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Flux Limiting Factors in Cross-flow Ultrafiltration of Invertase through an Asymmetric Inorganic Membrane

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ABSTRACT

Here the flow versus pressure characteristics of 0.1 to 5% w/w aqueous solutions of invertase, with a molecular weight of 270,000 dalton, are studied when they are tangentially filtered at 298 K through an inorganic asymmetric membrane of nominal pore radius 0.02 μm, with pressures going from 5 to 100 kPa, while the recirculation speed in the retentate loop is kept constant at 0.48 m/s. In such conditions, all these solutions are totally retained. The mass transfer coefficient is calculated, within the frame of the film layer theory for the concentration polarization phenomenon, by studying the volume flow as a function of concentration for several constant pressures in the above mentioned range. For low applied pressures, the concentrations giving zero volume flows can be interpreted as corresponding to osmotic pressure differences that balance the applied ones, and a power dependence of these pressures on concentration is proposed. For higher pressures the zero flow concentration is almost constant, probably due to a predominant gelification process.

INTRODUCTION

Ultrafiltration is amenable to both continuous and batch operations and offers several advantages over more traditional separation methods. For example, because there is no heat added, ultrafiltration is suitable for heat labile substances. In addition, the products are not subject to the chemical denaturation which can occur with solvent extraction (1, 2).

Therefore, ultrafiltration is being used increasingly as a concentration and purification process of macromolecular solutions. The soft treatment of the retentate is of special interest when dealing with labile proteinic

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enzymes; for example, invertase, whose concentration without denaturalization can be used as a step in saccharose hydrolyzation enzymatic reactors.

Once a macromolecular species is totally retained, high fluxes should be convenient for increasing the efficiency of an ultrafiltration application. Nevertheless, it is known that for high pressures the permeability decreases until a more or less flat plateau is reached. Hence, the volume flow cannot increase over a certain limit.

This reduction in flux has been attributed to the phenomenon of concentration polarization, i.e., the build up of rejected solute in the boundary layer near the membrane surface. The mechanisms by which flux reduction occurs have been variously thought to be: 1) a reduction in driving force, resulting from the increased osmotic pressure at the membrane surface; 2) the formation of a gel which offers hydraulic resistance in addition to that of the membrane; or 3) a fouling process.

The fouling caused by physical or chemical adsorption can be avoided or limited if the solute–surface interactions are minimized [for example, by controlling the pH level (3)] in order to decrease the membrane–protein affinity with subsequent modification in the protein activity.

Another way to obtain low fouling levels is to use a filtration device provided with a retentate chamber with a tangential flow. Thus, a high enough speed through the recirculation loop of the retentate should minimize the mean contact time of the protein on the solid surfaces and clean the membrane surface by also reducing the fouling due to pore clogging.

Here we will use a relatively high recirculation speed without changing the pH level (constant and almost neutral at 7.4).

Especially high fluxes can be obtained with inorganic membranes. Here we will use an alumina asymmetric filter made by Anopore whose nominal pore size is 0.02×10^{-6} m. The porosity of this filter is 50% according to the manufacturer. Here, this membrane will be called A002.

Our aim is to study the flow versus pressure characteristics of 0.1 to 5% w/w aqueous solutions of invertase whose molecular weight is 270,000 dalton. The volume flow against applied transmembrane pressure behavior will be analyzed when the protein solutions are tangentially filtered through the A002 membrane, with pressures going from 5 to 100 kPa, while the recirculation speed in the retentate loop is kept constant at 0.48 m/s.

The mass transfer coefficient will be calculated, within the frame of the film layer theory for the concentration polarization phenomenon, by studying the volume flow as a function of concentration for constant transmembrane pressures. The relevance of both the osmotic pressure and gelification processes will be analyzed.

EXPERIMENTAL

Materials and Experimental Setup

In order to avoid any irreversible change during operation, the membrane has to be pressurized at the highest pressure to be used for a sufficient length of time. Here, the asymmetric A002 filters were pressurized at 100 kPa for 45 minutes because pressurization for longer time periods gave the same results.

Invertase (EC 3.2.1.26) with an isoelectric point of 3.4 (4) and containing up to 40% glucose was obtained from Fluka and prefiltered through an A002 filter until all the glucose was eliminated according to a Fehling essay.

Aqueous solutions of this purified invertase were prepared at concentrations of 0.1001, 0.2003, 0.4010, 0.7997, 1.0023, 2.0010, and 4.9888% w/w. All concentrations were measured by using the Lowry-Folin assay (5) with a spectrophotometer set at 750 nm.

