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Modulation of immobilized enzyme activity by altering the hydrophobicity of nylon-grafted membranes Part 2: Non-isothermal conditions

M.M. El-Masry [a,1], A. De Maio [a], S. Di Martino [a,b], U. Bencivenga [a], S. Rossi [a], B.A. Manzo [a], N. Pagliuca [a], P. Canciglia [a,2], M. Portaccio [b], F.S. Gaeta [a], D.G. Mita [a,b,*]

[a] *International Institute of Genetics and Biophysics of CNR, via Guglielmo Marconi, 12, 80125 Naples, Italy*

[b] *Department of Human Physiology and Integrated Biological Functions of the Second University of Naples, via S.M. di Costantinopoli, 16, 80138 Naples, Italy*

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Abstract

Lactose hydrolysis by β-galactosidase immobilized on two nylon membranes, differently grafted, has been studied in a bioreactor operating under isothermal and non-isothermal conditions. One membrane (M_1) was obtained by chemical grafting of methylmethacrylate (MAA); the other one (M_2) by a double chemical grafting: styrene (Sty) and MAA. Hexamethylenediamine was used as a spacer between the grafted membranes and the enzyme. Both membranes have been physically characterized studying their permeabilities in presence of pressure or temperature gradients. Under non-isothermal conditions, the increase in activity of membrane M_2 was higher than that of membrane M_1. The α and β coefficients, giving the percentage of activity increase when a temperature difference of 1°C is applied across the catalytic membranes, have been calculated. Results have been discussed with reference to the greater hydrophobicity of membrane M_2 with respect to membrane M_1, the hydrophobicity being a prerequisite for the occurrence of the process of thermodialysis.

Keywords: β-Galactosidase; Grafted membranes; Immobilized enzymes; Lactose hydrolysis; Bioreactors

1. Introduction

It is well known from the thermodynamics of irreversible processes [1,2] that temperature gradients generate matter fluxes in bulk solutions (homogeneous systems) as well as across membranes (heterogeneous systems). In particular, when hydrophobic and unselective porous membranes are employed in a reactor to separate two

* Corresponding author. International Institute of Genetics and Biophysics of CNR, via Guglielmo Marconi, 12, 80125 Naples, Italy. Tel.: +39-081-2395887; fax: +39-081-2395887.

E-mail address: mita@iigbna.iigb.na.cnr.it (D.G. Mita).

[1] Permanent adress: Department of Polymers and Pigments-National Research Center-Dokki, Cairo-Egypt.

[2] Institute of General Physiology, University of Messina, Salita Sperone, 31, 98166, Messina, Italy.

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equal or different aqueous solutions kept at different temperatures, selective fluxes of solvent and solutes occur across it. This kind of mass transport has been called thermodialysis [3–7]. Water fluxes are directed towards the cold half-cell, while solute fluxes proceed in the opposite direction. Both solvent and solute fluxes have been found to be proportional to the temperature gradient applied across the membrane. It has been shown that the force driving matter transport is a thermal radiation force [9–11] associated to the heat flux crossing the system. Thus, the thermal radiation force is proportional to the temperature gradient.

When a hydrophilic catalytic membrane is coupled to a hydrophobic unselective membrane in a bioreactor operating under non isothermal conditions, the process of thermodialysis affects the enzyme reaction rate. As a consequence, a catalytic membrane activity higher than that measured under comparable isothermal conditions has been observed [11–15]. The activity increases range from 10% to 40%, depending on the nature of the enzyme and on the immobilization method used. These effects have also been observed when quiescent cells were immobilized and the catalytic activity of both cell wall and internal enzymes were studied under non-isothermal conditions [16,17].

The two-membrane system, one catalytic and the other hydrophobic, is not very suitable for industrial applications, particularly if hollow-fibre bioreactors have to be used. For this reason, in the last years our efforts were addressed to obtain single membranes, at the same time catalytic and hydrophobic. Such membranes should simultaneously give rise to catalysis and thermodialysis. Using the grafting technique by means of γ-radiation [18–26], Teflon or nylon membranes were copolymerized by us with suitable monomers and successively loaded with enzymes [25–33]. Some of these membranes were employed in non-isothermal bioreactors [29,33], giving increases of the enzyme reaction rate analogous to the ones obtained with the two-membrane system.

Grafting by γ-radiation is expensive, time-consuming, and allows only treatment of small samples and not of whole reactors. Thus, the use of chemical grafting appears to be more convenient [34–36].

The aim of this work is to study under non-isothermal conditions the behavior of a membrane, catalytic and hydrophobic, obtained by chemically grafting of methylmethacrylate (MAA) on nylon. Henceforth, this membrane will be called M_1. Since the hydrophobicity is an important prerequisite for the occurrence of the process of thermodialysis and the hydrophobicity of nylon is partially lost by the grafting treatment, this property was increased by a previous chemical grafting with styrene (Sty). The membrane thus obtained will henceforth be called M_2.

Both membranes M_1 and M_2 were loaded with β-galactosidase, used as enzyme model, in view of their applications in the process of lactose hydrolysis in milk. The isothermal behavior of these membranes is reported in a separate paper [37]. Here we report the results obtained when M_1 and M_2 membranes are employed in a non-isothermal bioreactor.

2. Apparatus, materials and methods

2.1. The bioreactor

The apparatus (Fig. 1) consisted of two cylindrical half-cells, 2.5 mm in depth and 35 mm in diameter, filled with the working solution and separated by the catalytic membrane. Substrate solutions were recirculated in each half-cell by means of a peristaltic pump through hydraulic circuits starting and ending in the common cylinder C. Each half-cell was thermostatted at a temperature T_i $(i = 1, 2)$. Under isothermal conditions T_1 was equal to T_2. Thermocouples, placed at 1.5 mm from each of the membrane surfaces, measured the temperatures inside each half-cell and allowed the calculation of the tem-

