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The process of thermodialysis in bioremediation of waters polluted by endocrine disruptors

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ABSTRACT

Endocrine disruptors are chemicals able to induce adverse effects into wildlife and humans owing to their ability of interfering with the endocrine system. Bisphenol A (BPA) has been chosen as model of endocrine disruptors. To reduce the BPA pollution in waters we proposed the employment of the process of thermodialysis. Two different catalytic membranes have been prepared by covalently immobilizing laccase (from *Trametes versicolor*) by means of a diazotation process or tyrosinase (from mushroom) by condensation. The support was a nylon membrane. The bioremediation power of both catalytic membranes has been analysed under isothermal and non-isothermal conditions.

The advantages in using non-isothermal bioreactors were discussed in terms of reduction of the bioremediation times.

1. Introduction

Under the name of thermodialysis [1–4] is recognized a selective matter transport across a hydrophobic porous membrane separating two aqueous solutions maintained at different temperatures. The driving force is the differential thermal radiation pressure associated to the heat flux and differently acting on the particles of solvent and solute confined in the membrane pores. Under these conditions each membrane pore constitutes a microscopic Soret cell into which a modified thermal diffusion occurs, the modifications being introduced on the water structure owing to its interaction with the walls of the pores.

As it is possible to see in Fig. 1, where it is illustrated the case of a two components solution, in presence of a temperature gradient, besides the heat flux, three matter fluxes are observed across the membrane: a macroscopic volume flux, J_{Volume}, from the warm to the cold side of the reactor; a drag solute flux, $J_{S,drag}$, associated to the volume flux, and a thermodiffusive solute flux, $J_{S,th}$, generally from the cold to the warm side.

The expressions for each of the three fluxes are:

$$\begin{cases} J_{Volume}\ (\mathrm{cm^3/cm^2\ s}) = D^*_{H_2O}\dfrac{\Delta T^*}{\Delta x} \\ J_{S,drag}\ (\mathrm{mol/cm^2\ s}) = \sigma J_{Volume} C_S \\ J_{S,th}\ (\mathrm{mol/cm^2\ s}) = D^*_{S,th} C_S \dfrac{\Delta T^*}{\Delta x} \end{cases} \quad (1)$$

where ΔT^* is the actual temperature difference across the membrane, Δx is the membrane thickness, C_S is the solute concentration expressed in $\mathrm{mol\,cm^{-3}}$, σ is the Staverman coefficient related to membrane selectivity, $D^*_{H_2O}$ and $D^*_{S,th}$ are the modified thermal diffusion coefficients (in $\mathrm{cm^2\ s^{-1}\ K^{-1}}$) for water and solute, respectively.

As it will be demonstrated later on, the temperature difference across the membrane ΔT^* is related to the temperature difference ΔT measured at the position of the thermocouples by the relationship $\Delta T^* = \beta \Delta T$, where β is a factor depending on the nature of the membrane and on the liquid phase filling the reactor. In the present case β was found to be equal to 0.11.

Endocrine disruptors [5–7] are chemicals that, when metabolized by living systems, mimic or block natural hormones, so disrupting their normal functions. Disruption occurs by altering normal hormone levels, blocking or stimulating the hormones production, changing the way by which hormones travel through the

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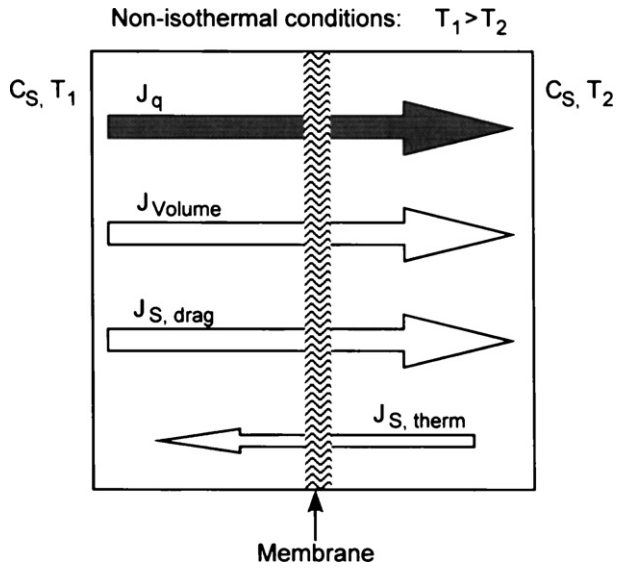

Fig. 1. Generalized matter fluxes driven by thermodialysis in the case of a two component aqueous solution.

body, thus affecting the functions that these hormones control. Chemicals recognized as endocrine disruptors are alkylphenols, phthalates, pesticides, flame retardants, dioxins, phytoestrogens, polychlorobiphenyles and some drugs. Exposure to endocrine disruptors occurs through direct contact or through ingestion of contaminated water, food or air. Endocrine disruptors are present in air or in water also as byproducts of many chemical and manufacturing processes. Several studies have found that some endocrine disruptors can also leach out of plastics, including baby's bottle or bags for blood transfusion. Many endocrine disruptors are persistent in the environment and accumulate in fat, so that the greatest exposures come from eating fatty food and fishes.

Many animal species, including the humans, show severe pathologies correlated to exposure to endocrine disruptors, in particular to Bisphenol A (BPA) [8–12].

To reduce the hazardous impact of endocrine disruptors on living organisms it is necessary their removal from the environment, in particular from the aquatic environment.

At this point some considerations are necessaries about the employment of a bioremediation process in place of a classical process of membrane remediation.

For problems of water treatment in ecosystems the traditional membrane-based processes are not useful since they alter the local life conditions. Ultrafiltration and reverse osmosis, for example, remove endocrine disruptors but, since the filtrate is pure water, its intake in the ecosystem alters the concentrations of the salts and bioelements necessary for the life. On the contrary, the selective removal of endocrine disruptors by means of enzyme treatment (bioremediation) appears suitable, since the treatment is effective only towards the harmful target, while the concentrations of the other components remain the same. Following this reasoning to bioremediate polluted waters in small ecosystems we have proposed, in place of reactors, the use of bioreactors, i.e. reactors in which enzymes, cells or antibody, are operating. In particular we suggested the use of non-isothermal bioreactors [13–22]. With these apparatuses we have found that with one degree centigrade of actual temperature difference across the catalytic membrane, i.e. with $\Delta T^* = 1\,^\circ C$, the enzyme reaction rate increases from 30% to 80% in comparison to the same reaction rate measured under comparable isothermal conditions. The increase of enzyme activity has been found to depend on: (a) the enzyme nature; (b) the immobilization method; (c) the substrate concentration; (d) the average temperature in the bioreactor; (e) the temperature difference across the catalytic membrane. From the industrial point of view, the main advantage in using non-isothermal bioreactors is the reduction of the production times, proportional to the size of the applied temperature difference across the catalytic membrane.

