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Recovery by Ultrafiltration of a Commercial Enzyme for Cellulose Hydrolysis

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Abstract

An enzymatic process of cellulose hydrolysis based mainly on the use of membrane techniques is under study. The proposed flow sheet assumes that during cellulose hydrolysis the enzyme is continuously separated from glucose and cellobiose and is recycled in the cellulose reaction vessel by membrane ultrafiltration. The ultrafiltration of Celluclast enzyme by Novo is performed in a DDS column module assembled with flat polysulfone membranes. Membrane polarization effects are studied in the 0.1–5% w/v enzyme concentration range under varying pressures up to 600 kPa. A partial loss of enzymatic activity is observed as a consequence of the ultrafiltration and membrane washing operations.

INTRODUCTION

Enzymatic hydrolysis is considered a viable, economic, and energy-saving process compared with chemical processes for cellulose transformation (*1*). However, this bioprocess still needs improvements concerning the availability of (a) more stable and active biocatalysts (enzymes), (b) cellulosic substrates more easily hydrolyzed, (c) simple and economic methods for enzyme recovery and recycling to reduce costs, and (d) methods for controlling the inhibition from hydrolysis products.

Membrane techniques are considered very promising for a positive solution of the two last points. A process under study (*2*, *3*) assumes that during cellulose enzymatic hydrolysis, the solution is continuously ultrafiltrated to recycle the enzyme and remove products (mono- and polysaccharides) which can inhibit the biocatalyst activity. The glucide solution is then converted by means of a suitable bioreactor (membrane immobilized enzyme) into the final products. In this paper the results obtained with the ultrafiltration of a commercial enzyme, Celluclast from Novo, for cellulose hydrolysis are discussed.

The conversion of cellobiose into glucose in a bioreactor formed by immobilization of whole cells of a yeast strain (*Hansenula henricii*) in a polysulfone hollow fiber ultrafiltration module has already been presented (*2*, *3*).

EXPERIMENTAL

Ultrafiltration experiments were performed with a feed solution of the commercial Celluclast (Novo) enzyme of cellulosic activity. Celluclast is obtained from *Trichoderma reesei* (*4*), and it was used in an aqueous citrate buffer at pH 4.4 in the concentration range 0.1–5% w/v. At the beginning of an experimental ultrafiltration run a mixture of glucose and cellobiose was added to the enzyme solution to obtain a final glucide concentration in the range 0.2–0.5% w/v, which reduced the inhibition effect on the enzyme.

Ultrafiltration was performed in a DDS module containing 8 flat polysulfone membranes (0.0175 m^2 each) according to the sketch of Fig. 1. Membranes with a 6000 Dalton constant cut-off were used. They had two different water permeabilities: four DDS-GS81PP more permeable membranes and four DDS-GR81PP less permeable membranes. The DDS column module allows the ultrafiltrate from each membrane couple to be collected. A constant axial flow rate of 7.5 L/min was maintained with driving pressures in the 100–600 kPa range at a fixed temperature of 20°C. Ultrafiltration tests were performed in a complete recycling mode, i.e., by mixing both unpermeate and permeate streams in the feed vessel in order to maintain a constant feed composition. Preliminary tests of the ultrafiltration concentration of the feed solutions have shown that the membranes completely retain the enzyme. No proteins were detected in the permeate. Proteins were tested by means of UV spectrophotometry at 277 nm after precipitation with trichloroacetic acid and centrifugation. Following the ultrafiltration tests, membranes were washed with water until no proteins were detected in the wash solution. Two batches of 5 L each were generally