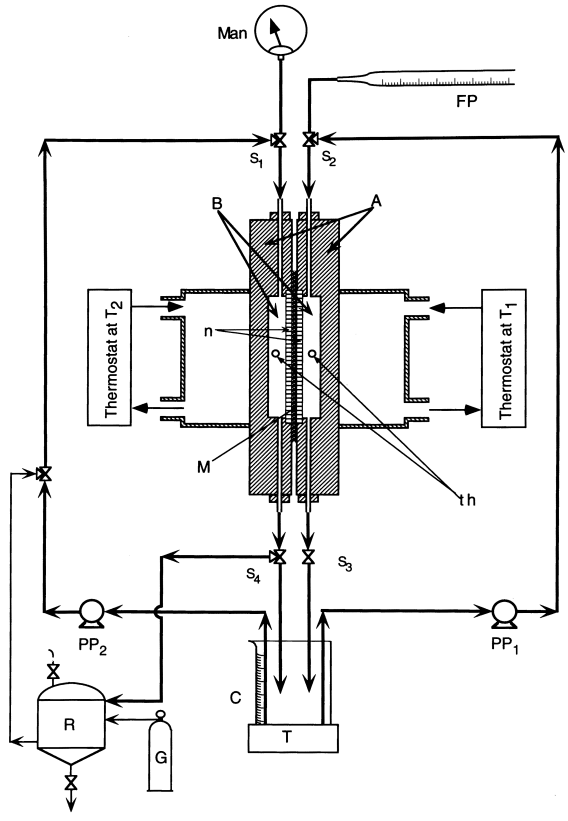

Fig. 1. Schematic (not to scale) representation of the bioreactor. (A) = half-cells; (B) = internal working volumes; (C) = external working volume; (M) = membrane; (n) = supporting nets; (th) = thermocouples; (S_i) = stopcocks; (T) = thermostatic magnetic stirrer; (PP_i) = peristaltic pumps; (Man) = manometer; FP = (flow-pipe); R = (reservoir containing the working solution); G = (pressurizing air tank).

perature profile across the catalytic membrane when the apparatus was kept under non-isothermal conditions. The temperatures read by the thermocouples will be indicated by T, while the ones calculated at the membrane surfaces is indicated by the symbol T^*. The values related to warm and cold sides will be indicated by the subscripts w and c, respectively. Under these assumptions $\Delta T = T_w - T_c$ and $\Delta T^* = T_w^* - T_c^*$, as well as $T_{av} = (T_w + T_c)/2$ and $T_{av}^* = (T_w^* + T_c^*)/2$. In non-isothermal experiments, $T_w^* < T_w$, $T_c > T_c$ and $\Delta T^* < \Delta T$.

The correlation between the temperatures read by the thermocouples and the actual temperatures on the catalytic membrane surfaces will be given in the following.

When the apparatus is employed to physically characterize the membranes, i.e., in the study of their hydrophobicity with measurements of transmembrane water transport under pressure and temperature gradients, the flow pipe and the pressure air tank are added and different hydraulic circuits are consequently used.

2.2. Materials

As solid support to be grafted, we used nylon Hydrolon membranes (Pall Italia, Milano, Italy). The membranes' characteristics are reported in Ref. [37].

All the chemicals, including the enzyme, were purchased from Sigma (Sigma Aldrich, Milano, Italy) and used without further purification. As monomer to be grafted, we used Sty and/or MMA. Hexamethylenediamine (HMDA) (70%

aqueous solution) was used as spacer between the grafted membrane and the enzyme. A 2.5% glutaraldehyde (Glu) aqueous solution was employed as bifunctional coupling agent for covalently binding the enzyme to the activated membrane.

The enzyme employed was a β-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae*. The enzyme hydrolyzes lactose in glucose and galactose.

2.3. Methods

2.3.1. Catalytic membrane preparation

Nylon/Poly-MMA and Nylon/Poly-Sty/Poly-MMA membranes were prepared according to the procedures described in Ref. [37].

The degree of grafting (X, %) was determined by the difference between membrane masses before, G_B, and after, G_A, the grafting by the formula:

$$X(\%) = \frac{G_A - G_B}{G_B} \times 100$$

Membrane activation was done by two successive treatments; each one for 1 h at room temperature. The first one was done by immersing the membrane in a 10% (w/v) hexamethylenediamine aqueous solution. After washing with running water to remove unreacted amines, the membranes were treated in a 2.5% (v/v) glutaraldehyde aqueous solution.

Enzyme immobilization was done by immersing for 16 h at 4°C the pre-activated membrane in a 0.1 M buffer phosphate solution containing the enzyme at a concentration 3 mg/ml, at pH 6.5. The overall process of membrane-grafting and enzyme-immobilization for both membranes has been represented in a schematic form in Ref. [37].

2.3.2. Determination of membrane activity and stability

Membrane activity was determined by sampling, at regular time intervals, the solution in the cylinder C with the membrane and measuring the glucose concentration by the GOD–Perid test according to Ref. [37]. Membrane activity, expressed as μmoles/min, is given by the slope of the linear plot of the glucose production as a function of time.

The time stability of the biocatalytic membranes was assessed by analyzing every day their activity under the same experimental conditions, i.e., 0.2 M lactose in 0.1 buffer phosphate solution, pH. 6.5 and $T = 30$°C. After 3 or 4 days, during which the membranes lost some activity, a stable condition was reached, remaining unchanged for over two months. No significant differences in approaching and maintaining the time stability were found between the M_1 and M_2 membrane. Only these stabilized membranes were used in the comparative experiments reported in the following.

Under the standard conditions defined above, the absolute membrane activity resulted to be 814 and 209 μmoles min^{-1} per m^2 of membrane surface area, for M_1 and M_2, respectively. When not in use, the membranes were stored at 4°C in 0.1 M buffer phosphate solution, pH 6.5.

2.3.3. Treatment of experimental data

Every experimental point reported in the figures represents the average value of four experiments performed under the same conditions. The experimental errors did not exceed 6%. Each experiment lasted 30 min, but only the initial reaction rates were accounted for in the construction of the figures.

3. Results and discussion

In a separate work [37] in which we used the same membranes, we have found that the amount of immobilized enzyme on the unit surface of M_1 was 0.545 ± 0.006 mg/cm^2, while on M_2 it was 0.509 ± 0.005 mg/cm^2. The grafting per-

cent values were (15 ± 2)% for the MMA, and (12 ± 3)% for Sty.

Before illustrating the results of lactose hydrolysis under non-isothermal conditions, let us characterize the catalytic membranes from the physical point of view.

