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Influence of the non-isothermal conditions on the activity of enzymes immobilized on nylon grafted membranes

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Abstract

β-Galactosidase was immobilized on a nylon membrane chemically grafted with butylmethacrylate. Hexamethylenediamine was used as spacer and glutaraldehyde as coupling agent. The catalytic behaviour of the membrane was studied under isothermal and non-isothermal conditions. It was found that in the presence of temperature gradients, the catalytic membrane exhibited higher activity and greater affinity for the substrate. The increases of the enzyme reaction rate was found to be proportional to the intensity of the applied temperature gradient. Percentage increases of the activity were also found to decrease with the increase of the average temperature and concentration. All the results are phenomenologically discussed in terms of substrate traffic across the catalytic membrane induced by the processes of diffusion or thermodialysis under isothermal and non-isothermal conditions, respectively. © 2000 Published by Elsevier Science B.V.

Keywords: Non-isothermal bioreactors; Thermodialysis; Catalytic membranes; Grafting; β-Galactosidase

1. Introduction

The employment of immobilized enzymes in industrial biotechnological processes as well as in analytical devices, such as biosensors, is increased in recent years [1–5]. Both applications are done in isothermal systems and utilize hydrophilic carriers, since this is the microenvironment in which the majority of the biocatalysts naturally work.

Even if the immobilization procedure reduces the specific activity of the catalyst, it offers the advantages of enzyme reuse, separation from reaction products and increase of thermal stability.

Some years ago, a "trick" was proposed by which it was possible to recover some of the specific activity of immobilized enzymes employing the catalytic membrane under non-isothermal conditions [6–18]. Increase of the enzyme reaction rate of about 10–40% was observed, when the catalytic mem-

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branes were interposed between two substrate solutions kept at different temperatures. These effects were found by immobilizing either purified enzymes or whole quiescent cells. In the latter case, the behaviour of internal or cell-wall-bound enzymes was studied.

The increase of the enzyme reaction rate was attributed to the process of thermodialysis [19–21] by which, under non-isothermal conditions, substrate is addressed to the catalytic site and reaction products are removed from it. Thermodialysis is the process by which selective and differential solvent and solute fluxes occur across unselective hydrophobic membranes, separating two solutions kept at different temperatures. Initial results [6–12] concerning the activity increase of enzyme reaction rates were obtained with a two-membrane system: one catalytic and the other hydrophobic, the latter being necessary to induce transport by thermodialysis. Similar results [13–18] were recently obtained employing a single membrane (having both catalytic and hydrophobic property at the same time) under non-isothermal conditions. These membranes were obtained by grafting (chemically or by γ-radiations) teflon or nylon membranes with suitable monomers.

The aim of this work is to investigate the effect of temperature gradients on the activity of β-galactosidase immobilized on a nylon membrane grafted with a hydrophobic monomer such as butylmethacrylate (BMA). The results are discussed in terms of substrate transport across the catalytic membrane by the process of diffusion, under isothermal conditions, and by the process of thermodialysis, under non-isothermal ones.

2. Apparatus, materials and methods

2.1. The bioreactor

The apparatus employed, shown in Fig. 1, consists of two metallic flanges in each of which a shallow cylindrical cavity, 70 mm in diameter and 2.5 mm deep, was bored. The two half-cells constituting the working volume, to be filled with the substrate solution, are separated and set in communication by means of the catalytic membrane. Independent external thermostats maintain the half-cells at predetermined temperatures. In isothermal experiments, the entire apparatus is controlled in parallel from a single thermostat. Thermocouples, positioned at 1 mm from the metallic basis of each half-cell, measure the solution temperature in each working volume. In this way, we can define the average temperature of the system (T_{av}), starting from the temperatures measured in the warm (T_w) and cold (T_c) half-cells as: $T_{av} = (T_w + T_c)/2$, since the system is symmetric. In the same way, we can define ΔT as $T_W - T_C$.

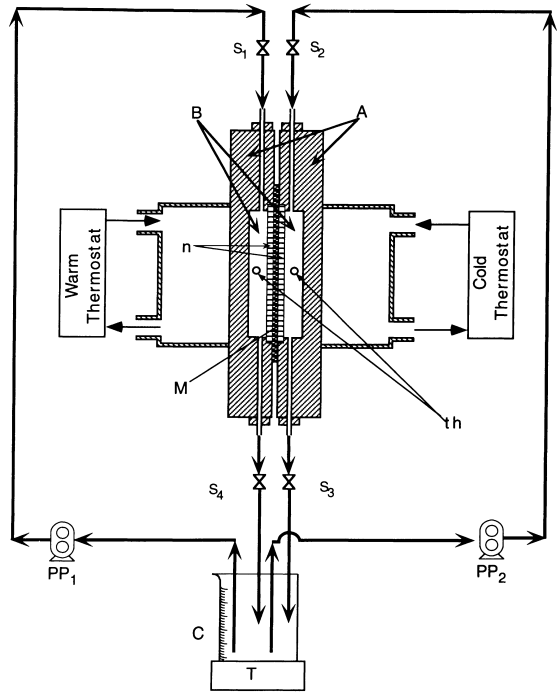

Fig. 1. Schematic (not to scale) representation of the bioreactor. A, half-cell; B, internal working volume; C, external working volume; M, membrane; n, supporting nets; th, thermocouples; S_i, stopcocks; T, thermostatic magnetic stirrer; PP, peristaltic pump.

Working solutions are recirculated through the two half-cells by means of hydraulic circuits which also ensure feeding and emptying of each half-cell in the same cylinder C. Under these conditions, the mixing undoes the effects of the differential water and solute transport by thermodialysis across the membrane, so that the solution composition in C remains constant during time whatever be the tem-

perature difference applied across the membrane. The use of catalytic membranes, therefore, allows to observe enzyme catalysis which alters only substrate and product concentrations in the treated liquid solution, without affecting the concentration of the other components even in presence of temperature gradients.

2.2. Materials

As solid supports to be grafted, nylon Hydrolon membranes by Pall (Pall Italia, Milano, Italy) were used. These hydrophobic membranes, 150 μm thick, have a nominal pore size of 0.2 μm. Pore size is defined as the size of the diameter of the smallest particles that can be retained by the membrane, since in the membrane there are no "classical" pores but irregular cavities, constituted by the interstities between the nylon fibers, spread cross the entire membrane thickness.