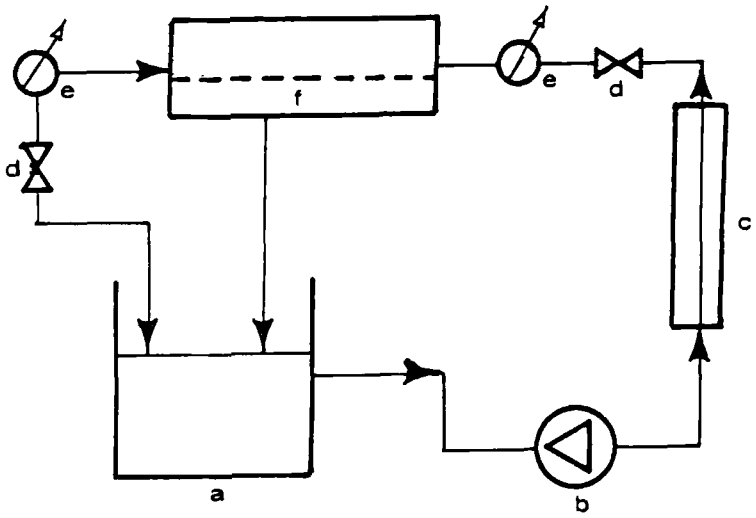

FIG. 1. Sketch of the experimental ultrafiltration apparatus: (a) feed tank, (b) recycling pump, (c) heat exchanger, (d) valves, (e) pressure gauges, (f) DDS ultrafiltration module.

recycled during 12–15 min at 200–300 kPa. The initial membrane water permeability was recovered after washing.

RESULTS AND DISCUSSION

Proteins in the Novo Celluclast enzyme are supposed to have a molecular weight in the 30,000–40,000 range, which is completely retained by the membranes with a cut-off of 6000 Dalton. During ultrafiltration at a given pressure and concentration, permeate flows initially decrease until a steady-state value is reached (Fig. 2) which is lower the higher the solution concentration. This polarization effect can be easily removed by washing with water.

The study of the polarization effect is best done under total recycle conditions (5) where the bulk feed concentration can be considered constant.

The steady-state values increase with the applied pressure difference until a limiting flux is obtained in the region where the permeate flow is pressure independent. The steady-state fluxes, as functions of the applied pressure, are plotted in Figs. 3 and 4 for the two DDS membranes with the water permeability of clean membranes. As expected, the more permeable membrane shows higher limiting fluxes at a given bulk concentration.

