BAG-IN-BOX TECHNOLOGY:
Membrane Filtration of Cucumber Fermentation Brine

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ABSTRACT

A major goal in producing process-ready, brined cucumbers is the reclaiming of the brine obtained from the fermentation for use in other products. This article summarizes research results obtained from the use of membrane filtration technology to remove microbial cells and other sediments from cucumber fermentation brine. The effects of process factors (filter pore size, flow rate, microbial cell concentration and pressure) on the rate of filtration are presented. In addition, a review of the applications of membrane filtration technology in food processing is given in the Appendix section of the article.

INTRODUCTION

A major benefit of producing process-ready, brined cucumbers, as proposed by Fleming et al. (2002), is the use of the entire container contents in finished products. The cucumbers are washed and blanched before they are conveyed into the bag. The brine components, including salt, are food-grade. The cucumbers are fermented by an added food-grade culture of lactic acid bacteria in a closed container, sealed from the environment. The low concentration of salt used does not require that it be leached from the fermented cucumbers before they are manufactured into finished products. Although the lactic acid content in the fermented cucumbers may be too high for certain products, strategies have been proposed for using the fermented cucumbers, as well as brine, in finished products (Johanningsmeier et al., 2002). Thus, the fermented brine is viewed as a valuable food component, rather than waste that must be treated before discharge into freshwater bodies, as in current technology.

For use of the fermentation brine in finished products, however, it must be clarified. This is because of cloudiness caused by cells of lactic acid bacteria used for fermentation. During fermentation, the bacterial cell population may reach over 1 billion cells per milliliter. It takes only about 10 million cells per milliliter to make the brine visually cloudy.

Various membrane separation processes have been used to separate components from liquids in the food industry, as is briefly reviewed in the Appendix of this paper. We chose to explore the potential of microfiltration/ultrafiltration for clarifying spent cucumber fermentation brines. We studied factors affecting the filtration of brine from one of our bag-in-box experiments. A mathematical model of the process was developed by Fasina et al. (2001).

MATERIALS AND METHODS

Brine

The brine of fermented cucumbers was obtained from experimental run no. 2 of the bag-in-box procedure (Fleming et al., 2002). Size 2B cucumbers (33-38 mm diameter) were washed, blanched, cooled, and transferred into a 300-gal bag (in box). The cover brine consisted of 4.4% NaCl (w/v), 118 mM acetic acid (as vinegar), 40 mM $Ca(OH)_2$, and 26.7 mM $CaCl_2$. Cucumbers occupied about 55% and brine 45% (w/v) of the contents of the bag. The brine-cucumber mass was inoculated with *Lactobacillus plantarum* MOP3 M6 (a culture that does not produce CO_2 from malic acid). After adding all components to the bag, the bag was heat-sealed and allowed to ferment/store at ambient temperature for 2 months. The cucumbers and brine were then removed from the bag. The cucumbers were processed into finished products by a commercial pickle company, and the brine was used for the current study.

Filtration System

A schematic diagram of the laboratory-scale, crossflow filtration system (model DC-10L, Amicon Co., Lexington, MA) used for the experiments is shown in Figure 1. The unit consisted of a 20-L reservoir and a variable speed, positive-displacement pump to pressurize and re-circulate the feed solution. Pressure and flow rates were controlled by means of 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, block-type flowmeter (model P-32462-00, Cole Parmer Instrument Co., Vernon Hills, IL) for the retentate and a turbine-type flometer (model S-111, McMillan Co., Georgetown, TX) for the permeate. The readings from the permeate flowmeter were automatically sent to and stored in a computer via a DaqBook Data Acquisition System

