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Downstream Processing of Lactic Acid-Whey Permeate Fermentation Broths by Hollow Fiber Ultrafiltration

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Index Entries: Cell harvesting; ultrafiltration; *Lactobacillus bulgaricus*.

INTRODUCTION

Fermentation processes are able to produce a broad range of chemical and biological products. One major limitation to the commercial application of biotechnology and fermentation is downstream processing. The primary downstream step is the separation of the microorganisms from the rest of the bioreactor constituents. The complexity and unique physicochemical properties of fermentation broths make separation of their components sometimes difficult. Not only do they contain a mixture of soluble and insoluble materials, but they also have a high water content and low specific gravity (*1*). The difficulty is enhanced when the desired product is present at low concentration and/or degrades easily at extreme temperature and pH (*2*).

The most common separation techniques are centrifugation and dead-end filtration, which have some limitations (*3,4*). Crossflow mem-

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brane filtration offers a number of advantages over conventional techniques and has been used to harvest microbial cells (*3–9*) and to increase productivity of fermentation processes (*10–12*).

The objective of this research was to evaluate the applicability of crossflow filtration for the continuous harvesting or separation of bacterial suspensions. The model system was a lactic acid fermentation broth with *Lactobacillus bulgaricus*, a homofermentative lactic acid bacteria, suspended in whey permeate, which is a byproduct of the ultrafiltration of cheese whey. Factors affecting the performance of the system, such as transmembrane pressure, velocity, and cell concentration, were investigated with a view toward minimizing fouling and concentration polarization effects and determining optimum operating conditions.

MATERIALS AND METHODS

Membrane Module

The membrane system used for these experiments was a pilot size hollow fiber ultrafiltration unit (Romicon Inc., Woburn, MA). The membrane cartridge's specifications are given in Table 1. The membrane was placed in a test loop consisting of a 50 gal-steam jacketed holding vat (the feed tank), a centrifugal pump, pressure gages, valves at the inlet and outlet of the module, flowmeters to measure flow rate (recycling rate) and permeate flux, and a thermocouple to measure temperature.

Experimental Design

The performance and fouling characteristics of the hollow-fiber membrane ultrafilter were investigated at several levels of cell concentration, velocity, and transmembrane pressure. Fouling and performance

Table 1
Hollow Fiber Module Specifications

Module designation	HF06-49-PM50
Membrane material	Polysulfone
Number of fibers	600–660
Length of cartridge	27 cm
Outside diameter of cartridge	7.6 cm
Potting material	Epoxy
Effective area	.56 m^2
Fiber inside diameter	1.2 mm
Maximum operating pressure	172.4 kPa
Maximum operating temperature	75°C
Nominal molecular weight cut off	50000
pH range	1–13

studies were conducted at 30°C and at constant feed concentration with both the permeate and retentate recycled back to the feed tank. Under these conditions, flux decline would be a result of membrane-solute interactions. For the concentration experiments, only the retentate was recycled back to the feed tank.

Flux (J), a measure of the filtration capacity of a membrane module, is expressed as volume of permeate per unit membrane surface area per unit time or liters per square meter per hour (LMH).

Membrane Cleaning

After each experiment, the membrane was flushed and backflushed with water at room temperature for 30–45 and 15 min, respectively. Following the water flush, an enzyme detergent solution (Tergazyme, Alconox Inc., NY) was circulated at 50°C for 5–10 min. The membrane was soaked in this solution overnight. After rinsing out the enzyme solution with warm water, the cleaning efficiency was checked by measuring the water flux under standard conditions. The cleaning procedure was repeated if necessary.

Fermentation Media

The media used in these experiments was reconstituted whey permeate powder (WPP, modified whey solids, Express Foods, Inc., Louisville, KY). It is the dried mineral/lactose fraction from the ultrafiltration of liquid whey. Its composition is (per 100 g): 79–81 g lactose, 8–10 g ash, 1–2 g moisture, and 2.5–4.5 g total nitrogen.