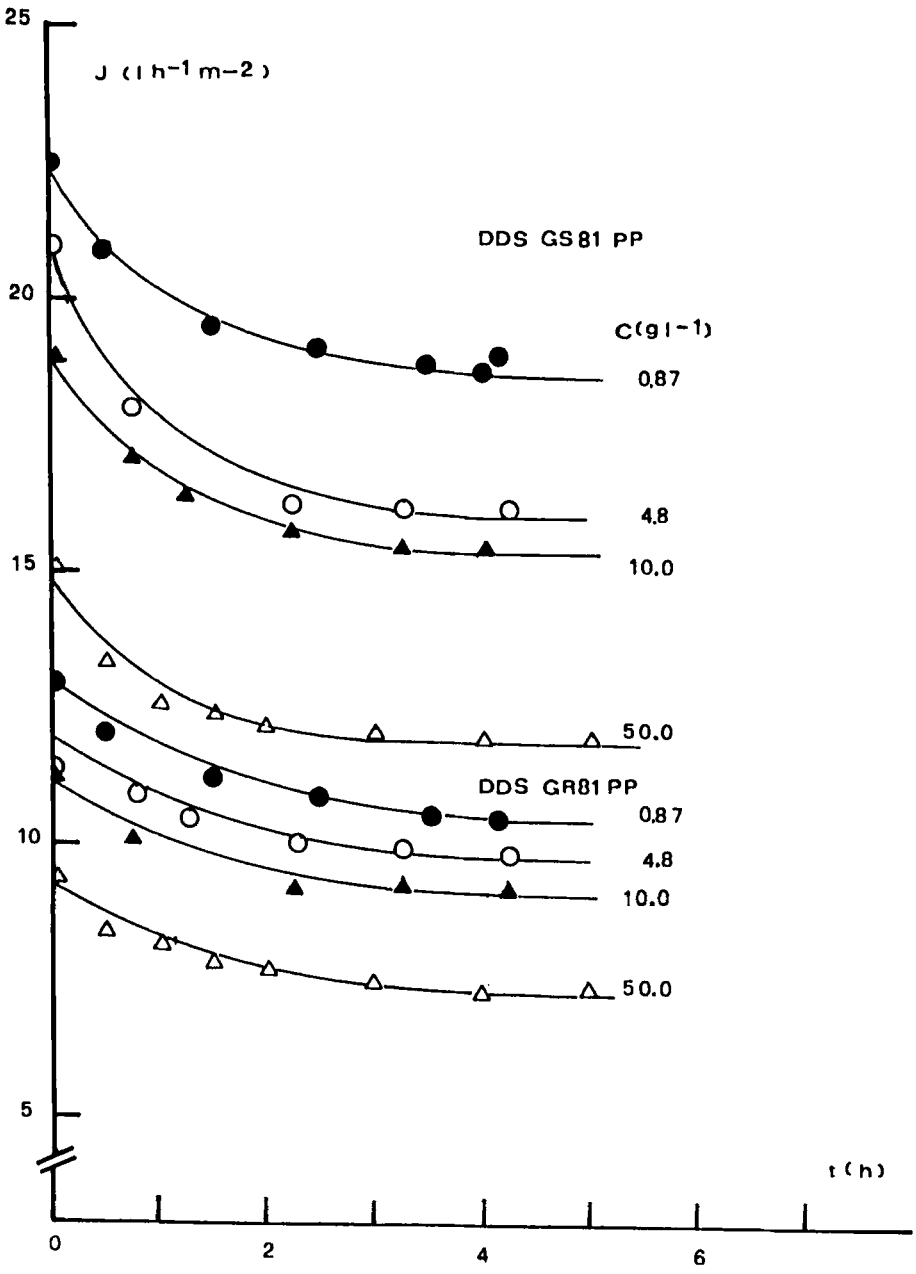

FIG. 2. Steady-state flows for GS81PP and GR81PP membranes as a function of the Celluclast concentration: pH = 4.4, T = 20°C, ΔP = 200 kPa.

