CROSSFLOW FILTRATION OF BRINE FROM CUCUMBER FERMENTATION+

O. O. Fasina, H. P. Fleming, E. G. Humphries, R. L. Thompson, L. R. Reina

ABSTRACT. *Due to environmental concerns, pickle companies are considering ways of reclaiming the brine obtained from cucumber fermentation. The removal of microbial cells is crucial in use of the brine in finished pickle products. The effects of transmembrane pressure (41 to 166 kPa), feed flow rate (7.8 to 15.5 L/min), pore size (500,000 NWCO and 0.2 μm), and cell concentration (optical density of 0.171 to 1.170 at 640 nm) on permeate flux during the crossflow filtration of brine obtained from bulk fermentation of cucumber were studied. Results indicate that the microfiltration membranes exhibited a large flux decline during the first 15 min of operation when challenged with the fermentation brine. The net decline in permeate flux increased with transmembrane pressure, flow velocity, pore size, and cell concentration. Filtration through filter pore size of 0.2 μm or smaller effectively removed the microbial cells present in the brine. Only the transmembrane pressure significantly affected the resistance of the cake formed at the filter surface. From the results obtained from the study, it is possible to use microfiltration to filter sediments and microbial cells from brine obtained from cucumber fermentation.*

Keywords. *Pickle, Permeate, Microfiltration, Bacteria.*

Brine fermentation is an important method for temporary preservation of pickling cucumbers because of sensory traits desired in certain products, processing strategies, and economic reasons (Fleming, 1982; Fleming and Moore, 1983). Close to one–third of the total U.S. production (550,000 tonnes annually) is preserved by this method (Fleming et al., 1995).

Conventionally, 5 to 8% salt (NaCl) is used during fermentation, which lasts between 10 to 14 days. More salt is added after fermentation to make 12 to 16% brine solution as a precaution against spoilage during storage. The high salt concentrations are specifically used to prevent microbial and enzymatic softening (Bell and Etchells, 1961). Since only 1 to 4% NaCl is desired in finished pickled products, the excess salt is removed from the pickles during further processing. Desalting of pickles is usually carried out by continuous flushing or successive washings with freshwater (Bomben et al., 1974). The disposal of the wastewater from this flushing stage has created environmental problems within the pickle industry because many companies cannot meet the 230 ppm of chloride limit set by the U.S. Environmental Protection Agency for discharge of wastewater into freshwater bodies (Anonymous, 1987; Humphries and Fleming, 1989).

To alleviate the problem of brining and storage of pickles at high salt content, our laboratory conducted research on controlled fermentation of cucumbers in anaerobic tanks (4438 L), which demonstrated the potential for storage at 2.7 and 4.6% NaCl (Fleming et al., 1988). Our current research involves application of this previous procedure in 'bag–in–box' (~1200 L) type containers. Figure 1 shows the schematic diagram of the traditional fermentation process and the modified fermentation process (Fleming et al., 2002). The process was successfully tested at the pilot scale level (in 300–gal containers) in the summers of 1999 and 2000. In addition to low–salt fermentation, one of the goals of the process is to reuse or recycle the fermentation brine. Reclamation of the brine will require that microbial cells from the brine be removed by a separation process such as crossflow filtration.

In crossflow filtration, the suspension to be purified is forced tangentially through a filter with microporous walls (Redkar and Davis, 1993). The flow on the retentate side of the filter exerts a shear on the membrane surface. The shear force diminishes the amount of fouling or cake layer on the filter surface and, thus, increased filtration rate is obtained in comparison to conventional dead–end filtration systems (Forman et al., 1990; Zeman and Zydney, 1996).

