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Author: Susana Luque José R. Álvarez F. Petrus Cuperus

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ESTER AMMONIOLYSIS IN AN ENZYMATIC MEMBRANE REACTOR

Susana Luque[1,*], José R. Álvarez[1] and F. Petrus Cuperus[2]

[1]*Department of Chemical and Environmental Engineering. University of Oviedo, 33071 Oviedo, Spain.*

[2]*SolSep BV. St Eustatius 65, NL-7335JE Apeldoorn, The Netherlands*

** Corresponding author: +Tel: +34 98 510 2998; Fax: +34 98 510 3434*

e-mail: sluque@uniovi.es

Highlights:

A gas-liquid enzymatic membrane reactor was implemented for direct ammoniolysis.

Ethyl octanoate was converted to octanamide by immobilized *C.antarctica* lipase B.

High enzyme stability and selectivity towards the amide (95% at 40 ºC) was achieved.

ABSTRACT

A form of generating amides is by reaction of esters with ammonia (ammoniolysis) or with amines (aminolysis). This reaction can be catalyzed by free or immobilized lipases. The aim of this work is to develop a gas-liquid enzymatic membrane reactor in which the ammoniolysis of ethyl octanoate, catalyzed by *Candida antarctica* lipase B (EC 3.1.1.3), is improved through immobilization of the biocatalyst onto a membrane surface which also serves as gas-liquid intephase. After the selection of a suitable membrane for the process, data show that the higher the initial substrate concentration, the lower the reaction rate. A Michaelis-Menten type model with competitive product inhibition correlated well with the kinetic data. In addition, the temperature dependence of the reaction rate goes through a maximum. The yield of the amide was always above 92 % in the range 25-55 ºC, with a maximum of 95 % at 40 ºC. At 40ºC, and after 24 h of reaction, values comparable to those reported in the literature on the free enzyme-catalyzed ammoniolysis were achieved, but with much lower enzyme/substrate ratio. Moreover, the immobilized enzyme kept its activity for a long period of time (three weeks).

KEYWORDS: *Candida antarctica;* ester ammoniolysis; lipase; membrane reactor

1. INTRODUCTION

The industrial use of enzymes is often limited by their cost because of the difficulty and expense of the recovery step when they are used in the freely soluble form. The use of enzymes is typically more cost-effective when they are immobilized. Immobilization of the enzyme on a support may have several advantages: the possibility of reusing the enzyme, a better control of the reaction (that allows a more feasible operation), the development of continuous large scale commercial processes and less pollution of the product by the residual biocatalyst. Moreover, it may also lead to a reduction of the inhibition and to the improvement of properties such as stability, activity, specificity and selectivity [1].

Lipases (Triacylglycerol acylhydrolases, EC 3.1.1.3) are widely distributed in nature, and are able to perform acyl-transfer reactions with a number of nucleophiles (i.e., water, alcohols, peroxides, amines, hydrazines and oximes) via an acyl-enzyme intermediate [2, 3]. Since water always competes with the desired nucleophile in a kinetically controlled process, water activity in the reaction mixture affects the selectivity of the reaction.

Enzyme use in the fat and oil industry has become important since they can catalyze reactions under mild conditions (i.e., the industrial hydrolysis of fats and oils or the manufacture of fatty acid amides) that otherwise would need high pressure and temperature, and consequently, expensive equipment. In addition, enzymatic methods have been developed in order to produce heat-sensitive amides, such as oleamide, without degradation. This degradation often results in the need on additional distillation steps to meet purity

specifications. Enzymes also allow achieving high specificities and, therefore, they can be used to obtain high-value chemicals for food and industrial uses at competitive production costs [4].

Amides are interesting chemicals since they can be employed in the peptide and lactam synthesis, and they have many applications in the textile, paper, wood, metal, rubber, plastic and coating industries. Fatty acid amides are produced in thousands of metric tons per year and have good lubricating and surfactant properties, with applications, i.e., in the plastic industry [5].

