Study of an Enzyme Membrane Reactor with Immobilized Fumarase for Production of L-Malic Acid

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Abstract: The conversion of fumaric acid into L-malic acid by fumarase immobilized in a membrane reactor was analyzed experimentally. The enzyme was entrapped in asymmetric capillary membranes made of polysulfone. The performance of the reactor was evaluated in terms of conversion degree, reaction rate, and stability. The influence of operating conditions, such as amount of immobilized enzyme, substrate concentration, residence time, and axial flow rate, were investigated. The kinetic parameters K_m, V_{max}, and k_{+2} were also measured. The stability of the immobilized enzyme was very good, showing no activity decay during more than 2 weeks of continuous operation. © 2001 John Wiley & Sons, Inc. *Biotechnol Bioeng* **72**: 77–84, 2001.
Keywords: membrane reactor; immobilized enzyme; fumarase; L-malic acid

INTRODUCTION

Membrane reactors using catalysts of biological origin represent a promising technology for production and processing in the pharmaceutical and food industry. The increased attention toward "natural-like" products and environmentally friendly processes makes them particularly attractive, because they do not require chemical additives, are able to work at mild conditions of pH, temperature, and pressure, and can help to reduce the formation of byproducts (Drioli, 1989; McConville, 1990). The catalytic action of enzymes is extremely efficient and selective with respect to ordinary chemical catalysts; that is, they show higher reaction rates, milder reaction conditions, and greater stereospecificity. The possibility of integrating biotransformations into productive cycles makes reliable the use of biocatalysts for production at large scale (Lopez and Matson, 1997; Takata, 1993). Membranes can be used not only for molecular separation but also for carrying out reactions, in the same way that living organisms achieve their biochemical transformations. The membranes' ability to compartmentalize makes them particularly suitable for bioreactor applications. The immobilized enzymes are retained in a defined reaction space and can be reused continuously. Furthermore, immobilization has also been shown to increase enzyme stability. Biocatalytic membrane reactors are of interest with respect to conventional ones, because they can combine selective mass transport with chemical reactions. The selective removal of products from the reaction site increases conversion of product-inhibited or thermodynamically unfavorable reactions (Wu, 1990, 1993).

Biocatalysts (such as enzymes, microorganisms, catalytic antibodies, etc.) can be used suspended in solution and compartmentalized by a membrane in a reaction vessel or immobilized within the membrane matrix itself. In the first case, the system might be comprised of a traditional stirred-tank reactor combined with a membrane separation unit. In the latter case, the membrane acts as a support for the catalyst and as a separation unit (Drioli and Giorno, 1999).

In the present work, the possibility of converting fumaric acid into L-malic acid using an enzyme membrane reactor, with fumarase entrapped in asymmetric capillary membranes, is investigated.

Fumaric acid is obtained using by-products resulting from the production of phthalic anydride. The application of fumaric acid in the industrial field is limited by its low solubility. In order to make it useful, it is enzymatically converted to L-malic acid, which is more soluble, and is used as an acidulant in fruit and vegetable juices, jams, infant foods, etc. (Giacobbe, 1980; Milson, 1987).

Fumarase, from various microorganisms, has been investigated in several conditions, free and immobilized, on various supports (Chibata et al., 1983, 1987; Petruccioli, 1996; Takata, 1979).

The reaction catalyzed by fumarase is as follows:

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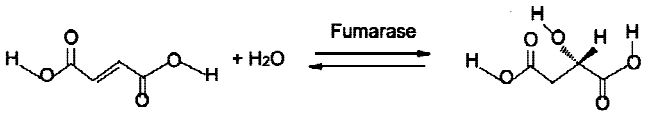

Scheme 1

The enzyme apparently possesses an absolute specificity for the substrates fumarate and L-malate (Chen et al., 1994, 1995; Irwin, 1997). At pH 7.5, the reaction on the right is faster than the one on the left, which is 100% at pH 9.

The activity of fumarase is extremely sensitive to temperature and to the concentration and type of anion in the assay mixture. The molecular weight of the native enzyme is about 200,000 Da.

In this work, the enzymatic production of L-malic acid is carried out using an enzyme membrane reactor with fumarase immobilized within the spongy layer of asymmetric membranes made of polysulfone (cut-off of 100,000 Da).

The reactor performance, in terms of conversion degree and reaction rate, is investigated as a function of various parameters, such as concentration of immobilized enzyme, substrate concentration, residence time, and axial flow rate. The kinetic parameters K_m, V_{max}, and k_{+2} were also measured. The enzyme membrane reactor showed no activity decay during more than 2 weeks of continuous operation.