Our attention in this paper is focused on biodegradation of BPA, a ubiquitous substance used in the production of epoxy resins and polycarbonate plastics. The latter are used in many foods and drink packaging applications, while the former are commonly used as lacquers coating food cans or in water supply pipes. Some polymers used in dental treatment also contain BPA. The estrogenic activity of this compound was known since 1938 [23].

To degrade BPA two different enzymes have been separately used: laccase from *Trametes versicolor* and tyrosinase from mushroom.

2. Experimental

2.1. The bioreactor

The apparatus employed is reported in Fig. 2a, while in Fig. 2b a schematic picture of its modus operandi is represented. The reactor consists of two metallic flanges in each of which it is bored a shallow cylindrical cavity, 70 mm in diameter and 2.5 mm depth, constituting the working volume filled with the aqueous solutions containing BPA. The catalytic membrane is clamped between the two flanges so as to separate and, at the same time, to connect the solutions filling the half-cells. Solutions are circulated in each half-cell by means of two peristaltic pumps through hydraulic circuits starting and ending in a common glass cylinder. The two half-cells are maintained at predetermined temperatures by means of independent thermostats. Thermocouples, placed 1.5 mm away from the membrane surfaces, measure the temperatures of the solution in that point of each half-cell. These measures allow the calculation of the temperature profile across the catalytic membrane.

To estimate the real effects of the temperature gradients on the activity of immobilized enzymes, the actual temperatures on the surfaces of the catalytic membrane (T^*_W and T^*_C) must be known. Subscripts "W" and "C" stay for warm and cold side, respectively. Being impossible to measure the temperatures on each membrane face, these were calculated from those measured at the position of the thermocouples (T_W and T_C), because the solution motion in the two half-cells, at a rate of 3.5 mL min^{-1}, was laminar. Under these conditions, the Reynolds number Re is lower than Re^{crit}, being Re lower than 10 [14,15,19]. It follows that heat propagation in the bioreactor occurs by conduction between isothermal liquid planes perpendicular to the direction of the heat flow. It follows that, by knowing the thermal conductivities and the thicknesses of both filling solutions and membrane, it is possible to calculate the temperatures on the membrane surfaces by means of the heat flux continuity principle:

$$J_q = -K_i \left(\frac{\Delta T}{\Delta x} \right)_i = \text{constant} \tag{2}$$

where K_i is the thermal conductivity of the ith medium crossed by the heat flux (solution or catalytic membrane), and $(\Delta T/\Delta x)_i$ is the temperature gradient existing in the same medium, Δx_i thick. Using computer simulation, the temperature profile into the bioreactor can be calculated. In this calculation as values of thermal conductivities of our solutions we have assumed those of pure water [24], whereas the thermal conductivity of the membrane was taken from Touloukian et al. [25]. It was found that the correlation between the temperatures read by the thermocouple and the ones on the

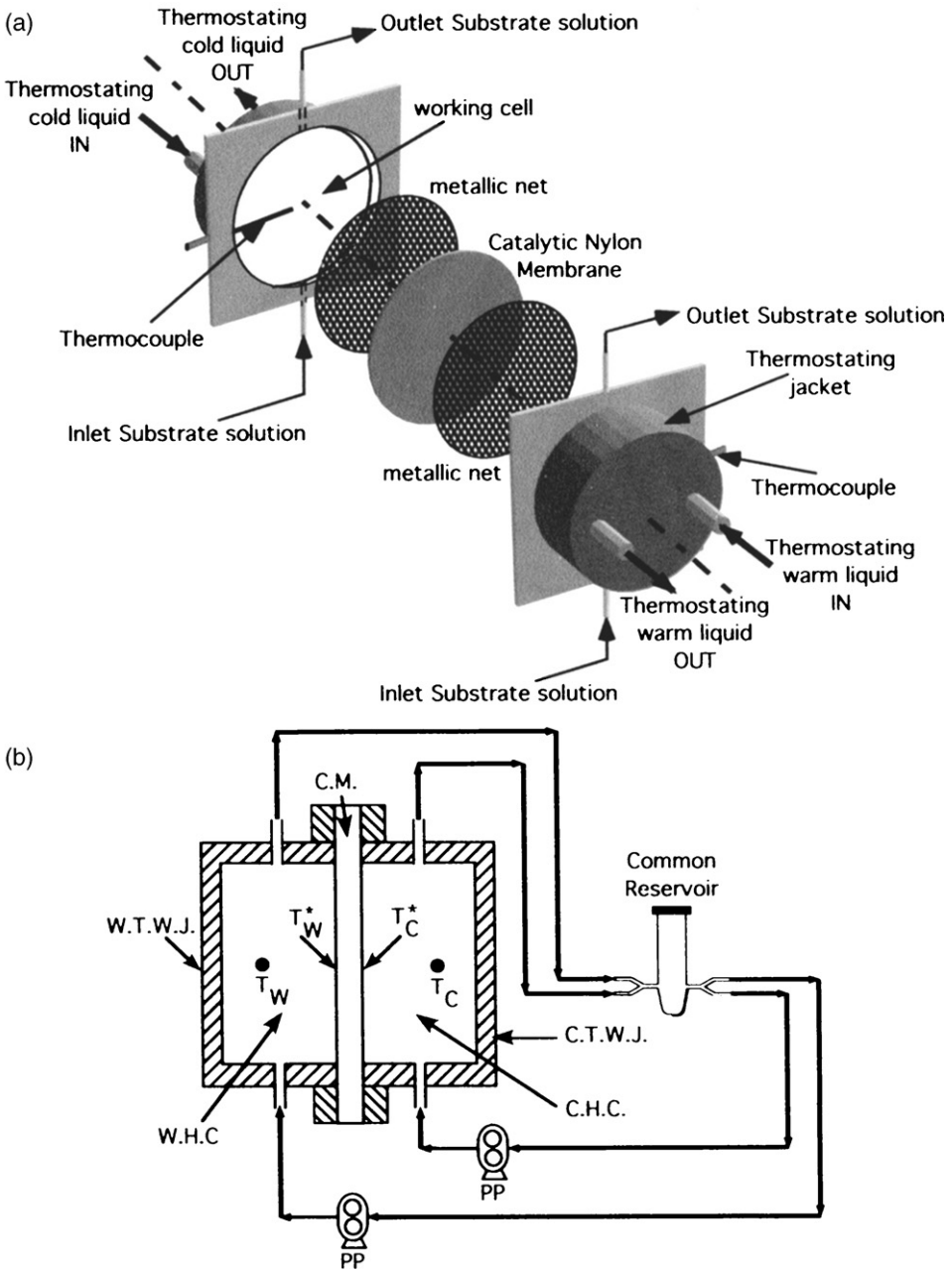

Fig. 2. Schematic representation of the bioreactor (a) and of its modus operandi (b).