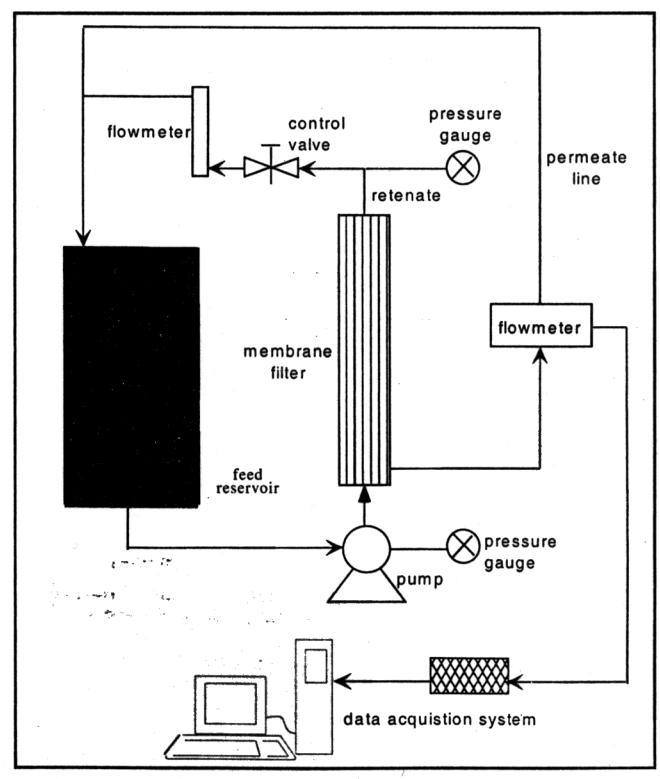

Figure 1. Schematic diagram of crossflow filtration system.

(IOtech, Inc., Cleveland, OH) every 15 sec. The flux rate was expressed in L per square m per hr (LMH) 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 cutoff) 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, MA). Each membrane was 63.5 cm in length and 3.2 cm in diameter, and had a total filtration area of 0.28 m^2. These membranes were chosen for our tests based on previous studies on removal of bacteria and yeasts from liquids (Merin et al., 1983; Nagata et al., 1989; Redkar and Davis, 1993).

Microbial and Chemical Analyses

General procedures for enumeration of microorganisms were carried out according to Fleming et al. (1992). High performance liquid chromatography (HPLC) analyses of organic acids and sugars were carried out by the procedures of McFeeters (1993). NaCl was determined by titration with standard $AgNO_3$ using dichlorofluorescein as an indicator (Fleming et al., 1992).

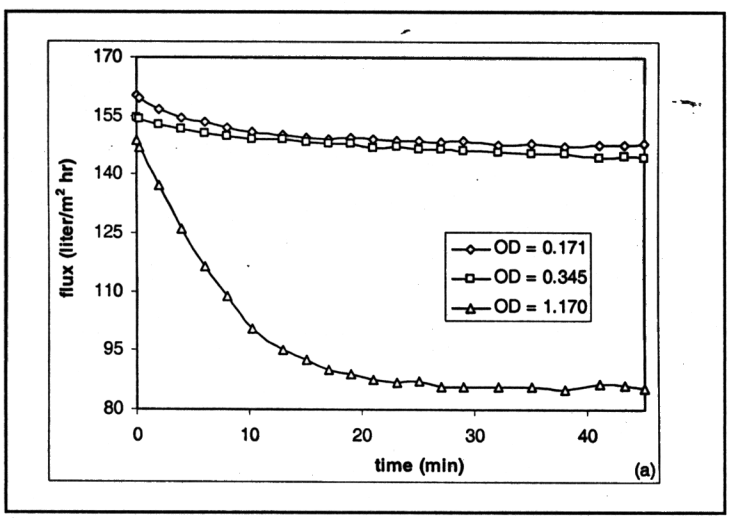

Figure 2. Flux decline curves of brine with different cell concentrations. Membrane pore size = 0.2 μm; transmembrane pressure = 103 kPa; flow rate = 11.6 L/min.

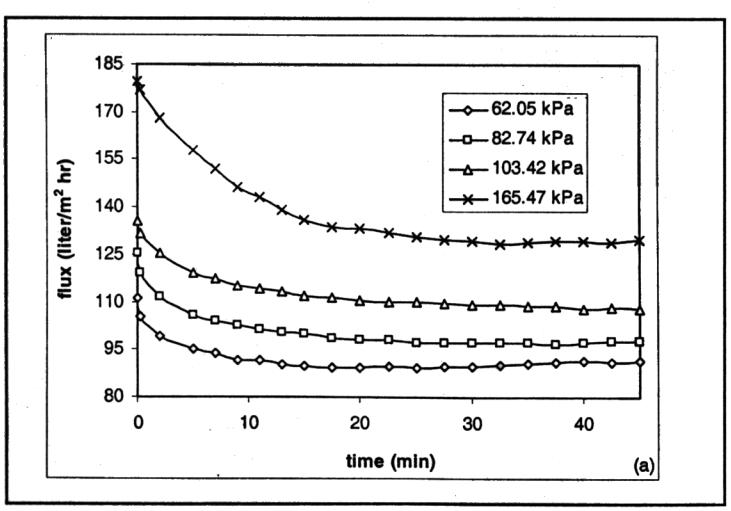

Figure 3. Flux decline curves at different transmembrane pressures for brine (OD = 0.171) filtered through 500,000 NWCO membrane; flow rate = 11.6 L/min.