Crossflow filtration is a viable alternative to centrifugation because centrifugation requires the existence of a suitable density difference between the two phases that are to be separated (Cheryan, 1998). Because of this advantage, crossflow filtration systems have been used in various

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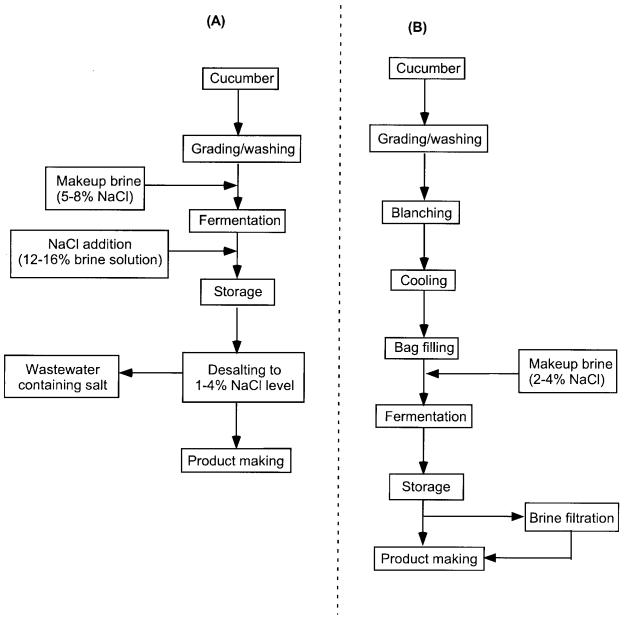


Figure 1. Flow chart of the traditional (A) and controlled (B) fermentation processes (Fleming et al., 2002).

biological applications such as in microbial cell harvesting and removal (Le and Atkinson, 1985; Caridis and Papathanasiou, 1997); the fractionation of cheese whey and the pre–concentration of milk for cheese manufacture (Rosenberg, 1995; Makardij et al., 1999; Jost and Jelen, 1997); in treatment of drinking and waste water; and in the clarification, concentration, and acidification of fruit juices and extracts (Cheryan, 1998).

To our knowledge, there is no documentation on the application of crossflow filtration to the removal of microbial cells from brine obtained from vegetable fermentation. Most of the applications of crossflow filtration in the fermentation industry involve cases where fermentation was carried out with a pure bacteria (Nagata et al., 1989; Tanaka et al., 1994; Caridis and Papathanasiou, 1997) or yeast culture (Patel et al., 1987; Redkar and Davis, 1993; Kavanagh and Brown, 1987). Merin et al. (1983) used microfiltration to remove microbial cells from concentrated NaCl brines (containing up to 106 CFU/mL of bacteria and 104 CFU/mL of yeasts and molds) used for the salting of cheese. Using a 0.2–μm pore size cartridge, the authors found that filtration resulted in a 3 to 4 log reduction of bacteria. Yeast and mold were not found in the permeate stream. The effectiveness of the crossflow filtration system was reduced when the cartridge pore size was increased beyond 0.2 μm.

About 10^6 to 10^8 CFU/mL consisting of lactic acid bacteria (Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus pentosaceous) and yeasts are usually found in brine from cucumber fermentation (Fleming, 1984; Daeschel and Fleming, 1981). In addition, the pH of cheese brine is about 5.1 to 5.3 in comparison to a pH of about 3.5 for pickle brine. The studies of Merin et al. (1983) were also carried out at a temperature of 37°C. For economical reasons, it is expected that filtration of pickle brine will be carried out at room temperature (~20°C) and at pH of 3.5.

The objectives of this study were (1) to investigate the effect of flow rate, transmembrane pressure, and membrane pore size on permeate flux during crossflow filtration of brine from cucumber fermentation, and (2) to compare the microbial load and chemical characteristics of resulting permeate to the unprocessed samples.

Materials and Methods

Brine used in this experiment was obtained by fermenting size 2B (33– to 38–mm diameter) cucumbers using the modified pickle fermentation procedure shown in figure 1. This procedure involved washing harvested cucumbers, blanching cucumbers at 82°C for 3 min., cooling the blanched cucumbers to room temperature (~ 23°C), filling the cucumbers (1400 lb) into a 300–gal (1162.5–L) bag, and adding fermentation culture (Lactobacillus plantarum) and brine into the bag. The make–up brine used to fill the bag consisted of the following ingredients: 4.4% NaCl, 118 mM of acetic acid, 40 mM $Ca(OH)_2$, and 26.7 mM $CaCl_2$. After brining the cucumbers, the bag was heat–sealed and fermented for 2 months. Thereafter, the fermented pickles were removed from the bag and the residual brine was used in this study. The residual brine was at a pH of 3.5 and contained 2.0% NaCl, 102 mM lactic acid, 64.2 mM acetic acid, and 5.8 mM malic acid.