surfaces of the catalytic membrane was given by

$$\begin{cases} T^*_W = T_W - a\Delta T \\ T^*_C = T_C + b\Delta T \end{cases} \quad (3)$$

with a and b numerical constants. Since our system is symmetric, $a = b$ and $\Delta T^* = (1-2a)\Delta T = \beta\Delta T$; $T_{av} = (T_W + T_C)/2 = T^*_{av} = (T^*_W + T^*_C)/2$. T_{av} and T^*_{av} are the average temperatures of the bioreactor and membrane, respectively. In the present case, we have found $a = b = 0.445$ and therefore $\Delta T^* = 0.11\Delta T$. It follows that in non-isothermal experiments $T^*_W < T_W$, $T^*_C < T_C$, and $\Delta T^* < \Delta T$.

To give one example of this calculation, in Table 1 we have reported the temperature values corresponding at the experimental condition employed in this research. It is easy to verify how also at the highest temperature values here used, i.e. at $T_{av} = 40\,^\circ$C and $\Delta T = 20\,^\circ$C, the T^*_W is equal to 41.10 °C, a temperature not deactivating the enzyme activity.

Table 1
Correspondence between the temperatures read by the thermocouple (T) and the ones calculated on the surfaces of the catalytic membranes (T^*).

$T_{av} = T^*_{av}$ (°C)	T_W (°C)	T_C (°C)	ΔT (°C)	T^*_w (°C)	T^*_C (°C)	ΔT^* (°C)
20	20	20	0	20.00	20.00	0
	25	15	10	20.55	19.45	1.1
	30	10	20	21.10	18.90	2.2
	35	5	30	21.65	18.35	3.3
30	30	30	0	30.00	30.00	0
	35	25	10	30.55	29.45	1.1
	40	20	20	31.10	28.90	2.2
	45	15	30	31.65	28.35	3.3
40	40	40	0	40.00	40.00	0
	45	35	10	40.55	39.45	1.1
	50	30	20	41.10	38.90	2.2

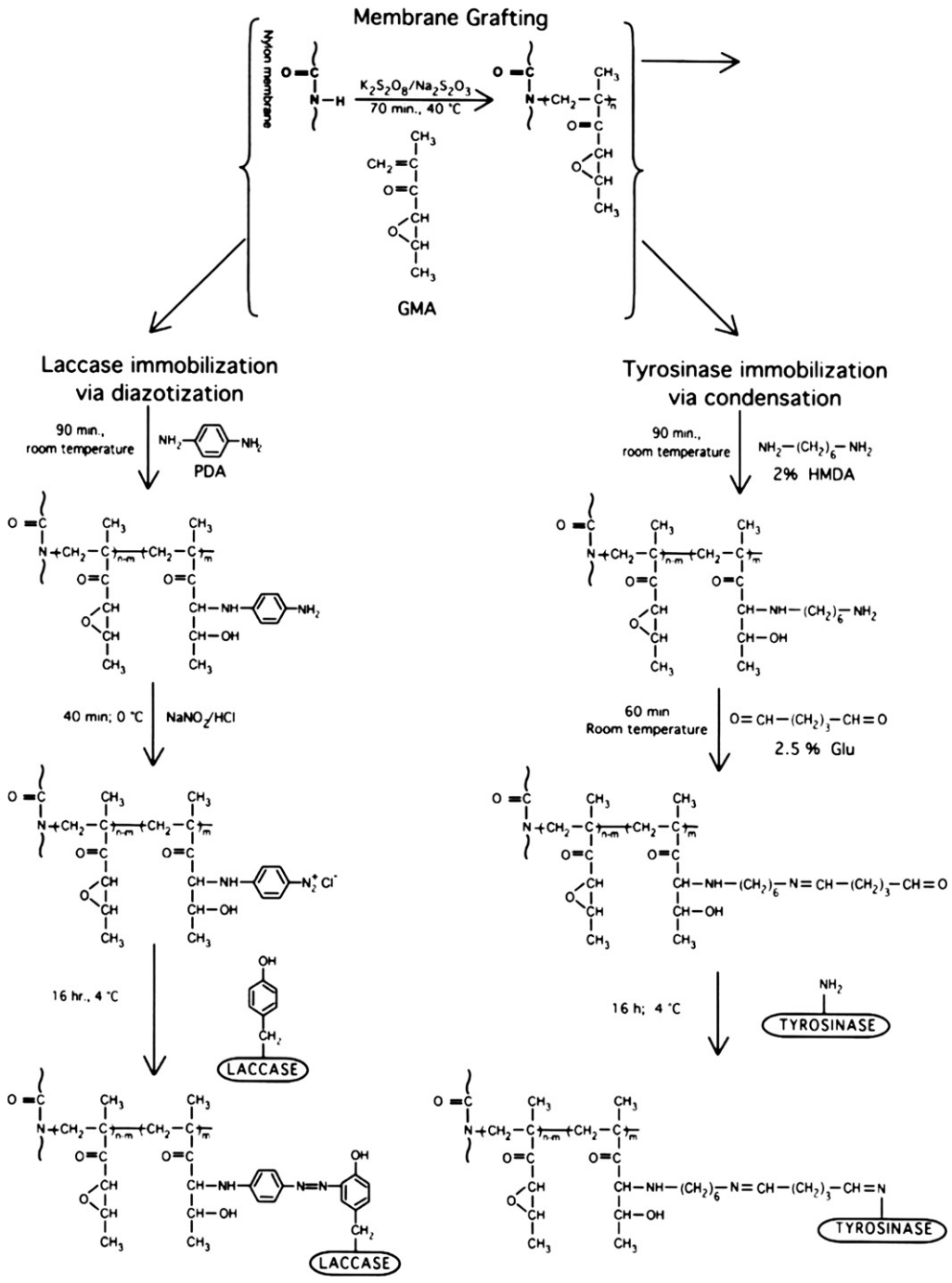

Fig. 3. Procedures for the preparation of the catalytic nylon membranes in the case of laccase (diazotization, left panel) or of tyrosinase (condensation, right panel).