3.1. Physical characterization of the membrane

Since the grafted membranes are physically different from the untreated ones, we have studied how the grafting process affects the transport parameters of the membranes. The hydraulic permeability coefficient, A, and the thermoosmotic coefficient, B, are two parameters controlling transmembrane mass transport. These coefficients are defined by the equations:

$$J_{\text{water}} = A\frac{\Delta P}{\Delta x} \tag{1}$$

$$J_{\text{water}} = B\frac{\Delta T}{\Delta x} \tag{2}$$

describing the volume flow (expressed in m^3 m^{-2} s^{-1}) across a membrane, catalytic or not, produced under isothermal conditions by a pressure gradient $\Delta P/\Delta x$ (measured in N/m^3) or under non-isothermal conditions by a temperature gradient $\Delta T/\Delta x$ measured in (K/m). A is expressed in m^4 s^{-1} N^{-1} and B in m^2 s^{-1} K^{-1}.

Hydraulic fluxes have been determined by pressurizing one half-cell by a gas cylinder and measuring by means of a graduated pipe the rate of water volume transport to the other half-cell. The temperature of the apparatus in these measurements was kept constant.

Thermoosmotic fluxes have been determined by measuring in the graduated pipe the rate of water volume transport from the warm to the cold half-cell, in absence of pressure gradients and in presence of temperature gradients.

In Fig. 2a and b, the values of the A and B coefficients as a function of temperature are respectively reported. From Fig. 2, it is possible to observe how the grafted membranes have greater A and smaller B with respect to the corresponding values of the untreated membrane. This means that somehow the treatment increases the hydraulic permeability and reduces the thermoosmotic permeability of the membrane, which however remains hydrophobic in as much as it still gives thermodialysis. In addition, the hydraulic permeability of M_1 is higher than that of M_2, while the contrary occurs for the thermoosmotic permeability. These results confirm the role of Sty in increasing the hydrophobicity of the grafted membranes.

Considering Eqs. (1) and (2), one calculates the values of the steady-state thermoosmotic pressure, $\Delta P_{\text{S-S}}$. This is the pressure that stops the water flux from the warm to the cold half-cell produced across the membrane by the applied temperature difference. From the same equations, one calculates also the γ coefficient, i.e., the pressure produced by a unit temperature difference across the membrane. In this way, we have:

$$\Delta P_{\text{S-S}} = \frac{B}{A}\Delta T \tag{3}$$

$$\gamma = \frac{\Delta P_{\text{S-S}}}{\Delta T} = \frac{B}{A} \tag{4}$$

The values of γ ($N\ m^{-2}\ K^{-1}$) are reported in Table 1. For $\gamma > 0$ thermoosmotic flow is observed, for $\gamma = 0$ no thermoosmotic flow occurs. To higher γ values, higher thermoosmotic fluxes correspond. This means that it is possible to foresee the yield of a separation process by thermodialysis for different membranes once their γ values have been measured. For example, starting with an equal initial lactose solution (0.2 M) in the two half-cells respectively kept at $T_w = 60°C$ and $T_c = 20°C$ and allowing water transport by thermodialysis, we have found after 1 h a lactose concentration ratio (C_W/C_C) which was equal to 2.5 for the un-

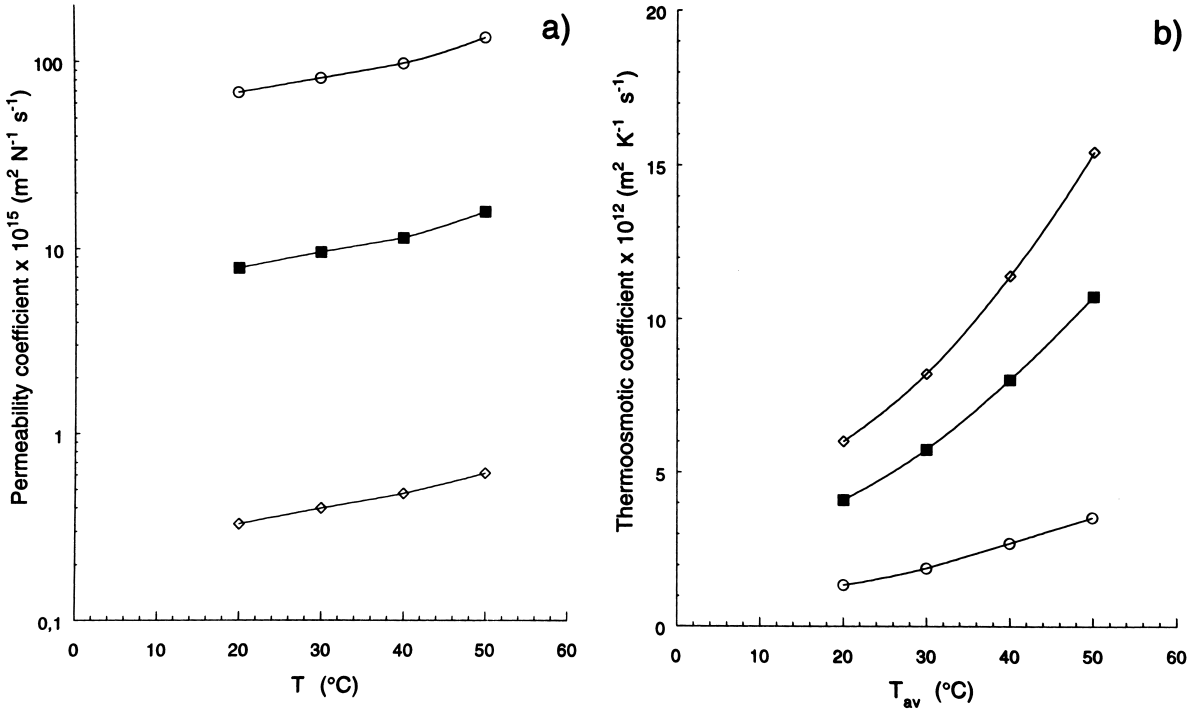

Fig. 2. (a) Hydraulic permeability coefficients as a function of temperature. (b) Thermoosmotic permeability coefficients as a function of average temperature. Symbols: (◇) = untreated nylon membrane; (○) = M_1 membrane; (■) = M_2 membrane.

treated membrane, to 1.4 for M_1 and to 0.9 for M_2. C_W and C_C were the lactose concentrations after 60 min in the warm and cold half-cells, respectively.