Filtration Experiment

A schematic diagram of the laboratory–scale crossflow filtration system (model DC–10L, Amicon Co., Lexington, Mass.) used for the experiments is shown in figure 2. The unit consisted of a 20–L reservoir and a variable speed positive–displacement pump to pressurize and recirculate the feed solution. Pressure and flow rates were controlled by a pump, a ball–type back pressure valve and two pressure gauges attached to the inlet and outlet of the membrane filter. Flow rates were measured with a direct reading valved block–type flowmeter (model P–32462–00, Cole Parmer Instrument Co., Vernon Hills, Ill.) for the retentate and with a turbine–type flowmeter (model S–111, McMillan Co., Georgetown, Tex.) for the permeate. The readings from the permeate flowmeter were automatically sent to and stored in a PC computer via a DaqBook Data Acquisition system (IOtech, Inc., Cleveland, Ohio) every 15 s. The flux rate was expressed in liters per square meter per hour and was numerically equal to the ratio of the flow rate to the membrane filter area.

Two hollow fiber polysulfone filtration membranes with pore sizes of 500K NMWC (500,000 nominal molecular weight cut off, approximately 0.05 μm) and 0.2 μm were used for the filtration experiment (models UFP–500–E–6A and CFP–2–E–6A, A/G Technology Co., Needham, Mass.). The membranes were 63.5 cm in length and 3.2 cm in diameter and have a total filtration area of 0.28 m^2. The brine solution used in this study (with optical density of 0.171) was obtained from the pilot scale fermentation experiment described in figure 1.

Each experiment consisted of passing deionized water through the filter for 30 min, followed by passing brine solution through the filter for 45 min or until the flux remained nearly steady state. To maintain constant concentration of the feed solution that was pumped to the filter, flux versus time experiments were performed in a total recycle mode of operation where permeate and retentate

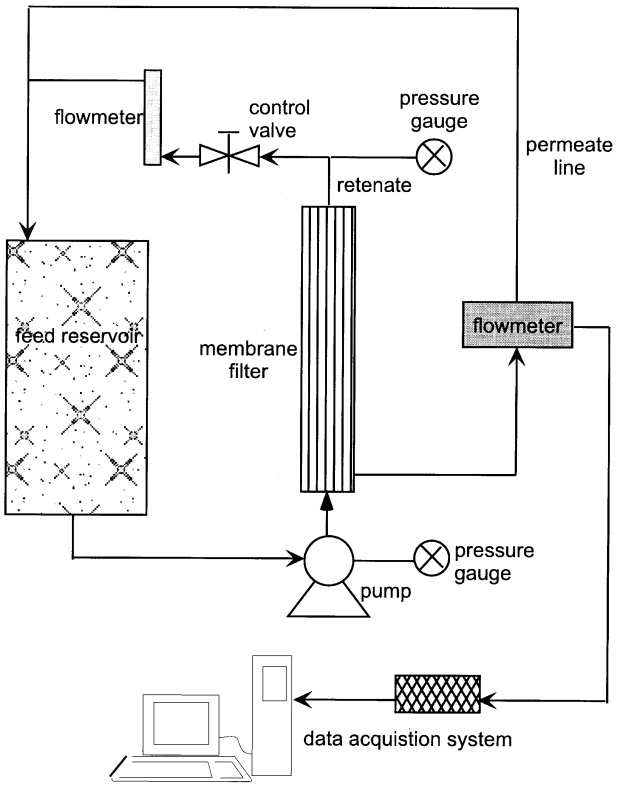


Figure 2. Schematic diagram of crossflow filtration process.

streams were returned to the feed reservoir, as illustrated in figure 2. All experiments were conducted at room temperature (~20°C). In between experiments, the filter was cleaned according to the recommendation of the manufacturer. To ensure that the membrane had been adequately cleaned, the flux with deionized water was checked under pre–selected operating conditions.