Distilled, degasified, and deionized (resistivity higher than 18 MΩ·cm) water was used. Its pH was kept constant at 7.4 by using HNa_2PO_3: H_2NaPO_3 at 8.1×10^{-3} N:1.9×10^{-3} N as the buffer, while NaN_3 at 0.02% w/w was added as a bactericidal agent.

The solutions were tangentially driven over the membrane and recirculated with a speed of 0.48 m/s, while the average transmembrane pressures were 5.2, 10.3, 15.4, 25.3, 35.3, 45.3, 55.4, 65.3, 75.5, 85.4, 95.2, and 102.5 kPa.

All the experiments were performed under isothermal conditions at 298 K by using a tangential ultrafiltration device that has been described elsewhere (6).

The solution was extracted from a thermostated reservoir by means of a regulatable impulsion pump. Two pressure transducers were placed before and after the membrane holder in the retentate loop. They have a range of 0–1000 kPa relative to the atmosphere and give a maximum error of ±0.25% full scale. Given that the permeate loop is open and the pressure loss along the hydraulic channel is small and almost linear, the transmembrane pressure can be taken as the average of the values given up and down the membrane cell.

In order to measure the retentate flow, an electromagnetic flowmeter was used. Its range is 1×10^{-6}–1×10^{-5} m^3/s, with errors lower than ±0.25% full scale. The speed and pressure in the retentate loop are independently controlled by means of pump regulation and a needle valve.

The membrane cell is made from methacrylate and is provided with four prismatic channels of $1.0 \times 5.25 \times 28.0$ mm on the membrane, whose

hydraulic diameter is $d_h = 1.68 \times 10^{-3}$ m, giving an effective membrane area of 9.00×10^{-6} m^2 and a total retentate loop cross-section of 5.25×10^{-6} m^2.

The permeate flux is measured by timing and weighting with a high precision balance with errors lower than $\pm 1 \times 10^{-7}$ kg.

Volume Flow and Permeability

The volume flow per unit of exposed area of the membrane, J_v, was measured as a function of transmembrane pressure for pure water (with the pH buffer and bactericidal agent) and gave a hydrodynamic permeability of 1.2047×10^{-8} m/Pa·s. J_v was also measured for all the solutions used. The concentration of the permeate is zero, giving total retention of the invertase.

The hydrodynamic permeability was measured after permeating the more concentrated solution, i.e., with a 5% w/w content of invertase, and rinsing the membrane with pure water (7). The dependence of J_v is then

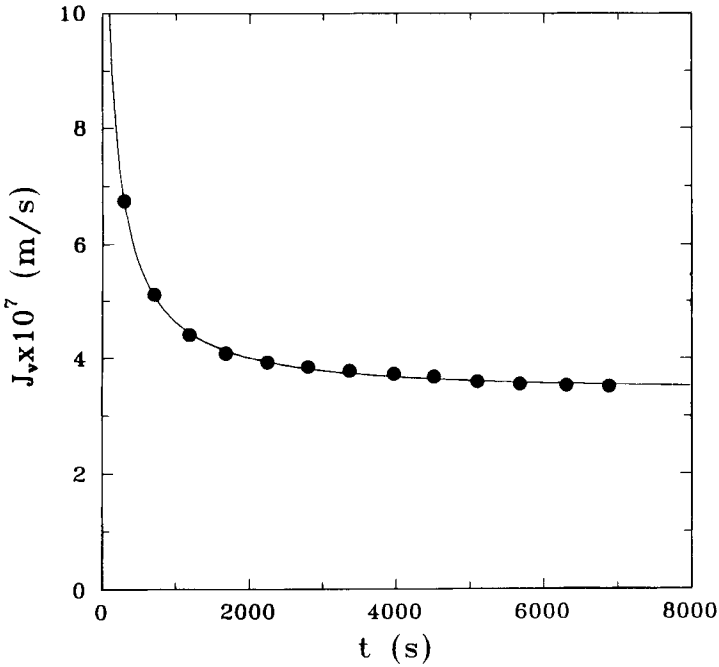

FIG. 1 Volume flow versus time for the 5% w/w solution of invertase and a transmembrane pressure of 5.3 kPa. The experimental results are fitted to $J_v = 3.45 + 52.4 \exp(-0.63t^{0.26})$. In this example, Δt is 530 seconds.

purely linear, and a value of 1.0703×10^{-10} m/Pa·s has been obtained for the hydrodynamic permeability.