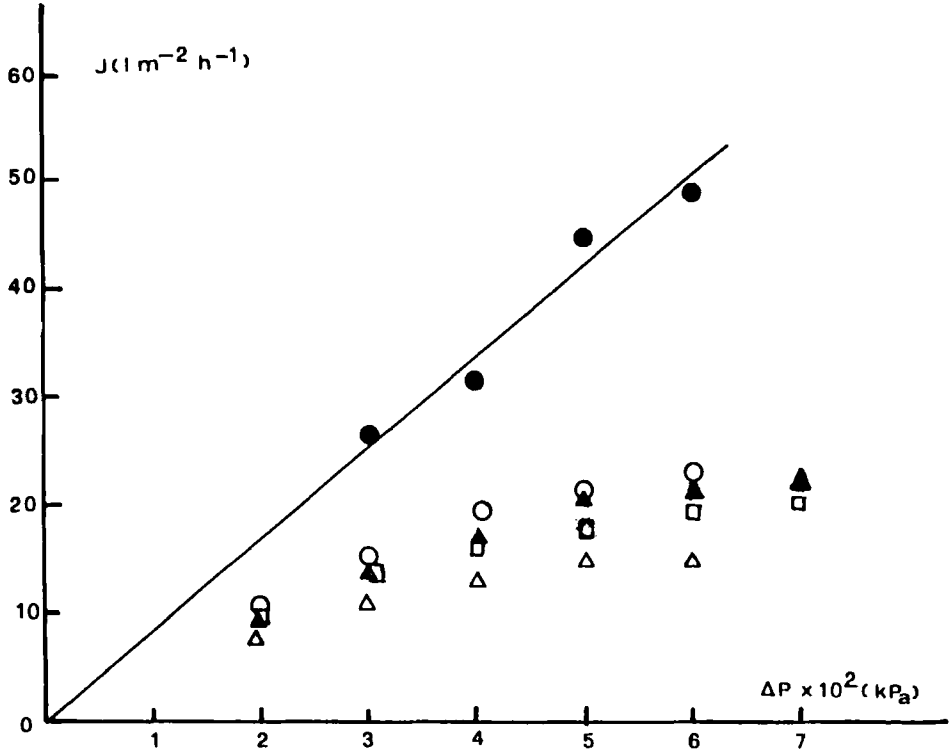

FIG. 3. Limiting fluxes for GR81PP membranes as a function of pressure and Celluclast concentration C_b (g/L): (●) water, (○) C_b = 0.87 (▲) C_b = 4.8, (□) C_b = 10, (△) C_b = 50. T = 20°C, pH = 4.4

This behavior, which is generally observed in protein ultrafiltration, is generally discussed in terms of the following models: film or gel model, osmotic model, and resistance model (*5–7*). However, only under special operating conditions (*6*) are small differences in model predictions observed; for example, the presence of a "fully" limiting flux is required for the gel model but not for the osmotic model. Moreover, this last model requires knowledge of the dependence of the osmotic pressure of the solution on the concentration of the dissolved macromolecules. Lacking these data, the polarization of DDS membranes during the ultrafiltration of Celluclast solutions will be discussed in terms of the mass transfer (gel) model and the hydraulic resistance model.

According to the mass transfer gel model, during the ultrafiltration of soluble macromolecules, which are completely retained by the membrane,