The operating conditions investigated were transmembrane pressure (TMP) (62 to 166 kPa for 500–K membrane, 41 to 103 kPa for 0.2–µm membrane) and feed velocity (7.8 to 15.5 L/min – 4.6×10^{-4} to 9.2×10^{-4} m/s). The high end of the operating conditions was chosen based on the manufacturer's recommendation for the maximum pressure/flow rate combination for the filter membranes. Preliminary experimentation was used to establish the minimum flow pressure/flow rate combination at which the brine could be filtered. Effect of feed concentration was tested with a 0.2–µm membrane operated at transmembrane pressure of 103 kPa and feed flow rate of 15.5 L/min. Brine samples with higher cell concentration were obtained by: (a) carefully collecting some of the cells at the bottom of the fermentation container (0.345 optical density); and (b) storing at room temperature and allowing the cells in the brine to multiply (1.170 optical density). Microbial and chemical analyses (see next section for methodology) were carried out on permeate samples collected from feed filtered at the following conditions: (1) membrane pore size of 500K NMWC, flow rate of 11.6 L/min, TMP of 166 kPa, and optical density of 0.171; and (2) membrane pore size of 0.2 µm, flow rate of 15.5 L/min., TMP of 103 kPa, and optical densities of 0.171, 0.345, and 1.170.

Microbial and Chemical Analyses

Procedures for enumeration of microorganisms were carried out according to Fleming et al. (1992). Media included standard methods agar (PCA, BBL Microbiology Systems, Cockeysville, Md.) for aerobes, violet red bile agar (BBL) + 1% glucose (VRBG) for Enterobacteriaceae and MRS broth (Difco Laboratories, Detroit, Mich.) + 1.5% agar + 0.02% sodium azide (MMRS) for lactic acid bacteria, and standard methods agar (BBL) + 0.1 mg/mL of chlortetracycline HCl + 0.1 mg/mL of chloramphenicol for yeasts. All pour plates were incubated at 30°C. Microbial colonies in VRBG plates were enumerated after 24 h, PCA and MMRS plates after 48 h, and YM plates after 72 h.

High–performance liquid chromatography (HPLC) analyses of organic acids and sugars were carried out by the procedures of McFeeters (1993). An Aminex HPX–87H column was used along with 3–mM heptafluorobutyric acid (Aldrich Chemical Co. Inc., Milwaukee, Wis.) as the mobile phase. Organic acids (malic and lactic acids) were detected with a conductivity detector (model CDM–2; Dionex Corp., Sunnyvale, Calif.), and sugars were detected by means of a refractive index detector. Data were collected and analyzed by using Chrom Perfect software (Justice Innovations, Inc., Mountain View, Calif.). NaCl was determined by titration with standard $AgNO_3$ using dichlorofluorescein as an indicator (Fleming et al., 1992).

Data Analysis

Several models (pressure–controlled, film–theory, resistance) have been proposed to describe the mechanism of transport through filtration membranes, but none are wholly satisfactory (Cheryan, 1998). The resistance model (eq. 1) is widely used and is based on the premise that fouling and concentration polarization add additional resistances to the membrane resistance (R_m).

$$J = \frac{3.6\times10^9 \Delta P}{\mu_p R_{total}} = \frac{3.6\times10^9 \Delta P}{\mu_p (R_m + R_g + R_f)} = \frac{3.6\times10^9 \Delta P}{\mu_p (R_m + R_c)} \quad (1)$$

where

J = flux (L per m^2 per h – LMH)
ΔP = pressure drop across the membrane (Pa)
µ = permeate viscosity (0.00104 Pa s – Fasina et al., 2002)
R_t = total resistance (m^{-1})
R_m = membrane resistance (m^{-1})
R_g = resistance due to concentration polarization (m^{-1})
R_f = fouling resistance at time t (m^{-1})
R_c = cake resistance, $R_f + R_g$ (m^{-1})

Membrane resistance (R_m) at any pressure drop across the membrane (ΔP) was calculated from the steady state flux (J_w) reached when only distilled water was passed through the filtration system.

$$R_m = \frac{3.6\times10^9 \Delta P}{\mu_w J_w} \quad (2)$$

where μ_ω is the viscosity of water (0.000911 Pa s – Fasina et al., 2002). The concentration polarization resistance (R_g) is a measure of the permeability and thickness of the cake

formed on the filter surface, and has been found to be a direct function of applied pressure (Cheryan, 1998).