Butylmethacrylate (BMA) was used as a hydrophobic monomer to be grafted on the nylon membrane. Hexamethylenediamine (HMDA) and glutaraldehyde (Glu) were used as spacer and coupling agent, respectively. β-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae* was used as a catalyst. This enzyme was chosen in view of the employment of these catalytic membranes in the process of lactose hydrolysis in milk.

All chemical products, including the enzyme, were purchased from Sigma Company (St. Louis, MO) and used without further purification.

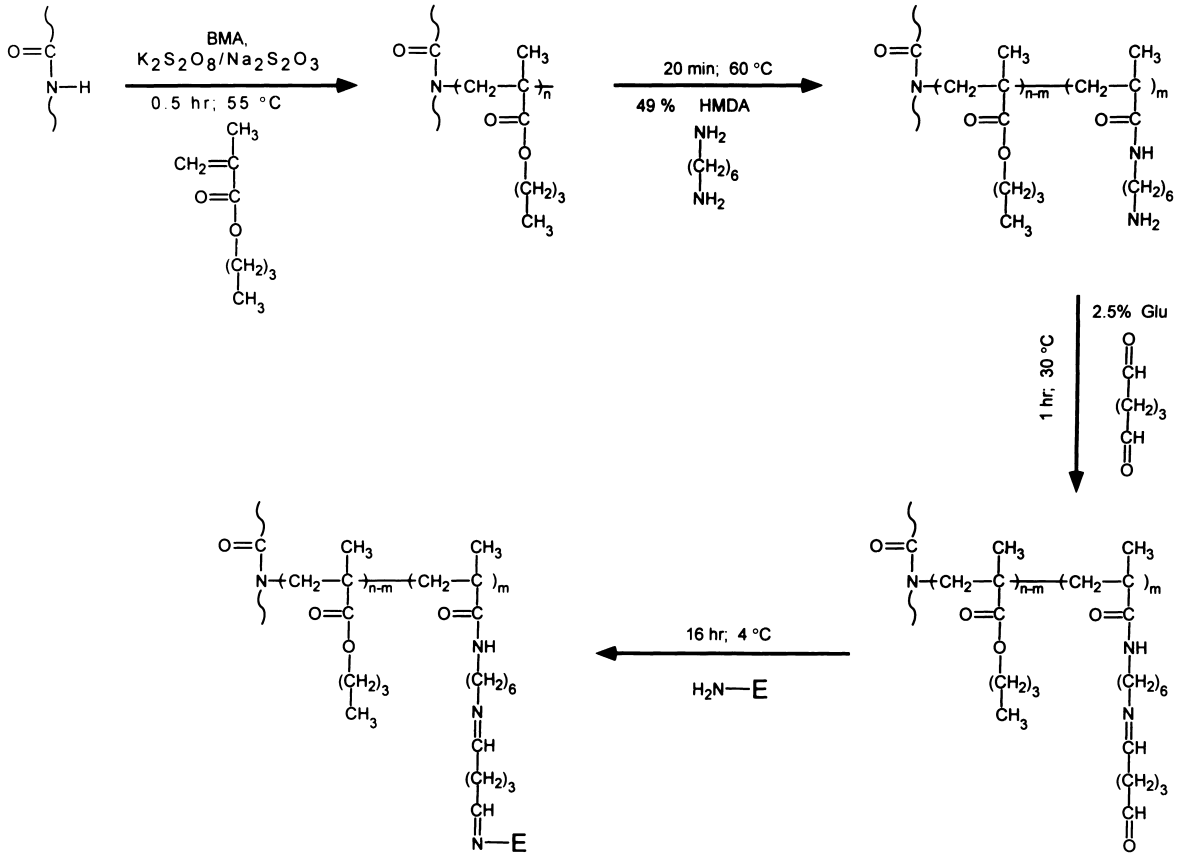

Fig. 2. Schematic picture of the experimental steps followed during the processes of grafting, membrane activation and enzyme immobilization.

2.3. Methods

2.3.1. Catalytic membrane preparation

Grafting copolymerization was carried out by using $K_2S_2O_8/Na_2S_2O_3$ in the ratio 1:2 as initiating system. The membranes were immersed, for 30 min at 55°C, in a reaction vessel containing 157.5 mM BMA, 12 mM $Na_2S_2O_3$ and 6 mM $K_2S_2O_8$ in a 1:1 water/ethanol solution. Later on, the membranes were treated with acetone to remove the produced homopolymer then dried at 60°C, until a constant weight was measured. At this point, a Nylon/Poly-BMA membrane was obtained. Grafting percentage, X (%), was determined by the difference between membrane masses before (G_B) and after (G_A) the grafting process by the expression:

$$X\,(\%) = \left(\frac{G_A - G_B}{G_B}\right) \times 100 \qquad (1)$$

A grafting degree of $14 \pm 3\%$ was obtained.

Membrane activation was performed in two successive steps. The first one was done by immersing the membranes in 49% hexamethylenediamine aqueous solution for 20 min at 60°C. After washing with water to remove unreacted amines, the membranes were treated for 1 h at 30°C in a 2.5% glutaraldehyde aqueous solution. After a new washing with double-distilled water and 0.1 M buffer phosphate solution at pH 6.5, they were treated for 16 h at 4°C with the same buffer solution containing β-galactosidase at a concentration of 3 mg/ml. At the end of this step, the membranes were washed with the buffer solution to remove unbound enzymes. The overall process of grafting, membrane activation and enzyme immobilization is schematically illustrated in Fig. 2.

2.3.2. Determination of catalytic membrane activity

Membrane activity was determined by sampling the solution interacting with the catalytic membrane, at regular time intervals and measuring the glucose concentration by the GOD–Perid test (Boheringer GmbH, Mannheim, Germany). This test uses a coupled enzyme reaction by which according to the scheme

$$\text{glucose} + O_2 + H_2O \overset{\text{GOD}}{\rightarrow} \text{gluconate} + H_2O_2$$

$$H_2O_2 + \text{ABTS} \overset{\text{POD}}{\rightarrow} \text{coloured complex} + H_2O$$

a coloured solution is obtained. The glucose concentration, proportional to the intensity of the solution colour, is spectrophotometrically determined. Reaction rate, expressed as micromoles per minute, is given by the angular coefficient of the linear plot of the glucose production as a function of time. The membranes were stored at 4°C in 0.1 M phosphate buffer solution, pH 6.5.