Organism

A selected strain of *Lactobacillus bulgaricus* was obtained from Chr. Hansen's Laboratory, Milwaukee, WI. The culture was first grown in 1 L of presterilized media containing 150 g/L WPP and 10 g/L yeast extract (Difco Lab, Detroit, MI). This seed culture was used to inoculate a 4-L batch, which was then used as seed for a 200-L batch. The 200-L batch media, containing 150 g/L WPP and 2–5 g/L yeast extract, was pasteurized at 63°C for 30 min, cooled to 45°C, and inoculated with the 4-L batch culture. The fermentations were done at 45°C, the optimum temperature for the bacteria, and the pH was controlled at pH 5.6 (± 0.2) using a pH controller (Model 5997-20, Horizon Ecology Co., Chicago, IL).

To obtain concentrated cell cultures, several cycles of fermentation-ultrafiltration-refermentation were conducted until the required cell density was obtained. Other cell concentrations were obtained by diluting this concentrated cell culture with prepasteurized media containing 150 g/L WPP.

RESULTS AND DISCUSSION

Water Flux

The water flux of the hollow fibers increased linearly with transmembrane pressure and was unaffected by feed flow rate. This relationship can be described by the Hagen-Poiseuille model for flow through channels (*3*). Water flux also increased with temperature. At 30°C and 1 atm pressure, the water flux was 244 LMH.

Fouling Studies

Flux of a solution or suspension is usually much lower than the pure water flux for several reasons, among them a change in feed solution properties, concentration polarization, and membrane fouling (*3*). Membrane fouling is manifested by a decline in flux with time. It differs from concentration polarization in the sense that fouling is an irreversible and time dependent process. It is probably a result of specific interactions between the membrane and fermentation broth components.

Our fouling studies were conducted under steady operating conditions, i.e., both the retentate and the permeate were recycled back to the feed tank in order to keep the bulk concentration constant. Therefore, any flux decay will be caused by membrane fouling. The fouling process is often assumed to follow a first-order reaction and can be expressed using power-type function, such as (*13*)

$$J_t = J_1 t^{-b} \quad (1)$$

where J_t is the flux at time t in *LMH*, J_1 is the flux at time $t = 1$ h, and b is the fouling index. The larger the b value, the greater the fouling rate.

Figure 1 shows typical fouling of the hollow fiber membrane by the lactic acid fermentation broth that was made up by using unclarified whey permeate as the media. The flux dropped quickly initially and then approached a quasisteady state over the next 20–24 h. In general, the higher the cell concentration, the lower the flux. Similar results were obtained with other combinations of velocity and concentration and different lots of WPP (*14*). The fouling index (b values) for this set of runs at a velocity of 2.1 m/s was 0.02–0.03, and J_1 was 51 LMH at 10 g/L and 43 LMH at 40 g/L cell concentration (*14*).

In order to clarify the contribution of the cells per se versus the rest of the broth components to the fouling phenomenon, similar fouling studies were conducted with the media itself with no cells. As seen in Fig. 2, the whey permeate media did show some fouling tendencies. This was surprising since whey permeate should contain only lactose, salts, and nonprotein nitrogen since it is the "permeate" from the ultrafiltration of sweet whey. Thus, little or no fouling was expected by whey permeate since all of its components should have been freely permeable. The effect of velocity on fouling by whey permeate is especially dramatic.