$$R_g = \phi \Delta P \quad (3)$$

where ϕ is an empirical constant and represents the intrinsic resistance of the polarized layer and the resistance of the boundary layer (Chiang and Cheryan, 1986). The relation below is obtained when equation 3 is substituted into equation 1.

$$J = \frac{3.6\times10^{9}\,\Delta P}{\mu_p (R_m + R_f + \phi \Delta P)} \quad (4)$$

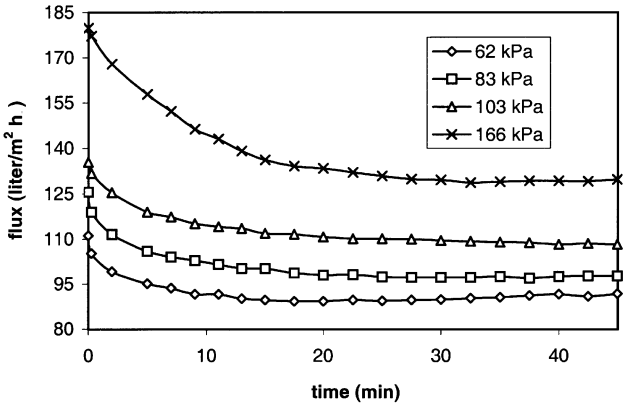
Parameters R_f and ϕ in equation 4 were estimated by means of the non–linear procedure (Sigma Plot Software, SPSS Inc., Chicago, Ill.) based on flux versus transmembrane pressure for each membrane. Statistical analysis was carried out using the analysis of variance procedure in SAS statistical software (SAS Institute Inc., Cary, N.C.).

Results and Discussion

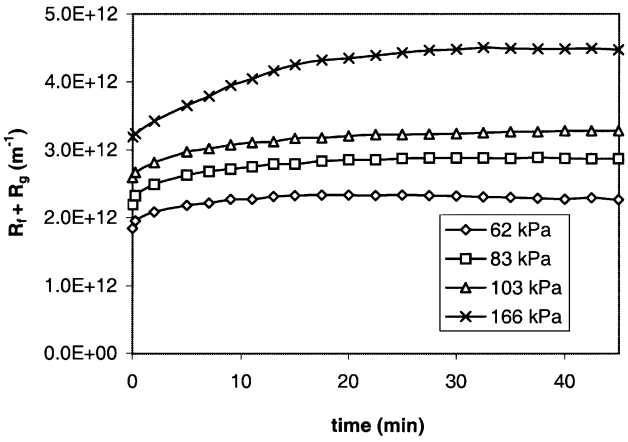
Filtration

Flux decline curves illustrating the effects of pore size, transmembrane pressure (TMP), cell concentration, and flow rate are given in figures 3a to 6a. In general, flux dropped significantly within the first 15 min of filtration and then continued to decline slowly. The net decline in flux increased with TMP, flow velocity, pore size, and cell concentration. This is similar to the results that have been reported on crossflow filtration of microbial cells (Taddei et al., 1990; Zahka and Leahy, 1985; Patel et al., 1987; Makardij et al., 1999). The decline in flux over the course of filtration is an indication that membrane fouling occurred during the filtration process.

As expected, resistance due to fouling and concentration polarization (eqs. 1 and 2) increased with TMP (figs. 3b and 4b) for the two filters used in the study. The increase in cake resistance with increase in TMP was caused by greater transport of particles to the membrane surface at higher applied pressures, which resulted in an increase in filter cake mass at the filter surface.

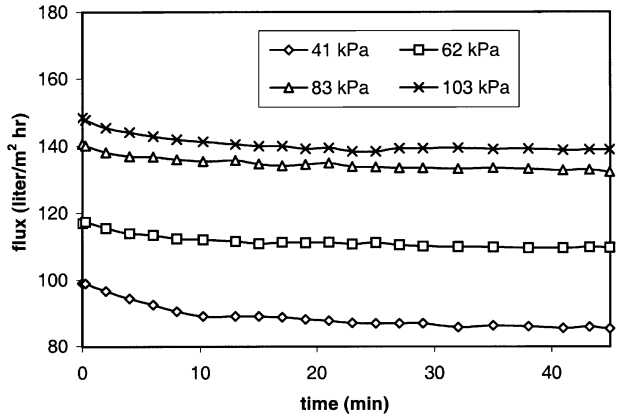


(a)