2.3.3. Temperature profile in the bioreactor

To estimate the real effects of temperature gradients on the activity of immobilized enzymes, the actual temperatures on the surfaces of the catalytic membrane must be known. Since it is impossible to directly measure these temperatures, these have been calculated from the temperatures registered by the thermocouples on the basis of the principle of heat flux continuity and knowing the thermal conductivities and thicknesses of both filling solutions and membrane, by means of the expression:

$$J_{qi} = K_i\left(\frac{\Delta T}{\Delta x}\right)_i = \text{constant} \qquad (2)$$

In this expression K_i is thermal conductivity of i-th medium crossed by the heat flux and ΔT is the temperature difference existing across the same medium of thickness Δx. Eq. (2) holds, provided

Table 1
Correspondence between the values of the temperature (in °C, indicated by the symbol T) read at the position of the thermocouples and the ones calculated at the surfaces of the catalytic membrane (indicated by T^*). Subscripts w and c refer to the warm and cold side, respectively

Correspondence between the temperatures T and T^*							
T_{av}	ΔT	T_c	T_w	T_c^*	T_w^*	T_{av}^*	ΔT^*
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

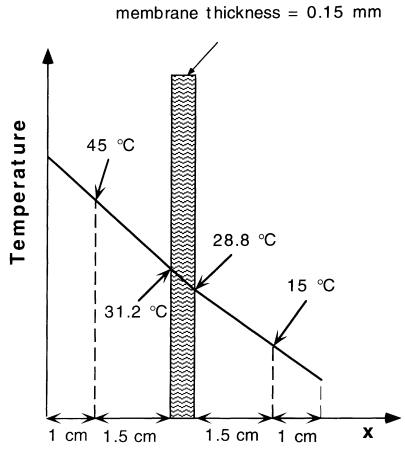

Fig. 3. Temperature profile in the bioreactor and across the catalytic membrane when $\Delta T = 30$°C and $T_{av} = 30$°C. Magnification along the x-axis is 10.

that solution motion in each half-cell is laminar. We have demonstrated in previous works [11,12] that under our experimental conditions in each half-cell of our bioreactor, the Reynolds number (Re) has values lower than R_e^{cri} and therefore fluid motion results laminar. Thus, heat propagation in the bioreactor occurs by conduction between isothermal liquid planes perpendicular to the direction of heat flow. This allows to approximate heat transport by Fourier law as in Eq. (2). Using computer simulation, we calculated the values of the temperatures at each point of the apparatus and hence, on the two surfaces of the catalytic membrane. In Table 1 the actual values of the temperatures at the surfaces of the catalytic membrane are reported for each of the experimental conditions used in this work. The only approximation made in this calculation is that thermal conductivity of the substrate aqueous solutions has been assumed to be equal to that of pure water. The temperatures on each membrane surface have been indicated with T^*, with the subscripts w or c for the surface facing the warm or cold half-cell, respectively. In this way, $\Delta T^* = T_W^* - T_C^*$ and $T_{av}^* = (T_W^* + T_C^*)/2$. Inspection of the temperature values reported in the Table 1 shows that, $T_W^* < T_W$, $T_C^* > T_C$ and,consequently, $\Delta T^* < \Delta T$.

Considering that our system is symmetrical and looking to the results of calculations reported in Table 1, it is possible to write a simple set of equations:

$$T_W^* = T_W - a\Delta T, \qquad T_C^* = T_C + a\Delta T,$$
$$T^* = \Delta T(1 - 2a) \tag{3}$$

In our case, $a = 0.46$.

Reference to these temperatures should be made to evaluate the actual extent of the effect of non-isothermal conditions on enzyme activity. In Fig. 3 the actual temperature profile in the bioreactor is reported for the case in which, $\Delta T = 30$°C and $T_{av} = 30$°C.

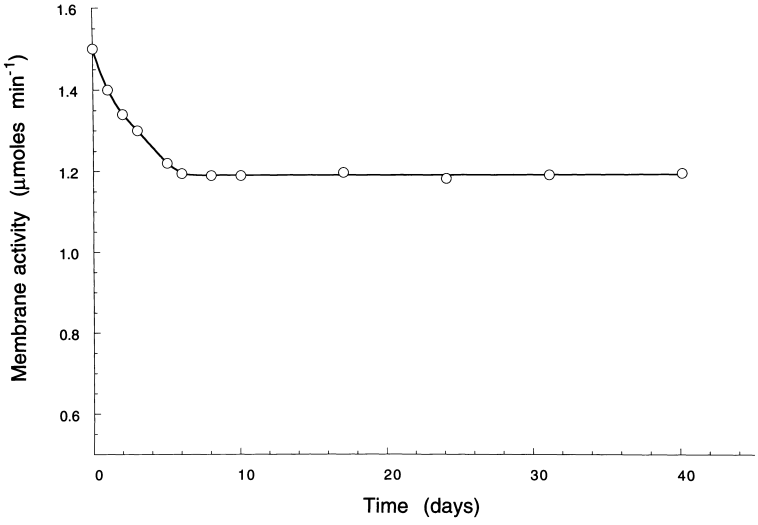

Fig. 4. Time stability of the catalytic membrane. Experimental conditions were: $T = 30$°C and 200 mM lactose concentration.

2.3.4. Treatment of experimental data

The time stability of the biocatalytic membranes was assessed by analyzing every day their activity under the same experimental conditions, i.e. under isothermal conditions at 30°C and 0.2 M lactose concentration. After a week, during which the membranes lost part of the activity, a stable condition was reached remaining unchanged for over one month, as reported in Fig. 4. Only these stabilized membranes have been used in the following experiments.

All points reported in the figures are the average of five independent experiments performed under the same conditions. The experimental errors never exceeded 4%.

3. Results and discussion

The parameters affecting the enzyme reaction rate are the substrate concentration [C], the average temperature T_{av} and the temperature difference ΔT. Isothermal experiments are a particular case in which $\Delta T = 0$. The pH dependence has been disregarded in this study.

Since there are three variables, it is possible to study the relation between any two of them, keeping the third constant. The results will concern the enzyme reaction rate: as a function of the substrate concentration at different average temperatures under isothermal and non-isothermal conditions; as a function of T_{av} at different initial substrate concentrations under isothermal and non-isothermal conditions; as a function of ΔT at different average temperatures and employing different initial substrate concentrations.

3.1. Case (i)

This section concerns the results obtained studying the enzyme reaction rate as a function of the initial substrate concentration at different average temperatures. Fig. 5 shows the results of this study. In particular, Fig. 5a refers to experiments performed under isothermal conditions, while Fig. 5b to experiments made under non-isothermal conditions with $\Delta T = 30$°C. In both figures, the average temperature is the parameter characterizing each curve. It must be observed that under isothermal conditions the temperature T has been identified with T_{av}, considering the isothermal conditions just a special case of the non-isothermal ones. Inspection of the figures shows that, in the temperature range studied, the enzyme reaction rates increase with the average temperature and that the ones under non-isothermal conditions are higher than those corresponding to comparable isothermal conditions.