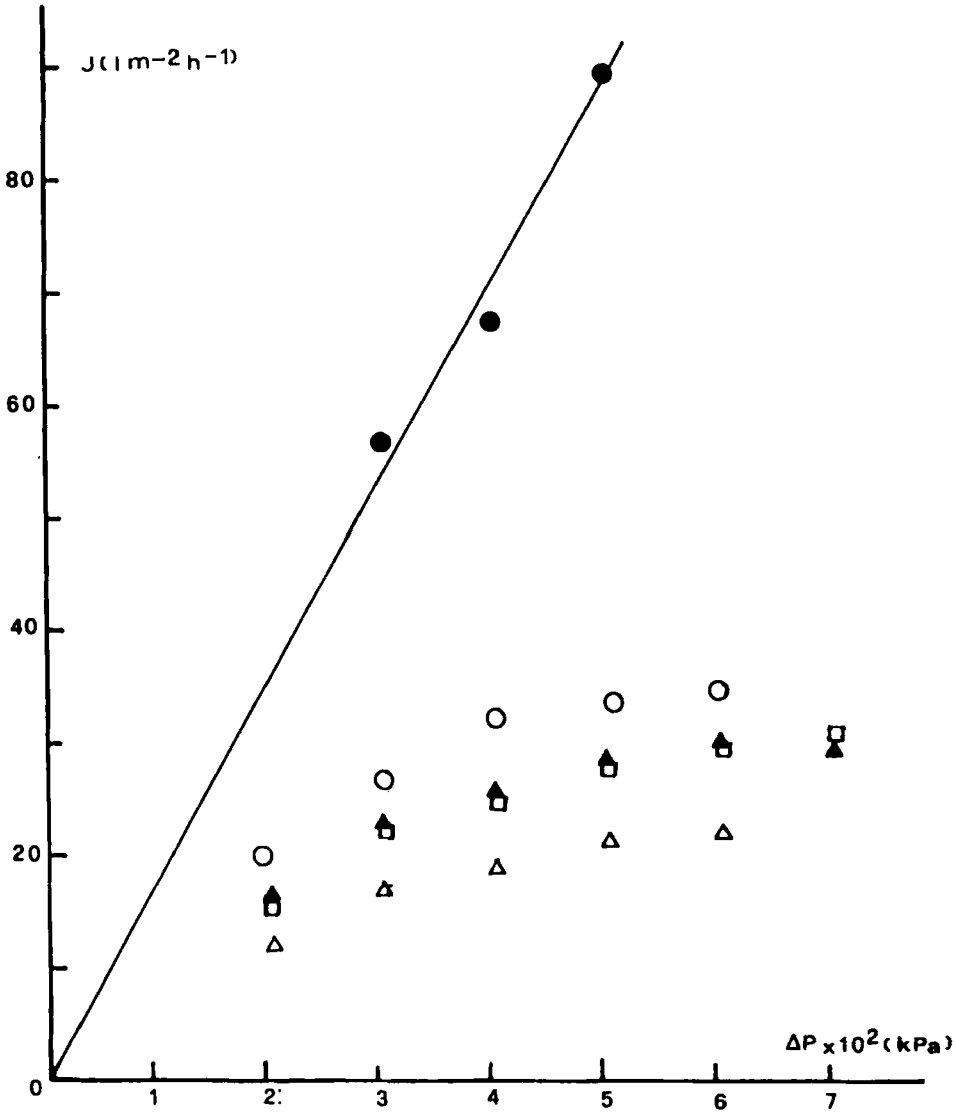

FIG. 4. Limiting fluxes for GS81PP membranes as a function of pressure and Celluclast concentration C_b (g/L): (●) water, (○) C_b = 0.87, (▲) C_b = 4.8, (□) C_b = 10, (△) C_b = 50. T = 20°C, pH = 4.4.

a steady-state value for the permeate flux is reached when the convective transport of the macromolecules toward the membrane high pressure surface (at which the concentration of macromolecules is $C_m > C_b$, the bulk solution concentration) is balanced by the backdiffusion of the macromolecules from the surface to the bulk solution. The backdiffusion is due to the concentration gradient built up in the boundary solution–membrane layer (thickness d). In these conditions the permeate flux is given by

$$J = (D/d) \ln (C_m/C_b) = k \ln (C_m/C_b) \quad (1)$$

where k $(=D/d)$ is the mass transfer coefficient and D is the diffusion coefficient of macromolecules in the boundary layer. If C_m increases up to the macromolecule solubility (C_g), a gel is formed on the membrane surface and a limiting value of the flux, J^*, is reached:

$$J^* = k \ln (C_g/C_b) \quad (2)$$

Under these conditions the flux is independent of the applied pressure. A plot of J^* vs ln C_b allows calculation of k (from the slope) and C_g (extrapolating the linear curve to $J^* = 0$, i.e., $C_b = C_g$). In Fig. 5 the dependence of the limiting flux J^* on the bulk concentration is shown for the two membranes. The resulting mass transfer coefficients are collected in Table 1.

The increase of the mass transfer coefficient with applied pressure, at a fixed recycling flow rate and assuming a constant average diffusion coefficient for the protein components of Celluclast, can be considered the result of a decrease of the thickness of the interfacial deposited layer which becomes thicker or denser. As a consequence, an increase in the overall membrane resistance (i.e., the true membrane resistance and the sum of the flow resistances due to the interfacial polarization and deposition layer effects) to the volume flow is expected.

The extrapolation of the J^* vs ln C_b curves is not acceptable due to the few available data. In fact, a tentative evaluation of the intercept on the x-axis gives C_g values exceedingly high and unrealistic. This point, and the very simple recovery of membrane water permeability after Celluclast ultrafiltration, suggest that a very loose "gel" is formed, and so the gel model can be only an approximation. An osmotic description of the polarization might be more realistic. However, as already said, the lack of data on the osmotic properties of Celluclast solution makes the use of such a model impossible.

