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Influence of the immobilisation process on the activity of β-galactosidase bound to nylon membranes grafted with glycidyl methacrylate Part 2. Non-isothermal behaviour

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Abstract

β-Galactosidase was immobilised via diazotisation or condensation on nylon membranes, chemically grafted with glycidyl methacrylate (GMA) and having identical physical transport properties, such as hydraulic and thermoosmotic permeabilities. Immobilisation by diazotisation involves the binding of the enzyme to the activated membranes through the tyrosine residues of the macromolecule, while immobilisation by condensation occurs through arginine residues.

The performance of both membranes types was studied as a function of substrate concentration, average temperature and temperature gradients in a bioreactor operating under non-isothermal conditions. Both membrane types exhibit apparent K_m values of the immobilised form higher than that of the free enzyme. Under non-isothermal conditions the apparent K_m of the immobilised β-galactosidase approached values more close to that of the free enzyme, recovering in this way the loss of affinity consequent to the immobilisation process. Moreover, membranes obtained via diazotisation resulted more efficient than those obtained via condensation, showing higher percentage activity increases with respect to all the experimental variables studied. This behaviour has been attributed to the different immobilisation technique, since the multipoint enzyme attachment through the arginine residues of the macromolecule affects the catalytic structure of the catalytic site more than the attachment by diazotisation.

Activity increases under non-isothermal conditions were found to decrease with the increases of substrate concentration and average temperature. The values of the yield increases with 1 °C of temperature difference across both catalytic membranes confirm the useful employment of the technology of the non-isothermal bioreactors in productive biotechnological processes.

Keywords: β-Galactosidase; Graft membranes; Enzyme immobilisation; Diazotisation; Condensation; Bioreactors

1. Introduction

The use of insoluble catalytic carriers is increasing in industrial applications [1,2]. The advantage of multiple reuses supplies the disadvantage of the apparent

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decrease in the affinity for the substrate. The principal cause of loss of specific activity is the limitation to the diffusion of substrate and reaction products towards or away from the catalytic site due to the steric hindrance offered by the carrier structure and the adjacent Nernst layers. To overcome this drawback some tricks have been attempted, such as enzyme immobilisation onto soluble–insoluble matrices [3–8] or in hydrogels thermally reversible [9,10] or pressure sensitive [11]. Some years ago [12] we proposed a new approach to overcome the diffusion limitation problem. This approach consisted in using a catalytic membrane in a bioreactor operating under non-isothermal conditions. Under these conditions the activity of a catalytic membrane results higher than that measured under comparable isothermal conditions [13–19]. The activity increases, ranging from 10 to 30%, depend on the nature of the enzyme, on the type of graft membrane and on graft monomer. The physical cause responsible for the activity increase has been identified in the process of thermodialysis [20–22]. This process describes the non-isothermal mass transport across a hydrophobic and unselective porous membrane separating two aqueous solutions at different temperatures. Under these conditions differential water and solute fluxes take place. Water fluxes are directed towards the cold half-cell, while solute fluxes proceed towards the cold or the warm half-cell according to their nature. Both solvent and solute fluxes increase with the temperature gradient applied across the membrane.

When a catalytic and hydrophobic membrane separate two substrate solutions kept at different temperatures, substrate fluxes produced by thermodialysis add to the diffusive ones so that the enzyme immobilised onto the membrane encounters a concentration higher than that encountered under comparable isothermal conditions. These effects were observed [13–19] provided that the catalytic membranes were hydrophobic. Membrane hydrophobicity is the prerequisite for the occurrence of the process of thermodialysis. A collateral application of the technology of the non-isothermal bioreactors has been made by designing and constructing non-isothermal biosensors [23–25]. Bearing in mind the role of membrane hydrophobicity in our previous studies attention was paid to the construction of hydrophobic and catalytic membranes, disregarding the effects of the immobilisation method used.

In this paper we report the non-isothermal activity of two different catalytic nylon membranes, having the same hydrophobicity and loaded with the same amount of enzyme, but different for the immobilisation method used: diazotisation or condensation.

In a separate paper [26] the behaviour of both these membranes in a bioreactor operating under isothermal conditions have been discussed.

2. Apparatus, materials and methods

2.1. The bioreactor

The apparatus (Fig. 1) was the same used for the isothermal experiments reported in [26], where technical constructive details have been also done. Thermocouples, placed 1.5 mm from each of the membrane surfaces, measured the temperatures inside each half-cell and allowed to calculate the temperature profile across the catalytic membrane under non-isothermal conditions. The temperatures read by the thermocouples will be indicated by T, while the ones calculated at the membrane surfaces by T^*. Moreover, the temperatures of the warm or cold side are indicated by the subscript w or c, respectively. Under these assumptions, it follows that $\Delta T = T_w - T_c^*$, $\Delta T^* = T_w^* - T_c^*$, $T_{av} = (T_w + T_c)/2$, $T_{av}^* = (T_w^* + T_c^*)/2$, $T_w^* < T_w$, $T_c^* > T_c$, $\Delta T^* < \Delta T$, and, being our system symmetric, $T_{av} = T_{av}^*$.

The two working half-cells, separated by and communicating through a membrane, were thermostatted at the desired temperature T_i ($i = 1, 2$) by means of water recirculating in external jackets and coming from two thermostatic baths.

2.2. Materials

Nylon hydrolon membranes, gift of Pall Italia (Pall Italia srl-Milano-Italy), were used as solid support to be grafted. These membranes, 150 μm in thickness are hydrophobic and have a nominal pore size of 0.2 μm.