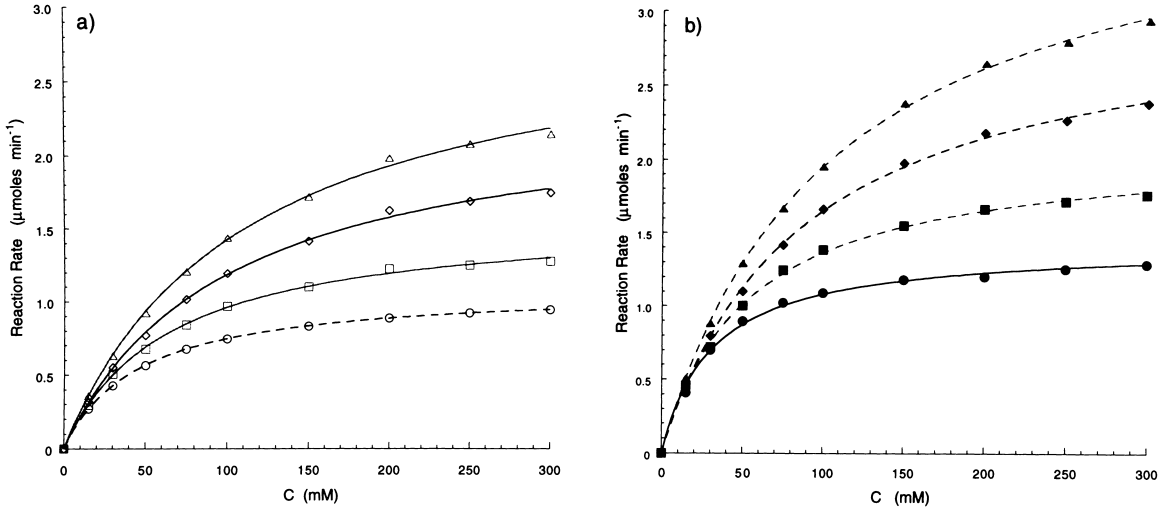

Fig. 5. Enzyme reaction rate as a function of the initial substrate concentration under isothermal (a) and non-isothermal (b) conditions. Curve parameter is the average temperature. Symbols: $T_{av} = 25$°C (○) and (●); $T_{av} = 30$°C (❑) and (■); $T_{av} = 35$°C (◇) and (◆); $T_{av} = 40$°C (Δ) and (▲).

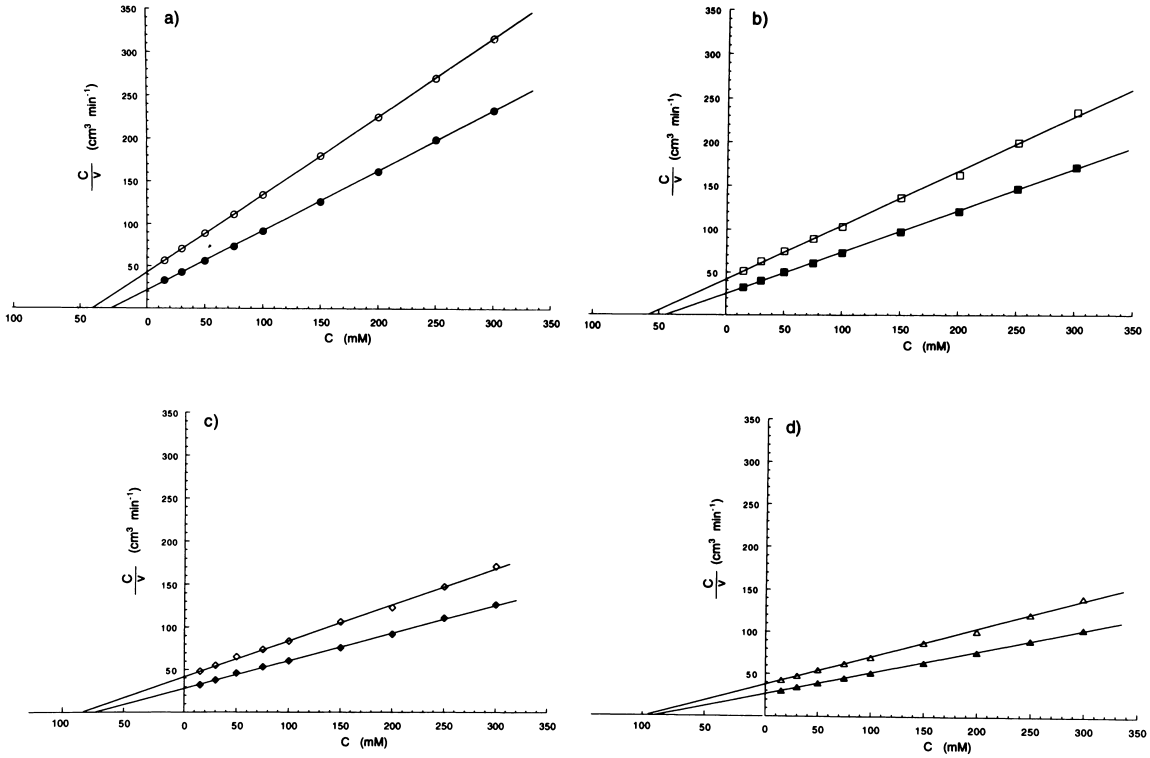

Fig. 6. Hanes plots relative to experiments performed under isothermal (open symbols) and non-isothermal (full symbols) conditions. Experimental conditions: a) $T_{av} = 25°C$; b) $T_{av} = 30°C$; c) $T_{av} = 35°C$; d) $T_{av} = 40°C$.