MATERIALS AND METHODS

Fumaric acid was provided by Pantochim (Belgium). Fumarase (EC 4.2.1.2) from porcine heart (F-1757) and L-malic acid were purchased from Sigma Co. (St. Louis, MO). Fumarase was present in solutions and each lot measured 6 mL with a protein concentration of 2.2 mg/mL. A bicinchoninic acid protein assay (BCA) to measure protein concentration was purchased from Pierce.

The reactions were carried out at pH 7 in phosphate buffer (Na_2HPO_4/NaH_2PO_4) at concentrations of between 0.01 and 0.5 *M*, as indicated in what follows; sodium fumarate was then used by the enzyme as substrate. The reaction rate was evaluated by measuring the decrease of substrate concentration during that time. The concentration of fumaric acid was measured spectrophotometrically at 310 nm. Calibration curves where prepared using a standard concentration of fumaric acid in the range of 20 to 120 m*M*. This wavelength was selected by the spectra of fumarate and malate at between 400 and 200 nm. As reported by Colowick (1955), fumarate shows good absorption in the ultraviolet region and at up to 320 nm, whereas malate has negligible absorption between the ultraviolet and visible range. To avoid interference with malate, we chose the 310-nm wavelength.

In addition, to confirm that this method is accurate and no interfence was taking place, the concentration of malate was also detected by high-performance liquid chromatography (HPLC). The following conditions were used: a Prontosil C_{18} AQ column, 120 Å, 5 mm, 250 × 4.6 mm (Alltech, Inc.), with a mobile phase of H_3PO_4 0.05 m*M*, flux 0.7 mL/min, T = 25°C, pressure 72 bar, λ = 205 nm. The external standard method was applied. For each set of measurements, a calibration curve with two standard concentrations was constructed and each standard was injected three times. The samples to be detected were also injected three times.

High-performance liquid chromatography (HPLC) confirmed that the spectrophotometric method was accurate and, due to its simplicity, spectrophotometry was used routinely, whereas HPLC was used randomly to confirm the data.

Preliminary experiments were also carried out with the enzyme free in solution to assess native performance. The reactions took place in a stirred-tank reactor using 50 mL of the total volume of reaction mixture. The reaction tank was kept at 25°C in a thermostatic bath. Complete mixing was done using a magnetic stirrer.

Capillary membranes made of polysulfone (PS; Romicon, Inc.) were used. The membranes had an asymmetric structure with a spongy layer on the shell side and a thin layer on the lumen side. The nominal molecular weight cut-off (NMWCO) of the selective layer was 100,000 Da, and the inner and outer diameters were about 1 and 1.75 mm, respectively. The membrane module had an effective length of 180 mm, the external membrane surface area was 25 ± 2.5 cm^2 or 34 ± 3 cm^2, and the void volume was 0.96 or 0.7 cm^3, as detailed in what follows. The enzyme was immobilized within the porous spongy layer by crossflow ultrafiltration from shell to lumen.

Experimental Set-Up

The enzymatic conversion of fumaric acid into L-malic acid was investigated at the laboratory scale. The equipment used to carry out the experiments is shown in Figure 1. and

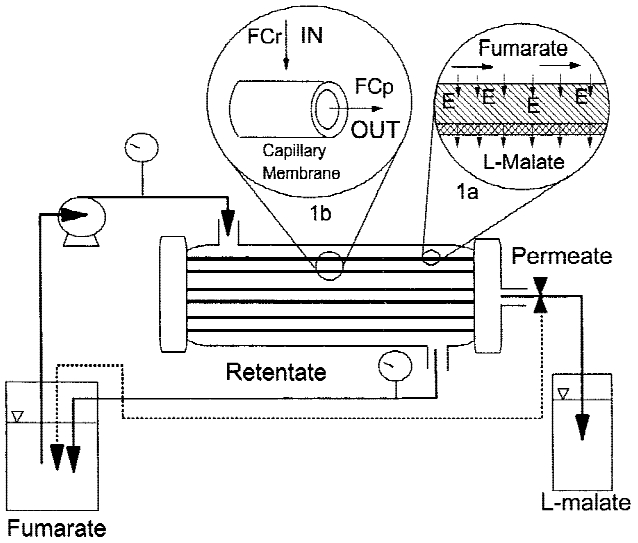

Figure 1. Experimental apparatus (*Cr* and *Cp*, concentration of the retentate and permeate, respectively; F, flow rate; E, enzyme).

consisted of a peristaltic pump (PP; flow rate range 4.8 to 500 mL/min) used to feed substrate solutions to the enzyme membrane reactor; manometers (M) used to measure inlet and outlet pressures from the membrane module, which are needed to calculate the transmembrane pressure [TMP = $(P_{in} + P_{out})/2$]; and a membrane module (MM) made of a Pyrex cylinder containing capillary ultrafiltration membranes encased in a parallel configuration. A thermostatic bath was used to keep the temperature constant at 25°C.