All chemicals, including the enzyme, were purchased from Sigma (Sigma Aldrich srl-Milano-Italy) and used without further purification. As monomer to be grafted we used glycidyl methacrylate (GMA). Phenylenediamine (PDA) was used as spacer between

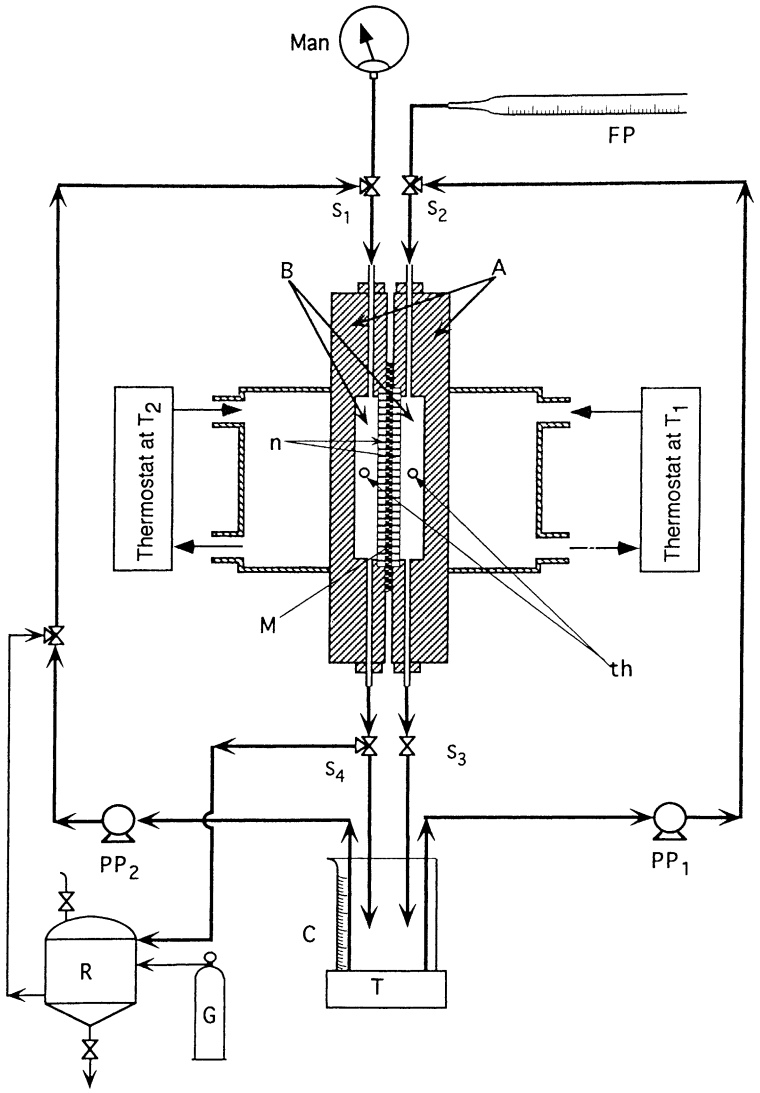

Fig. 1. Schematic (not to scale) representation of the bioreactor. A: half-cells; B: internal working volumes; C: external working volume; M: membrane; n: supporting nets; th: thermocouples; S_i: stopcocks; T: thermostatic magnetic stirrer; PP_i: peristaltic pumps; Man: manometer; FP: flow-pipe; R: reservoir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

the grafted membrane and the enzyme. For the preparation of the catalytic membrane via condensation a 2.5% glutaraldehyde aqueous solution was also employed.

The enzyme employed was a β-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae*. β-Galactosidase has been used in view of the employment of these catalytic membranes in the process of lactose hydrolysis in milk as well as in the treatment of the waste waters coming from dairy industry.

2.3. Methods

2.3.1. Catalytic membrane preparation

Two different membrane types were prepared for this research.