3.2. Effect of the non-isothermal conditions on enzyme activity

In Fig. 3a and b typical experimental results of lactose hydrolysis under isothermal and non-isothermal conditions are reported. In Fig. 3a, the glucose production of membrane M_1 as function of time is shown, while Fig. 3b shows the same parameter for membrane M_2. The slopes of the lines fitting the experimental points give the activity of the catalytic membranes under the different experimental conditions.

Since the ratio between the isothermal glucose production of membrane M_1 to that of membrane M_2 is 3.9, i.e., higher than the ratio between the enzyme amount immobilized on membrane M_1 to that immobilized on membrane M_2 which is 1.07, it is possible to give evidence to the role of Sty which, owing to its hydrophobicity, creates the conditions of a restricted diffusion of substrate toward the catalytic site. The role of Sty, which is negative under isothermal conditions, becomes positive under non-isothermal conditions since, for instance, the ratio of glucose production by M_1

Table 1
γ values at different average temperatures
γ values, calculated by means of the Eq. (4), applied to results in Fig. 4.

$T_{average}$ (°C)	$\gamma_{Untreated\ membrane}$ (N m^{-2} K^{-1})	$\gamma_{Membrane\ M_1}$ (N m^{-2} K^{-1})	$\gamma_{Membrane\ M_2}$ (N m^{-2} K^{-1})
20	18182	19.51	517.7
30	20500	22.77	593.6
40	23750	27.11	689.7
50	24839	25.62	666.2

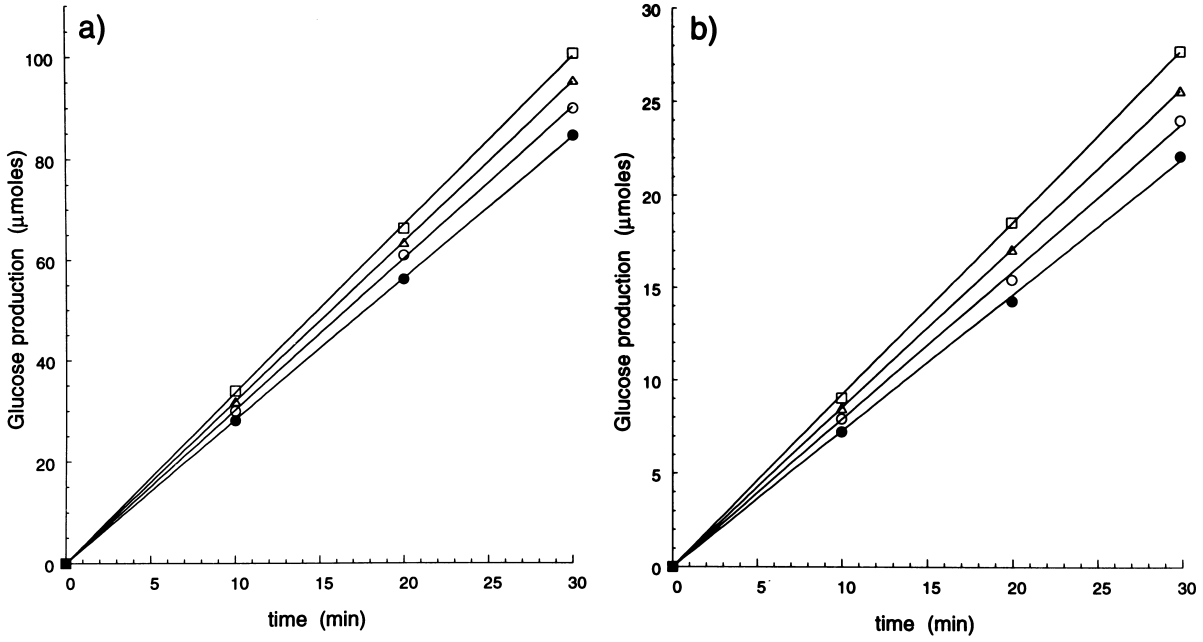

Fig. 3. Lactose production by membrane M_1 (a) and membrane M_2 (b) as a function of time. Symbols: (●) $\Delta T = 0$°C; (○) $\Delta T = 10$°C; (△) $\Delta T = 20$°C; (□) $\Delta T = 30$°C.

and M_2 in presence of a ΔT of 30°C becomes 3.5.

The experimental points reported in Fig. 4 are obtained from experiments done with the

