrifuged under 8000*g* for 10 min to remove cells, pasteurized, and then formulated with water, sugar, and flavorings to simulate a popular drink in the Taiwanese local market that contains citric acid, oligosaccharides, and *in vivo* Bifidobacteria activation agents. Pair comparisons were made between the test and the reference (mini-Oligo, Gold Cart Co., Ltd., Chungli, Taiwan) samples to primarily measure the acceptance level of the test sample by a small panel of 12 food science students (Kramer and Twigg, 1974).

Sample Analysis. Crude proteins and titratable acids were determined by AOAC methods (AOAC, 1984). Sugars were analyzed by modifying the Macrae and Zand−Moghaddam's HPLC method (1978). The system consisted of a Hitachi intelligent pump L-7100, a chromato-integrator D-7500 (Hitachi, Tokyo, Japan), a refractive index detector (RI-8110, Bishoff GMBH, West Germany), and a sample injector (Model 7251, Rheodyne, Cotati, CA). The separation column was a LiChrosorb-NH_2 (E. Merck, Darmstadt, Germany) eluted with acetonitrile:water (85:15) at 1.0 mL/min flow rate. The residual sugars in the broth were determined by the DNS method (Miller, 1959). COD was measured according to the Standard Methods of NIEA W515.50A (NIEA, 1993). A color differentiometer (JP7100F, Juki Co., Japan) was used to measure the color of membrane-treated streams. The growth of microorganisms during fermentation was monitored by a spectrophotometer (U-2000, Hitachi, Tokyo, Japan) at 600 nm and expressed as optical density (OD). Each data point obtained was the average of three replicates.

RESULTS AND DISCUSSION

Membrane Performance. Reverse osmosis and nanofiltration membranes were first tested separately with the pretreated soybean soaking water within the temperature range of 20−40 °C and pressure range of 1000−4000 kPa. Data (Figure 2) were fitted with an empirical equation, $J = A(\Delta P_T - \Delta\pi)$ (Cheryan, 1986), where J is the permeate flux; A is the permeability coefficient; $\Delta\pi$ is the osmotic pressure; ΔP_T is the transmembrane pressure. Results (Table 2) show that permeability coefficients A increased while osmotic pressure $\Delta\pi$ decreased as the temperature increased. These observations follow the thermodynamic predictions for permeate flux. As temperature increased, the chemical potential of the solvent moving through the NF and RO membrane increased resulting in an increase in A and thus permeate flux J. An Arrhenius plot shown in Figure 3 was employed to calculate the temperature-dependent energy barriers for permeation (ΔH) using the Arrhenius equation $A = A_0 e^{-\Delta H/RT}$, where A_0 is the permeation coefficient at Kelvin temperature

Table 2. Permeability Coefficients and Osmotic Pressures of Soybean Soaking Water with NF and RO under Various Temperatures Using the Empirical Equation $J = A(\Delta P_T - \Delta\pi)$

operating temp (°C)	permeability coefficient, A (L m^{-2} h^{-1} kPa^{-1})		osmotic pressure $\Delta\pi$ (kPa)	
	NF	RO	NF	RO
20	0.0287	0.0051	263	548
30	0.0291	0.0054	247	491
35	0.0300	0.0080	240	477
40	0.0307	0.0096	232	443

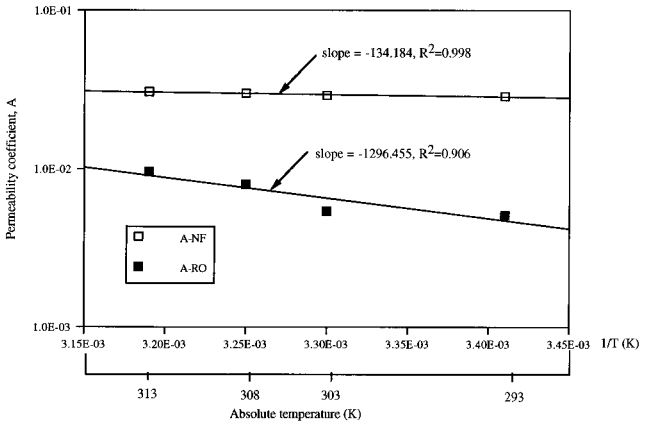

Figure 3. Arrhenius plots of permeability coefficients of NF and RO versus reciprocals of absolute temperature.

of absolute zero; R is the universal gas constant; T is the absolute temperature. ΔH, calculated from the slope of the linearly regressed line, was 2.57×10^3 and 2.48×10^4 kJ/kg·mol for NF and RO, respectively. These data indicate that more energy is needed for RO than NF to increase its permeation and that permeate flux of RO is more temperature-dependent than NF.

Decreases in $\Delta\pi$ due to increases in temperature could be attributed to the decrease in solution viscosity or the increase of the solvent chemical potential. However, back diffusion of solutes around the membrane surface to the bulk solution could have increased with temperature, which also helps reduce the negative effect of concentration polarization and, in turn, $\Delta\pi$.