In Fig. 6, the results of Fig. 5 are represented in the form of Hanes plots to derive the apparent kinetic parameters, K_m and V_{max}, under isothermal and non-isothermal conditions. Fig. 6a refers to the results obtained at $T_{av} = 25°C$, while Fig. 6b, c and d to those obtained at $T_{av} = 30°C$, $T_{av} = 35°C$ and $T_{av} = 40°C$, respectively. The K_m and V_{max} values derived from Fig. 6 are reported in Table 2. Inspection of these values shows that the K_m values increase with the temperature and that the ones obtained under non-isothermal conditions are smaller than the corresponding values under isothermal conditions, indicating in the former case an improved apparent enzyme affinity for the substrate. We explain this behaviour with the circumstance that the presence of a temperature gradient increases (by means of the process of thermodialysis) substrate and products fluxes across the membrane thus reducing the diffusion limitations introduced by the hydrophobic barrier created by the BMA branched chains. A further

Table 2
Apparent kinetic parameters calculated under isothermal and non-isothermal conditions throughout the experiments reported in Figs. 3 and 4

	Isothermal		Non-isothermal	
T_{av} (°C)	$K_{m,app}^{\Delta T=30°C}$ (mM)	$V_{max,app}^{\Delta T=0°C}$ (μmoles min^{-1})	$K_{m,app}^{\Delta T=30°C}$ (mM)	$V_{max,app}^{\Delta T=30°C}$ (μmoles min^{-1})
25	52.2	1.10	25.3	1.42
30	70.4	1.60	51.1	2.10
35	105.0	2.41	84.5	3.05
40	112.0	3.00	98.8	3.97

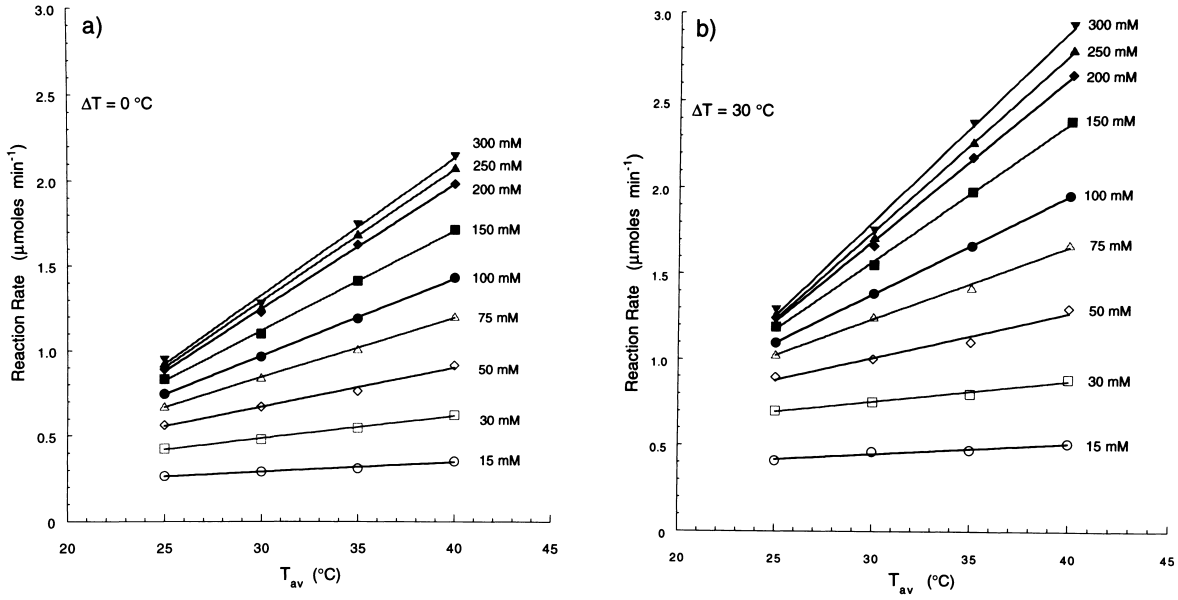

Fig. 7. Enzyme reaction rate as a function of T_{av} under isothermal (a) and non-isothermal (b) conditions. Curve parameter is the substrate concentration. Symbols: 15 mM (O); 30 mM (□); 50 mM (◇); 75 mM (Δ); 100 mM (●); 150 mM (■); 200 mM (◆); 250 mM (▲); 300 mM (▼).

consequence is the increase of enzyme activity under non-isothermal conditions directly related to a possible increase of K_{cat}. When the K_m values are plotted as a function of average temperature, straight lines (nearly parallel) interpolate the experimental points relative to both isothermal and non-isothermal conditions. The correlation coefficient for both straight lines is 0.99.

3.2. Case (ii)

This section concerns the study of the dependence of the enzyme reaction rate on T_{av} at different substrate concentrations under isothermal and non-isothermal conditions. The results of this experimentation are reported in Fig. 7. Fig. 7a refers to experiments performed under isothermal conditions, while Fig. 7b refers to experiments done under non-isothermal conditions at $\Delta T = 30$°C. Both figures show, for each substrate concentration, a linear dependence of the reaction rate on the temperature.

An Arrhenius plot of the experimental points of Fig. 7 allows to calculate for each substrate concentration, the activation energy of the process. The values of these energies, reported in Table 3, do not evidence significant differences between isothermal and non-isothermal conditions while they evidence an increase with the increase of the substrate concentration. This means that at low substrate concentration the enzyme process is diffusion limited.

Table 3
Activation energies (kcal mole^{-1}) calculated for different initial lactose concentrations under isothermal and non-isothermal conditions

Activation energies		
[Lactose] (mM)	Isothermal (ΔT = 0°C)	Non-isothermal (ΔT = 30°C)
15	3.45	2.39
30	4.78	2.88
50	5.92	4.41
75	7.16	5.91
100	8.05	7.13
150	8.98	8.69
200	10.00	9.78
250	10.15	9.95
300	10.22	10.31