After the membrane module was prepared, it was characterized by measuring the pure water permeability (using distilled and filtered water). The permeate flux in time as a function of different transmembrane pressure values was measured; the steady-state values of flux were then plotted versus TMP and, from the slope of the straight line obtained, the permeability was calculated. ($J = L_p \cdot \Delta P$, where J is the permeate flux [liters per hour per square meter], L_p is the permeability [liters per square meter per hour per bar], and ΔP is the transmembrane pressure [bar]. The membrane pure water permeability was about 700 ± 20 $L/m^2 \cdot h \cdot bar$). The reason for measuring this parameter pertains to the need to check membrane performance for subsequent use of membrane modules after enzyme removal.

The permeability of membranes containing the enzyme decreases as a function of amount of immobilized enzyme. For example, enzyme-loaded membranes at 0.22 mg, 0.44 mg, and 1.3 mg had a permeability of 403 $L/m^2 \cdot h \cdot bar$, 333 $L/m^2 \cdot h \cdot bar$, and 116 $L/m^2 \cdot h \cdot bar$, respectively.

Immobilization Procedure

The immobilization procedure was started after membrane characterization. Enzyme solutions were prepared by adding the desired amount of fumarase solution (2.2 mg/mL) to the phosphate buffer in order to obtain a final volume of 150 mL. The solution was recirculated along the shell side at a flow rate of 140 mL/min, with a transmembrane pressure of 0.2 bar; hence, the enzyme solution permeated from shell to lumen. In this way, the fumarase (MW 200,000 Da) entered the spongy layer but could not pass through the thin layer (cut-off 100,000 Da) because of the relative size. The permeate was collected and filtered again three times. It was then removed and the circuit was rinsed with distilled and prefiltered water as long as no additional protein was detected (by measuring optical density at 280 nm) in the retentate stream. The circuit was dried with nitrogen and the substrate solution was fed to the enzyme-loaded membrane. The entire process was carried out at 25°C. The amount of immobilized fumarase (m) was determined by mass balance between the enzyme present in the initial solution (C_iV_i) and that present in the permeate (C_pV_p) and retentate (C_rV_r). The enzyme concentration was measured using BCA protein assay reagent (Pierce). In general, 10% of the mass present in the initial solution was immobilized. During the reaction, samples were taken and a protein test done to determine whether there was any enzyme leakage.

This immobilization method provides a random distribution and orientation of the protein within the membrane. Nevertheless, it allows one to obtain a reproducible performance from the enzyme-loaded membrane from preparation to preparation; in fact, starting with the same concentration and volume of enzyme solution, and carrying out the immobilization for a similar period (generally 2 h), the observed reaction rate and conversion (using the same substrate concentration) were the same for the different tests done (with a difference of about 1% to 2% between experiments).

Reaction Mode Operation

After immobilization, the substrate solution (fumaric acid in phosphate buffer at pH 7.00 ± 0.02) was circulated along the shell side of the membrane module and permeated through the membrane wall (where the enzyme was loaded) with an applied transmembrane pressure. As the fumaric acid permeated through the enzyme-loaded membrane it was converted to L-malic acid (Fig. 1b). Because the molecular weights of fumaric and malic acids are 116 and 134, respectively, both permetated through the membrane (which has a cut-off 100,000 Da). This means that the permeate recovered from the lumen side of the membrane contained both reaction product (L-malic acid) and unconverted substrate (fumaric acid). By measuring the decrease of fumaric acid concentration in the permeate during the operation, it was possible to calculate the reaction rate and the degree of conversion.

If the enzyme is immobilized only within the pores, and not on the membrane surface, the concentration of the substrate in the retentate stream does not change, because only the solution that permeates through the membrane is in contact with the biocatalyst. The substrate is then continuously recycled to the tank and fed to the reactor at a constant concentration.

If the enzyme is also present on the membrane surface, some decrease of substrate concentration in the retentate stream is also observed. A blank experiment confirmed that when no enzyme was present on the membrane no conversion occurred.

The conversion degree was calculated as follows:

$$\text{Conversion} = \frac{C_r - C_p}{C_r} \tag{1}$$

where C_r is the concentration of fumaric acid in the retentate solution and C_p is the concentration of fumaric acid in the permeate solution. This gives the conversion at each passage through the membrane.

When the enzyme is also immobilized on the membrane surface, it is necessary to take into the account the conversion of the substrate in the retentate solution; then the total conversion is calculated as:

$$\text{Total conversion} = \frac{(C_r - C_p) + (C_i - C_r)}{C_i} \quad (2)$$

where C_i is the initial substrate concentration.