According to the membrane performance, in order to run the system with a larger A and a smaller $\Delta\pi$ of RO, the membrane system should be arranged in a NF−RO series as described in Figure 1. Pretreated soybean soaking water was first pumped through the NF membrane, and then the permeate of NF was fed to the RO. Working conditions for NF and RO were 2500 kPa and 30 °C. Gradual flux decay observed in NF and RO proved the combination was helpful in prolonging the operating cycle of RO as shown in Figure 4.

Recovery of Soluble Materials and Water. Retentates of NF and RO were collected as their weight concentration ratio reached 7 and 6, respectively, or a total weight concentration ratio of 3.5. Compositions of all streams were determined (Table 1). Data of the RO retentate revealed that almost all of the solubles were recovered for further utilization. Recovered RO water was clearly clarified with a Hunter L^* value above 90 and good for reuse in the soaking process. The existence of the slight amount of COD in the permeate may have been due to the effect of pore size distribution of the RO membrane and the contaminants in tubing and containers for analysis. With the described process, over 70% of the soybean soaking water could be recycled in the production site.

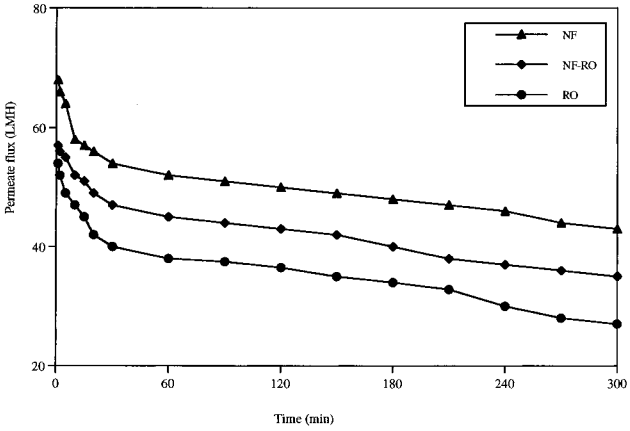

Figure 4. Performance curves of NF and RO membranes using soybean soaking water as feed under 30 °C and transmembrane pressure of 2500 kPa. (NF-RO, RO uses permeate from NF as feed; LMH, L m^{-2} h^{-1}.)

Lactic Acid Fermentation. The recovered retentates from the combined NF−RO system were mixed and utilized without added nutrients as the medium for lactic acid fermentation. Composition of the combined retentate includes total sugars 0.70%, crude protein 0.28%, and ash 0.15%. The retentate was inoculated with the MRS cultivated strains, CCRC 11847, CCRC 10695, or a mixed culture of CCRC 11847 + 10695. Regardless of the types of strains, changes in OD, titratable acidity, and pH during fermentation are similar (Figures 5 and 6).

Results (Figures 5 and 6) indicate that residual sugars and pH decreased drastically, while OD increased rapidly during the first 8 h of fermentation. At this stage, sugars may have been utilized mostly to increase the cell population, although acids were produced gradually. After 16-h fermentation, OD of the broth became a plateau with a slight increase when reaching 48-h fermentation. However, pH and titratable acidity continued to respectively gradually decrease and increase up to 24-h fermentation, as with the case of residual sugars. At this stage, most sugars may have been converted into acids. Afterward, sugars left in the broth dropped to the level of 1000 ppm and remained constant, as did OD, pH, and titratable acidity in spite of prolonging fermentation period. Most likely, the high acidity of the broth at this point had an inhibitory effect on cell growth (Montelongo et al., 1993). This reasoning found support from the optimal pH range (5.0−6.5) for the growth of lactic acid bacteria (FIRDI, 1992; Huang, 1992). The mixed culture of CCRC 11847 + 10695 exhibited a slightly greater ability in acid production and reached a total organic acid concentration of 7.5 g/L at 48-h fermentation (Figure 5). HPLC analysis of organic acids (Gancedo and Luh, 1986) showed that about 80% of the acids harvested from soybean soaking water was lactic acid (Hsieh et al., 1995).

Test Beverage from Recovered Lactic Acid. Lactic acid has many applications in food and other industries. Methods of lactic acid recovery from fermentation broth have been extensively studied (Vickroy, 1985; Wang, 1991; Chaudhuri and Pyle, 1992; Eyal and Bressler, 1993; Reisinger and Marr, 1993). The most popular application is to make cheese and yogurt through lactic acid fermentation. Reportedly, lactic acid bacteria produced pleasant volatiles to mask the beany flavor in the soy milk fermentation (Mital and Steinkraus, 1979). Beverages containing organic acids and oligosaccharides or *in vivo* Bifidobacteria activation