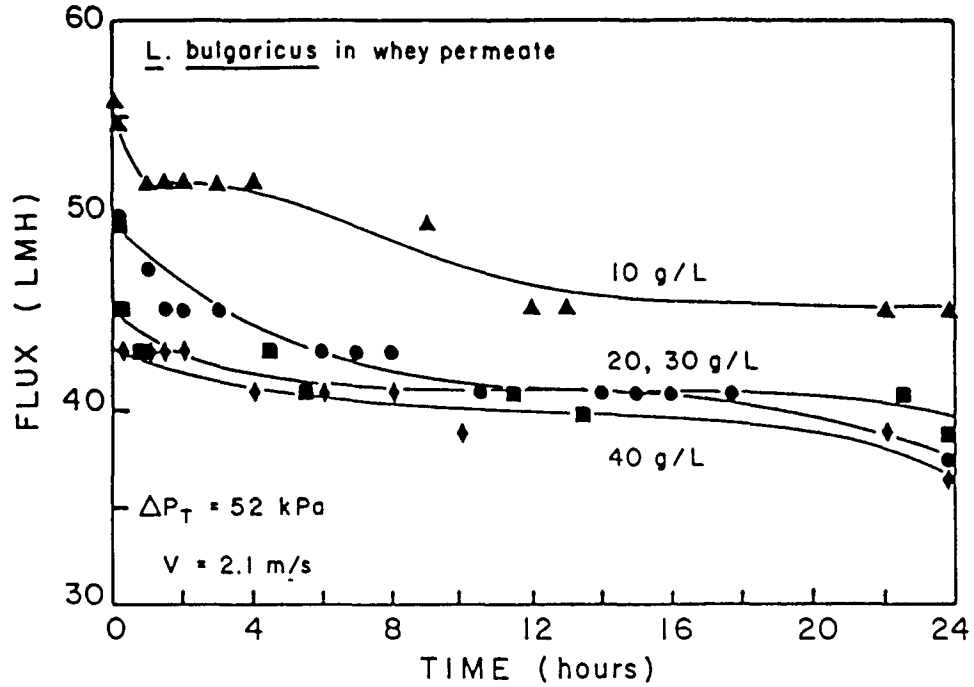

Fig. 1. Fouling of hollow fibers by *L. bulgaricus* fermentation broth: effect of cell concentration. The transmembrane pressure (ΔP_T) was 52 kPa and the velocity (V) was 2.1 m/s.

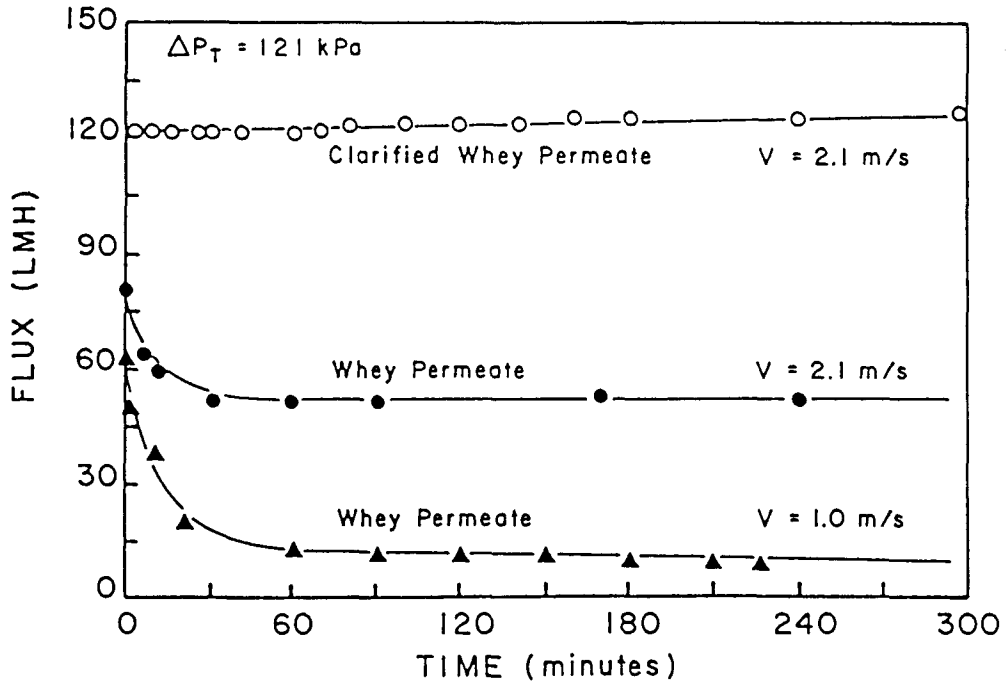

Fig. 2. Effect of velocity on fouling by whey permeate and clarified whey permeate. Cell concentration = 0.