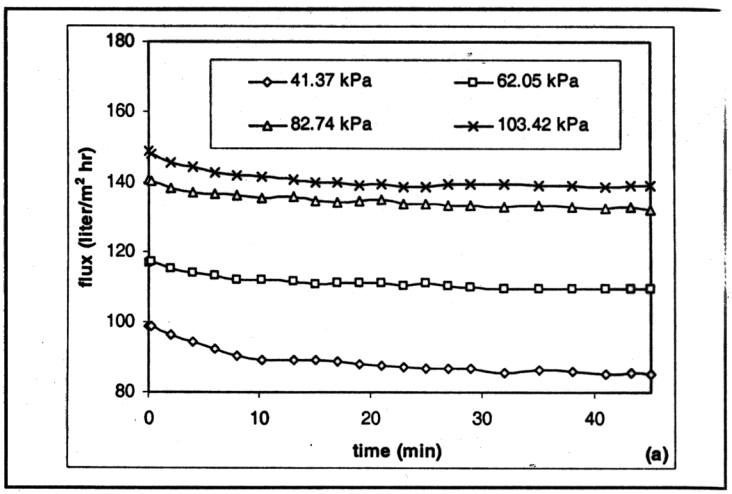

Figure 4. Flux decline curves at different transmembrane pressures for brine (OD = 0.171) filtered through 0.2 μm membrane; flow rate = 11.6 L/min.

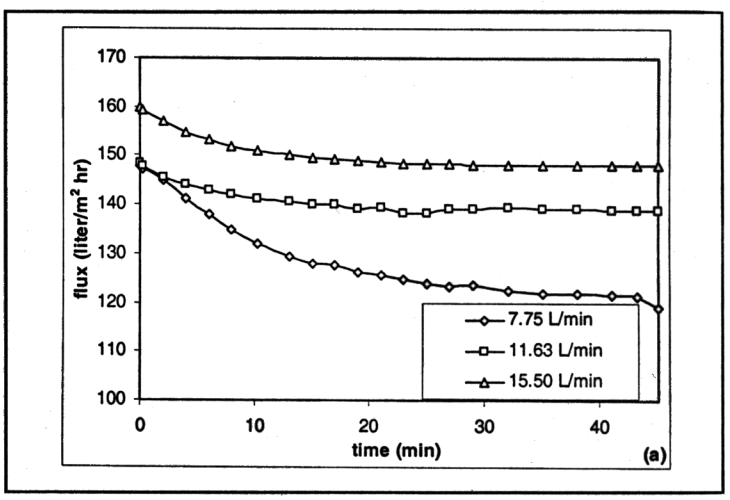

Figure 5. Flux decline curves at different flow rates for brine (OD = 0.171) filtered through 0.2 μm membrane. Transmembrane pressure was 103 kPa.

RESULTS AND DISCUSSION

Many factors may influence the rate at which cucumber fermentation brine is clarified by membrane filtration. Important factors, which we studied, are pore size of the membrane, pressure on the membrane (or transmembrane pressure, TMP), microbial cell concentration, and brine flow rate. In general, the brine flux dropped most significantly within the first 15 min of filtration, and then continued to decline slowly as illustrated in Figures 2-5. The decline in flux over the course of filtration is due to fouling or clogging of the panes of the membrane during the filtration process.

Cell density was a major factor influencing brine flux (Fig. 2). At OD = 1.170 (5.4 x 10^8 CFU/mL), flux decreased greatly within the first 15 min, compared to OD = 0.171 (4.5 x 10^7 CFU/mL) or OD = 0.345 (2.35 x 10^8 CFU/mL). This demonstrates the importance of reducing cell density in the brine before filtration. Simply allowing cells in the brine to settle may greatly facilitate filtration. Also, choosing a fermentation culture that readily settles can be helpful.

Pore size of the membrane greatly influences flux, as illustrated in Figure 3 (500,000 NWCO membrane) and Figure 4 (0.2 μm membrane pore size). The 0.2 μm membrane should be adequate for

filtering out bacteria and yeasts that are likely to be present in cucumber brines. Brine flux was greater at higher transmembrane pressure and higher flow rates (Figs. 4 and 5). In all cases, the brine flux over a 40-min period was very small (<25%). It seems that initial cell concentration is more influential on brine flux than transmembrane pressure. This is because, as the initial cell concentration increases, more solutes accumulate at the filter surface, thus reducing permeate flux.

Both pore size membranes tested were effective in removing bacterial and yeast cells from the brine (Table 1). The chemical composition of the brine was not significantly affected by filtration (Table 2). The filtered brine was crystal clear, with a light amber color (Fig. 6). The brine was used to acidify and flavor pickle products which were evaluated for sensory qualities (Johanningsmeier, 2002). Membrane porosities used in these studies are unlikely to remove enzymes, such as polygalacturonases responsible for cucumber softening. Membranes are available for such purposes, but likely would be less efficient in removing microbial cells. Such enzymes should not create a problem in pasteurized finished products.