2.2. Materials

Laccase (EC. 1.10.3.2) (20 Units/mg) from *T. versicolor* and tyrosinase from mushroom (EC. 1.14.18.1) (1500 Units/mg) were used as catalyst. Laccase and tyrosinase are polyphenol oxidases catalyzing the reaction of several organic substances, such as phenols, with concomitant reduction of oxygen to water, accompanied by the oxidation of the phenolic substrate. The same occurs in our case with BPA. Since BPA is weakly soluble in water, we prepared our aqueous solutions by dissolving BPA in ethyl alcohol stock solutions.

As solid support on which to immobilize the enzyme, nylon "Hydrolon" membranes (150 μm thick and with 0.2 μm nominal pores) from Pall (Pall Italia, Milano, Italy) were used. Since the nylon membranes are hydrophobic and unable to bind enzymes, their surfaces were activated through the chemical grafting technique. Glycidyl methacrylate (GMA) was used as monomer to be grafted, while phenylenediamine (PDA) or hexamethylenediamine (HMDA) was used as spacer in the case of laccase and tyrosinase, respectively. The presence of the spacer was required to minimize the effect of the negative electric charges of the Nylon support on the enzyme structure and on the microenvironment in which the catalytic site was operating.

All chemicals, including the enzymes and BPA, were purchased from Sigma (Sigma Italia, Milano, Italy) and used without further purification.

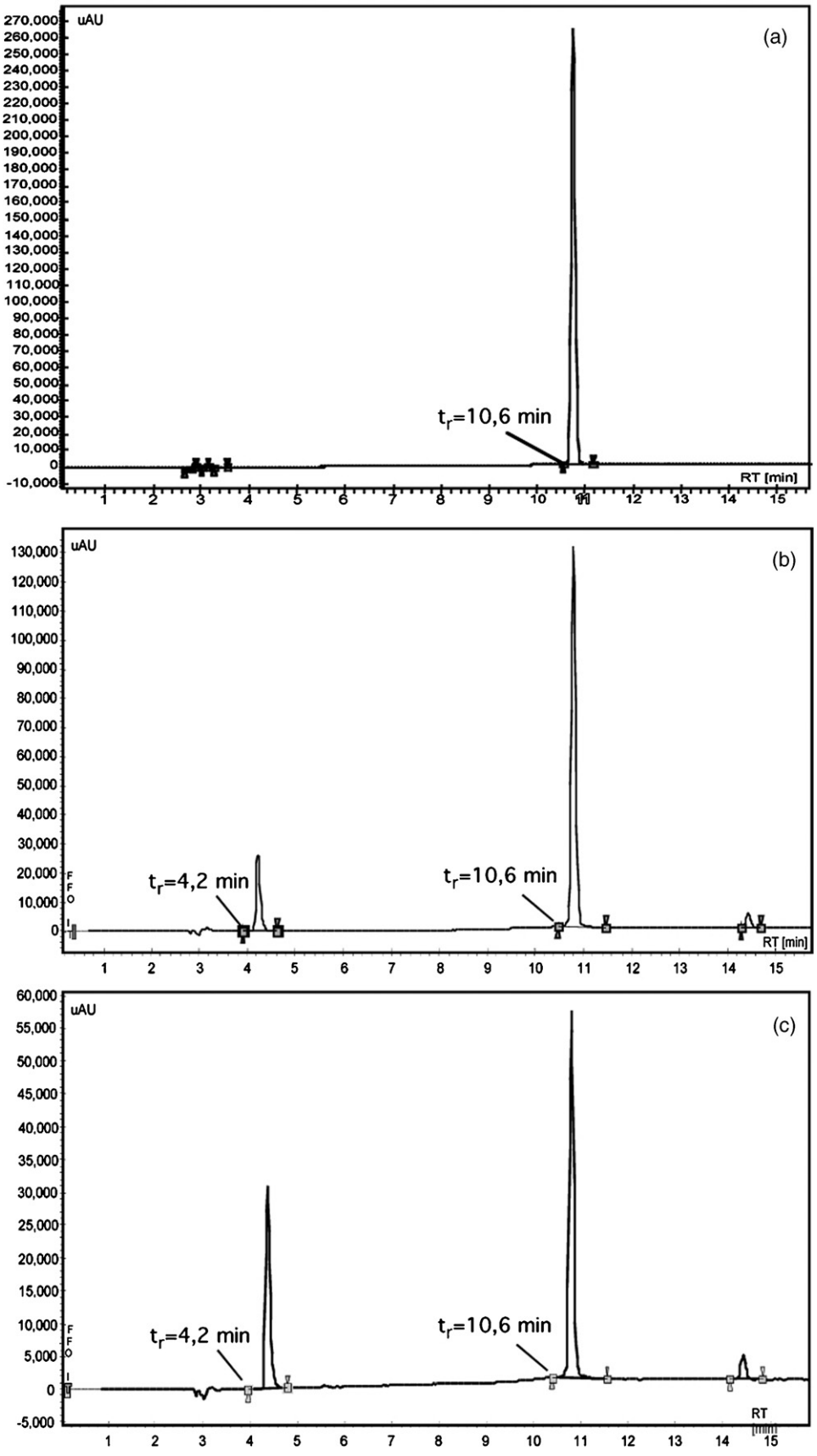

Fig. 4. Time evolution of BPA and reaction product concentration by using immobilized laccase in the common container of the bioreactor at three different time: t_0 = 0 min (panel a); t_1 = 30 min (panel b); t_2 = 60 min (panel c). The scales on the ordinate axis are different.

2.3. Methods

2.3.1. Catalytic membrane preparation

The preparation of the catalytic membranes was carried out by means of two steps: (a) grafting copolymerization and (b) enzyme immobilization.