This permeability is much lower than that for a totally new membrane, probably due to a decrease in porosity resulting from a reduction in the pore radii caused by protein adsorption on the pore walls and a decrease in the number of open pores per surface unit due to some pore clogging.

It is known that the volume flow decreases with time until a stationary state is reached when we are dealing with protein adsorption (8). This is also true here, as shown in Fig. 1 for 5% w/w and 5.3 kPa. The criterion for stationarity is

$$\frac{J_v(t - \Delta t) - J_v(t)}{J_v(t)} < 0.01 \tag{1}$$

where Δt is a suitable time span between two consecutive measurements.

The stationary values of J_v for each pressure and concentration are shown in Fig. 2. They show that a plateau is reached for high pressures

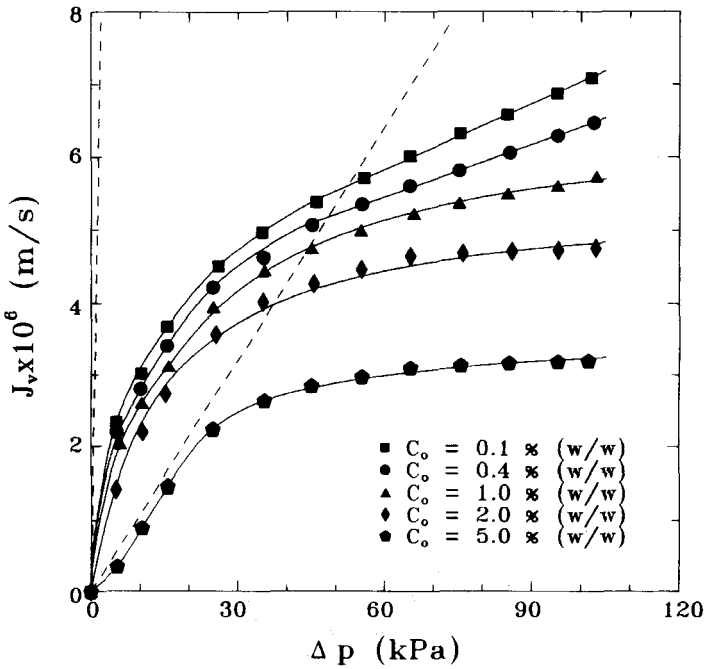

FIG. 2 Volume flow versus the transmembrane pressure for several concentrations of invertase within the analyzed range. The dashed lines correspond to the hydrodynamic permeabilities for a new membrane and one through which a 5% w/w solution has permeated.

for all concentrations, i.e., a limit of J_v is obtained which is lower for higher concentrations.

In Fig. 2 the pure water volume flow versus Δp curves for both clean and used membranes are also shown.

RESULTS AND DISCUSSION

Mass Transfer Coefficient

Due to the so-called concentration polarization, it has to be assumed that in contact with the membrane there is a concentration of $c_m > c_0$ due to an accumulation phenomenon resulting from the balance of convection through the membrane and back-diffusion. This can be studied by following the so-called film-layer model (9) which assumes there is a zone where the concentration decreases from c_m on the membrane to c_0 at a distance δ inside the retentate phase. This hypothesis leads to (10–12)

$$J_v = K_m \ln \frac{d_m - c_p}{c_0 - c_p} \tag{2}$$

where K_m ($= D/\delta$) is the mass transfer coefficient and D is the diffusion coefficient.

Given that invertase is totally retained, Eq. (2) leads to

$$J_v = K_m(\ln c_m - \ln c_0) \tag{3}$$

Thus, when c_m is constant, the volume flow must be linear with the feed concentration with a slope of $-K_m$. In fact, the concentration of the solution in contact with the membrane should be constant only if a gelification process was completed and $c_m = c_g$.

The volume flow is shown versus the feed concentration in Fig. 3 for some transmembrane pressures. It can be seen that, for any Δp, the plot seems to be almost linear for high concentrations. Therefore, a more or less significant K_m can be obtained for any Δp. Actually, J_v is only truly linear with high concentrations when the transmembrane pressures are also high.

In Fig. 4 the slopes so obtained for high concentrations are shown versus Δp. These slopes increase with pressure until ~50 kPa and then, above this transmembrane pressure, the slope is constant for any Δp.

Given that K_m is the ratio between the diffusion coefficient and the thickness of the concentration polarization layer, this increase could be attributed to a decrease of δ, i.e., to an increasing compaction of the film layer.