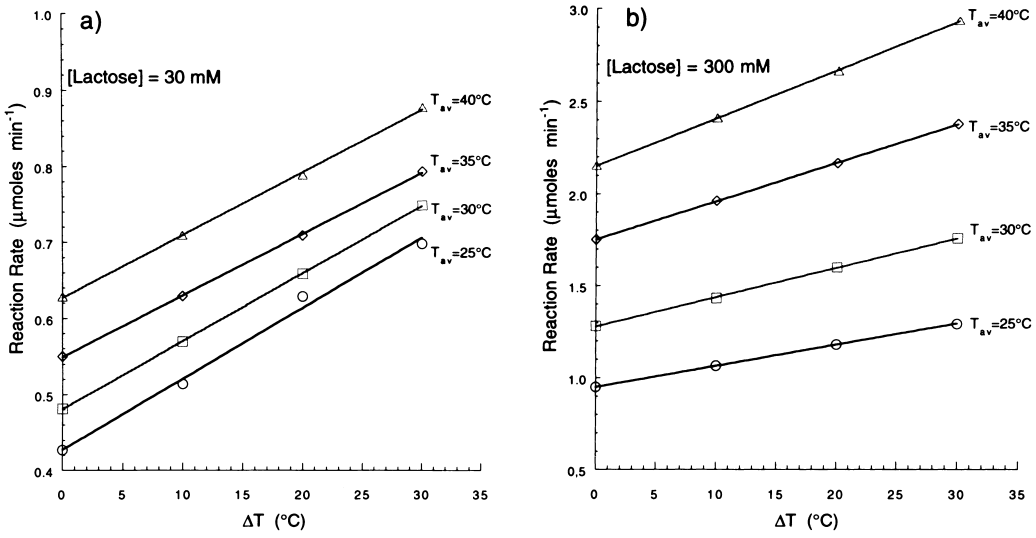

Fig. 8. Enzyme reaction rate as a function of the applied ΔT for a 30 mM (a) and 300 mM (b) lactose concentration. Curve parameter is the average temperature. Symbols: $T_{av} = 25°C$ (O); $T_{av} = 30°C$ (□); $T_{av} = 35°C$ (◇); $T_{av} = 40°C$ (Δ).

3.3. Case (iii)

This section concerns the dependence of the enzyme reaction rate on the applied ΔT at constant substrate concentration and variable T_{av}, or at fixed T_{av} and variable substrate concentration.

Fig. 8 refers to results obtained at constant substrate concentration and variable T_{av}. For the sake of

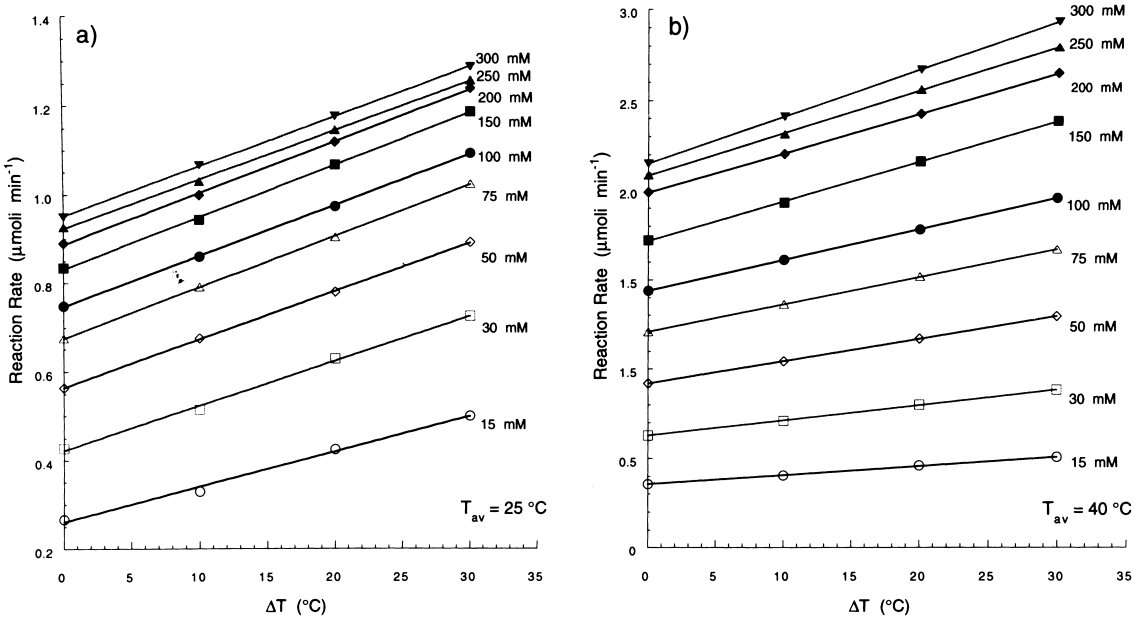

Fig. 9. Enzyme reaction rate as a function of the applied ΔT at $T_{av} = 25°C$ (a) and $T_{av} = 40°C$. Curve parameter is the substrate concentration. Symbols: 15 mM (O); 30 mM (□); 50 mM (◇); 75 mM (Δ); 100 mM (●); 150 mM (■); 300 mM (♦); 250 mM (▲) 300 mM (▼).

simplicity, the behaviour of only two substrate concentrations have been reported: one at low concentration (Fig. 8a) and the other at high concentration (Fig. 8b). Both figures evidence an activity increase, which is a linear function of the applied temperature difference, and at each average temperature a greater activity increase is observed at lower concentration. Similar results were obtained for all the other substrate concentrations studied in this work.

In Fig. 9, the results obtained at constant T_{av} and variable concentrations have been reported. Fig. 9a refers to results obtained at the lowest average temperature used in this experimentation (i.e. 25°C), while Fig. 9b to those obtained at the highest average temperature (i.e. 40°C). Also in this case, a linear dependence of the enzyme reaction rate on the applied ΔT is observed. The angular coefficients of the straight lines at low concentrations are higher than those corresponding at high concentrations. Similar results were obtained at all the other average temperatures studied.

The linearity of results in Figs. 8 and 9 allows to write equations of the type:

$$\mathrm{RR}(C)|_{\Delta T \neq 0}^{T_{av}} = \mathrm{RR}(C)|_{\Delta T = 0}^{T_{av}}(1 + \alpha \Delta T) \qquad (4)$$

where, $\mathrm{RR}(C)|_{\Delta T \neq 0}^{T_{av}}$ and $\mathrm{RR}(C)|_{\Delta T = 0}^{T_{av}}$ are the enzyme reaction rate at the concentration C and the temperature T_{av} under non-isothermal and isothermal conditions, respectively. The α coefficient, at each average temperature and concentration, represents the increase of the enzyme reaction rate when a temperature difference of 1°C is read by the thermocouples. The values of the α coefficients calculated from the experiments reported in Figs. 8 and 9, as well as from the ones obtained under different experimental conditions, are reported in Table 4. Inspection of the table shows that the α coefficients decrease with the increase of the concentration and average temperature.

It is interesting to observe that the α-values found with this membrane are similar to the ones obtained with the two-membrane system or with other membranes, grafted with different monomers.

The α-values found in these experiments support the idea of employing the technology of non-isothermal catalysis in industrial processes using immobilized enzymes or cells. It is easy to see, indeed, how an increase of the enzyme reaction rate under non-isothermal conditions turns out in a decrease of the production times of a productive process.