Table 1. Microbial load of retentate and permeate from filtration of brine.

OD[1]	Retentate, CFU/mL[2]			Permeate,[2] CFU/mL		
	TA	LAB	YM	TA	LAB	YM
0.171[3]	4.5×10^7	4.3×10^6	<200	<10	<10	<10
0.171[4]	1.4×10^8	2.5×10^7	<200	<10	<10	<10
0.345[4]	2.4×10^8	3.5×10^7	<200	<10	<10	<10
1.170[4]	5.4×10^8	5.3×10^7	2.5×10^2	<10	<10	<10

[1]Optical density, 630 nm.

[2]TA = total aerobes, LAB = lactic acid bacteria; YM = yeasts and molds. The retentate was enumerated by Spiral Plating, with 200 CFU/mL being the minimum number for detection. The permeate was enumerated by Petri film, with 10 CFU/mL being the minimum number for detection.

[3]Pore size of 500,000 NWCO, flow rate of 11.6 L/min, TMP of 166 kPa.

[4]Pore size of 0.2 μm, flow rate of 15.5 L/min, TMP of 103 kPa.

Table 2. Chemical composition of filtered and unfiltered brine.

Chemical	Retentate	Permeate
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

Summary

It can be summarized from the study that: (a) crossflow filtration 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; (b) the chemical composition of the brine was not affected by the filtration process; and (c) flux of the permeate from the membranes was affected by transmembrane pressure, feed velocity, pore size, and cell concentration.

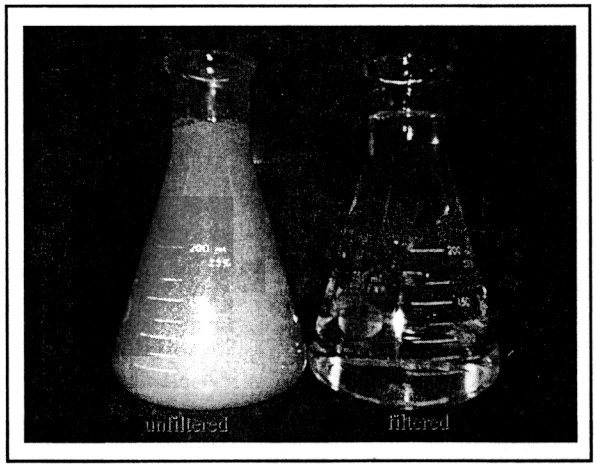

Figure 6. Picture of unfiltered and filtered brine. Filtered brine was crystal clear with a light amber color.

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APPENDIX

Membrane Technology and Food Processing

INTRODUCTION

The application of membrane technology in the clarification of brine obtained from cucumber fermentation was described in the main section of this paper. The intent of this Appendix is to provide a broader view of the application of various membrane separation methods in the food processing industry. Some of these other methods also may have application in the pickle industry. Membrane processes are generally used to concentrate or fractionate a liquid to yield two liquids that differ in composition. Some other industrial uses of membrane separation process include separation of mixtures of gases and vapors, miscible liquids (organic and aqueous/organic mixtures and solid/liquid and liquid/liquid dispersions), and dissolved solids and solutes from liquids (Rosenberg, 1995).

The feature that distinguishes membrane separations from other separation techniques is the provision of another phase, the membrane. This phase, either solid, liquid, or gaseous, introduces an interface(s) between the two bulk phases involved in the separation, and gives the advantage of efficiency and selectivity. Transport of selected species through the membrane is achieved by applying a driving force (pressure difference, temperature difference, electrical potential gradient and concentration/activity gradient) across the membrane (Field, 1996; Scott and Hughes, 1996).

In a membrane separation process (Fig. 1A), the feed mixture is separated into a retentate (that part of the feed that does not pass through the membrane—i.e., is retained) and a permeate (that part of the feed that passes through the membrane).

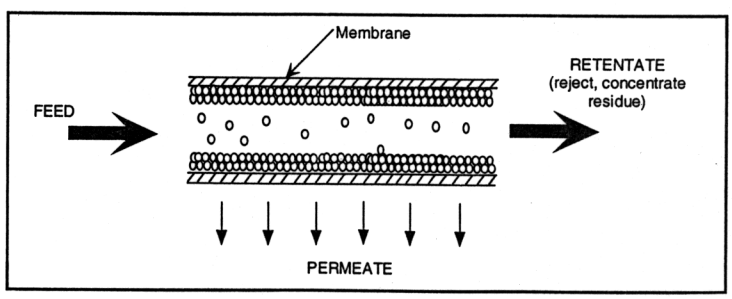

Figure 1A. Schematics of a general membrane process (Davis, 1992).