The reaction rate wquation was derived by the balance equation at steady state (Fig. 1c):

$$FC_f - FC_p + v_r \cdot V = 0$$

Resolving for v_r, the following is obtained:

$$V_r = \frac{F(C_f - C_p)}{V} \quad (3)$$

where v_r is the reaction rate (millimoles per cubic centimeter per minute), F is the permeate flow rate (cubic centimeters per minute), C_f is the feed concentration (millimoles per cubic centimeter), C_p is permeate concentration (millimoles per cubic centimeter), and V is the volume (cubic centimeters).

RESULTS AND DISCUSSION

Using the equipment and the procedures described, the performance of fumarase from porcine heart free in solution and immobilized in the UF-capillary membrane reactor was studied. The various parameters are discussed in the following sections.

Activity of Free Enzyme

The activity of free fumarase has been widely reported in the literature using different substrate and operating conditions (Takata and Tosa, 1993). Nevertheless, the activity of the free fumarase from porcine heart has been investigated to assess the performance of native enzyme as compared with the immobilized enzyme in the same conditions of temperature, pH, buffer concentration, substrate, etc. Experiments were carried out using 80 m*M* fumaric acid in 0.5 *M* phosphate buffer (pH 7.00), with T = 25°C and an enzyme concentration of 0.0132 mg/mL. As shown in Figure 2, a conversion degree of 80 ± 2% was reached, and the initial reaction rate was about 0.29 ± 0.01 mmol/cm^3 per hour. Because the enzyme is present in the reaction mixture, it needs to be separated to be reused. Immobilization of the enzyme in a membrane reactor allows for continuous reuse.

Activity of Immobilized Enzyme and Stability

The first series of experiments was intended to measure the activity of fumarase immobilized on the polysulfone membrane. Immobilization of fumarase was carried out starting from 200 mL of protein solution made with 5 mL of enzyme (2.2 mg/mL) in 195 mL of pure water (distilled and prefiltered water). A membrane module with a surface area of 25 cm^2 and a void volume of 0.7 cm^3 was used; the amount of immobilized protein was about 1.2 mg/cm^3. Experiments were carried out with 80 m*M* fumarate at 25°C (pH 7), axial flow rate 140 mL/min, and TMP = 0.2 bar.

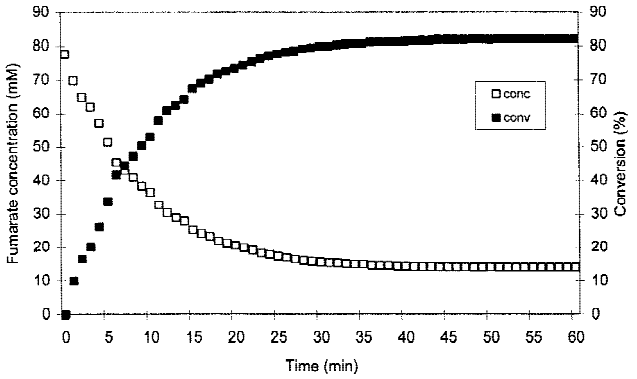

Figure 2. Time course of fumarate concentration and conversion for fumarase used free in the stirred-tank reactor. (Operating conditions: T = 25 (±1)°C; fumarate 80 m*M* in 0.5 *M* phosphate buffer [pH 7.0]; total reaction volume 50 mL.)

In Figure 3, the time course of substrate concentration (fumarate) in the retentate and permeate streams is reported. The figure shows that, at each passage through the enzyme-loaded membrane, the substrate solution was converted at 80%. During the experiment, the reaction rate was about 2 mmol/cm^3 per hour and the enzyme activity was about 1.42 mmol/h per gram. These experiments were carried out with the enzyme present only within the pores of the membrane.

The behavior of fumarate concentration in the retentate and permeate stream and the total conversion degree for a reactor where the enzyme is also immobilized on the membrane surface are shown in Figure 4. As can be seen, the presence of fumarase on the surface causes a decrease of substrate concentration in the retentate stream as well. Nevertheless, the enzyme is not deactivated, which is evidenced by the fact that, when replacing the substrate solution, the conversion starts from the initial performance. This is also confirmed by the fact that total conversion remains constant during the entire operation period.

Experiments have also been carried out on operation in

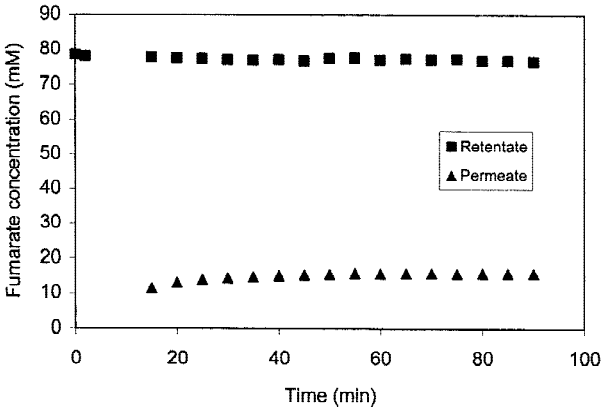

Figure 3. Time course of fumarate concentration in the retentate and permeate streams in a reactor with enzyme immobilized within the membrane pores. (Operating conditions: T = 25 ± 1°C fumarate 80 m*M* in 0.5 *M* phosphate buffer [pH 7.0]).