2.3.1.1. Grafting copolymerization. Grafting copolymerization was carried out by dissolving as initiating system $K_2S_2O_8$ and $Na_2S_2O_3$ in a 1:1 (v/v) water/ethanol mixture. To obtain the nylon-poly(GMA) membranes, the untreated nylon membranes were immersed, for 60 min at 40 °C, in a reaction vessel filled with the water/ethanol solution containing 600 mM GMA, 8 mM $K_2S_2O_8$, 8 mM $Na_2S_2O_3$ and 0.03 mM $CuCl_2$. At the end of the grafting process, the membranes were treated with acetone to remove the produced homopolymer, and dried until a constant weight was reached. Grafting percentage G (%) was determined by the difference between the membrane masses after (M_a) and before (M_b) the grafting process through the expression:

$$G\ (\%) = \frac{M_a - M_b}{M_b} \times 100 \quad (4)$$

All membranes used in these experiments had a grafting percentage value of 13.3 ± 2.2%.

2.3.1.1.1. Laccase immobilization. Laccase immobilization was carried out through a diazotization process, involving the phenolic group of tyrosine residues. Grafted membranes were treated, for 90 min at room temperature, with a 2% (w/v) PDA solution in 0.1 M sodium carbonate buffer, pH 9.0. PDA was used to obtain aminoaryl derivatives on the grafted membranes. Once washed with water, the membranes, constituted by aminoaryl derivatives, were treated for 40 min at 0 °C with an aqueous solution containing 2 M HCl and 4% $NaNO_2$. At the end of this treatment, the membranes were washed at room temperature with double distilled water and 0.1 M citrate buffer solution, pH 5.5, and then treated for 16 h at 4 °C with the same buffer solution containing laccase at concentration of 3 mg mL^{-1}. At the end of this step, membranes were washed with the 0.1 M citrate buffer solution pH 5.5 to remove the unbound enzymes.

2.3.1.1.2. Tyrosinase immobilization. Tyrosinase was immobilized using glutaraldehyde in a condensation process involving their NH_2-group. For this purpose, HMDA was used as a spacer. Poly(GMA)-HMDA membranes were obtained by immersing the GMA grafted membranes in a 2% (v/v) HMDA aqueous solution for 30 min at room temperature. After this stage, the membranes were washed with water to remove the unreacted amines, and were then treated for 1 h at room temperature with a 2.5% (v/v) glutaraldehyde (Glu) aqueous solution. After further washings with twice distilled water and 0.1 M phosphate buffer solution, pH 6.5, the membranes were treated for 16 h at 4 °C with the same buffer solution containing tyrosinase at the concentration of 3 mg mL^{-1}. At the end of this step, membranes were washed with the phosphate buffer solution to remove the unbound enzyme.

The amount of immobilized enzyme was calculated by subtracting the amount of the enzyme recovered in the solution at the end of immobilization process and into the washing solutions from the amount of enzyme initially used for the immobilization. Protein concentration was measured with the method of Lowry [26].

Processes of membrane grafting and enzyme immobilization are reported in graphical form in Fig. 3.

2.3.2. Enzyme activity measurements

Enzyme reaction rate has been followed by measuring at regular time intervals and by means of an HPLC, the BPA concentration in the common glass container. Fig. 4 shows one example of chromatograms obtained at the beginning of an experiment (panel a: only BPA is present) and after two different times of treatment with laccase (panels b and c: BPA and product are present). From Fig. 4 it is possible to appreciate how during the enzyme treatment the BPA concentration decreases, while the product concentration increases. So, through a calibration curve, it is possible to know the time dependence of BPA concentration in the common container. Once known the changes of BPA concentration during the time, the initial reaction rate (expressed in μmol min^{-1}) is obtained from the value of $(dBPA/dt)_{t=0}$ multiplied by the volume of the treated solution. Just to give one example of the followed methodology in Fig. 5 we have reported the changes in BPA concentration measured during the reaction with the free laccase. We used free laccase since this form is more susceptible to deactivation in respect to the insoluble form and since it has been reported that free laccase in presence of BPA show some suicide effect [27,28]. The experimental conditions of Fig. 5 were: BPA concentration 1 and 3 mM, 20 mL reaction volume, laccase 0.3 mg mL^{-1}, and room temperature. Both results in figure show that the BPA concentration fall to zero in a very short time following a monophasic exponential curve of the type $C(t)/C_0 = e^{-kt}$, where $C(t)$ and C_0 are the BPA concentration at t and zero time, and k (s^{-1}) is a rate constant which depends from the C_0 value. When a suicide effect is present, as demonstrated by Kim and Nicell [26] a biphasic curve of the BPA changes during the time is expected, the first part showing a rapid decrease of the BPA concentration (indicating a higher enzyme activity) and a second part where the decrease of BPA concentration is slower and with a saturation effect. This behaviour is analytically expressed by

$$\frac{C(t)}{C_0} = a\ \exp(-k_1 t) + b\ \exp(-k_2 t),$$

where now a and b are numerical adimensional constant characterized by the circumstance that $a + b = 1$. Looking at Fig. 5, our results indicate that no suicide effect is present in our case since both our curves are monosphasic. If this is true for the free enzyme, it is also true for the immobilized laccase since it is well known that the immobilization process strengthens the properties of the free enzyme. Similar behaviour has been observed when tyrosinase was used in place of laccase.

To directly compare the laccase and tyrosinase activities the initial reaction rate must be referred to 1 mg of immobilized enzyme taking also in mind the specific activity (Units/mg) of the free enzyme.

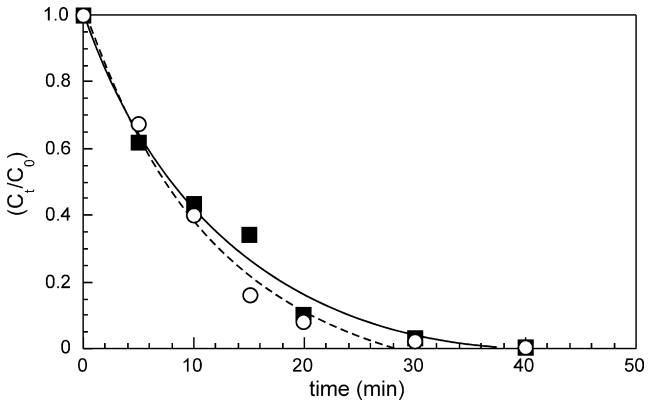

Fig. 5. BPA normalized concentration as a function of the enzyme reaction time. Experimental condition: 20 mL reaction volume, laccase 0.3 mg mL^{-1}, room temperature symbols: (○) [BPA] = 1 mM; (■) [BPA] = 3 mM.