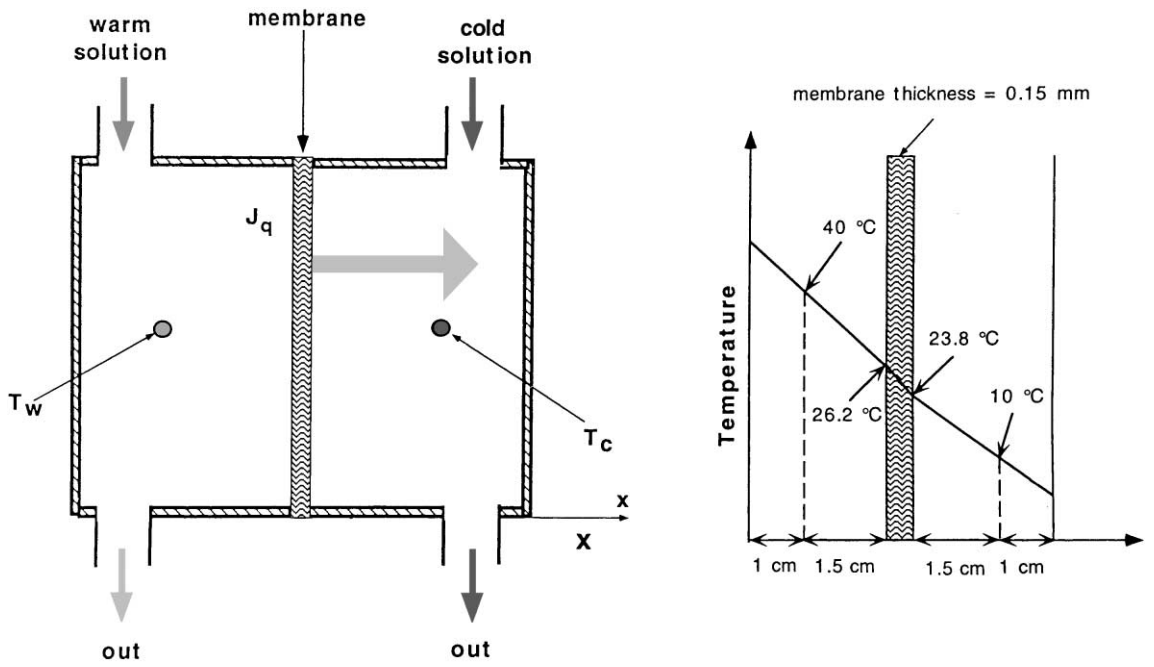

Fig. 2. (a) Side view of the cell showing position of thermocouples, membrane and heat flux (J_q). (b) Temperature profile in the bioreactor when the temperatures read by the thermocouples are $T_w = 40\,^{\circ}C$ and $T_c = 10\,^{\circ}C$. Magnification along the *x*-axis is 10.

The first type, named M_1, was prepared by immobilizing β-galactosidase on the nylon membrane, grafted with GMA, via diazotisation, i.e. by binding the enzyme to the activated supports through the tyrosine residues of the macromolecule.

The second type, named M_2, was prepared by immobilizing the enzyme on the preactivated supports through the arginine residues of the macromolecule.

The preparation of the catalytic membranes was done according to the procedure described in [26]. In Figs. 2 and 3 of the same reference it is possible to find a picture of the steps involved in the processes of grafting, membrane activation and enzyme immobilisation.

Grafting degree (G.D.) was determined according to the expression reported in [26].

When not used, the membranes were stored at 4 °C in a 0.1 M phosphate buffer solution, pH is 6.5.

2.3.2. Determination of membrane activity

Membrane activity and stability were determined according to the procedures described in [26].

2.3.3. Temperature profile across the catalytic membrane

To estimate the real effects of temperature gradients on the activity of immobilised enzymes, the actual temperatures on the surfaces of the catalytic membrane must be known. Since it is not possible to measure the temperatures at each face of the membrane, they can be calculated from the temperatures measured at the position of the thermocouples. By assuming that the solution motion in the two half-cells is not turbulent, and by knowing the thermal conductivities and thicknesses of both filling solutions and membrane, it is possible to calculate these temperatures by means of the heat flux continuity principle through the equation

$$J_q = -K_i \left(\frac{\Delta T}{\Delta x}\right)_i = \text{constant} \qquad (1)$$

where J_q is the heat flux, K_i the thermal conductivity of the *i*th medium and $(\Delta T/\Delta x)_i$ is the temperature gradient existing in the same medium having thickness Δx_i. Since in each half-cell the solution is recirculated at a flow rate of 2.5 ml min^{-1} through two

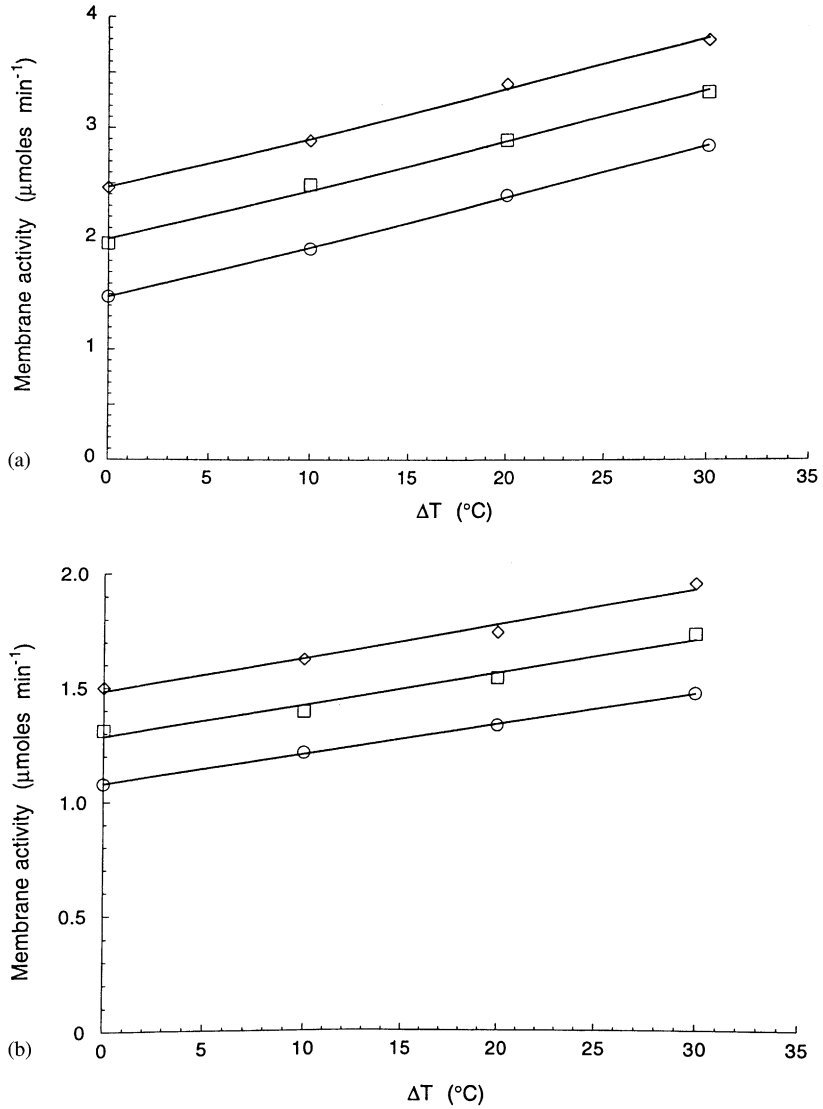

Fig. 3. Membrane activity as function of temperature difference. (a) Membrane M_1; (b) membrane M_2. Symbols: (○) $T_{av} = 25\,^{\circ}C$; (□) $T_{av} = 30\,^{\circ}C$; (◇) $T_{av} = 35\,^{\circ}C$.