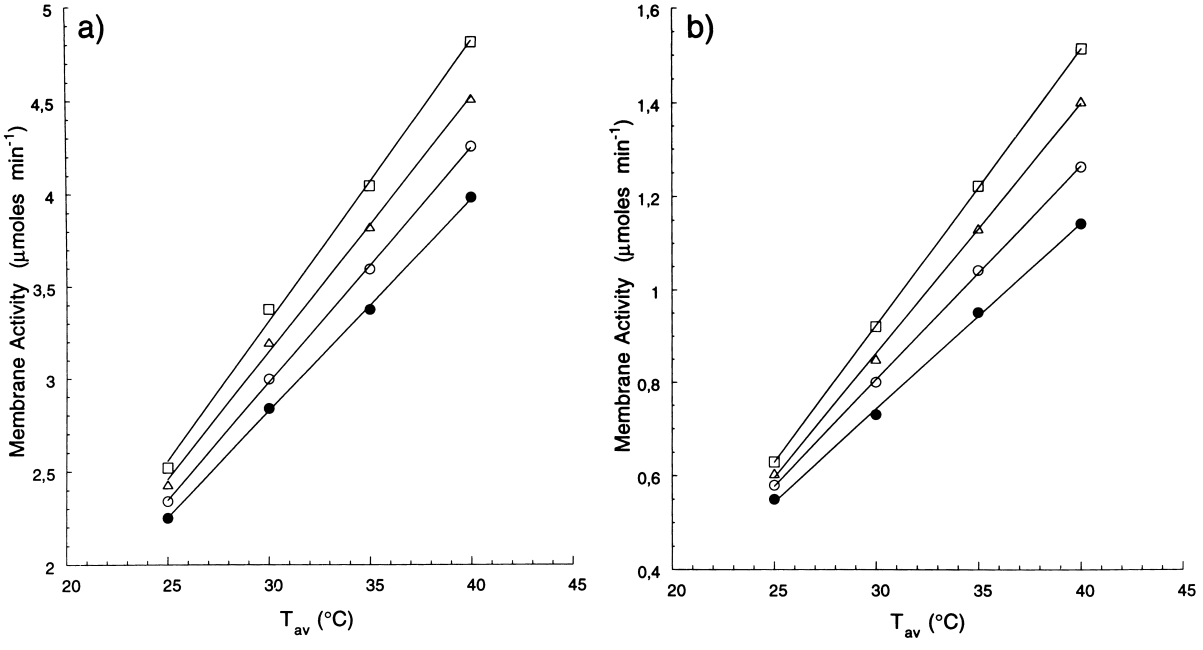

Fig. 4. Catalytic activity of membrane M_1 (a) and membrane M_2 (b) as a function of the average temperature, T_{av}. Symbols: (●) $\Delta T = 0$°C; (○) $\Delta T = 10$°C; (△) $\Delta T = 20$°C; (□) $\Delta T = 30$°C.

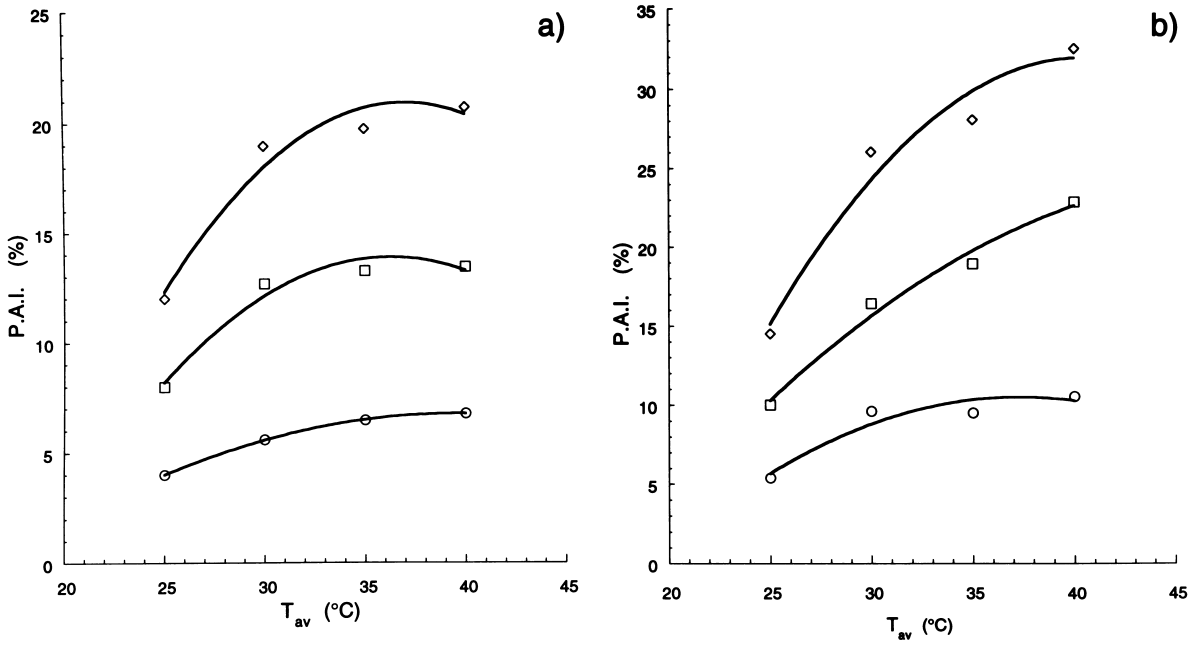

Fig. 5. Percentage activity increase for membrane M_1 (a) and membrane M_2 (b) as a function of the average temperature, T_{av}. Symbols: (○) $\Delta T = 10$°C; (□) $\Delta T = 20$°C; (◇) $\Delta T = 30$°C.

same methodology. Fig. 4a and b show the catalytic activity of membrane M_1 and M_2, respectively, as a function of average temperature. The curve parameter is the temperature difference read by the thermocouples. When $\Delta T = 0$°C, i.e., under isothermal conditions, $T_{av} = T$.

Both figures show that, keeping T_{av} constant, membrane activity increases with the temperature difference and, hence, with the temperature gradient applied. This means that the same lactose hydrolysis can be obtained either at low T_{av} and high ΔT or at high T_{av} and low ΔT.

A parameter giving direct indication on the usefulness of non-isothermal bioreactors in industrial processes is the percentage activity increase (P.A.I.) defined, at every average temperature, as:

$$\text{P.A.I.} = \frac{[\text{Activity}]_{T_{av}}^{\Delta T \neq 0} - [\text{Activity}]_{T=T_{av}}^{\Delta T = 0}}{[\text{Activity}]_{T_{av}}^{\Delta T = 0}} \times 100 \quad (5)$$

where $[\text{Activity}]_{T_{av}}^{\Delta T \neq 0}$ and $[\text{Activity}]_{T=T_{av}}^{\Delta T = 0}$ are the catalytic membrane activities, at a given $T = T_{av}$, under non-isothermal and isothermal conditions, respectively. In Fig. 5a and b, the P.A.I. values, calculated through Eq. (5) from the experimental points of Fig. 6a and b, are reported as a function of T_{av}. Each curve in the figures refer to a specific value of ΔT. Fig. 7a and b show P.A.I. values which, at each average temperature, increase with the applied ΔT, and that, under a given ΔT, increase with the average temperature. Since matter fluxes produced by the process of thermodialysis follow the same behavior, as one can also see from the Fig. 2b, it is possible to correlate the increase of the enzyme activity with the presence of the process of thermodialysis. This correlation becomes more stringent by observing that the percentage activity increases are higher for membrane M_2, which produces higher fluxes than membrane M_1 under thermodialysis.