2.3.3. Membrane stability

The stability of the catalytic membranes has been operatively determined by measuring, every day, the enzyme activity under standard conditions (pH, temperature and substrate concentra-

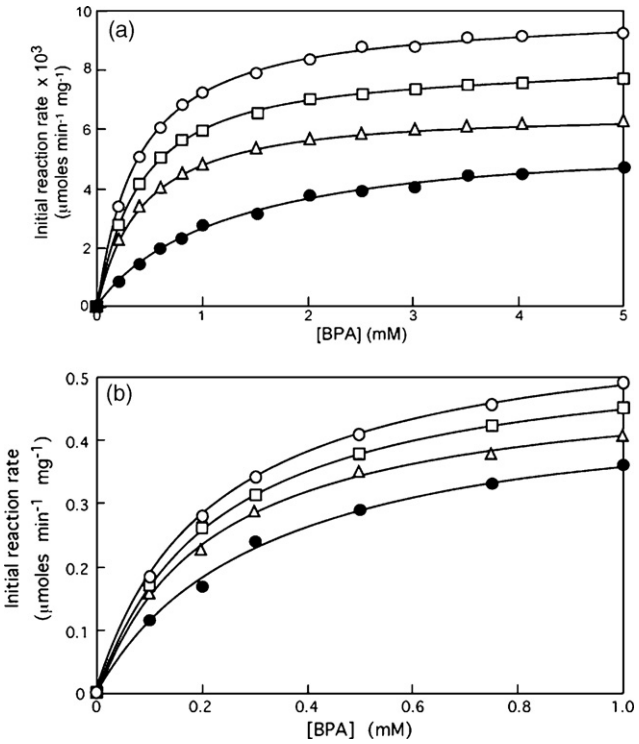

Fig. 6. Initial reaction rates as a function of BPA concentration in the case of immobilized laccase (a) or immobilized tyrosinase (b). Symbols: (●) at $\Delta T = 0\,^{\circ}C$, (△) at $\Delta T = 10\,^{\circ}C$, (□) at $\Delta T = 20\,^{\circ}C$ and (○) at $\Delta T = 30\,^{\circ}C$.

tion). When the lost of activity reached the 5% of the stabilized value, i.e. not before of a month of operation, the catalytic membrane was discarded. Also this high time stability ensures the absence of suicide effects by reaction products on laccase [27,28] or tyrosinase [29,30].

When not used, the membranes were stored at 4 °C in their appropriate buffers.

3. Results and discussion

In order to verify the advantage of non-isothermal bioreactors in bioremediation processes, we have explored the BPA biodegradation up to a concentration of 5 mM. In Fig. 6 the initial reaction rates, expressed as $\mu mol\,min^{-1}\,mg^{-1}$ of immobilized enzyme, are reported as a function of BPA concentration. Fig. 6a refers to experiments carried out with laccase and Fig. 6b to experiments with tyrosinase. The experimental conditions were: $T_{av} = 20\,^{\circ}C$, and $\Delta T = 0$, 10, 20, 30 °C. Each run lasted 60 min. It is interesting to observe that the higher values of the initial reaction rate for tyrosinase are due to the high specific activity of the free enzyme (1500 Units/mg) in respect to the specific activity of laccase (20 Units/mg).

Inspection of Fig. 6 shows that for both enzymes: (i) the initial reaction rate depends on the substrate concentration and

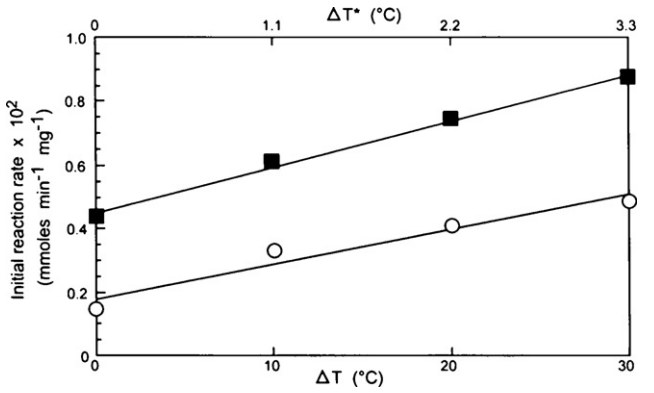

Fig. 7. Initial reaction rate of immobilized laccase as a function of the macroscopic applied ΔT (lower abscissas scale) or of the actual transmembrane ΔT^* (upper abscissas scale) at BPA concentration equal to 0.4 mM (○) or 4.0 mM (■). The average temperature is 20 °C.

exhibits a Michaelis–Menten behaviour under isothermal and non-isothermal conditions; (ii) at each BPA concentration the initial reaction rates are higher under non-isothermal conditions. The kinetic parameters relative to these experiments are listed in Table 2. Data in Table 2 show that the K_m values under non-isothermal conditions are lower than those of the corresponding isothermal condition. This result indicates that the non-isothermal conditions increase the apparent affinity of immobilized laccase for BPA. This is not a surprise, since we obtained qualitatively and quantitatively similar results in our previous papers [13–22]. The explanation is based on the circumstance that, as illustrated in Fig. 1, in the presence of a temperature gradient the immobilized enzymes in the unit of time "encounter" more substrate molecules, since additional thermodiffusive fluxes add to the diffusive ones. This means that under non-isothermal conditions the immobilized enzymes face in the microenvironment around their catalytic site a substrate concentration higher than in the bulk solution. This is a new kind of "partitioning effect" due to the presence of the temperature gradient.

It is interesting at this point to quantify the effect of the temperature gradients on the initial reaction rate at each BPA concentration. As demonstrated in our previous works and with other enzyme/substrate systems, it is easy to show that at each average temperature and at each substrate concentration it is possible to find a linear relationship between the enzyme reaction rate and the temperature gradients existing across the catalytic membrane. In order to verify the existence of this relationship in the present case too, we have analysed, as one example, what occurred with immobilized laccase at two BPA concentrations different for an order of magnitude: 0.4 mM (empty symbols) and 4 mM (full symbols). These results are shown in Fig. 7 where the initial enzyme activities are reported as a function of the macroscopic temperature differences ΔT (lower abscissa scale) or of the actual temperature differences ΔT^* across the membrane (upper abscissa scale). Results in Fig. 7 show that at each of the two substrate concentrations the initial activities linearly increase with the applied ΔT and

Table 2
Kinetics parameters of immobilized laccase and tyrosinase.