fins with rounded tips, the Reynolds number has values lower than 10 and consequently the fluid motion is laminar [17–19,27]. Thus, heat propagation in the bioreactor occurs by conduction between isothermal liquid planes, which are perpendicular to the direction of the heat flow (Fig. 2a). This allowed us to approximate heat transport by the Fourier law, as in Eq. (1). Using computer simulation, we calculated the values of the temperatures at each point of the apparatus, and at the two surfaces of the catalytic membrane. In this way the temperature profile in the bioreactor and in the catalytic membrane can be known. In the calculation we assumed as value of the thermal conductivity of our solution that of pure water, whereas the value of thermal conductivity of the membrane was taken out from Touloukian et al. [28]. We assumed also that the grafting process did not change thermal conductivity of the untreated membrane. In Table 1, we

Table 1
Correspondence between the temperature read by the thermocouples (indicated by T) and the temperature calculated (indicated by T^*) at the surfaces of the catalytic membranes

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

report the temperatures read at the thermocouple positions and the corresponding ones on the surfaces of the catalytic nylon membrane interposed between the substrate solutions maintained at different temperatures. In Fig. 2b, the real temperature profile for one of the cases reported in Table 1 is illustrated.

Inspection of temperature values in Table 1 allows us to write the following empirical equations:

$$T_w^* = T_w - a\,\Delta T, \qquad T_c^* = T_c + a\,\Delta T,$$
$$\Delta T^* = \Delta T(1 - 2a) \tag{2}$$

where a is a constant. In our case $a = 0.445$.

2.3.4. Treatment of experimental data

Every experimental point reported in the figures represents the average value of five experiments performed under the same conditions. Each experiment lasted 30 min, but only the initial reaction rates were accounted for in the construction of the figures. The duration of each experiment as well as the hydrophobic nature of the membrane excluded the occurrence of membrane fouling. In any case, to avoid membrane fouling owing to membrane reuse, a cleaning solution, 0.1 M phosphate buffer at pH 6.5 was recirculated for 20 min through the bioreactor and the membrane between two subsequent experiments. Effects due to concentration polarisation, even present, have been disregarded considering membrane hydrophobicity. The experimental errors did not exceed 4%.

3. Results and discussion

In a separate paper [26], we have demonstrated that membranes M_1 and M_2 from a physical point of view were sufficiently comparable since they had practically equal hydraulic, A, and thermoosmotic, B, permeability coefficients. In the same paper we have demonstrated that from a biochemical point of view the two membranes were different, since the membranes prepared via diazotisation, i.e. membranes of M_1 type, had a specific and absolute activity higher than those prepared by condensation, i.e. the membranes of M_2 type. Some of these properties are listed in Table 2. During the measurements of hydraulic and thermoosmotic permeabilities concentration polarisation on/or fouling of the membranes were absent since the experiments were carried out with pure, double-distilled water. From this table it clearly emerges that both

Table 2
Physical and biochemical parameters characterizing membrane M_1 and M_2, respectively

Membrane	Grafting percentage (%)	A ($m^4\,N^{-1}\,s^{-1}$)	B ($m^2\,K^{-1}\,s^{-1}$)	$C = B/A$ ($N\,m^{-2}\,K^{-1}$)	Amount of immobilised enzyme (mg)	Absolute membrane activity ($U\,cm^{-2}$)	Specific membrane activity ($U\,mg^{-1}$)
M_1	15.9	3.05×10^{-14}	3.7×10^{-12}	121	1.89	0.021	0.78
M_2	15.3	2.95×10^{-14}	3.9×10^{-12}	132	1.73	0.015	0.64

membranes have values of the C coefficient higher than zero. C, defined as the ratio between the coefficients B and A, is a measure of the pressure difference ΔP to be applied to the cold half-cell to stop the thermoosmotic water flux from the warm to cold side of the bioreactor in the presence of a temperature difference ΔT measured at the position of the thermocouples. C is also a measure of the effects produced by the process of thermodialysis across hydrophobic membranes, catalytic or not. For $C \neq 0$ thermodialysis take places, for $C = 0$ it is impossible to observe thermodialysis. Higher values of C correspond to higher matter fluxes produced by the process of thermodialysis. As a consequence of all the above considerations C can be considered a parameter related to the membrane hydrophobicity. From the above observations it follows that for $C = 0$, i.e. in the absence of the process of thermodialysis, the yield of a catalytic membrane should be the same under isothermal or non-isothermal conditions. For $C > 0$, i.e. with membranes giving matter transport by thermodialysis, the reaction rate of a catalytic membrane under non-isothermal conditions increases with the increase of the C value in comparison to that observed under isothermal conditions.

Since both our catalytic membranes have C values higher than zero, they appear to be candidate for an increase of their enzyme activity under non-isothermal conditions.

The more simple way to study the effect of temperature gradients on the activity of membranes M_1 and M_2 is to measure, at a fixed substrate concentration, the dependence of their catalytic activity as a function of the temperature difference applied to the bioreactor, keeping the average temperature constant. The results of this investigation are reported in Fig. 3a and b for membranes M_1 and M_2, respectively. The curve parameter in both figures is the average temperature. All experiments were carried out with a 200 mM lactose concentration in 0.1 M phosphate buffer solution at pH 6.5. Results in Fig. 3a and b show that at each average temperature the activity of both catalytic membranes linearly increases with the applied temperature difference ΔT.