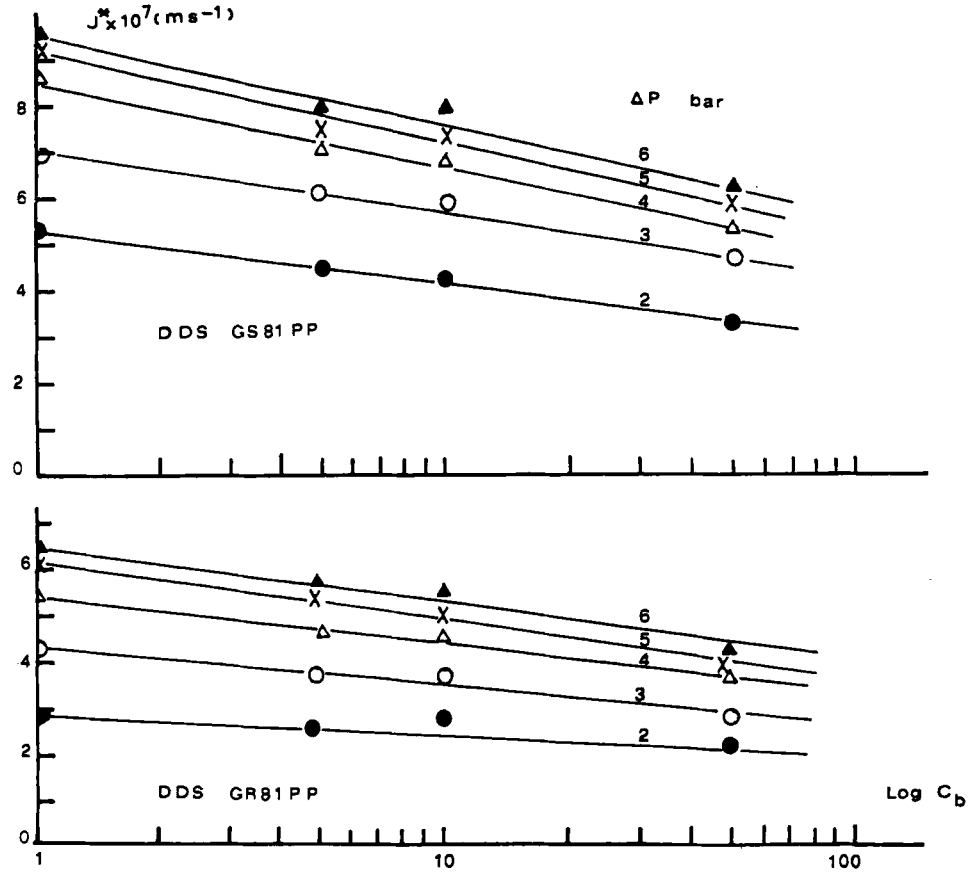

FIG. 5. Evaluation of the mass transfer k (m/s) coefficient according to the gel model. $T = 20°C$.

TABLE 1
Mass Transfer Coefficients k (m/s) for Celluclast and DDS Polysulfone Membranes

P (kPa)	$k \times 10^7$ DDS-GR81PP	$k \times 10^7$ DDS-GS81PP
200	1.87	5.48
300	3.09	6.97
400	4.44	9.31
500	4.98	8.83
600	5.73	8.90

TABLE 2
Polarization R^* Resistances during Celluclast Ultrafiltration through DDS Polysulfone Membranes

	DDS-GR81PP		DDS-GS81PP	
P (kPa)	$J^* \times 10^7$ (m/s)	$R^* \times 10^{-14}$ (L/m)	$J^* \times 10^7$ (m/s)	$R^* \times 10^{-14}$ (L/m)
		C_b = 0.87 (g/L)		
200	2.85	6.6	5.20	3.7
300	4.19	6.7	6.95	4.1
400	5.29	7.1	8.60	4.5
500	5.98	7.9	9.20	5.2
600	6.39	8.9	9.38	6.2
		C_b = 4.8 (g/L)		
200	2.53	7.5	4.46	4.3
300	3.68	7.7	6.07	4.7
400	4.60	8.3	6.99	5.5
500	5.47	8.7	7.45	6.5
600	5.75	10.0	7.82	6.5
700	6.21	10.8	8.00	6.6
		C_b = 10 (g/L)		
200	2.76	6.8	4.23	4.5
300	3.77	7.5	5.98	4.8
400	4.46	8.5	6.76	5.7
500	5.00	9.6	7.36	6.6
600	5.52	10.4	8.19	7.1
700	5.98	11.3		
		C_b = 50 (g/L)		
200	2.07	9.2	3.27	5.9
300	2.76	10.4	4.60	6.3
400	3.68	10.4	5.24	7.4
500	4.14	11.7	5.98	8.2
600	4.14	14.1	6.07	9.8