The fouling index (b) is typically 0.44–0.48 at a velocity of 1 m/s, whereas it is 0.02–0.09 at 2.1 m/s. Higher velocities reduce the rate of fouling because fouling layers are removed by higher shear stress.

It appears that commercial WPP contains some suspended matter that is not permeable and fouls the membrane, or at least causes severe polarization effects. To confirm this, the whey permeate feedstream was "clarified" of the suspended matter by ultrafiltration and the fouling study repeated. As shown in Fig. 2, the remarkably stable and high flux obtained was a clear indication that the whey permeate powder obtained from commercial sources contains some fouling components of a macromolecular or colloidal nature. Comparing the fouling parameters (b and J_1 values) with and without cells, it appears that the media components have almost as much of an effect on fouling rates as the cells.

Performance Study

Figure 3 shows the flux–transmembrane pressure relationship for our system. The data were taken when the system had reached an apparent steady state (after 24 h of operation). Concentration polarization phenomenon is exhibited by this system, in that the permeate flux initially increases with pressure but becomes independent at higher pressures. Similar curves were obtained at all cell concentrations studied (*14*).

Figure 4 shows the effect of velocity (expressed as Reynolds number)

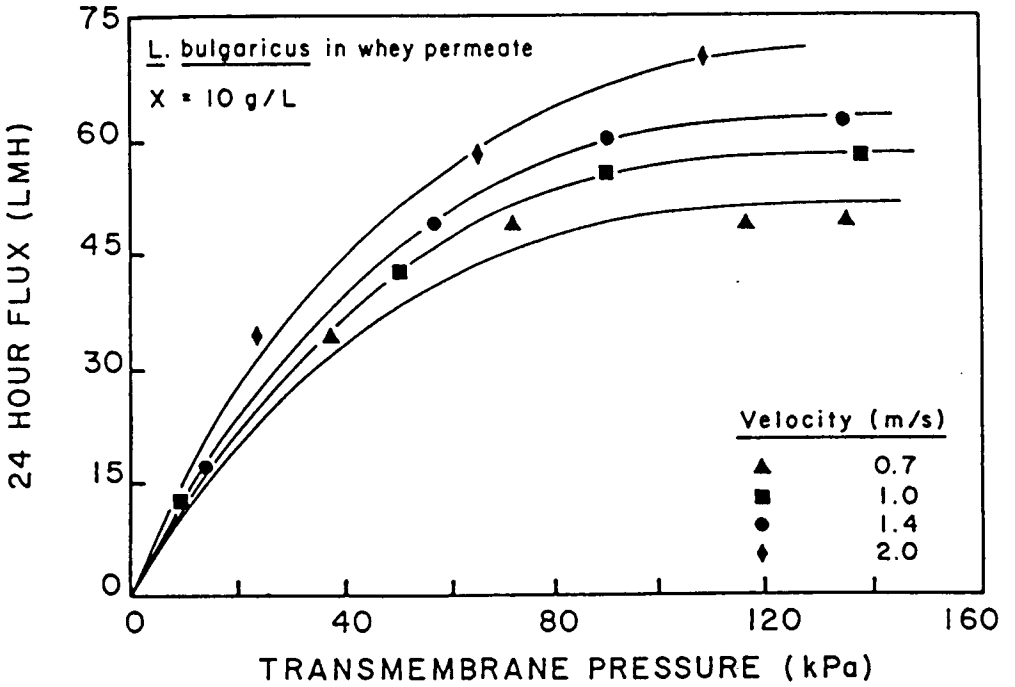

Fig. 3. Effect of transmembrane pressure on flux at a cell concentration of 10 g/L. Variable is linear velocity in m/s.