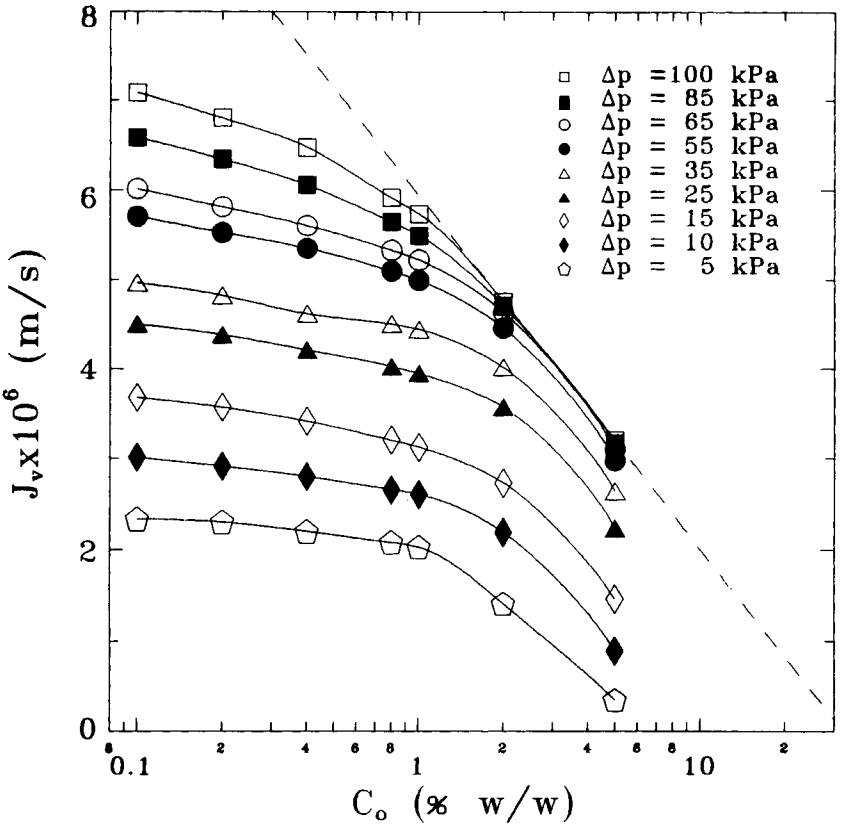

FIG. 3 J_v as function of log c_0 for some transmembrane pressures from 5 to 100 kPa.

Actually, as mentioned above, the slope of J_v versus c_0 should only be independent of Δp if $c_m = c_g$, and then it should be identified with the mass transfer coefficient.

The concentration in contact with the membrane, c_m, increases with c_0, but we can conclude that at low pressures c_m is lower than c_g for all feed concentrations and gelification is not reached. For higher transmembrane pressures, c_m increases with c_0 until $c_m = c_g$ and then is constant. The plot is linear with a slope given by K_m.

Concentrations and Osmotic Pressures

On the other hand, for each Δp there is a value of the feed concentration that gives zero J_v; this concentration will be called $\bar{c}_m$ and calculated as

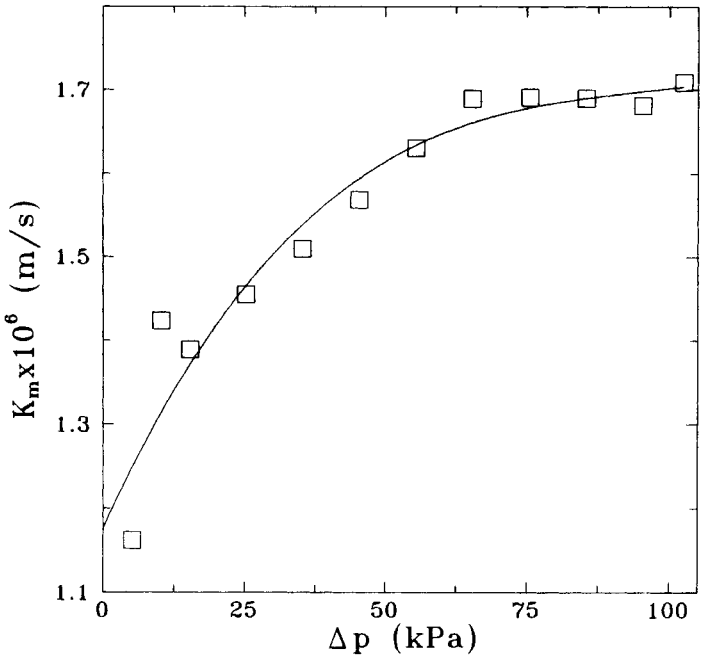

FIG. 4 Mass transfer coefficient as a function of the transmembrane pressure.

the intercept of the high concentration curve with the x-axis. These transmembrane pressures can be plotted as a function of $\bar{c}_m$, as can be seen in Fig. 5.