The decrease of the mass transfer coefficient can be discussed in terms of polarization resistance at the membrane/solution interface. According to the resistance model (*7*), the permeate flux can be written as

$$J = \Delta P/\eta(R_m + R^*) \quad (3)$$

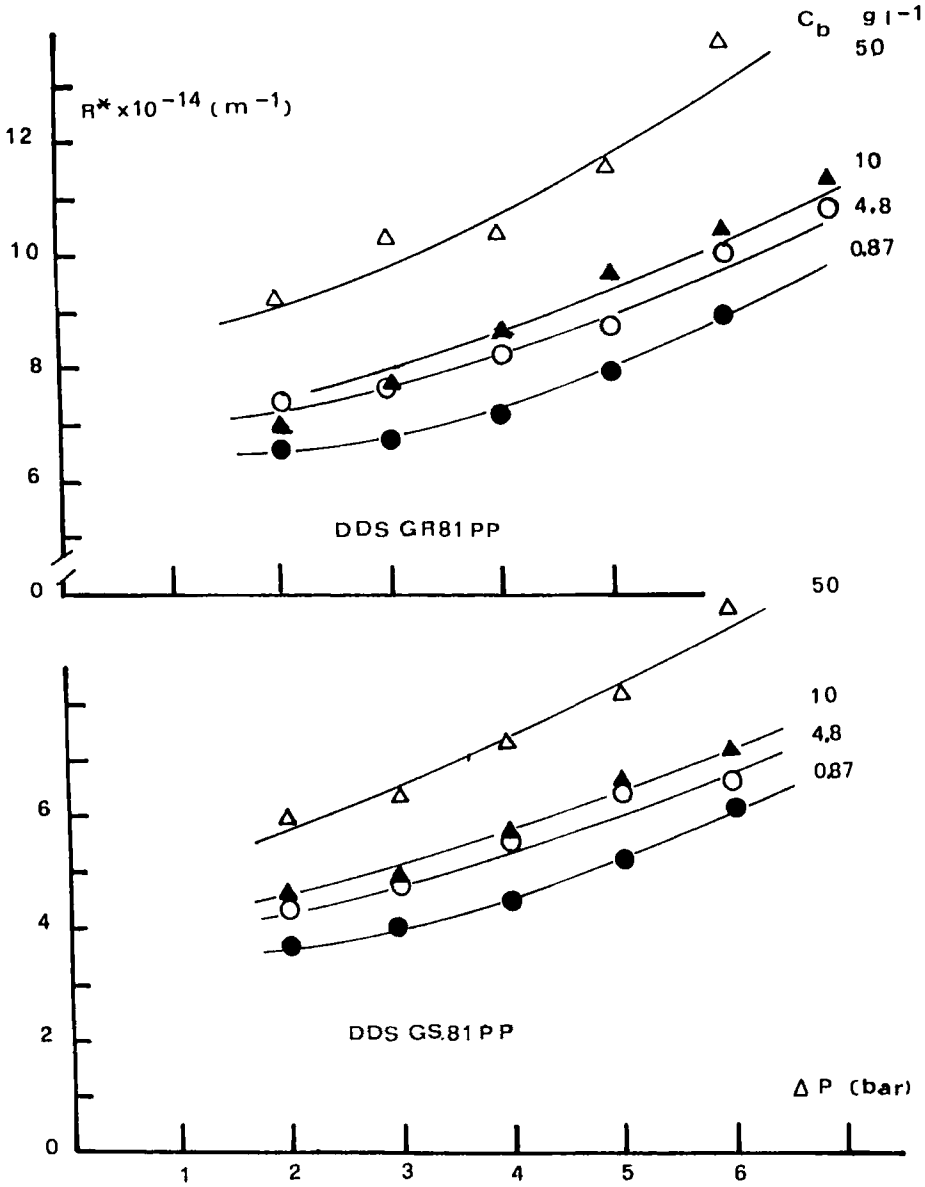

FIG. 6. Dependence of the polarization resistance R^* (m^{-1}) on pressure and Celluclast solution concentrations. T = 20°C.

where η is the viscosity, R_m is the membrane resistance, and R^* is the resistance due to all the possible polarization effects at the solution–membrane interface.