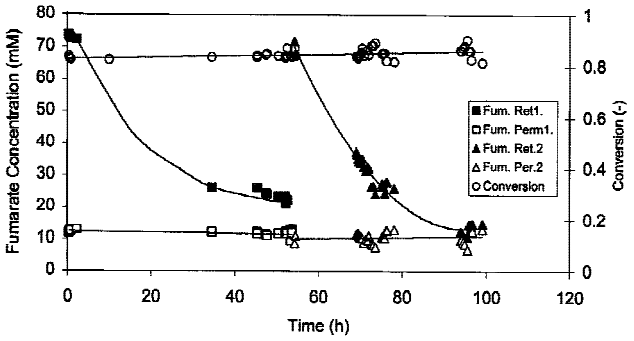

Figure 4. Behavior of fumarate concentration and conversion in a reactor with enzyme also immobilized on the membrane surface. (Operating conditions: T = 25 ± 1°C; fumarate 80 m*M* in 0.5 *M* phosphate buffer [pH 7.0]).

the batch recycle mode; that is, recycling the permeate in the retentate stream. In this case, the reaction rate can be calculated by the decrease in fumaric concentration over time, and the conversion degree as the ratio $(C_i - C_t)/C_i$, where C_i and C_t are the fumaric concentration at initial (i) and t timepoints. The time courses of fumaric concentration and conversion are illustrated in Figure 5. The experiments clearly show that fumarase is not inhibited by the product. No enzyme leakage was detected. This was verified by performing a protein test of the samples during the operation time.

It is conventional to measure the enzyme stability in terms of half-life time ($t_{1/2}$) — the time at which enzyme activity is reduced to half its initial value.

The stability of the enzyme was measured using 1.1 and 1.3 mg of immobilized enzyme in 0.7 cm^3 (1.57 and 1.85 mg/cm^3, respectively) of 80 m*M* fumaric acid in 0.5 *M* phosphate buffer at pH 7.00. Experiments were carried out continuously for 2 weeks, at an axial flow rate of 140 mL/min and a TMP of 0.2 bar. During the experiments there was no evidence of activity decrease in the reactor, and the conversion was constant at about 82 ± 2% for both series of experiments (Fig. 6). To be able to calculate the half-life experiments will need to be carried out for a longer time; nevertheless, the results show that the immobilized enzyme maintained good stability for at least 15 days.

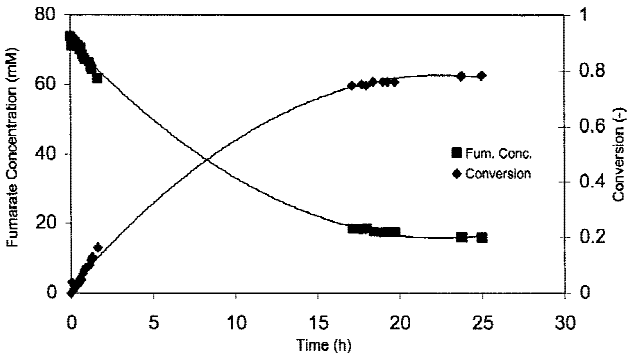

Figure 5. Behavior of fumarate concentration and conversion during batch recycle mode operation.

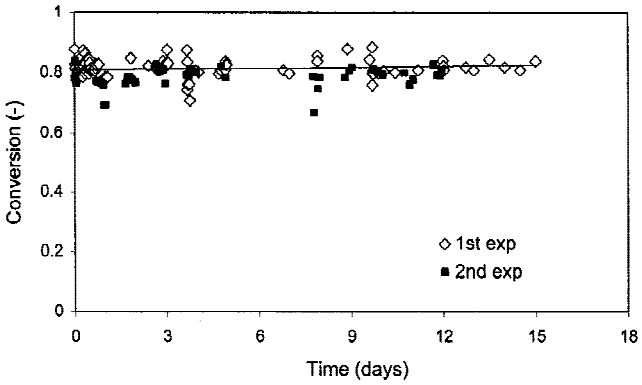

Figure 6. Enzyme stability during continuous operation. (◇) First experiment, amount of immobilized enzyme 1.1 mg; (◆) second experiment, amount of immobilized enzyme 1.3 mg. All other operating conditions are as previously indicated (fumarate 80 m*M*, in 0.5 *M* phosphate buffer [pH 7.0]).