Membrane Separation Types

The most common types of membrane separation processes are microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO),

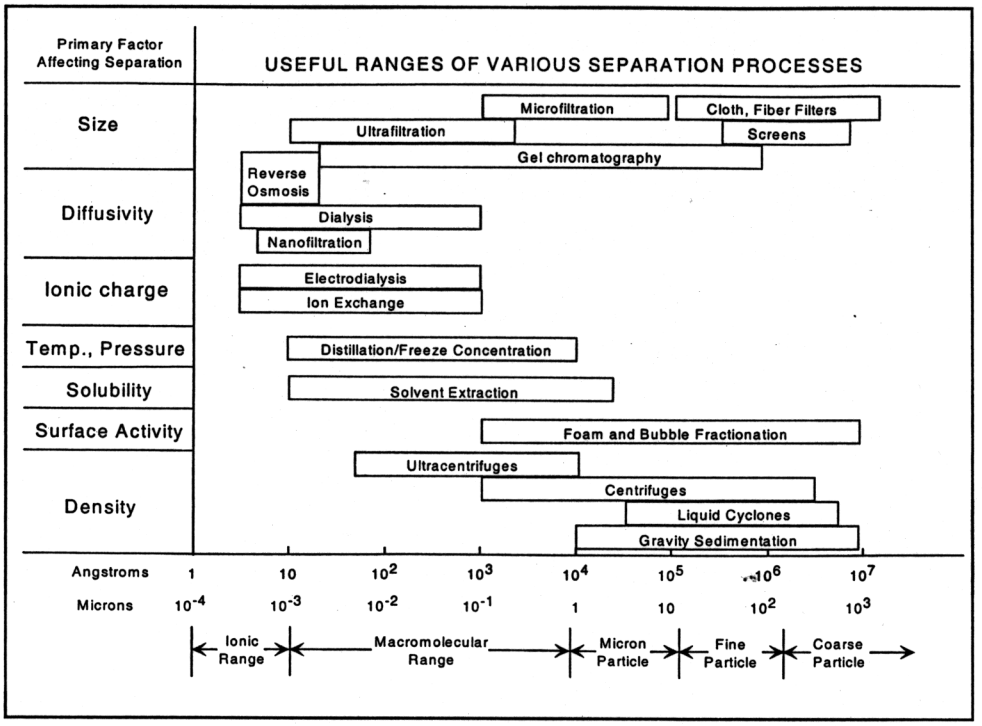

Figure 2A. Comparison of membrane separation processes to other separation processes (Cheryan, 1998).

Table 1A. Features of membrane separation process (Strathmann, 1992).

Membrane separation process	Driving force	Application
MF	Hydrostatic pressure difference of 50-100 kPa	Separation of suspended materials
UF	Hydrostatic pressure difference of 100-1,000 kPa	Concentration, fractionation, and cleaning of macromolecular solutions
RO/NF	Hydrostatic pressure difference of 1,000-10,000 kPa	Concentration of components of low molecular weight

Table 2A. Features of membrane modules (Strathmann, 1992; Humphrey and Keller, 1997; Cheryan, 1998).

Item	Membrane module			
	Hollow fiber	Spiral wound	Plate and frame	Tubular
System cost ($/m^2)	1,000-1,500	300-1,000	500-1,000	2,000-6,000
Membrane replacement cost ($)	300-600	40-100	50-100	~1,000
Energy consumption (W/m^2)	80-700	40-130	-	700-2,000
Membrane area per volume (m^2/m^3)	~15,000	800-1,000	400-600	20-30
Resistance to fouling	Poor	Moderate	Good	Very good
Ease of cleaning	Poor	Fair	Good	Excellent

nanofiltration (NF) and dialfiltration (DF). Other types of membrane separation processes are pervaporation, dialysis, liquid membranes, gas separation, electrodialysis, and membrane distillation. The discussion in this review will be limited to MF, UF, RO, NF, and DF since they are the most common types of membrane separation processes employed in food processing operations.

MF is used for clarification and sterile filtration in a wide range of industries, including the food and biochemical industries. Membranes for MF typically have pore size of 0.1 to 2 μm and can selectively separate particles with molecular weights greater than 200 kDa. UF involves the use of membranes with a molecular weight cutoff in the range of 1-200 kDa. UF is used to remove particles in the size range of 0.001-0.02 μm. The principal application of UF is in the separation of macromolecules with size retention in the molar mass range of 300 to 300,000. MF, in combination with UF, can solve almost any problem involving particulate material and macromolecules. The market areas for UF are in the food and dairy industries, biotechnology, water purification, and effluent treatment.