Inspection of the Fig. 3a and b also shows that the values of the catalytic activities of membrane M_1 are higher than the corresponding values of membrane M_2, when identical conditions of average temperature and temperature difference are compared.

Having ascertained the effect of temperature gradients on the catalytic activity of both enzyme derivatives at one fixed substrate concentration, we extended this study at different lactose concentration up to 300 mM. In Fig. 4a and b, the activity of both catalytic membranes is reported as a function of substrate concentration. Fig. 4a refers to results obtained with membrane M_1, while Fig. 4b refers to membrane M_2. Experimental conditions were $T_{av} = 25\,^{\circ}C$ and different ΔT's values, which are the curve parameter. Results in Fig. 4a and b show that for both catalytic membrane types the enzyme reaction rates under non-isothermal conditions are higher than those found under comparable isothermal conditions, and that at each substrate concentration the reaction rates increase with the increase of the applied ΔT.

At this point the problem arises whether it is possible to compare the results obtained under two different physical situations, i.e. in the presence or in the absence of temperature gradients, particularly when large temperature gradients are applied. Such a comparison is possible since the actual temperature difference across the catalytic membrane is reduced to small values. The temperatures between the two membrane surfaces vary linearly within the membrane thickness (Fig. 2b). Also in the presence of high temperature differences measured by the thermocouples, they are slightly different from the temperature at the centre of the membrane which is equal to the macroscopic average temperature, as one can see in Table 1. This implies that, in the absence of specific effects induced by the temperature gradients and by considering only the dependence of the activity on the temperature, the total activity of the catalytic membrane under non-isothermal conditions can be considered equal to that obtained at the corresponding T_{av}. In fact, by considering the enzyme distribution uniform through the membrane thickness, the loss of activity at temperatures lower than the average one is balanced by that obtained at temperatures higher than the average. Accordingly, the activity of the membrane under non-isothermal condition should be considered equal to that corresponding to the average temperature. These considerations allow the comparison between the results obtained under non-isothermal conditions with those obtained under isothermal conditions at the corresponding average temperature.

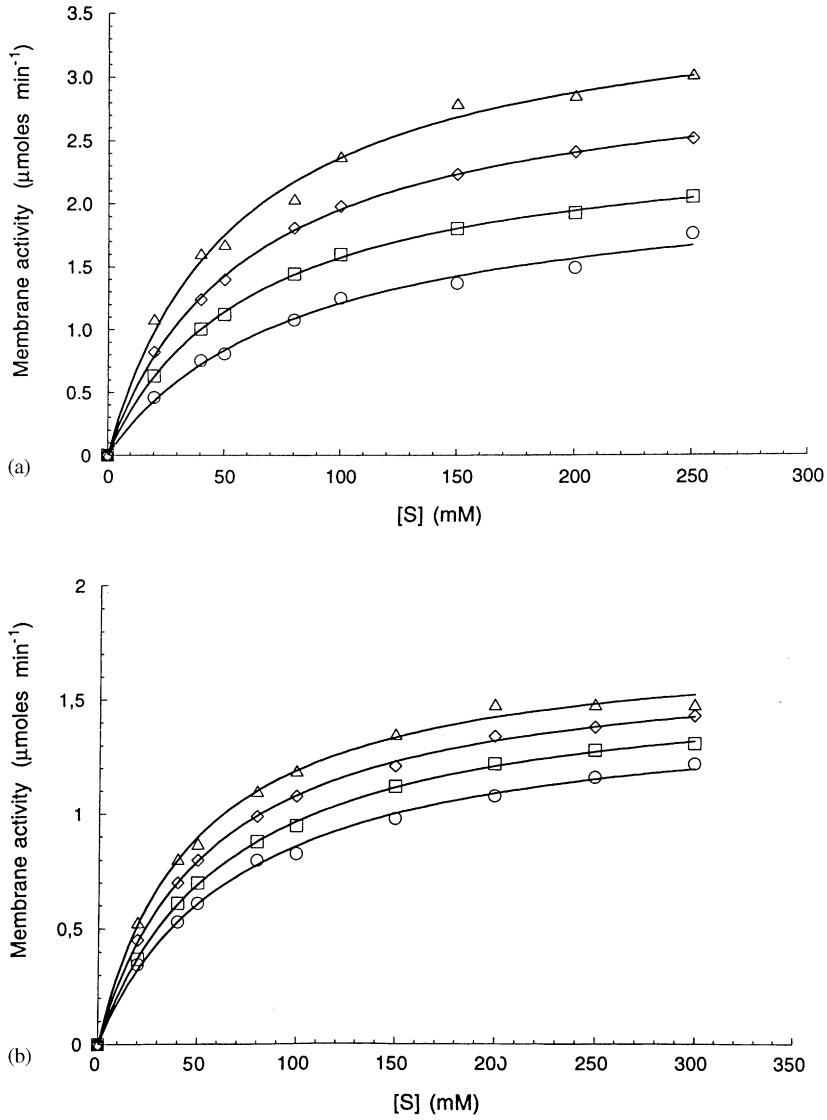

Fig. 4. Membrane activity as function of substrate concentration. $T_{av} = 25\,^{\circ}C$. (a) Membrane M_1; (b) membrane M_2. Symbols: (○) $\Delta T = 0\,^{\circ}C$; (□) $\Delta T = 10\,^{\circ}C$; (◇) $\Delta T = 20\,^{\circ}C$; (△) $\Delta T = 30\,^{\circ}C$.