The volume flow should be given by

$$J_v = \frac{\Delta p - \Delta \pi}{R_m} \tag{4}$$

where $\Delta \pi$ is the osmotic pressure drop and R_m is the membrane resistance, which is the inverse of the hydraulic permeability

$$R_m = 1/L_p \tag{5}$$

If the osmotic pressure is assumed to follow a power dependence on the concentration in contact with the membrane, Eq. (4) could be modified to

$$J_v = \frac{\Delta p - a c_m^b e^{bJ_v/K_m}}{R_m} \tag{6}$$

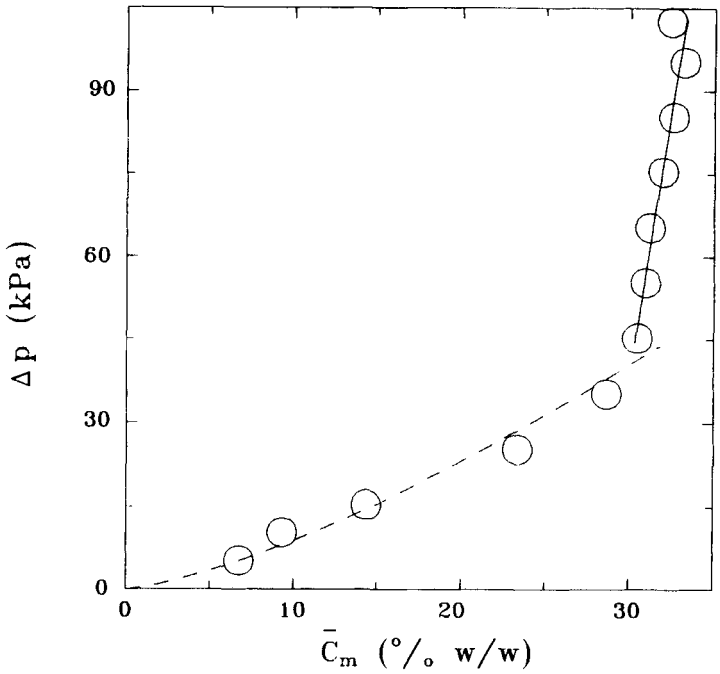

FIG. 5 The pressure versus the concentration that gives zero J_v. The experimental results up to 30.5% w/w are fitted to $\Delta p = 364.2\bar{c}_m^{1.39}$.

Hence the concentration $\bar{c}_m$ should correspond to an osmotic pressure $\Delta\pi = \Delta p$ (13, 14). Then Fig. 5 can be used to fit a dependence of the osmotic pressure as a function of the concentration. This has been done by using

$$\Delta p = a\bar{c}_m^b \tag{7}$$

for concentrations until 30.5% w/w and pressures up to 45.3 kPa, giving $a = 364.2$ and $b = 1.39$.

For higher pressures, $\bar{c}_m$ should be constant and equal to c_g. The mean value of this gelification concentration is 32.2% w/w according to Fig. 5.

If Eq. (7) is used to calculate the osmotic pressure, according to Eqs. (4) and (5) the volume flow should be linear with $\Delta\pi$ with a slope of $-L_p$ and an intercept with the y-axis given by $L_p\Delta p$. If this is done for pressures of less than 45.3 kPa, what we obtain is shown in Fig. 6, where it is seen that the plot is again linear only for high osmotic pressures or concentrations. The values of L_p are shown in Table 1.