Influence of Residence Time on Reactor Performance

The conversion of fumaric to L-malic acid occurs within the membrane pores, where the enzyme is present, during the permeation of the substrate solution through the membrane (the mass transport is obtained by convection). In this type of operation mode, the permeate velocity is an important parameter, and it influences the degree of conversion. In fact, high permeate velocity means low residence time of substrate solution within the pores. The residence time (τ) of substrate solutions was obtained as:

$$\tau = \frac{l}{v_p}$$

where l is the membrane thickness (liters) and v_p is the velocity of permeate (liters per residence time). The residence time has also been assessed as a function of immobilized enzyme. Experiments were carried out using five different concentrations as summarized in Table I. The enzymatic solutions were prepared using the desired milliliters of fumarase (2.2 mg/mL) in 150 mL (total volume) of 0.05 *M* phosphate buffer. Each solution was used to immobilize the enzyme, as described previously.

The membrane modules were prepared using three hollow fibers (only the fifth one had four fibers) with an inner diameter of 0.96 ± 0.04 mm and an outer diameter of 1.755 ± 0.005 mm.

The reactions were carried out using 80 m*M* fumaric acid in 0.5 *M* phosphate buffer at an axial flow rate of 140 mL/min; the values of permeate flux examined were between 7 and 50 L/h per square meter, corresponding to residence times between 500 and 30 s, respectively. The time courses of conversion as a function of permeate flux for the different quantities of immobilized enzyme were measured. For example, in Figure 7, the data measured for the reactor containing 1.171 mg/cm^3 are reported. For each enzyme membrane reactor, the steady-state value of the

Table I. Conditions for different enzyme concentrations immobilized on membrane reactors.

Enzyme solution (mL)	Initial mass (mg)	Buffer solution (mL)	Initial enzyme concentration (mL/min)	Membrane void volume (cm^3)	Immobilized enzyme (mg/cm^3)
1	2.2	149	0.0146	0.708	0.310
2	4.4	148	0.029	0.751	0.621
4	8.8	146	0.058	0.70	1.171
6	13.2	144	0.088	0.712	1.854
5	11	145	0.073	0.96[a]	1.140

[a]Module with 4 fibers.

permeate fluxes were then plotted versus conversion (as illustrated in Fig. 8). From these values, the corresponding residence times were calculated as reported in Table II. The results clearly show that, at the lower residence time, conversion decreased, and this effect was particularly significant for small amounts of immobilized enzyme.

Influence of Enzyme Concentration on Conversion Degree

As noted previously, the concentration of biocatalyst is another important parameter to consider. There is evidence in the literature that enzymes work better in dilute solutions rather that in concentrated ones. When the enzyme is immobilized this effect is even more accentuated. The reason for this is that when too much enzyme is in the support the mass transport resistance is increased and reagents cannot reach (substrate) or release (product) the reaction site, with an overall effect of reducing reactor performance.

The results obtained for fumarase in the membrane reactor used are shown in Figure 9. The best performance was attained using a concentration of protein within the membrane pores of about 1.2 mg/cm^3. At higher concentrations, the protein formed a gel layer on the membrane surface, resulting in the effects shown in Figure 4.

Influence of Axial Flow Rate on Reactor Performance

The effect of axial flow rate of the retentate stream was investigated using a reactor with 1.8 mg/cm^3 of immobilized fumarase. As in the previous cases, the experiments were carried out with solutions of 80 m*M* fumaric acid in 0.5 *M* phosphate buffer at pH 7.00. The results reported in Figure 10 demonstrate that, for the range of values studied, the axial flow rate did not influence reactor performance. (The slight decrease observed at the higher axial flow rate was due to the fact that, when increasing axial flow rate, the transmembrane pressure also increased, and this caused an increase in the permeate flux with a corresponding decrease in residence time and then of conversion.)

Within the range of values in which the axial flow rate did not have an effect on the transmembrane pressure, the conversion, reaction rate, and enzyme activity were constant. This obviously depends on the fact that, because mass transport through the membrane occurs by convection and the solutes are not retained by the membrane, the effects of axial flow rate on mass transfer coefficient are not significant.

Measure of Kinetic Constants

The kinetic constants (K_m, V_{max}, k_{+2}) were measured using an enzyme membrane reactor with about 1.3 mg of immobilized fumarase in a membrane module with a surface area of 34 cm^2 and a void volume of 0.96 cm^3. Experiments were carried out at 25°C and pH 7.00 ± 0.02. The experiments at different substrate concentrations were carried out with the

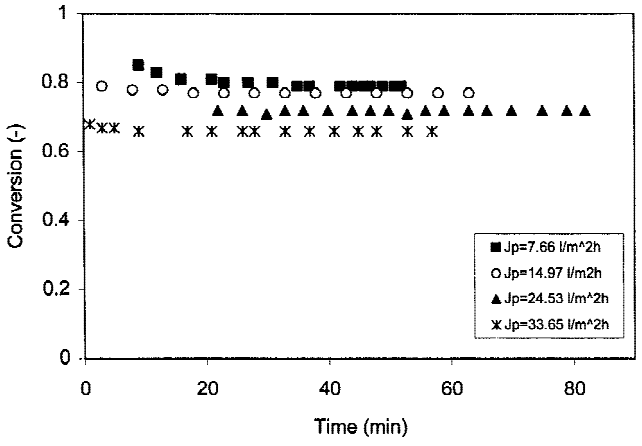

Figure 7. An example of steady-state conversion as a function of permeate flux.