T_{av} (°C)	ΔT (°C)	Laccase		Tyrosinase	
		K_m (mM)	V_{max} ($\mu mol\,min^{-1}\,mg^{-1}$)	K_m (mM)	V_{max} ($\mu mol\,min^{-1}\,mg^{-1}$)
20	0	1.20	0.0057	0.323	0.476
	10	0.41	0.0067	0.223	0.500
	20	0.40	0.0083	0.222	0.550
	30	0.39	0.0096	0.225	0.600

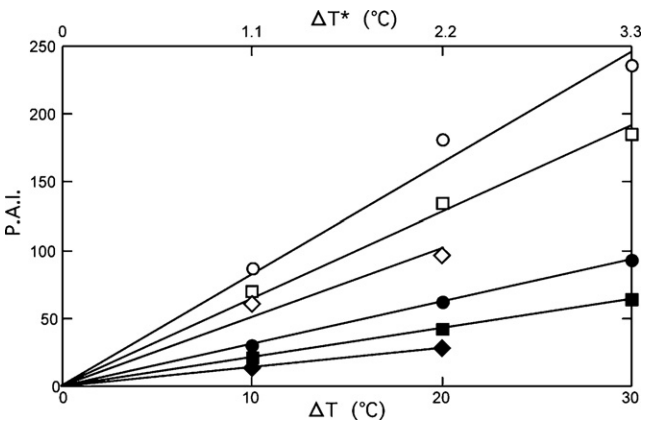

Fig. 8. Percentage activity increase (P.A.I.) as a function of the applied $\Delta T(\Delta T^*)$ in the case of BPA concentration equal to 0.4 mM (empty symbols) or 4.0 mM (full symbols). Curve parameter is the average temperature. Symbols: (◊) and (♦) = T_{av} 40 °C; (□) and (■) = T_{av} 30 °C; (○) and (●) = T_{av} 20 °C.

that each straight line fitting the experimental points is represented by an equation of the type:

$$RR_{C,\Delta T\neq 0}(T_{av}) = RR_{C,\Delta T=0}(T_{av})\left(1+\frac{\alpha}{100}\Delta T\right) \quad (5)$$

where $RR_{C,\Delta T=0}(T_{av})$ and $RR_{C,\Delta T\neq 0}(T_{av})$ are the initial activities under isothermal ($\Delta T = 0$) and non-isothermal ($\Delta T \neq 0$) conditions at fixed concentration C and T_{av}. Under these assumptions α (%, °C^{-1}) represents the percentage activity increase (P.A.I.) when the temperature difference between the thermocouple positions is equal to 1 °C. The expression for α is

$$\alpha = \frac{RR_{C,\Delta T\neq 0}(T_{av}) - RR_{C,\Delta T=0}(T_{av})}{RR_{C,\Delta T=0}(T_{av})}\frac{100}{\Delta T} = \frac{\text{P.A.I.}}{\Delta T} \quad (6)$$

We must note that in the absence of a specific effect of the temperature gradient and considering only the temperature dependence of the enzyme activity, the α coefficient should be equal to zero. This is an obvious consideration remembering the small differences in the temperature values on the two faces of the catalytic membrane and considering that what is gained in activity on the warm surface of the catalytic membrane is exactly lost on its cold surface.

When the P.A.I. are considered for laccase, one obtains the results reported in Fig. 8 relative to the data in Fig. 7 and to analogous experiments, not reported here, carried out at T_{av} = 30 and 40 °C. From Fig. 8 it is possible to infer that the non-isothermal conditions are more effective at low average temperatures than at high temperatures. Fig. 8 also shows that 3.3 °C of actual temperature difference across the catalytic membrane are enough to produce, at 4 mM BPA and at T_{av} = 20 °C, a percentage activity increase equal to 95%. Similarly, at 0.4 mM BPA concentration and a T_{av} = 20 °C, a ΔT^* = 3.3 °C produces an increase of enzyme activity equal to 250%. Results in Fig. 8 clearly indicate also that the temperature gradients are more effective at lower concentrations. The explanation of these results is that the additional BPA fluxes driven by the temperature gradient are more effective when the isothermal reaction rate is low. The slopes of the straight lines in Fig. 8 directly give the values of the coefficient α (%, °C^{-1}) or α^* (%, °C^{-1}) which are indicative of the percentage activity increases when 1 °C of temperature difference is read at the thermocouples position (α) or 1 °C of temperature difference is actually applied across the catalytic membrane (α^*). Of course $\alpha^* = \alpha(\Delta T/\Delta T^*) = \alpha/\beta$. In Table 3 the α and α^* values relative to the experimental results of Fig. 7 are reported for each of the average temperature employed.

When this approach is applied to all the experimental points in Fig. 6, one obtains the α^* values reported in Fig. 9 as a function of the BPA concentration. Calculations have been reported only for the common BPA concentration range explored with laccase and tyrosinase. The points in Fig. 9 have been calculated by taking the values on the theoretical Michaelis-Menten curves in Fig. 6. Inspection of the data in the Fig. 9 shows that the α^* values decrease with the increase of BPA concentration. This behaviour finds explanations by considering that the additional BPA fluxes driven by the temperature gradient are more effective when the immobilized enzymes work far from their maximal working capability (i.e. far from the saturation). On the contrary, when the enzymes work at saturation the additional substrate fluxes affect in a reduced way the reaction rate.

Table 3
α and α^* values calculated from the experiments reported in Fig. 5.