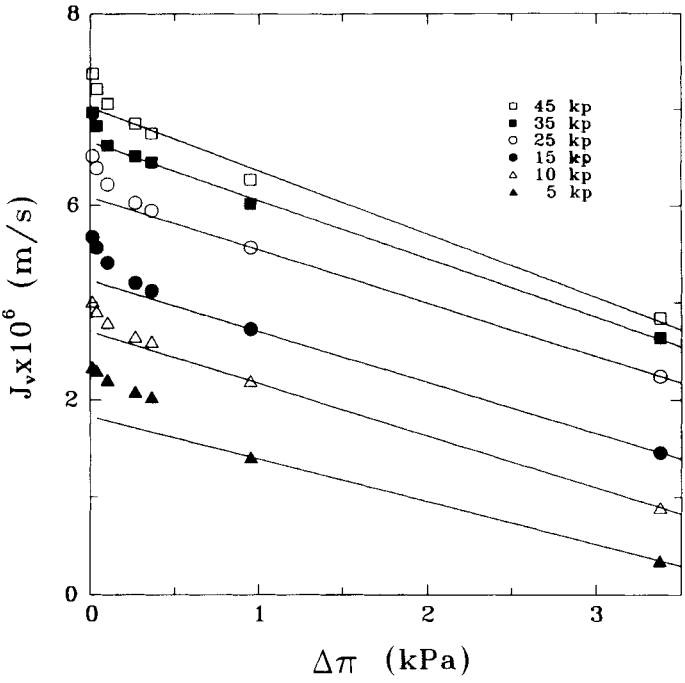

FIG. 6 J_v as function of the osmotic pressure for several applied transmembrane pressures.

TABLE 1
The Hydraulic Permeability of A002 as Obtained from Fig. 6

Δp (kPa)	L_p (10^{-10} m/s·Pa)
5.2	4.38
10.3	5.37
15.4	5.24
25.3	5.49
35.3	6.56
45.3	6.63

The mean value of these hydraulic permeabilities is 5.61×10^{-10} m/s·Pa, which is in between the corresponding values of hydrodynamic permeabilities for clean and 5% w/w permeated membranes.

CONCLUSIONS

The total retention of invertase should be interpreted in terms of the gyration radius of the protein. According to a phenomenological correlation between the molecular weight and the equivalent radius of the macromolecules polyethylene glycols, polyvinylpyrrolidone, dextrans, sugars, and proteins in aqueous solutions (16), invertase should correspond to an approximate mean gyration radius of 0.008 μm, while the nominal pore radius is 0.010 μm. In fact, the intermolecular interactions and the pore reduction due to adsorption should justify total retention in spite of the fact that we seem to be placed just outside the limit of retention.

For low applied pressures, the osmotic pressure difference generated by the concentration of invertase in contact with the membrane, taking into account that the concentration of the permeate is zero, seems to be the fundamental factor limiting the volume flow. This leads to a continuous increase of c_m until $\Delta\pi$ balances the applied transmembrane pressure Δp, which establishes $\bar{c}_m$. This is why the plot of J_v against c_0 is not totally linear for any concentration for these low pressures.

The osmotic pressure dependence on concentration obtained is of the usual power kind and its coefficients are within the typical ranges.

On the other hand, for higher applied pressures, c_m increases to a limiting value (c_g) which is constant for all high pressures. Once this limiting value of c_m is reached, the volume flow decreases linearly with the feed concentration with the same slope for every high Δp. Actually, this slope is the only one that can be fully interpreted as the mass transfer coefficient.

Therefore, both the gelification and the osmotic pressure difference through the membrane play some role in limiting the volume flow, but each of them predominates in a different pressure range.

The hydraulic permeability of the membrane is calculated for each Δp (in the low range where osmotic pressure seems to be the main phenomenon) by Eq. (4) and shown in Table 1. The mean value of these permeabilities is substantially equal to the average of the slopes of J_v vs Δp for low applied pressures. Therefore, the osmotic pressure limit seems to be a reduction in the thermodynamic force acting through the membrane without any relevant change in the membrane resistance.

The slight increase in L_p with Δp can be attributed to the possible loss of rigidity of the invertase molecules whose complex structure could be

simpler for high pressures, leading to an easier permeation through a significant portion of pores.

SYMBOLS

a	first coefficient of the osmotic pressure versus concentration relation
b	second coefficient of the osmotic pressure versus concentration relation
c_m	membrane concentration in contact with the high pressure interface (mol/m^3)
$\bar{c}_m$	concentration in contact with the membrane when the volume flow is zero (mol/m^3)
c_g	gelification concentration (mol/m^3)
c_p	permeate concentration (mol/m^3)
c_0	feed concentration (mol/m^3)
d_h	diameter of the hydraulic channel (m)
D	diffusion coefficient (m^2/s)
J_v	volume flow per unit of area and time through the membrane (m/s)
K_m	mass transfer coefficient (m/s)
L_p	hydraulic permeability (m/Pa·s)
R_m	membrane resistance (Pa·s/m)
δ	thickness of the concentration polarization film layer (m)
Δp	pressure drop through the membrane (Pa)
$\Delta \pi$	osmotic pressure drop through the membrane (Pa)
Δt	time lap between two consecutive measurements of J_v (s)

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