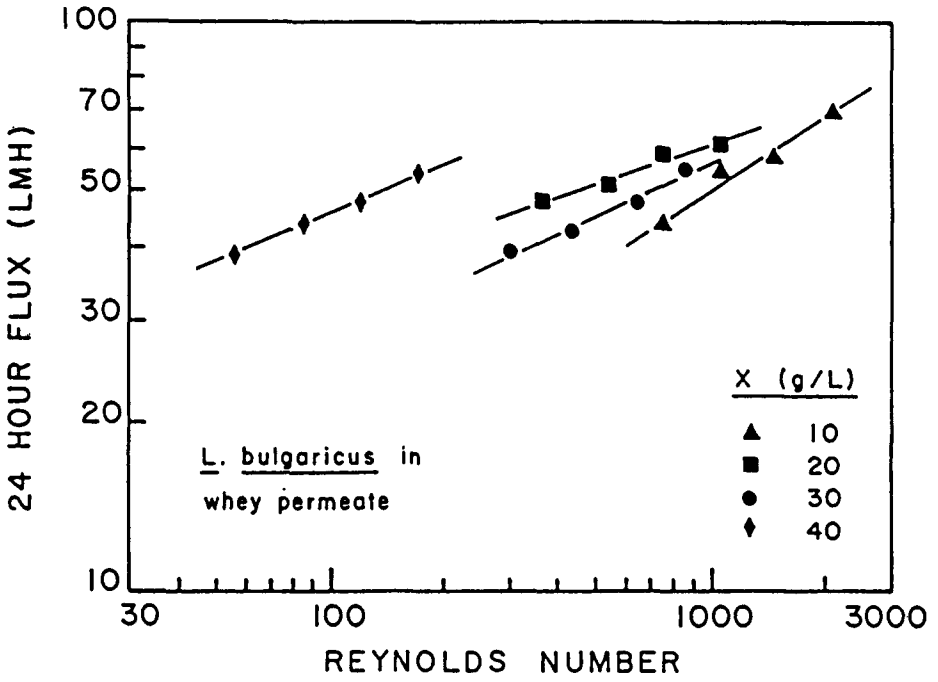

Fig. 4. Effect of turbulence (expressed as Reynolds number) on flux.

on permeate flux. The hollow fiber system was operated under laminar flow conditions, since the Reynolds numbers were between 50 and 2500. The data can be represented by an equation of the form

$$J = A(Re)^{a}(Sc)^{\beta} \ln (C_b/C_g) \quad (2)$$

where *Re* is the Reynolds Number, *Sc* is the Schmidt Number, and A, a, β are constants depending on the state of turbulence and development of the velocity profile. C_b is the bulk concentration of retained solids in the feed stream and C_g is the theoretical maximum concentration of the retained solids. For a particular cell concentration at a constant temperature, we can simplify the model to

$$J = A'(Re)^{a} \quad (3)$$

The value of a varied from 0.25 to 0.34 (Table 2). In theory, for a lami-

Table 2
Values of Constants (a and A') in the Flux Model (Eq. [3])

Cell concentration, g/L	a	A'
10	.34	.72
20	.25	1.05
30	.31	.85
40	.26	1.16
Average	.29	.95

nar flow system such as the hollow fibers, the value of a should be 0.33–0.50, depending on the state of development of the velocity profile (*3*).

Concentration of L. bulgaricus Fermentation Broth

The effect of cell concentration on permeate flux is shown in Fig. 5 for one transmembrane pressure and four velocities. As cell concentration increased, the permeate flux declined rapidly in a semilogarithmic manner. Extrapolating these lines to zero flux gives an average C_g value of 800 g/L. However, in practice, such high cell concentrations result in sharp increases in viscosity (Fig. 6) that would make pumping difficult and would require pressures greater than the burst pressure of the hollow fibers. Our studies (*14*) indicate that cell concentrations in this hollow fiber system would be limited to about 100–150 g/L before the flux became too low to be economical.