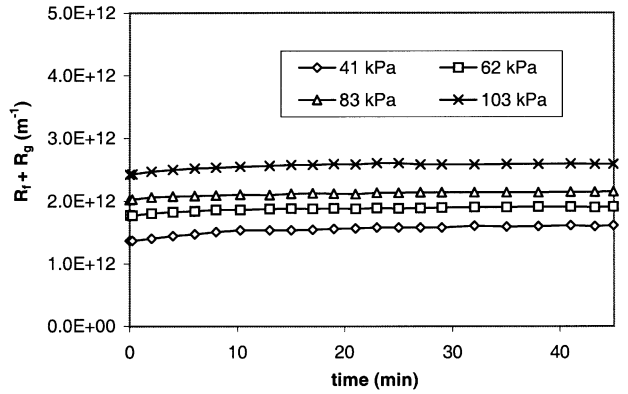


(b)

Figure 3. Flux decline curves and cake resistance at different transmembrane pressures for brine (OD = 0.171) filtered through 500,000 NMWC membrane. Flow rate was 11.6 L/min.



(a)



(b)

Figure 4. Flux decline curves and cake resistance at different transmembrane pressures for brine (OD = 0.171) filtered through 0.2–µm membrane. Flow rate was 11.6 L/min.

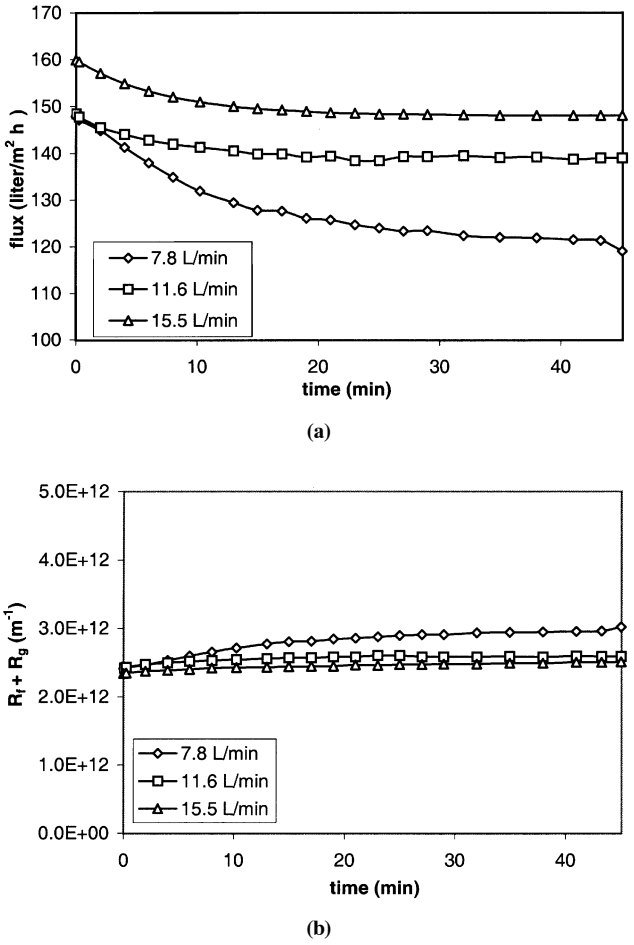


Figure 5. Flux decline curves and cake resistance at different flow rates for brine (OD = 0.171) filtered through 0.2–µm membrane. Transmembrane pressure was 103 kPa.