The membrane resistance R_m can be calculated from the membrane permeability to water. From the data of Figs. 3 and 4, 4.3 and 2.0×10^{13} m^{-1} for the R_m resistance of GR81PP and GS81PP membranes are obtained. The steady-state fluxes at different concentrations and pressures allows calculation of the R^* values of Table 2 (from Eq. 3), which are plotted in Fig. 6.

According to these data, at a given bulk Celluclast concentration there is a small variation of the polarization resistance when the applied pressure increases. This is expected because the polarization resistance due to the gel layer and the associated boundary layer is pressure dependent (5), and an increase in pressure makes the layer deposited on the membrane surface thicker or denser. However, because the water permeabilities of the membranes are easily recovered by solvent washing, the deposited layer is not strongly bound to the membrane surface and no permanent fouling is produced.

Celluclast Inactivation

Membranes are supposed to represent a good opportunity for the separation of biomolecules because membrane techniques do not require drastic operating conditions. However, it is necessary to check the effect of recycling enzyme solutions under pressure on their hydrolytic activity. For this reason, specimens from recycled feed solutions have been tested at different times during an ultrafiltration, i.e., at different concentration levels.

The feed solution, 0.2% in enzyme and 0.2% w/v in glucose (25 L volume), was concentrated at 15 and 6 L, and the enzyme activity of the three solutions was tested. The concentrated solutions have glucose added

TABLE 3
Celluclast Enzymatic Activity after Concentration by Recycling Ultrafiltration

Concentration factor	Glucose production (mg/L)				Inactivation (%)
	t (h): 0	10	21	40	
1.0	2.02	2.30	2.76	3.06	—
1.7	2.00	2.31	2.77	3.02	2
4.2	1.98	2.27	2.67	2.80	9

to restore the initial concentration of the feed (standard solution). Activity tests were performed on a 100-mL solution (acetic acid–acetate buffer: 0.1 *M*) following the conversion of 2 g of pure cellulose during 42 h under stirring and at a controlled temperature (40°C). Glucose production (mg/L) was measured at different times. The results (Table 3) show a slight inactivation of the enzyme complex as a consequence of the ultrafiltration concentration in the case of medium-high concentration factors.

CONCLUSION

The recovery of a Celluclast enzyme complex from a hydrolytic batch in which cellulose is degradated can be accomplished by means of ultrafiltration with membranes with a 6000 cut-off. A specific model for the observed polarization effects cannot be demonstrated from our data on the ultrafiltration of Celluclast solutions with polysulfone DDS membranes. However, the unrealistic high values of the concentration of a possible "gel" formed on the membrane surfaces and the very simple washing of the membranes after ultrafiltration suggest two possibilities: the formation of a very loose deposit on the membranes (low Celluclast protein interaction with polysulfone) or the osmotic origin of polarization effects. Lack of osmotic pressure data on Celluclast solutions prevents the demonstration of the last hypothesis.

From a practical point of view, the resistance model seems more useful. The small changes in the values of the polarization resistance with Celluclast concentrations in the 1–50 g/L range and the small dependence on the pressure allows us to conclude that Celluclast recovery with concentration factors in the 10–20 range could be performed with small changes in the steady-state fluxes.

A very slight inactivation of enzyme activity is observed if the feed solution is concentrated by a factor of 10 while the glucides produced by hydrolysis are recovered.

Membrane polarization, especially with GS81PP (a highly permeable membrane), is easily removed with water washing, and the original membrane water permeability is recovered.

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