[BPA] (mM)	T_{av} (°C)	α (%°C^{-1})	α^* (%°C^{-1})
4	20	3.26	29.67
4	30	2.35	21.37
4	40	1.75	15.94
0.4	20	7.71	70.10
0.4	30	5.89	53.58
0.4	40	5.28	48.00

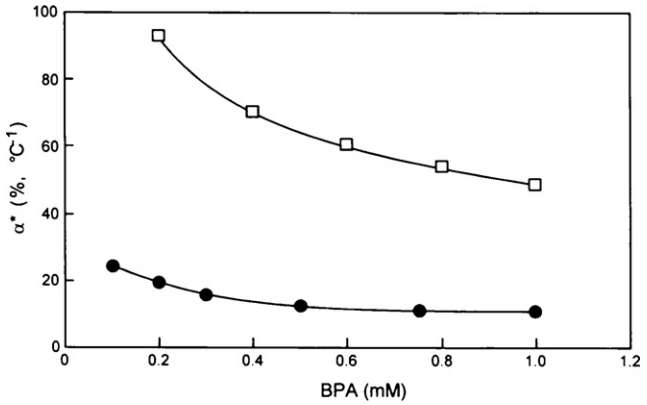

Fig. 9. α^* values for immobilized laccase (□) or tyrosinase (●) as a function of BPA concentration. T_{av} = 20 °C.

4. Conclusions

All the above results demonstrate that: (1) it is possible to bioremediate aqueous solutions polluted by BPA; (2) the bioremediation rate is increased when the enzyme reaction is carried out under non-isothermal conditions; (3) under non-isothermal conditions the laccase is more efficient than tyrosinase in degrading BPA.

It is interesting, at this point, to correlate the α^* values with the reduction of the bioremediation time, τ_r, defined as

$$\tau_r\ (\%) = \frac{\tau_{iso} - \tau_{non\text{-}iso}}{\tau_{iso}} \times 100 \quad (7)$$

where $\tau_{non\text{-}iso}$ and τ_{iso} are the times required to obtain the same percentage of biodegradation under non-isothermal and isothermal conditions, respectively. To correlate τ_r with the applied ΔT^*, we must consider the time course of BPA degradation under isothermal and non-isothermal conditions. The same value of BPA degradation will be reached when:

$$RR_{C,\Delta T=0}\tau_{iso} = RR_{C,\Delta T\neq 0}\tau_{non\text{-}iso} \quad (8)$$

or, according to Eq. (5), when:

$$RR_{C,\Delta T=0}\tau_{iso} = RR_{C,\Delta T=0}\left(1+\frac{\alpha}{100}\Delta T\right)\tau_{non\text{-}iso} \quad (9)$$

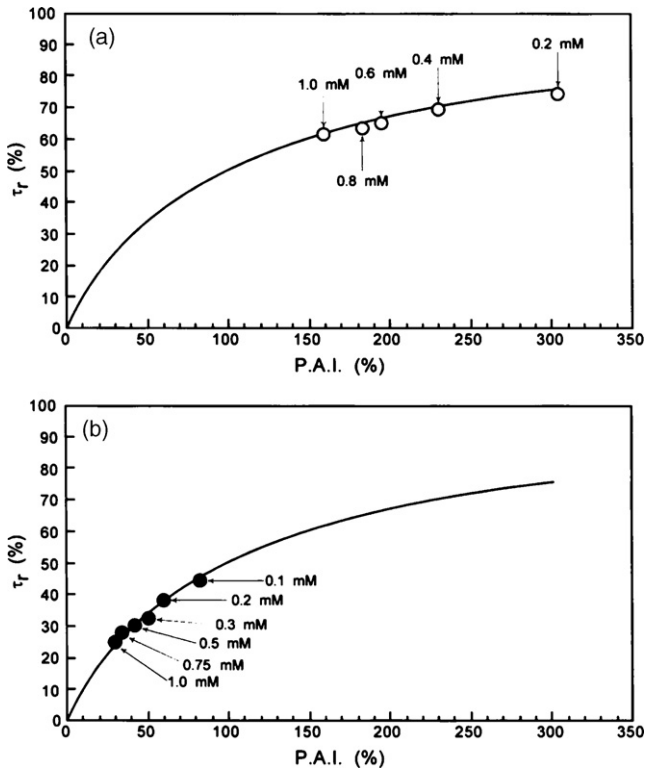

Fig. 10. Bioremediation reduction times as a function of P.A.I. in the case of immobilized laccase (a) or immobilized tyrosinase (b). T_{av} = 20 °C and ΔT = 30 °C.

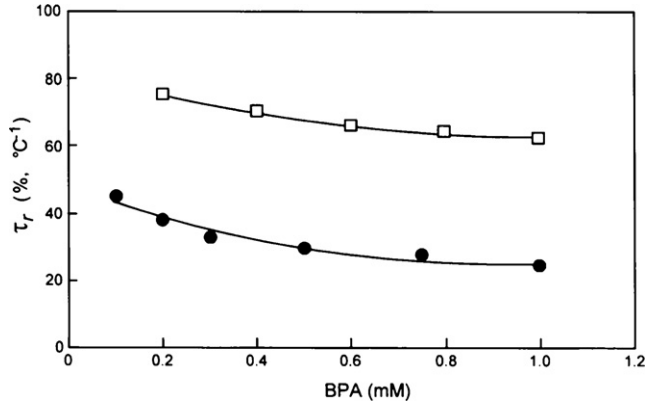

Fig. 11. Bioremediation reduction times as a function of BPA concentration. Experimental conditions: T_{av} = 20 °C and ΔT = 30 °C. Symbols: (□) = laccase and (●) = tyrosinase.

From this equation and from equation (6) it follows:

$$\tau_r\ (\%) = \left(\frac{\alpha\Delta T}{\alpha\Delta T + 100}\right) 100 = \left(\frac{\alpha^*\Delta T^*}{\alpha^*\Delta T^* + 100}\right) 100 = \left(\frac{\text{P.A.I.}}{100 + \text{P.A.I.}}\right) 100 \quad (10)$$

A theoretical plot of this equation is given in Fig. 10, where we have reported also the τ_r values of the P.A.I.'s obtained at the BPA concentrations reported in Fig. 9 with a ΔT = 30 °C. As expected the values of the reduction of the bioremediation times are function of the percentage increase of the enzyme activity (P.A.I.) and, therefore, of the temperature difference applied across the catalytic membrane.

Before concluding it is interesting to consider also how the reduction of bioremediation times depends on the BPA concentration. This is done in Fig. 11 where the reduction of the bioremediation times are reported as a function of BPA concentration when immobilized laccase or tyrosinase are employed in bioreactors operating under non-isothermal conditions. As expected, the reductions of the bioremediation times decrease with the increase of BPA concentration.

From the industrial point of view this observation is very important since the concentrations studied in this work are in any case higher than those really found in polluted waters. Indeed some authors [31], analysing the BPA concentration in streams and rivers in Japan, Europe and United States, estimated for BPA an average value of 2×10^{-9} M.

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