A correct evaluation of the magnitude of the observed effects requires to bear in mind that

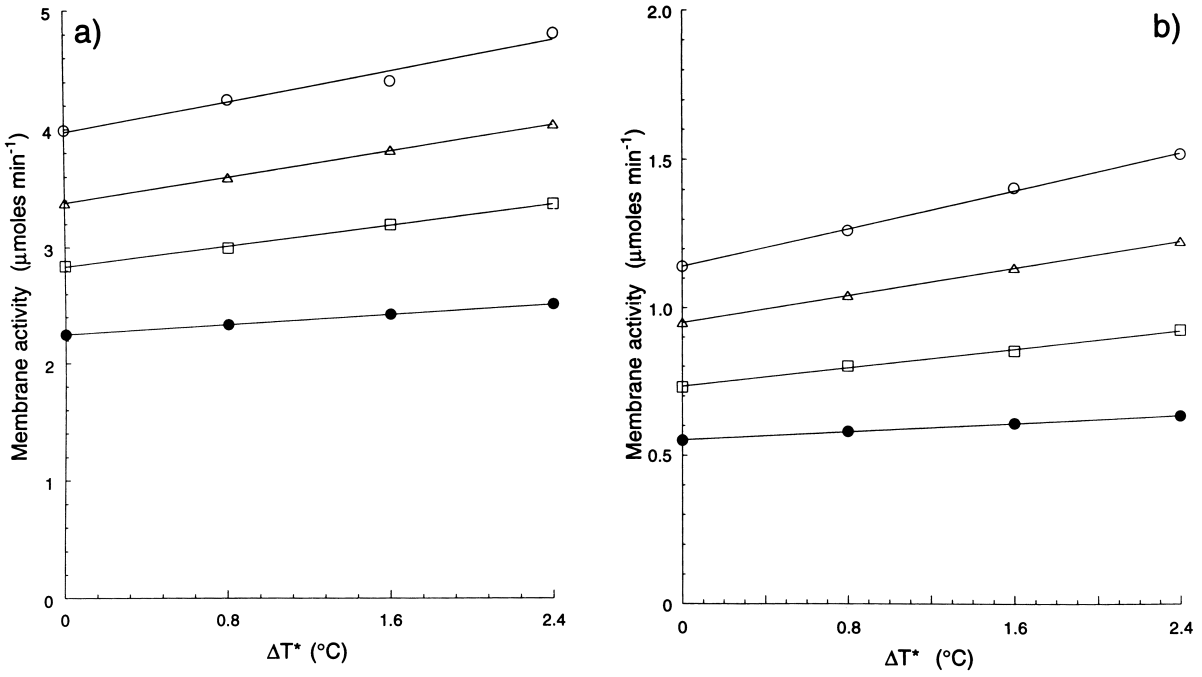

Fig. 6. Membrane activity as a function of the actual temperature differences ΔT^* across membranes M_1 (a) and M_2 (b). Curve parameters are the membranes average temperatures. Symbols: (●) $T_{av} = 25°C$; (□) $T_{av} = 30°C$; (△) $T_{av} = 35°C$; (○) $T_{av} = 40°C$.

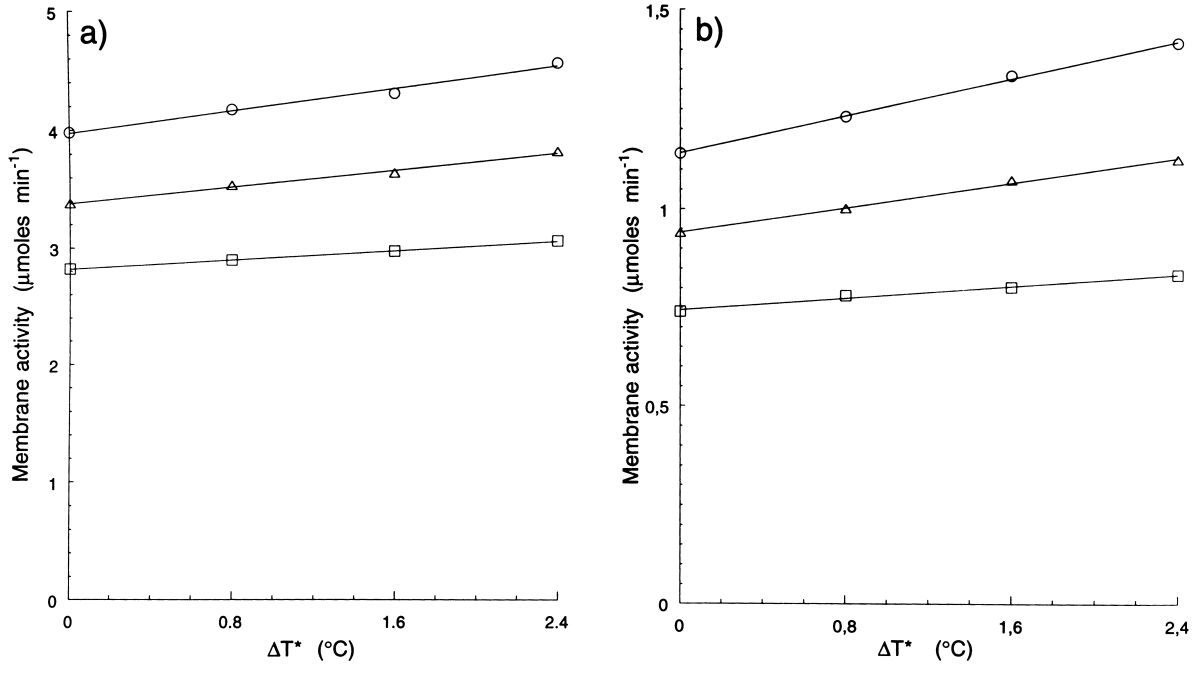

Fig. 7. Membrane activity as a function of the actual temperature differences ΔT^* across membranes M_1 (a) and M_2 (b). Curve parameters are the actual temperatures of the warm surface of the membrane. Symbols: (□) $T_w = 30°C$; (△) $T_w = 35°C$; (○) $T_w = 40°C$.

under non-isothermal conditions a flux of heat is present in the apparatus. Thus the actual temperatures on the catalytic membrane surfaces are different from the ones registered by the thermocouples. As a consequence, $T_w^* < T$ and $T_c^* > T_c$, so that $\Delta T^* < \Delta T$. As specified under Materials and methods the apex $(^*)$ relates to the corresponding values at the membrane surfaces. Being impossible to measure the temperatures on the membrane surfaces, we can calculate them considering that under our experimental conditions the solution flow into each half-cell is laminar being $(R_e \ll R_e^{\text{critical}})$ [14,15,17] and consequently the heat flow propagates through the solution and the catalytic membranes according to Fourier's law. The calculation is done by means of the expression:

$$J_q = K_i \left(\frac{\Delta T}{\Delta x} \right)_i = \text{constant} \tag{6}$$

where K_i is thermal conductivity of the i-th medium crossed by the heat flux and $(\Delta T/\Delta x)_i$ the temperature gradient existing in that medium.