Membrane Deterioration

In our studies, the original water flux was never fully regained even with rigorous cleaning procedures using 0.1*N* NaOH or 25% ethanol. Figure 7 shows that after 300 h of use, the water flux was only about 80% of its "new" value. Sims and Cheryan (*5*) also observed a gradual decay in membrane water flux with the Membrana microporous tubular membrane that had been used for harvesting *Aspergillus niger*, perhaps a re-

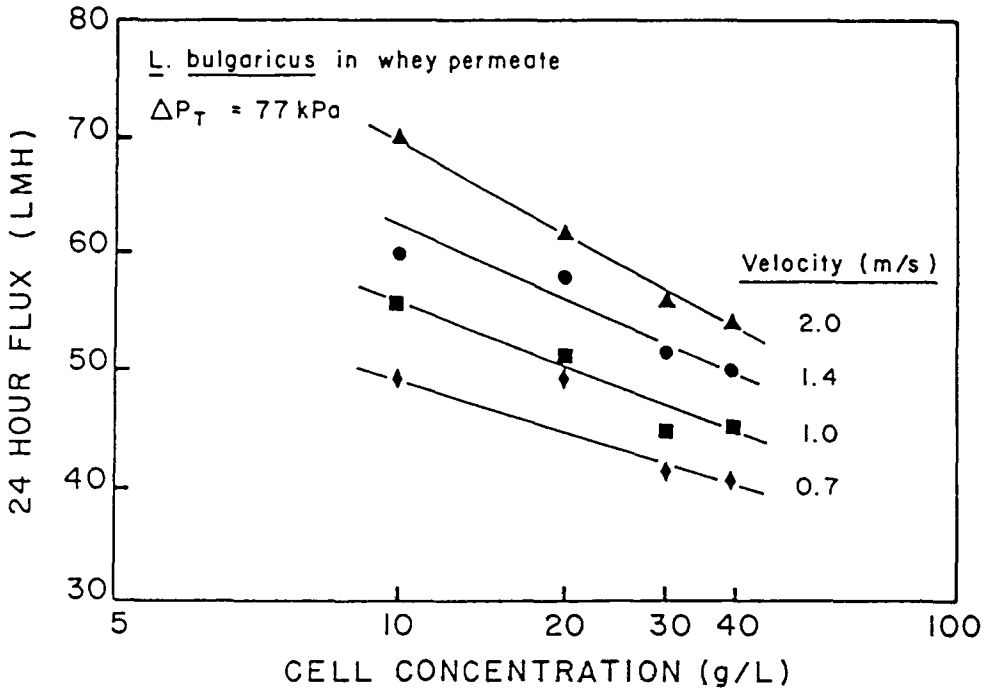

Fig. 5. Effect of cell concentration and velocity on flux (ΔP_T = 77 kPa).