RO is used to separate ionic solutes and macromolecules from aqueous streams. Unlike MF, the mechanism of separation is based not only on size but on shape, ionic charge, and interactions with the membrane itself. RO membranes can, therefore, essentially separate all solute species (both inorganic and organic) from solution. The particle size range for applications of RO is approximately 0.0001 to 0.001 μm (1-10 A). Complete separation is possible with solutes of molar masses greater than 300 Da. NF is similar to RO except that it is operated at a lower pressure than RO. NF is used when high sodium rejection is not needed, but where salts with divalent ions (e.g., Mg and Ca) are to be removed. The molecular weight cutoff for NF membrane is around 200. DF is used to improve the recovery of membrane-permeable solutes during UF or MF (Scott, 1996). The process consists of diluting the concentrate, usually with water, and

continuation of the separation process until a satisfactory extent of solute removal is achieved (Rosenberg, 1995). Features of the different membrane separation processes are given in Table 1A.

The useful ranges of membrane separation processes in relation to other separation processes are given in Figure 2A. Roughly speaking, MF has the capability of performing separations equivalent to those obtained in a high speed centrifuge (5,000-10,000 g). UF is equivalent to ultracentrifugation (10,000-100,000 g). Since centrifugal forces are not capable of separating ions from water, there is no equivalent for RO and NF (Porter, 1997).

Membrane Separation Efficiency

The efficiency of membrane technology is quantified by the amount of fouling that occurs during process operation. Fouling is the decline in flux (volume of permeate obtained per hr per square m of membrane area) with time of operation. The factors that affect the extent of fouling can in general be categorized into equipment-dependent factors and operating factors. The equipment-dependent factors include membrane design, membrane properties, pore size, and shape. Some characteristics and costs of some membrane module design characteristics are given in Table 2A. The major operating variables are transmembane pressure, temperature, concentration of solute(s), feed type, and turbulence provided on membrane surface (i.e., use of crossflow in an industrial module). See Dr. Cheryan's book (Cheryan, 1998) on UF and MF for an excellent review of the role of these factors on the efficiency of the membrane separation process.

Advantages and Limitation of Membrane Technology

When versatility is considered, centrifugation is the only method that can match membrane technology. Two factors that have limited the widespread use of centrifugation in the food industry are: (1) the existence of a suitable density difference between the two phases that are to be separated, and (2) the two phases must be immiscible. Membrane separation processes have no such requirements. Membrane separation processes, therefore, permit separation of dissolved molecules down to the ionic range, provided the appropriate membrane is used (Cheryan, 1998). Other advantages of the membrane separation process include.

(1) **Ambient temperature operation.** Separation using membrane technology are often used to reduce the water content of liquid products. Conventional de-watering processes such as evaporation and freezing require a change in phase or state of the solvent during the de-watering process. This requires that the product be heated (in case of evaporation) or frozen (in the case of freezing). This is often not needed in membrane separation processes and, thus, the thermal, oxidative, and texture degradation problems common to evaporation, freezing, and other de-watering processes are avoided.

(2) **Absence of phase change.** As mentioned previously, membrane processes during de-watering do not require phase change. This has a direct impact on de-watering cost. For example, evaporation requires energy input of about 1,000 BTU/lb (2,259 KJ/kg) of water evaporator, while energy input of 144 BTU/lb (325.5 KJ/kg) is needed during the freezing of water. Membrane separation processes generally require energy inputs of less than 33.2 BTU/lb (84 KJ/kg). Apart from savings in energy requirements and costs, no complicated heat transfer and heat-generated equipment are needed for membrane separation processes. Membrane operation requires only electrical energy to drive the pump motor (Cheryan, 1998). A comparison of energy requirements and cost between evaporation and membrane technology for four processes are given in Table 3A.

(3) **Separation selectivity.** Membranes can be produced, which in many cases, can be designed and manufactured to be selective for the components to be separated.

Some factors that have limited the usage of membrane technology include difficulty in obtaining more than one pure product, inability to obtain substantial savings in cost when the process is scaled up, and membrane fouling.