The results reported in Fig. 4, in addition, show catalytic activities of membrane M_1 higher than those of membrane M_2, when identical conditions of substrate concentration and temperature difference are compared. Fig. 4 also evidences a Michaelis–Menten behaviour for the enzyme activities of membranes M_1 and M_2 either in the presence or in the absence of temperature gradients, therefore it is possible to calculate the apparent values of the kinetic constants K_m and V_{max} for each of the two physical situations. In Fig. 5, the experimental points of Fig. 4 are reported in forms of Hanes plots to calculate the values of the apparent K_m and V_{max}. These values are listed in Table 3 together with the ones relative to the free enzyme [29]. The data in Table 3 show that: (i) the immobilised enzymes have apparent K_m values higher

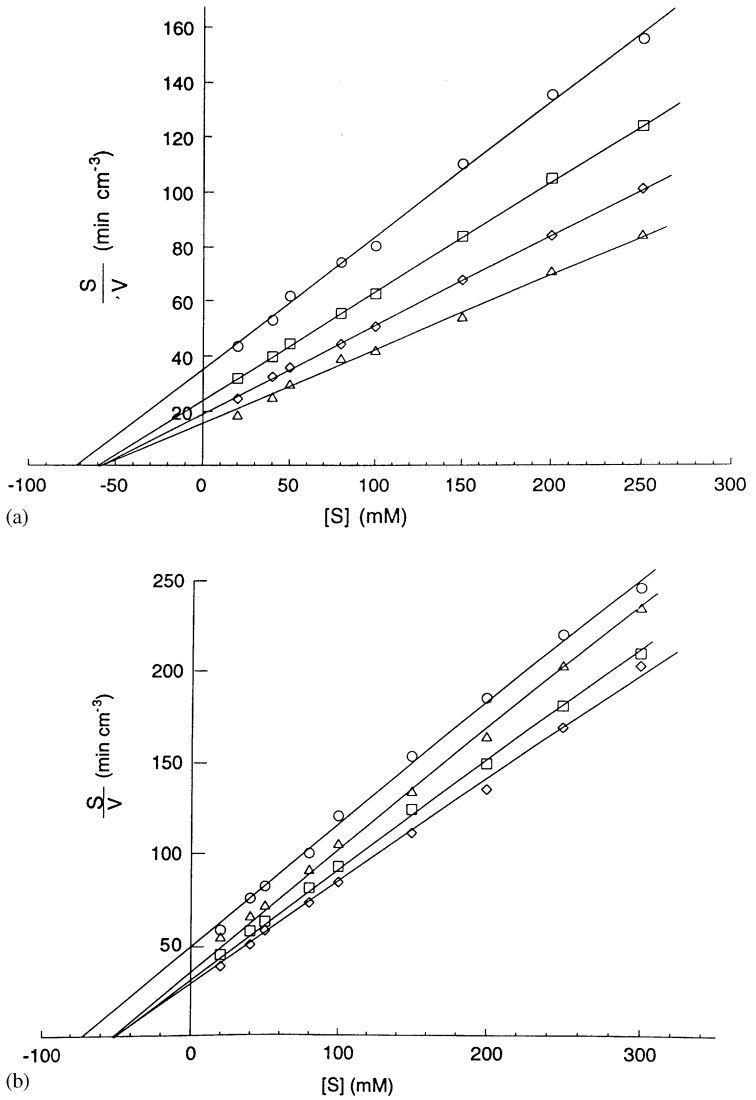

Fig. 5. Hanes plots relative to the experimental point reported in Fig. 4. (a) Membrane M_1; (b) membrane M_2. Symbols: (○) $\Delta T = 0\,^{\circ}C$; (□) $\Delta T = 10\,^{\circ}C$; (◇) $\Delta T = 20\,^{\circ}C$; (△) $\Delta T = 30\,^{\circ}C$.

than that of the free counterpart; (ii) both membrane types have apparent values of K_m under isothermal conditions higher than the corresponding values under non-isothermal conditions; (iii) for each membrane type the K_m values under non-isothermal conditions appear independent of the macroscopic temperature difference measured by the thermocouples.

The explanation to the first observation can be found in the diffusive limitations introduced by the immobilisation process to substrate and products traffic towards or away from the catalytic site.

The explanation to the second observation can be found in the circumstance that the temperature gradient increases substrate and product fluxes across the catalytic membrane reducing, in this way, the diffusion limitations for these substances during their movement towards or away from the catalytic site. Moreover the increases of the enzyme reaction rates with the applied

Table 3
Kinetic parameters relative to the free and immobilised β-galactosidase

Enzyme status	Experimental conditions	K_m (mM)	V_{max} (μmol min^{-1})
Free	$T_{av} = 25\,^{\circ}C$; $\Delta T = 0\,^{\circ}C$	21.4	3.2
Membrane M_1	$T_{av} = 25\,^{\circ}C$; $\Delta T = 0\,^{\circ}C$	72.0	2.00
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 10\,^{\circ}C$	60.0	2.53
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 20\,^{\circ}C$	60.0	3.24
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 30\,^{\circ}C$	60.0	4.00
Membrane M_2	$T_{av} = 25\,^{\circ}C$; $\Delta T = 0\,^{\circ}C$	73.0	1.52
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 10\,^{\circ}C$	52.4	1.54
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 20\,^{\circ}C$	52.4	1.87
	$T_{av} = 25\,^{\circ}C$; $\Delta T = 30\,^{\circ}C$	52.4	1.94

ΔT find explanation with the analogous increases with the applied ΔT of transmembrane mass fluxes induced by the process of thermodialysis [20–22].