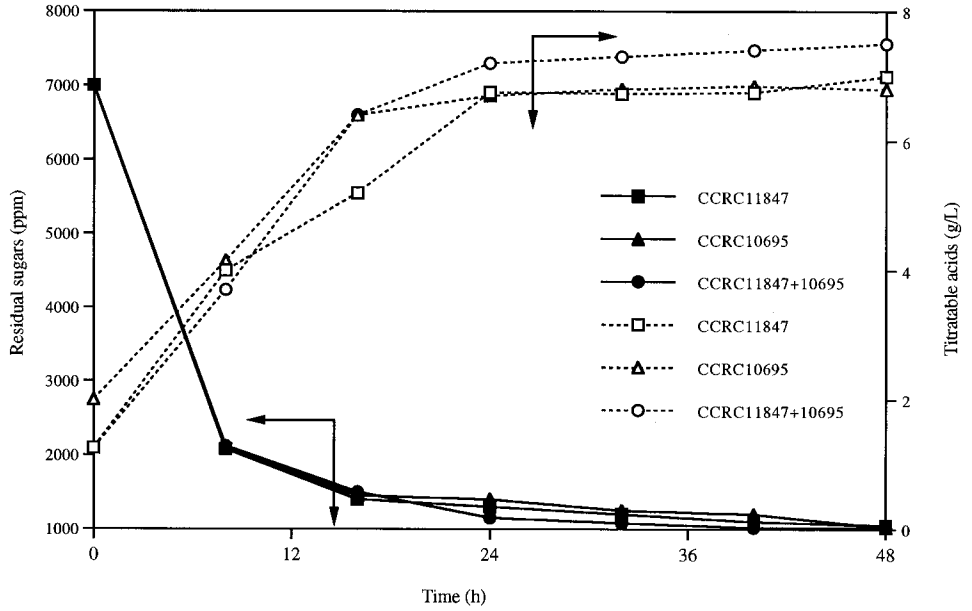

Figure 5. Changes of titratable acids and residual sugars during lactic acid fermentation using the recovered NF and RO retentates from soybean soaking water.

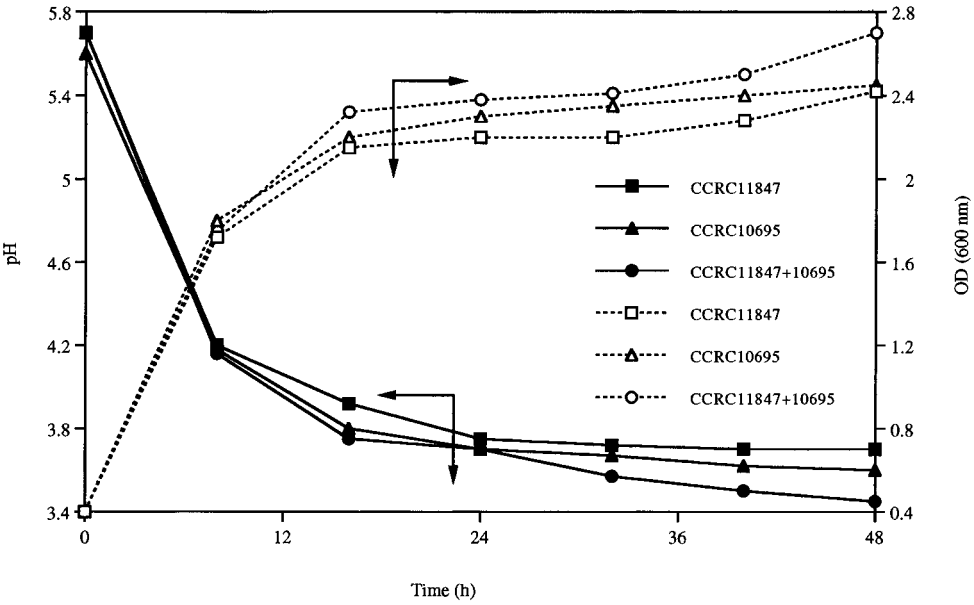

Figure 6. Changes of pH and optical density during lactic acid fermentation using the recovered NF and RO retentates from soybeansoaking water.

agents have gradually become popular in the local market. Thus, each lactic acid bacteria-fermented broth from the recovered solubles of soybean soaking water was formulated with corn syrup and flavorings to simulate a popular Bifidobacteria activation beverage available in the local market. Pair comparisons were conducted with the test and market samples to measure the acceptance level of the test. Results were analyzed from 25 replicates for each test beverage. The test beverages were as acceptable as the market sample ($p < 0.05$), having the acceptability scores of 7.6−8.2 out of a 10-point hedonic scale. Test beverage from the mixed culture of CCRC 11847 + 10695 had the least beany flavor and was rated first (best liked) among the three test samples.

Conclusions. A combined NF−RO membrane system was found efficient in separating solubles and recovering water for reuse from soybean soaking water. Lactic acid fermentation could be a feasible way to utilize the membrane recovered solubles. Selected probiotic strains, *L. acidophilus* CCRC 10695 and *B. longum* CCRC 11847, were both capable of converting the recovered solubles into lactic acid, which might be suitable for formulating lactic acid beverage. When both strains were equally mixed in the fermentation of the recovered retentates, the acceptance level of the test beverage was rated best over those prepared from the individual strains. However, further study should be conducted to investigate the conversion of the recovered solubles from the soybean soaking water to lactic acid by other probiotic strains and the addition of other nutrients to enhance fermentation efficiency.

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