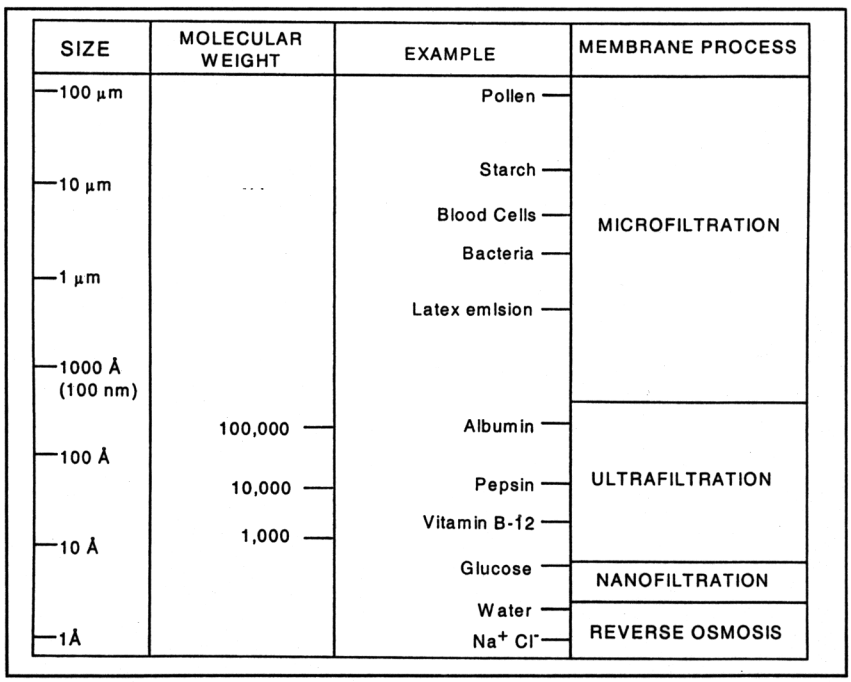

Figure 3A. Typical examples of solutes separated by membrane processes (Porter, 1997).

Industrial Applications of Membrane Technology

Improvements and advances in membrane technology over the last two decades have expanded the application of membrane technology in many industrial sectors such as chemical, food, petrochemical, pharmaceutical, biotechnology, pulp, paper, electronics, and water. This has put membrane separations in competition with other physical methods of separation. Therefore, membrane technology is now used in numerous applications from medicine to wastewater treatment. Examples of representative commercial uses for membrane processes are given in Figure 3A and Tables 4A and 5A.

Table 3A. Comparison of energy requirements and costs between evaporation and membrane technology (Cheryan, 1998).[1]

Process	Evaporation	Membrane technology
Whole milk (2.2x)	577.4 KJ/kg (MVR)	71.1 KJ/kg (RO)
Cheese whey (3x)	$380,000/yr (double effect)	$130,000/yr (RO)
Corn steep liquor (6-50% TS) - 300 gpm	$1.2 million/yr (MVR)	$390,000/yr (RO to 14% TS, then MVR)
Gelatin (2-18% TS) - 20 tons/hr	$516,200/yr (4 effect)	$186,750/yr (UF)

[1]Type of evaporation process in parentheses. MVR = multiple vapor recompression; TS - total solids; 2.2x = material concentrated 2.2 times.

Table 5A. Sales of membrane industry (million U.S. $) in 1990 (Strathmann, 1992).

Application	MF	UF	RO	Others	Total
Medicine	20	130	-	900	1050
Water treatment	310	60	120	60	490
Food industry	95	44	15	15	169
Chemical industry	35	15	10	140	200
Biotechnology	-	-	-		20

Table 4A. Industrial applications of membrane separation processes

RO and NF

- Recovery of freshwater from seawater and brackish water
- Treatment of wastewater to remove a wide variety of impurities
- Treatment of surface and ground water
- Recovery of sugars in food processing
- Concentration of milk and whey for cheese production
- Removal of alcohol from beer and wine

UF

- Concentration and recovery of latex particles from wastewaters
- Concentration and fractionation of proteins
- Separation of wax components from lower-molecular-weight hydrocarbons
- Recovery of vaccines and antibiotics from fermentation broth
- Clarification of fruit juice and brine

MF

- Removal of micron-sized particles from a wide variety of liquid streams
- Concentration of fine solids
- Separation of mammalian cells from a liquid
- Clarification and biological stabilization of beverages
- Removal of bacteria
- Fractionation of milk proteins

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ABOUT THE COVER:

Bulk storage in brine has been an economic means of extending the processing season of pickling cucumbers since before the 1930's (1). When larger sizes of cucumbers began to constitute a higher proportion of the crop in the 1960's, bloater formation resulted in buoyancy force sufficient to rupture tank heading timbers (2), but purging of CO_2 from the brine reduced bloater damage and buoyancy forces within the tank (3). However, use of high concentrations of salt in brine storage requires washing of the excess from the brine-stock before conversion to finished products, which requires the use of aeration ponds to biodegrade the organic matter (4), but still results in problems in the handling of salt and other non-biodegradable wastes. The use of fiberglass and polyethylene tanks (5) has reduced salt leakage that was prominent with wooden tanks (1-3), but relatively high salt concentrations are still used to serve as insurance against vagaries of nature due to tanks being open to the atmosphere. Closed tanks have been considered by the industry (6), but various factors have resulted in modernized brine yards of open-top, fiberglass and polyethylene tanks and a waste handling system (7). This issue of the journal is devoted largely to summarizing efforts to design and test a pilot system (8) for preserving "process-ready," brined cucumbers with improved quality and reduced wastes, and with intended benefits to the producer and processor of pickling cucumbers.