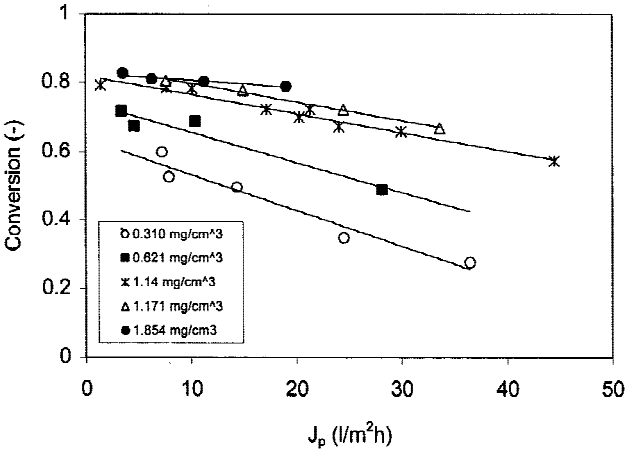

Figure 8. Behavior of conversion versus permeate flux for different amounts of immobilized enzyme.

Table II. Comparison of permeate velocity, residence time, and conversion for membrane reactors with different amounts of immobilized enzyme.

Immobilized enzyme (mg/cm^3)	Membrane thickness (m) × 10^4	Permeate flux ($L/m^2 \cdot h$)	Permeate velocity (m/s) × 10^6	Residence time (s)	Conversion (−)
0.310	3.775	7.26	2.016	187	0.599
		7.90	2.194	171	0.528
		14.37	3.99	94	0.497
		24.53	6.81	55	0.348
		36.50	10.13	37	0.275
0.621	3.775	3.38	0.938	401	0.719
		4.62	1.283		
		10.42	2.894	130	0.688
		28.17	7.825	48	0.489
1.140	3.750	1.413	0.754	497	0.793
		7.7	0.392	175.39	0.786
		10.12	2.138	133	0.784
		17.18	2.811	78.57	0.724
		20.30	4.772	66.50	0.701
		21.32	5.638	63.23	0.724
		24.10	5.922	56.01	0.675
		30.00	6.694	45	0.660
		44.50	8.333	30.33	0.575
1.171	3.750	7.66	2.127	177	0.806
		14.97	4.158	90	0.777
		24.53	6.813	55	0.722
		33.65	9.347	40	0.670
1.854	3.850	3.54	0.983	392	0.827
		6.30	1.75	220	0.810
		11.26	3.13	123	0.803
		19.04	5.28	72.7	0.790

same membrane module, and at the end an experiment with the initial concentration was carried out to verify that enzyme activity was unchanged.

Experiments were carried out after preparing substrate concentrations of 8 m*M*, 10 m*M*, 20 m*M*, 30 m*M*, 40 m*M*, 60 m*M*, 80 m*M*, and 10 m*M*. (The buffer concentration was increased proportionally with the substrate concentration, from 0.05 *M* to 0.5 *M*, in order to dissolve the fumaric acid and keep a constant pH 7.0. It was previously experimentally confirmed that the activity of fumarase was not affected by the buffer concentration in the range used.) The effective value of each initial substrate concentration was measured after preparation of the solutions and resulted in concentrations of 8.4 m*M*, 10.3 m*M*, 19.02 m*M*, 29.44 m*M*, 39.6 m*M*, 60.7 m*M*, and 81.7 m*M*. The reaction rate value obtained for each substrate concentration was 0.310 ± 0.06 $mmol/cm^3$ per hour, 0.390 ± 0.09 $mmol/cm^3$ per hour, 0.750 (±0.02) $mmol/cm^3$ per hour, 1.125 (±0.02) $mmol/cm^3$ per hour, 1.50 (±0.03) $mmol/cm^3$ per hour, 2.125 (±0.02) $mmol/cm^3$ per hour, and 2.375 (±0.03) $mmol/cm^3$ per hour, respectively.