The third observation, concerning the independence of the K_m values on the macroscopic temperature difference across the catalytic membrane, can be justified with changes in the protein structure and dynamics induced in the immobilised enzyme by the flux of thermal energy associated to the presence of the temperature gradient. In our opinion the temperature gradients play on enzyme structure the same role than positive effectors. We must remember that specific positive effectors affect the K_m value of the enzyme reaction, and consequently the structure and dynamics of the protein, independently from their concentration. In this analogy the size of the temperature gradients results equivalent to the size of the effector concentrations.

It is appropriate at this point to introduce a parameter giving information on the usefulness of employing non-isothermal bioreactors in industrial processes. This parameter is the percentage activity increase (P.A.I.) defined as

$$\text{P.A.I.} = \frac{\text{RR}|_{T_{av}}^{\Delta T \neq 0} - \text{RR}|_{T=T_{av}}^{\Delta T=0}}{\text{RR}|_{T=T_{av}}^{\Delta T=0}} \quad (3)$$

where $\text{RR}|_{T=T_{av}}^{\Delta T=0}$ and $\text{RR}|_{T_{av}}^{\Delta T \neq 0}$ are the enzyme reaction rates at $T = T_{av}$ under isothermal and non-isothermal conditions, respectively. The P.A.I. values, calculated from the results of Fig. 3, are reported in Fig. 6 as a function of the applied temperature difference ΔT. Fig. 6a refers to results obtained with membrane M_1, while Fig. 6b refers to results obtained with membrane M_2. In both figures the curve parameter is the average temperature. The results in Fig. 6 show that: (i) the P.A.I. values for both membrane types linearly increase with the increase of the applied ΔT; (ii) the P.A.I. values decrease with the increase of the average temperature; (iii) the P.A.I values of membrane M_1 are higher than the corresponding values of membrane M_2. This last observation clearly shows the influence of the immobilisation method on membrane activity not only when the membranes operate under isothermal conditions [26], but also when they are operating under non-isothermal conditions.

The linear dependence of the P.A.I. values on the temperature difference ΔT measured at the thermocouples position allows us to define a new parameter α, which is indicative of the effect of the non-isothermal conditions on the activity of the catalytic membranes. This parameter α is the angular coefficient of the straight lines in Fig. 6 and represents the P.A.I. when a temperature difference of $1\,^{\circ}C$ is measured at the thermocouple positions. The α coefficients relative to the results of Fig. 6 are reported in Fig. 7 as a function of average temperature. Now the curve parameter is the membrane type. Results in figure indicate that the α values decrease with the average temperature. Similar results were found by us with other catalytic membranes [17–19], prepared with different enzymes, supports and grafted monomers.

By applying the same methodology to results reported in Fig. 4 one obtains the results of Fig. 8, where the α values for each membrane type are reported as a function of substrate concentration. Results in figure show that: (i) the α values of both

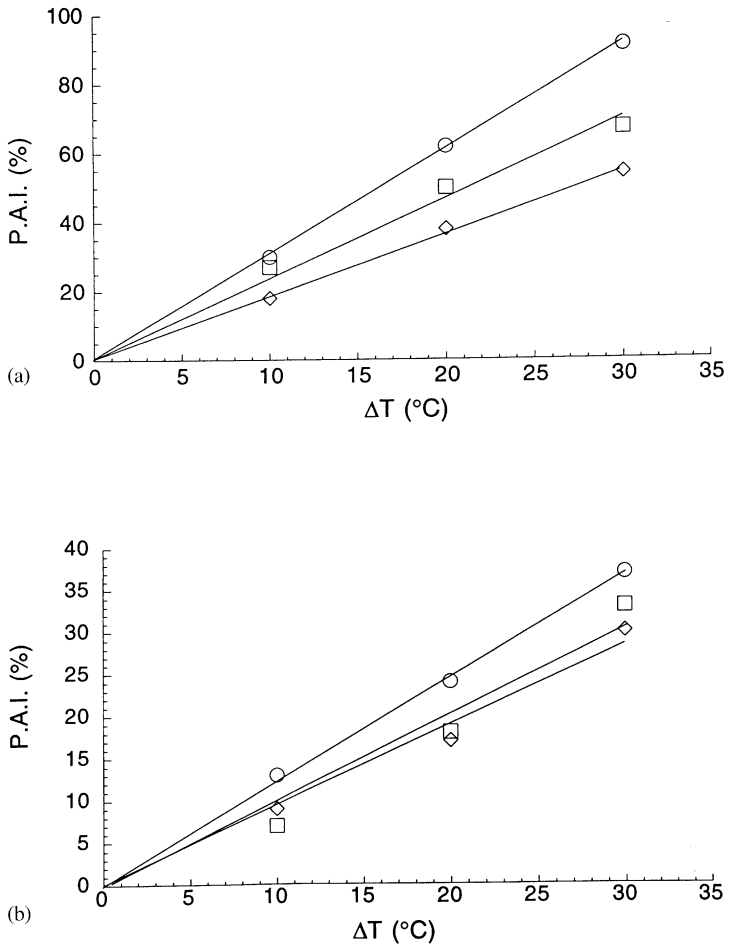

Fig. 6. Percentage activity increase (P.A.I.) calculated from results of Fig. 3. (a) Membrane M_1; (b) membrane M_2. Symbols: (○) $T_{av} = 25\,^{\circ}C$; (□) $T_{av} = 30\,^{\circ}C$; (◇) $T_{av} = 35\,^{\circ}C$.

membrane types decrease with the increase of substrate concentration; (ii) at each lactose concentration the α values relative to membrane M_1 are higher than those relative to membrane M_2. The decrease of the α values with the increase of substrate concentration has already been found by us with β-galactosidase [17,18] and urease [19] separately immobilised on differently grafted membranes. A qualitative explanation for the decrease of the α values with the increase of the substrate concentration is that when the enzymes work at their maximum rate, i.e. at saturating substrate concentrations, the modulation of the reaction rate by temperature gradients results less effective. A quantitative explanation of this behaviour has been recently published by us [17–19]. In these papers, starting from a substrate balance into the catalytic membrane under isothermal and non-isothermal conditions, the profiles of substrate concentration into the membrane have been derived. From these profiles the α parameter has been deduced as a function of the initial substrate concentration.