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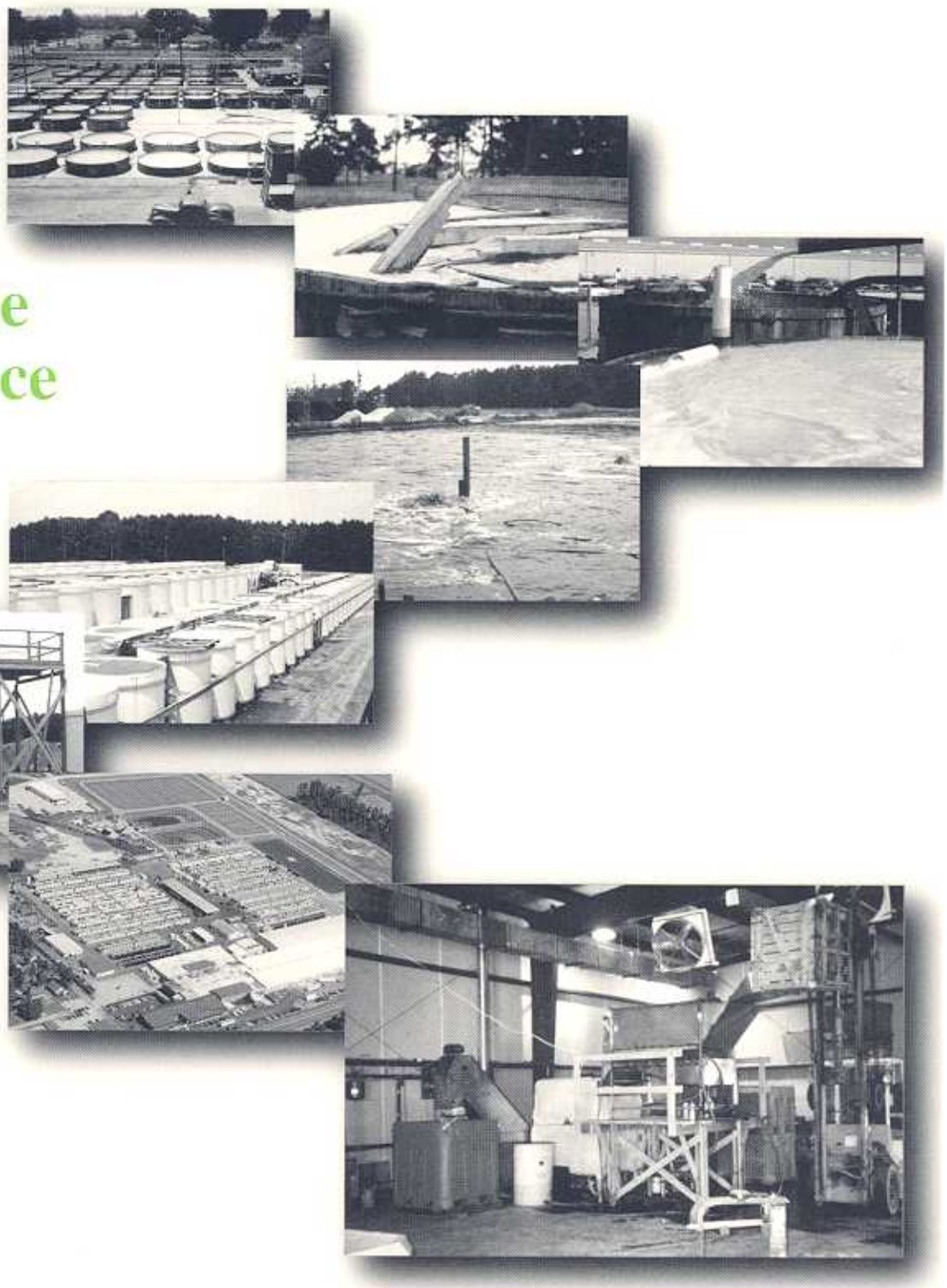
SCIENCE

Bulk Storage in Brine Since the 1930's



A journal reporting research relating to brined, salted and pickled vegetables and fruit.