The Michaelis–Menten and Lineweaver–Burk plots (Bailey and Ollis, 1986) for these reaction conditions were also constructed. From the Lineweaver–Burk equation, V_{max} and K_m were calculated from the slope and intercepts of the straight line obtained to the *y* and *x* axes, as illustrated in

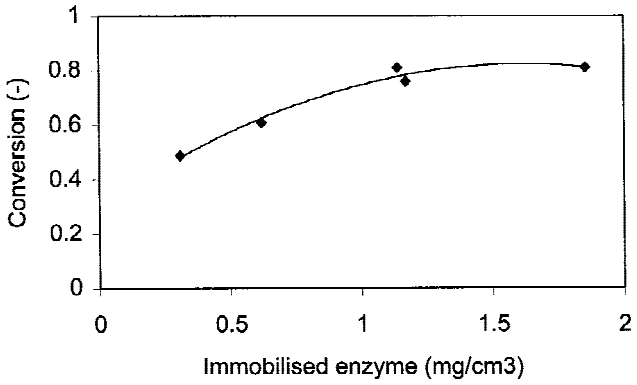

Figure 9. Conversion as a function of immobilized fumarase.

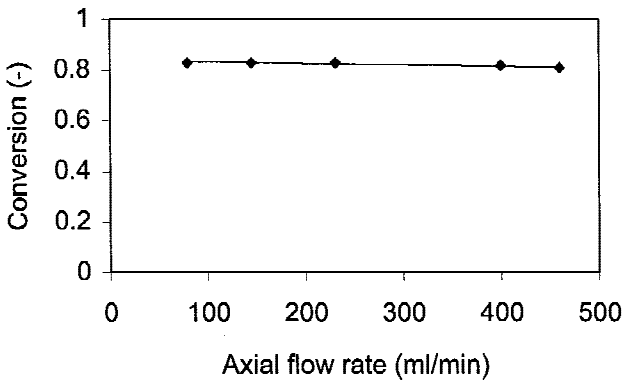

Figure 10. Influence of axial flow rate on conversion.

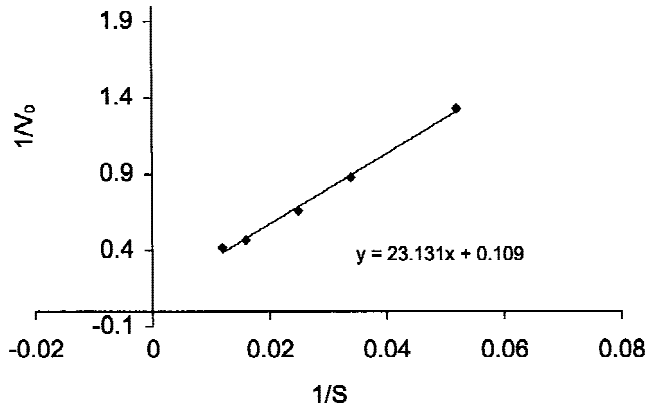

Figure 11. Evaluation of kinetic constants from the Lineweaver–Burk equation (T = 25 ± 1°C).

Figure 11. V_{max} was 9 mmol cm^{-3} h^{-1} and K_m was about 212 m*M*.

The value of k_{+2} was calculated by: $V_{max} = k_{-2} \times [E]_{tot}$, with $[E]_{tot} = 5.2 \times 10^{-6}$ mmol/cm^3 and $k_{+2} = 5 \times 10^2$ s^{-1}. This value is the same order of magnitude for k_{+2} reported in the literature for free fumarase (8×10^2 s^{-1}) (Voet and Voet, 1995).

CONCLUSIONS

In this study, an enzymatic membrane reactor for the conversion of fumaric acid into L-malic acid was investigated. The influence of various parameters (such as activity, stability, permeate flux, and residence time, amount of immobilized enzyme, and axial flow rate) on reactor performance was also investigated. The kinetic parameters (K_m, V_{max}, k_{+2}) of the reaction system were measured. The kinetic properties of the enzyme in immobilized form were not significantly changed with respect to the native form.

On the basis of the results obtained at the laboratory scale, the enzyme membrane reactor for this application seems to be of value in terms of stability and conversion degree. The stability of the enzyme-loaded membrane system showed very good results, with no activity decay during the 15-day period of continuous operation. A steady-state conversion degree of 80% was reached.

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NOMENCLATURE

- C_f feed concentration (M L^{-3})
- C_p concentration of fumaric acid in the permeate solution (M L^{-3})
- C_p permeate concentration (M L^{-3})
- C_r concentration of fumaric acid in the retentate solution (M L^{-3})
- F permeate flow rate (L^3 T^{-1})
- J flux (L^3 T^{-1} L^{-2})
- l membrane thickness (L)
- L_p permeability (L^3 T^{-1} L^{-2} bar^{-1})
- V volume (L^3)
- v_p permeate velocity (L T^{-1})
- v_r reaction rate (M L^{-3} T^{-1})
- Δ transmembrane pressure (TMP) (bar)

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