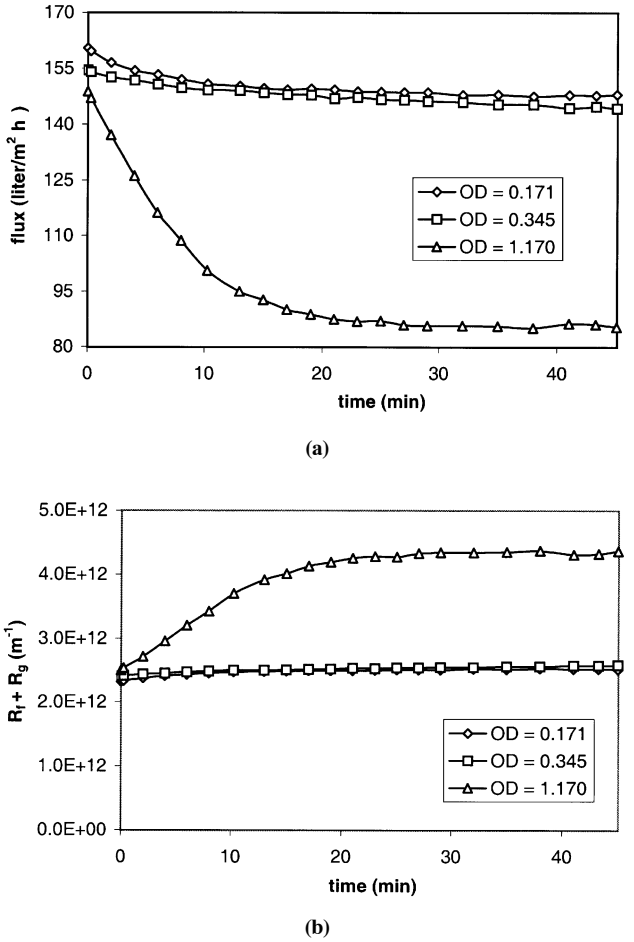


Figure 6. Effect of cell concentration on flux decline curves and cake resistance. Membrane pore size = 0.2–µm, transmembrane pressure = 103 kPa, flow rate = 11.6 L/min.

Figure 5b shows that the rate of cake formation on filter surface decreased slightly with increase in flow rate despite the increase in permeate flux with increased flow rate. Cheryan (1998) suggested that increased agitation at the filter surface is obtained when the flow velocity is increased. Accumulated solutes are therefore swept away from the filter surface thereby reducing the formation of the cake on filter surface.

Cake resistances obtained from brine samples with optical densities of 0.171 and 0.345 were similar (fig. 6b). The cake resistance of brine samples with an optical density of 1.170 was significantly higher than the other two brine samples. The brine with OD of 1.170 contained a significant amount of yeast (see results of microbial analysis). This high yeast concentration would not be found in brine samples obtained from properly fermented pickles, which have an expected optical density of 0.345 or less. It can be said that feed concentration will not significantly affect permeate flux when filtering brine obtained from pickles that were properly fermented.

Standard error of estimate and correlation coefficients obtained from the non–linear fit of equation 4 to experimental data on flux versus transmembrane pressure are given in tables 1 and 2. Parameter ϕ was estimated to be 1.88×10^7 and 1.55×10^7 m^2 s/m^3 for the 500K NMWC and 0.2–µm membrane filters, respectively. As expected, the individual resistances (R_f and R_g) obtained for the 0.2–µm filter were lower than those for the 500K NMWC filter. The lower the filter pore size, the more the amount of particles retained on the filter surface and the higher the cake resistance.

For both filters, fouling resistance (R_f) was not significantly ($P < 0.05$) affected by transmembrane pressure. When skim milk was ultrafiltered, Chiang and Cheryan (1986) also found that R_f was relatively independent of operating parameters (temperature, protein content, and feed velocity). The values of R_f in tables 1 and 2 are similar to those reported by Boyaval et al. (1996) during the crossflow microfiltration of a cell culture of lactic acid bacteria (*Lactobacillus leveticus*) with a cell concentration of 4×10^9 CFU/mL.

Table 1. Effect of transmembrane pressure on fouling parameters for brine (optical density of 0.171) filtered at 11.6 L/min through 500,000 NMWC membrane.

TMP (kPa)	J_{ss} (L m^2/h)	R_{total}[a] ($m^{-1}\times10^{-11}$)	R_m ($m^{-1}\times10^{-11}$)	R_g ($m^{-1}\times10^{-11}$)	R_f ($m^{-1}\times10^{-11}$)	R_{total}[b] ($m^{-1}\times10^{-11}$)
62	90.49	24.88	1.69	11.65	12.42	25.76
82	97.52	30.54	1.71	14.72	12.40	28.83
103	108.40	34.40	1.57	19.42	12.51	33.50
166	133.49	44.62	1.22	31.08	12.87	45.17

[a] Obtained from experimental data.
[b] Obtained from estimated values of R_m, R_g, and R_f.
Standard error of estimate (s.e.) = 3.97, R^2=0.97.