The usefulness of employing this technology appears more interesting, if attention is paid to the effective temperature difference existing across the

Table 4
α and α' coefficients[a] calculated by means of the Eq. (4) applied to experimental results reported in Figs. 6 and 7

Percentage activity increases under non-isothermal conditions			
T_{av} (°C)	Concentration (mM)	α (°C^{-1})	α' (°C^{-1})
25	15	2.91	37.01
30	15	1.90	23.75
35	15	1.65	20.62
40	15	1.40	17.50
25	30	2.37	29.70
30	30	1.85	22.12
35	30	1.49	18.62
40	30	1.34	16.75
25	50	2.01	25.14
30	50	1.60	20.00
35	50	1.43	17.87
40	50	1.34	16.75
25	75	1.74	21.78
30	75	1.58	19.75
35	75	1.31	16.37
40	75	1.26	15.75
25	100	1.55	19.40
30	100	1.40	17.50
35	100	1.29	16.12
40	100	1.19	14.87
25	150	1.41	17.61
30	150	1.36	17.00
35	150	1.34	16.75
40	150	1.17	14.62
25	200	1.31	16.43
30	200	1.16	14.50
35	200	1.16	14.50
40	200	1.10	13.75
25	250	1.20	16.05
30	250	1.21	15.12
35	250	1.11	13.87
40	250	1.13	14.12
25	300	1.18	15.84
30	300	1.21	15.12
35	300	1.18	14.75
40	300	1.20	15.00

[a] α'-values are obtained by means of the expression $\alpha' = \alpha(\Delta T/\Delta T^*)$. Values for experimental conditions not reported in Figs. 6 and 7 are also listed.

catalytic membrane. Inspection of Table 1 shows that a macroscopic temperature difference of $\Delta T = 30°C$ read by the thermocouples reduces to ΔT^* of 2.4°C across the catalytic membrane. On the basis of this consideration the α-values listed in Table 4 must be corrected to account for the effective temperature difference across the membrane. The new values, defined as α', are reported in Fig. 10. Now the influence of the non-isothermal conditions on the activity of immobilized enzymes appears more pronounced. Fig. 10 shows that the activity percentage increase of the catalytic membrane exponentially decreases with the initial substrate concentration. At the same time the α'-values decrease with the increase in the average temperature. The reasons for this behaviour will become clear, if substrate (and products) transport is considered under isothermal and non-isothermal conditions.

Under isothermal conditions, the substrate traffic across the catalytic membrane is due to diffusion only, according to Fick law

$$J_s^{diff} = D\frac{\Delta C}{\Delta x} \quad (5)$$

where J_s^{diff} is expressed in moles $cm^{-2}\,s^{-1}$, D in $cm^2\,s^{-1}$, $\Delta C/\Delta x$ in moles $cm^{-4}\,s^{-1}$. D is the isothermal diffusion coefficient and $\Delta C/\Delta x$ is the concentration gradient existing across the membrane, having thickness Δx.

Under non-isothermal (ni) conditions a volume flux (water flux) is observed from warm to cold region together with a substrate flow in the opposite direction. Both fluxes are due to the process of thermodialysis.

The volume flux, practically coincident with the water flux, is given by

$$J_{vol}^{ni} = J_w^{ni} = B\frac{\Delta T}{\Delta x} \quad (6)$$

and the substrate flux by

$$J_s^{ni} = D'C\frac{\Delta T}{\Delta x} \quad (7)$$

In these equations, J_{vol}^{ni} is expressed in moles $cm^{-2}\,s^{-1}$, B in $cm^2\,s^{-1}\,K^{-1}$, $\Delta T/\Delta x$ in $K\,cm^{-1}$, J_s^{ni} in moles $cm^{-2}\,s^{-1}$, D′ in $cm^2\,K^{-1}\,s^{-1}$, C in moles cm^{-3}. B is a coupling thermodynamic coefficient, $\Delta T/\Delta x$ the temperature gradient across the membrane thickness Δx, D' the thermal diffusion coefficient, C the substrate concentration.

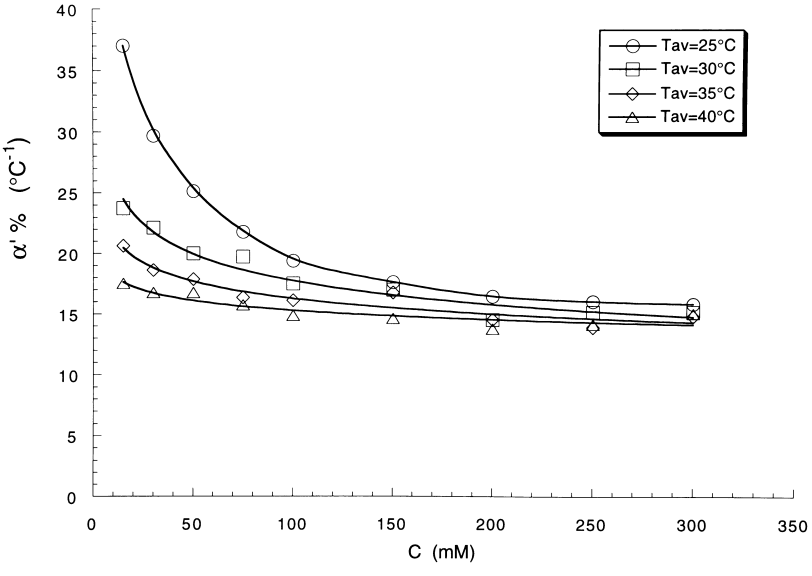

Fig. 10. Percentage increase of the enzyme reaction rate as a function of substrate concentration. Curve parameter is the average temperature. Symbols: $T_{av} = 25°C$ (O); $T_{av} = 30°C$ (□); $T_{av} = 35°C$ (◇); $T_{av} = 40°C$ (Δ).

Associated with the volume transport, there is a substrate transport (from warm to cold), known as solute drag transport, given by

$$J_s^{drag} = C_W J_{vol}^{ni} \quad (8)$$

where C_w is the actual concentration in the warm half-cell, from which the volume flux is coming. Of course, diffusive substrate fluxes are still present.

A schematic picture of substrate fluxes occurring across the catalytic membranes is represented in Fig. 11 for the isothermal (a) and non-isothermal (b) conditions.

As a consequence of the above reasoning under isothermal conditions, only diffusive substrate flux according to the Eq. (5) occurs across the catalytic membranes; while under non-isothermal conditions diffusive flux (Eq. (5)) adds to non-isothermal diffusive flux (Eq. (7)) and drag flux (Eq. (8)). For these reasons, under non-isothermal conditions the enzyme immobilized on and into the membrane faces a concentration higher than under isothermal conditions and, hence, the activity of the catalytic membrane increases. This means that the substrate concentration profiles are different in the membrane not only, under isothermal and non-isothermal conditions, but also if catalysis is considered or not. These profiles are qualitatively represented in Fig. 12. The increase of substrate concentration affects the reaction rate at low concentration, while it is less effective at substrate concentrations saturating the reaction. This is the reason, for which the percentage increase of the enzyme reaction rate is higher at lower substrate concentration.