Under these conditions, knowing the thermal conductivities and thicknesses of both filling solutions and membrane, it is possible to calculate the effective temperatures on the membrane surface. The results of this calculation are reported in Table 2. Inspection of Table 3 and the observation that our system is symmetric, allow us to write a simple equations system:

$$\begin{cases} T_w^* = T_w - a\Delta T \\ T_c^* = T_c + a\Delta T \\ \Delta T^* = \Delta T(1 - 2a) \end{cases} \tag{7}$$

In our case, a is equal to 0.46.

Considering these calculations, the results of Fig. 4a and b can be examined with reference to the average temperature of the membrane and to the actual temperature difference across it. This has been done in Fig. 6a and b. In these figures, catalytic membrane activity is reported as function of the actual temperature difference ΔT^* existing across it. Each line refers to a given membrane average temperature, T_{av}^*. This temperature, since our system is symmetric, is coincident with the average temperature of the bioreactor. From the figures, it is evident how, at each membrane average temperature, membrane activity increases with the actual temperature difference existing across the warm and cold membrane surfaces.

Table 2
Correspondence between the temperatures T and T^*
Correspondence between the temperature values read at the thermocouples (indicated by the symbol T) and the ones calculated at the surfaces of the catalytic membrane (indicated by T^*). Subscripts w and c refer to the warm solution and to the cold solution, respectively.

T_{av} (°C)	ΔT (°C)	T_c (°C)	T_w (°C)	T_c^* (°C)	T_w^* (°C)	T_{av}^* (°C)	ΔT^* (°C)
25	10	20	30	24.6	25.4	25	0.8
25	20	15	35	24.2	25.8	25	1.6
25	30	10	40	23.8	26.2	25	2.4
30	10	25	35	29.6	30.4	30	0.8
30	20	20	40	29.2	30.8	30	1.6
30	30	15	45	28.8	31.2	30	2.4
35	10	30	40	34.6	35.4	35	0.8
35	20	25	45	34.2	35.8	35	1.6
35	30	20	50	33.8	36.2	35	2.4
40	10	35	45	39.6	40.4	40	0.8
40	20	30	50	39.2	40.8	40	1.6
40	30	25	55	38.8	41.2	40	2.4

Table 3
α coefficients at different average temperatures
α coefficients calculated by means of Eq. (8) applied to results in Fig. 8.

Membrane system	T_{av} (°C)	$\alpha \times 100$ (°C^{-1})
M_1	25	5.15
	30	8.48
	35	8.49
	40	8.50
M_2	25	6.00
	30	11.00
	35	12.20
	40	14.00

The linear increase of membrane activity with the temperature difference allows to write, for each value of T_{av}, the equation:

$$\left[A(T) \right]_{\Delta T^* \neq 0}^{T = T_{av}^*} = \left[A(T) \right]_{\Delta T^* = 0}^{T = T_{av}^*} \{1 + \alpha \Delta T^*\} \quad (8)$$

where $A(T)$ indicates the membrane activity at $T = T_{av}^*$, under isothermal ($\Delta T^* = 0$) and non-isothermal ($\Delta T^* \neq 0$) conditions. The coefficient α represents, at each given average temperature, the percentage of membrane activity increase when a temperature difference of 1°C is applied across the catalytic membrane. The α values calculated through the experimental results of Fig. 6a and b by means of Eq. (8) are reported in Table 3. Inspection of the α values shows that the ones of membrane M_2 are higher than those of membrane M_1, confirming in this way that hydrophobicity is an important parameter for thermodialysis and, consequently, the role of this process in affecting the enzyme reaction rate.

A more significant representation of our results is obtained considering the actual temperature of the warm membrane surface under non-isothermal conditions. This is done in Fig. 7 where catalytic activities of membrane M_1 (a) and membrane M_2 (b) are reported as a function of the actual temperature differences applied. Now each line is referred at a given temperature of the warm membrane surface. The linear increases of the enzyme activity of both membranes also allow us to write in this case an equation similar to Eq. (8), this new equation being:

$$\left[A(T) \right]_{\Delta T \neq 0}^{T = T_w^*} = \left[A(T) \right]_{\Delta T = 0}^{T = T_w^*} \{1 + \beta \Delta T\} \quad (9)$$

Here, the β coefficient is the percentage increase of catalytic membrane activity when the temperature of the warm membrane surface is kept constant and the temperature of the cold membrane surface is lowered by 1°C. The β values calculated through Eq. (8) and applied to the results of Fig. 7 are listed in Table 4. Also with respect to the β values, the positive role of the hydrophobicity of membranes operating under non-isothermal conditions is confirmed.

Let us see, now, how all the experimental results reported above can be explained in terms of matter transport induced by the process of thermodialysis. This will be done analyzing the substrate (and products) traffic across the catalytic membrane under isothermal and non-isothermal conditions.

Under isothermal conditions only diffusion occurs. The diffusive substrate flux (mol m^{-2} s^{-1}) is expressed by the equation:

$$J_S^D = D^* \frac{\Delta C}{\Delta x} \quad (10)$$

where D^* is the restricted diffusion coefficient (m^2/s) in the membrane pores and $\Delta C/\Delta x$ is the concentration gradient (mol/m^4).

Table 4
β coefficients at different average temperatures
β coefficients calculated by means of Eq. (9) applied to results in Fig. 8.