Table 2. Effect of transmembrane pressure on fouling parameters for brine (optical density of 0.171) filtered through 0.2–μm membrane. Flow rate of 11.6 L/min.

TMP (kPa)	J_{ss} (L m^2/h)	R_{total}[a] ($m^{-1}\times10^{-11}$)	R_m ($m^{-1}\times10^{-11}$)	R_g ($m^{-1}\times10^{-11}$)	R_f ($m^{-1}\times10^{-11}$)	R_{total}[b] ($m^{-1}\times10^{-11}$)
41	85.61	17.40	1.42	6.41	9.56	17.39
62	109.67	20.40	1.33	9.61	9.65	20.59
83	124.38	23.95	1.00	12.81	9.98	23.79
103	138.88	26.81	0.90	16.02	10.08	27.00

[a] Obtained from experimental data.
[b] Obtained from estimated values of R_m, R_g, and R_f.
Standard error of estimate (s.e.) = 1.12, R^2 = 0.99.

Membrane resistance (R_m) was largely unaffected by filtration conditions. The values of R_m that we obtained are typical for crossflow filtration membranes (Davis, 1992) and varied from 0.90×10^{11} to 1.71×10^{11} m^{-1}.

The results show that microfiltration with a pore size of 0.2 μm or lower can be effectively used to remove microbial cells and sediments in brine obtained from cucumber fermentation. The choice of membrane of size (i.e. 0.2 μm or 500K NMWC) will be dependent on the process economics with respect to energy consumption and desired process capacity. Either of the two membranes can be used to filter brine obtained from a cucumber fermentation process.

Permeate Microbial and Chemical Retention

The optical density of the permeate was not significantly affected by processing conditions. Average optical density of 0.003±0.001 was obtained, an indication that the permeate was free of microbial cells. Further microbiological analyses on selected permeate samples (table 3) showed that crossflow filtration effectively removed the microbial cells in the brine samples under the conditions tested in this study. In addition, the filtration process did not affect the chemical composition of the brine samples (table 4).

Conclusions

It can be concluded from this study that crossflow filtration using filters with pore size of 0.2 μm or lower can be effectively used to remove the microbial cells present in brine obtained from cucumber fermentation. Flux of the permeate from the membranes was affected by transmembrane pressure, feed velocity, pore size, and cell concentration while only the transmembrane pressure affected the resistance of cake at the filter surface. The chemical composition of the brine was not affected by the filtration process.

Table 3. Microbial load of retentate and permeate from filtration of brine.

	Retentate			Permeate[a]		
OD[b]	TA[c]	LAB[d]	YM[e]	TA	LAB	YM
0.171[f]	4.5×10^7	4.3×10^6	<2.3	<10	<10	<10
0.171[g]	1.35×10^8	2.5×10^7	<2.3	<10	<10	<10
0.345[g]	2.35×10^8	3.5×10^7	<2.3	<10	<10	<10
1.170[g]	5.4×10^8	5.3×10^7	2.5×10^2	<10	<10	<10

[a] Ten is the minimum detection level of the petri film used to count the number of microorganisms. No visible colonies were seen on the film.
[b] Optical density.
[c] TA – total aerobic bacteria count (cfu/mL).
[d] LAB – lactic acid bacteria count (cfu/mL).
[e] YM – yeasts and molds count (cfu/mL).
[f] Pore size of 500,000 NMWC, flow rate of 11.6 L/min, TMP of 166 kPa.
[g] Pore size of 0.2 μm, flow rate of 15.5 L/min, TMP of 103 kPa.

Table 4. Chemical composition of filtered and unfiltered brine.

Sample	Retentate[a]	Permeate[a]
Malic acid (mM)	5.8	6.2
Lactic acid (mM)	102.2	102.5
Acetic acid (mM)	64.2	64.3
Salt (%)	2.02	1.98

[a] Each value reported is the average for the samples listed in table 3.

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