A quantitative treatment of the influence of these substrate fluxes on the substrate concentration profile in the catalytic membrane is discussed in a parallel

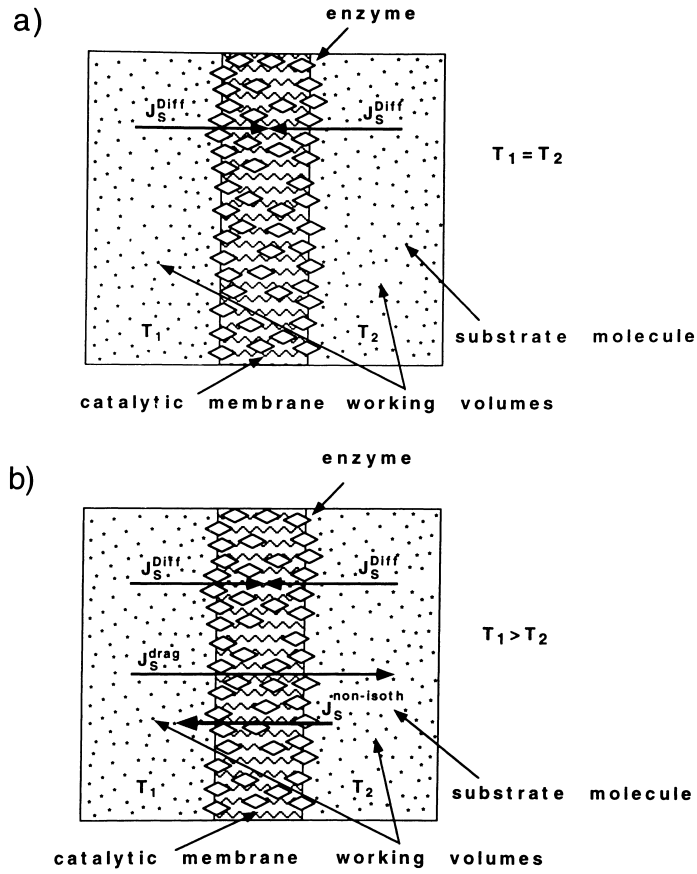

Fig. 11. Substrate traffic across a catalytic membrane induced by the processes of diffusion (a) and thermodialysis (b).

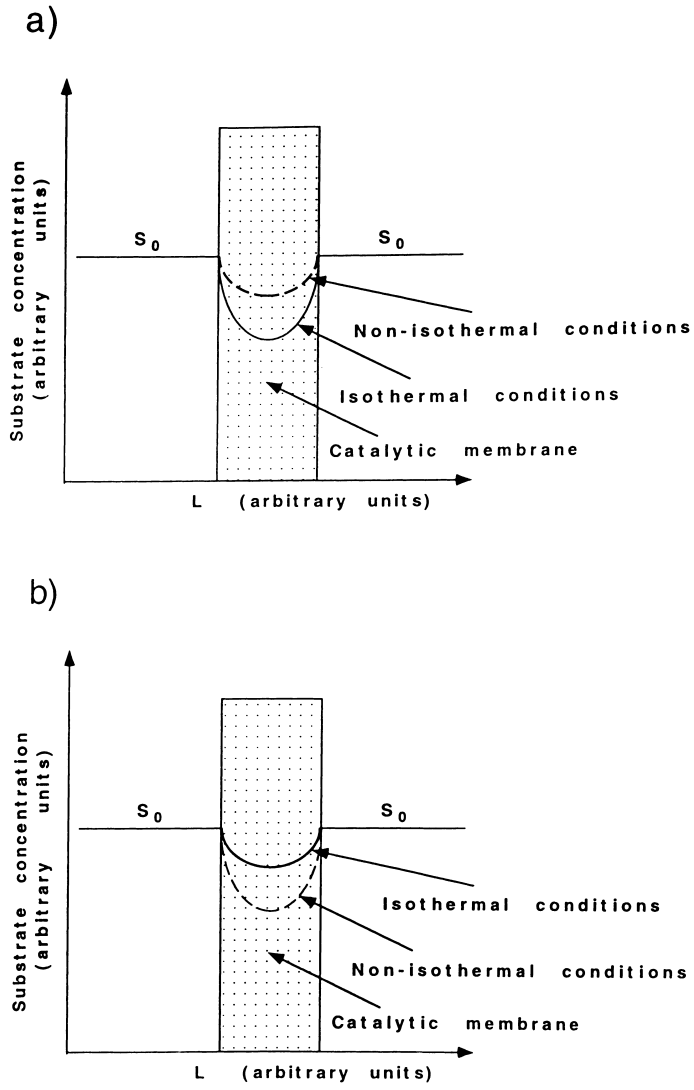

Fig. 12. Qualitative representation of the substrate concentration profile in absence (a) or in presence (b) of catalysis under isothermal and non-isothermal conditions.

paper [22], where diffusion and thermodialysis experiments with lactose aqueous solutions are reported in view of this analysis.

4. Conclusions

All the experimental data reported here, confirm the influence of the temperature gradient on the activity of immobilized enzymes. The substitution of the two-membrane system with one membrane, catalytic and hydrophobic, does not affect the increases of the enzyme reaction rate under non-isothermal conditions, which are of the same order of magnitude of those found with other membrane systems.

The study of the enzyme activity as a function of substrate concentration has shown that the K_m values under non-isothermal conditions are smaller than the values under the isothermal conditions and linearly increase with the average temperature.

The most important result appears to be the dependence of the percentage activity increases on substrate concentration. These increases became smaller on increasing either the concentration or the average temperature, following an exponential-like law with a fast decreasing part at substrate concentration lower than the K_m value and an asymptotic constant part at concentration higher than that of the K_m.

The role of the thermodialysis process on the increase of the enzyme reaction rate has been qualitatively indicated. A detailed and quantitative analysis of the results here illustrated is reported in a paper [22], where the substrate concentration profile in the catalytic membrane is calculated when catalysis occurs under isothermal and non-isothermal conditions.

The use of non-isothermal bioreactors in industrial biotechnological processes appears promising, particularly in the case in which a geometry different from the planar one employed in this research is used. Experiments in this direction are in progress in our laboratory.

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