Membrane system	T_w (°C)	$\beta \times 100$ (°C^{-1})
M_1	30	3.85
	35	5.50
	40	6.19
M_2	30	4.95
	35	8.25
	40	10.31

Under non-isothermal conditions, i.e., when a temperature gradient is applied across a hydrophobic membrane, catalytic or not, the membrane is crossed by differential solvent and solutes fluxes, produced by thermodialysis. Both matter fluxes are directly proportional to the magnitude of the temperature gradient applied. Under these conditions two separate matter fluxes are observed: a volume flux (water) from warm to cold, measured in (m^3 m^{-2} s^{-1}) and expressed by Eq. (2) and a solute flux from cold to warm, measured in moles m^{-2} s^{-1}, and expressed by the equation:

$$J_S^{\text{therm}} = D'^{*} C_c \frac{\Delta T}{\Delta x} \tag{11}$$

In these equations, $\Delta T/\Delta x$ is the temperature gradient across the membrane (K/m), C_c is the actual concentration of solute in the cold half-cell (mol/m^3) and D'^{*} is the restricted thermal diffusion coefficient (m^2 s^{-1} K^{-1}) in the membrane pores.

Associated with the volume flux there is a solute flux, due to the solvent drag, given by:

$$J_S^{\text{drag}} = J_{H_2O} C_w = V_{H_2O} C_w \tag{12}$$

where C_W is solute concentration in the warm half-cell, from which the water flux is coming and V_{H_2O} is the rate of water transport in m/s.

Of course also under non-isothermal conditions the contribution of isothermal substrate diffusion still remains.

Summing up, under isothermal conditions the only substrate traffic is given by that represented by Eq. (10), while under non-isothermal conditions the substrate traffic across the membrane is due to three distinct contributions. The first one is expressed by Eq. (10), and the others by Eqs. (11) and (12). A picture of the substrate traffic across the catalytic membrane operating under isothermal and non-isothermal conditions is given in Fig. 8a and b. On the basis of all these consideration it is easy to conclude, therefore, that under non-isothermal conditions the enzyme immobilized onto the hydrophobic–catalytic membrane faces a higher substrate traffic and hence interacts with a substrate concentration higher than under isothermal conditions. A qualitative picture of this situation is given in Fig. 8c.

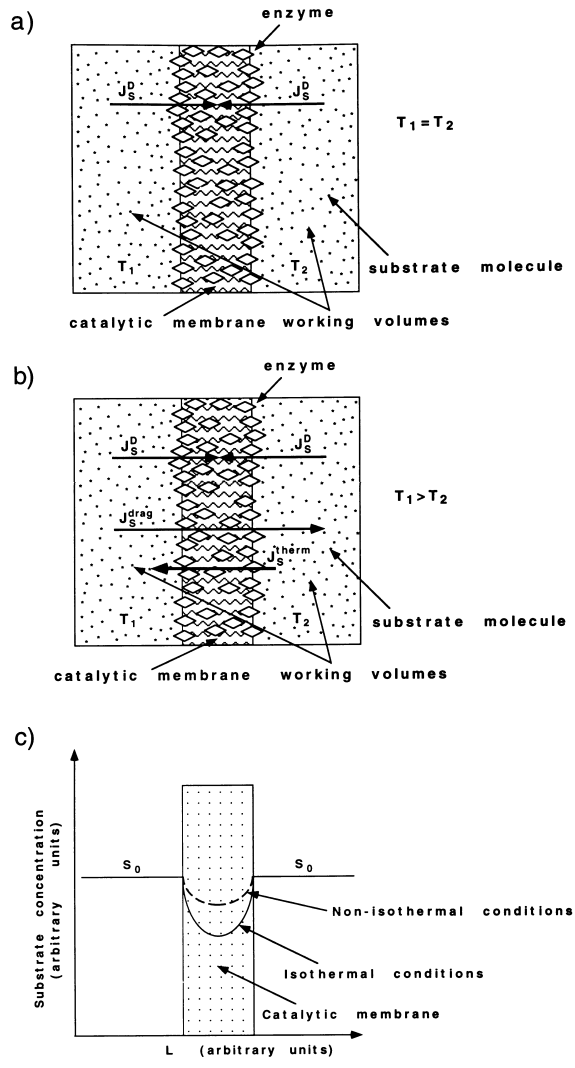

Fig. 8. A descriptive representation of substrate traffic across the catalytic membrane operating under isothermal (a) and non-isothermal (b) conditions. Substrate concentration profile (c).

The same reasoning is applicable to the product removal from the active site. Consequently, the presence of a temperature gradient increases the apparent turnover number of the enzyme

reaction, thus increasing the rate of the enzyme reaction.

4. Conclusions

The principal aim of this work has been reached since two membranes, giving catalysis and thermodialysis, have been successfully constructed.

When employed in bioreactors operating under non-isothermal conditions, both membranes have shown increases of the enzyme reaction rates proportional to the applied transmembrane temperature gradient. In particular membrane M_2, having higher hydrophobicity than M_1, exhibited highest efficiency increases, according to the role of thermodialysis in modulating the activity of catalytic membranes employed in non-isothermal bioreactors.

One negative aspect coming from present results is that α and β values found for M_1 and M_2 membranes are lower than those found with other membrane system. This is due to two circumstances.

The first one consists in the presence of an untreated Teflon membrane when a two membrane system was used. In this case volume and substrate fluxes produced by the process of thermodialysis across the Teflon membrane (and the coupled catalytic membrane) were highest owing to the highest hydrophobicity of the Teflon. This, of course, increased the concentration of substrate and the rate of product removal in the catalytic membrane, thus increasing the α and β values for the catalytic system.

The second circumstance is that in the present work we used a lactose concentration which is saturating the enzyme reaction rate for both membranes, insomuch as the apparent K_m values found for the enzyme immobilized on M_1 and M_2 were 94 and 46 mM, respectively [37]. It is clear that it becomes difficult to induce high increases in the yield of a process when the catalyst works at saturation. A paper describing the dependence under non-isothermal conditions of the percentage increase of the enzyme reaction rate on the substrate concentration is in advanced state of preparation.

Since the M_2 membranes provide the greatest efficiency increases under non-isothermal conditions, experiments are in progress in our laboratory to increase the hydrophobicity of this membrane, increasing the amount of grafted Sty.

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