The α values in Figs. 7 and 8, obtained with reference to the macroscopic temperature difference ΔT measured in the bioreactor at the thermocouple positions, support the idea of employing non-isothermal bioreactors in biotechnological processes. This tech-

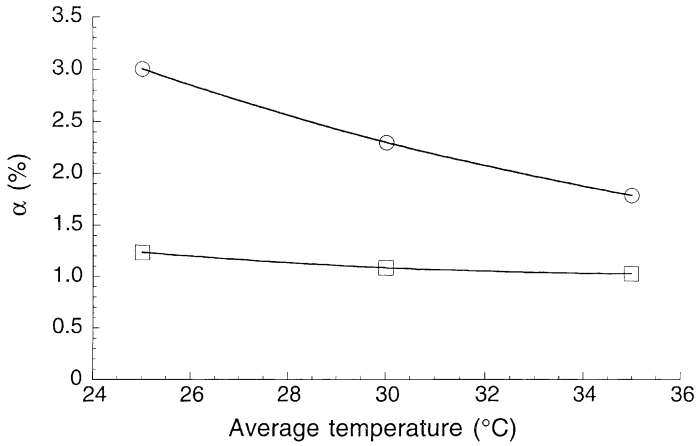

Fig. 7. The α coefficient as a function of T_{av}. Lactose concentration was 200 mM. Symbols: (○) membrane M_1; (□) membrane M_2.

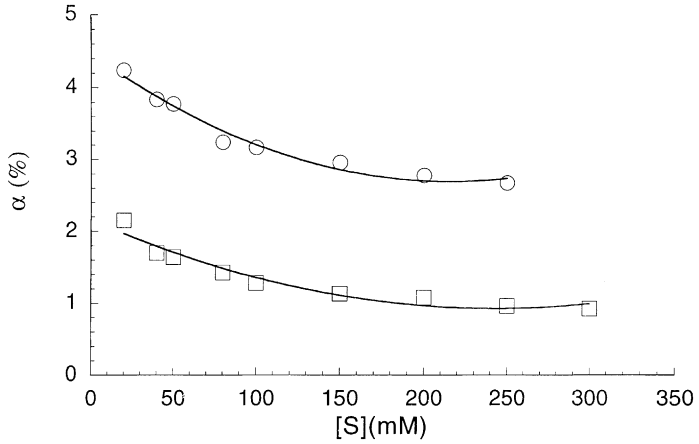

Fig. 8. The α coefficient as a function of lactose concentration. $T_{av} = 25\,^{\circ}C$. Symbols: (○) membrane M_1; (□) membrane M_2.

nology becomes more interesting if attention is paid to the actual temperature difference across the membrane, according to the values listed in Table 1. Bearing this observation in mind it is possible now to define a new coefficient α', related to the α coefficient by means of the equation

$$\alpha' = \alpha \frac{\Delta T}{\Delta T^*} = \frac{\text{P.A.I.}}{\Delta T^*} \quad (4)$$

In this case, α' represents the percentage increase of the enzyme reaction rate when a unit temperature difference is applied across the catalytic membrane. The α' values relative to the results in Fig. 8 are listed in Table 4. Once again the α' values of membrane

Table 4
The α' values have been calculated from the data of Fig. 8 through Eq. (5)

Lactose concentration (mM)	α' (%, $^{\circ}C^{-1}$) (membrane M_1)	α' (%, $^{\circ}C^{-1}$) (membrane M_2)
20	53.0	26.9
40	47.9	21.2
50	47.2	20.5
80	40.5	17.8
100	39.6	15.9
150	37.0	14.1
200	34.7	13.5
250	33.4	12.0

M_1 are higher than the corresponding value of membrane M_2. This circumstance, together with all other results showed in this work, confirms the dependence of the catalytic yield of a enzyme membrane under non-isothermal conditions on the immobilisation method used for its preparation.

The way by which temperature gradients affect the reaction rate of immobilised enzyme has been analytically discussed elsewhere [17–19].

4. Conclusions

The enzyme activity of two different types of catalytic membranes, having the same physical and biochemical properties, has been studied in a non-isothermal bioreactor to demonstrate the dependence of their performances on the method used for enzyme immobilisation. The first membrane type was obtained by immobilizing β-galactosidase via diazotisation, the second one by immobilizing β-galactosidase via condensation.

Results have demonstrated that the activity increases, which are independent of the specific activity of the enzyme, depend on the immobilisation method. P.A.I. values of membrane M_1, as well as the α and α' parameters, are higher than the corresponding values of membrane M_2. This means that immobilisation via diazotisation results more useful than immobilisation via condensation when the former membrane type is employed in a bioreactor operating under non-isothermal conditions.

All the results reported in this paper, moreover, show that in the presence of temperature gradients the immobilised enzymes recover their affinity for the substrate through a reduction of the diffusion limitation problems. Consequently the technology of non-isothermal bioreactors candidate as an useful tool in biotechnological productive processes.

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