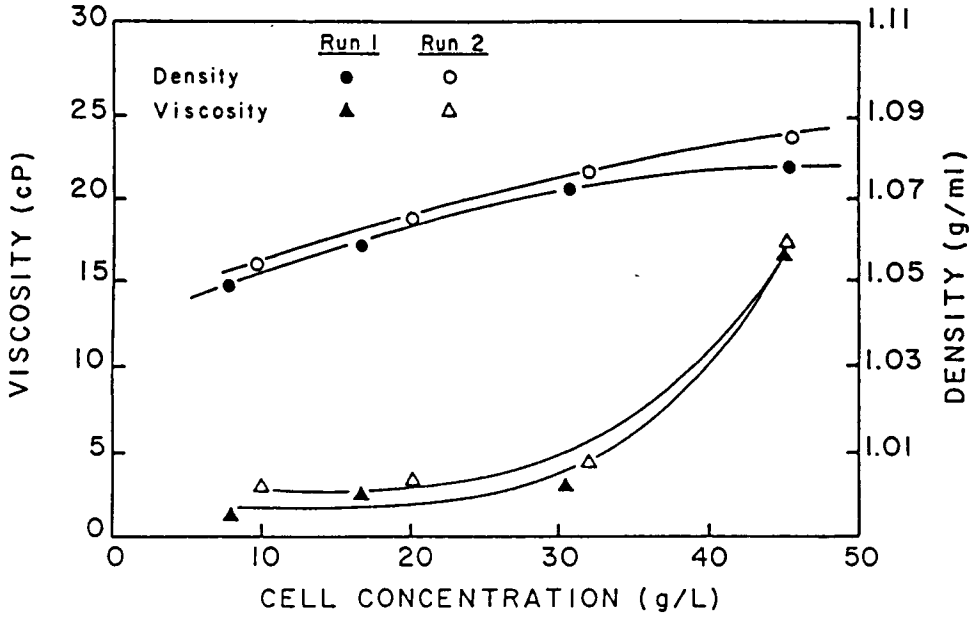

Fig. 6. Effect of cell concentration on viscosity and density at 30°C. Viscosity was measured by the Hoeppler falling ball viscometer.

sult of long-term exposure to acidic conditions. This apparent change in the intrinsic permeability of the membrane affected the performance with our fermentation broth. For example, after 300 h of use, the fermentation broth flux had declined by almost 50% under otherwise identical operating conditions (*14*). This deterioration must be considered when designing a crossflow membrane system for harvesting of *L. bulgaricus*.

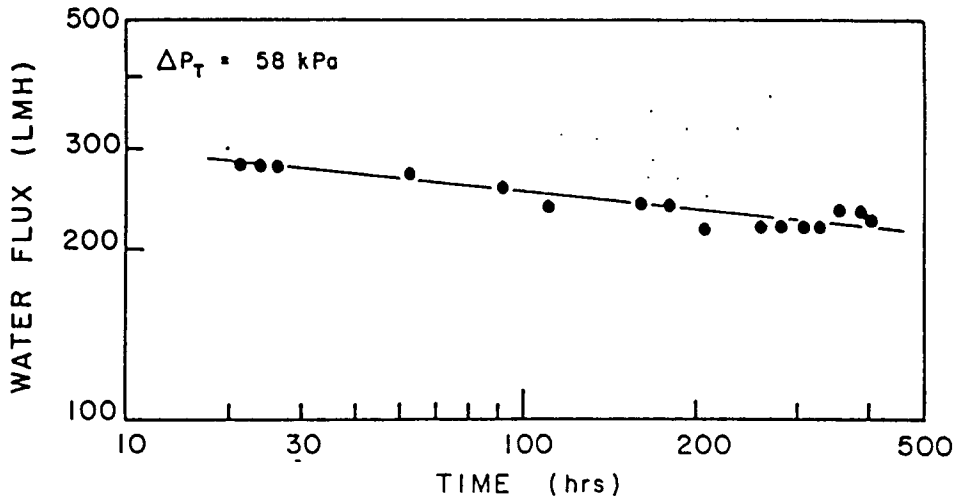

Fig. 7. Decline in hollow fiber membrane performance with use. Each point represents the water flux of the cleaned membrane after the indicated hours of processing the *L. bulgaricus* fermentation broth. Water flux was measured at 30°C and 58 kPa.

CONCLUSIONS

Membrane filtration is a suitable method for cell harvesting and clarification of fermentation broths. Hollow fiber ultrafilters gave essentially 100% rejection of *L. bulgaricus* cells from a whey permeate fermentation broth. A combination of low pressures and high velocity generally gave the best permeate flux. Fermentation media components (in this case, from the whey permeate) contributed significantly to fouling. Considering the pressure limitations of the current generation of asymmetric hollow fiber modules and the changes in physical properties of the fermentation broths, a cell concentration of 100–150 g/L could be obtained with the flux still relatively high (above 20 *LMH*), although the chemical compatibility of the membrane module itself under long-term exposure to high acid conditions should be considered.

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