Lipase-catalyzed ammoniolysis reactions are those where ammonia serves as the nuclophile [6,7], and have been successfully accomplished with high conversions under mild conditions with, i.e., esters [3, 8 – 13], 3-substituted glutarates [14, 15] and amino acid esters [16, 17] and in the preparation of carboxylic amides from carboxylic acids [18, 19]. One-pot lipase-catalyzed procedures for the conversion of carboxylic acids into substituted amides via in-situ formation of the ethyl ester and subsequent ammoniolysis have also been reported [20, 21]. Other sources for the NH_2 group have been employed, such as urea [22], ammonium bicarbonate [23], ammonium carbamate and several ammonium salts [24, 25]. If amines are used, the reaction that takes place is an aminolysis instead of ammoniolysis, and secondary amides can be prepared [6, 7, 10, 14, 15, 21, 26, 27]. Enantioselective biocatalyzed ammoniolysis has also received significant attention in the last decade [28 – 31].

However, all of these aforementioned references use either free enzymes or enzymes immobilized on particulate matter, mostly the commercial ones, prepared on porous support made of polypropylene. The aim of the present paper is to develop an enzymatic membrane reactor in which the enzyme-catalyzed ammoniolysis of a carboxylic acid ester can be improved by means of biocatalyst immobilization onto a membrane surface.

Ethyl octanoate was selected as substrate. The free enzyme-catalyzed ammoniolysis of this substrate has been reported [3, 8], and the best results were obtained when *Candida antarctica* lipase B (CALB) was used with an enzyme/substrate ratio of 20 mg/mL, bubbling ammonia gas to a mixture of ethyl octanoate (0.5 mL) in dry tert-butanol (5 mL). The selectivity of this reaction set was a function of the excess ammonia used. After 24 hours at 40°C, a 1:1 ratio (1.0 equivalent of ammonia per equivalent of octanoate) resulted in 43% of the substrate to converted in to octanamide, whereas with an excess of ammonia (5:1 equivalents), 95 % of ethyl octanoate became amide. Fig. 1 shows a scheme of the reactions involved.

2. MATERIALS AND METHODS

2.1. Materials

Two different dense membranes (designed for gas separation) in hollow-fiber arrangement were used, one made of polyetheretherketone (PEEK), with an area of 0.004 m^2 and the other made of a 0.1 μm silicon rubber (SIR) layer supported on polyetherimide, with an overall area of 0.04 m^2. The PEEK membrane module was produced at the Institute of Membrane Technology- ITM-CNR in Calabria (Italy), and had the characteristics previously reported by Giorno et al. [32] and the PEI membrane module was fabricated at the University of Twente, following the protocol described by Roesink et al. [33].

Freeze-dried CALB and a frozen solution containing 28 g/L CALB were kind gifts of NovoNordisk (Amersfoort, NL). The former showed less activity (120 μmol tributyrin/mg enzyme·min) than the latter (275 μmol tributyrin/mg enzyme·min), as measured by the

tributyrin test [34]. Glycerol (≥99%), ethyl octanoate (> 98 %), tert-butanol (> 99 %) and octanoic acid (>98%) were purchased from Merck, Netherlands. Bovine serum albumin (lyophilized powder, ≥98%), hexane (for HPLC, ≥97%) and 1-hexadecanol (≥99%) were supplied by Sigma Aldrich. The ammonia was essentially water free (Hock Lios, T404 E, >99.95 %).

2.2. Enzyme immobilization

The enzyme was immobilized according to the procedure of Macrae et al. [35]. This immobilization is based on physical adsorption on the outside (spongy layer) of the fibers, which is an easy procedure with small associated costs. It basically consisted of a preliminary wetting of the membrane by pumping an aqueous solution containing 5 % glycerol to the shell side of the module for 24 h at negligible transmembrane pressure. This was followed by a thorough rinsing with deionized water to remove the excess glycerol. Alcohol wetting led to membrane swelling. Then, a 1000 ppm bovine serum albumin solution was fed to the outside of the fibers at a transmembrane pressure of 2 kPa and room temperature for 8 h, allowing the development of a first adsorbed layer of protein on the membrane surface, which favored enzyme immobilization. This adsorption is enhanced by the previous alcohol swelling step. The membrane was rinsed again with deionized water and the enzyme solution was placed in the feed tank and recycled in the system (through the shell side as well) with a retentate pressure around 4 kPa at room temperature. There was no observable permeate flux during the whole process. Additionally, immobilization took place only on the spongy layer of the fibers because the membranes were dense (i.e. non porous) and therefore, they did not allow the passage of proteins nor enzymes.

The amount of active lipase adsorbed with time was calculated by mass balance of the initial activity in the feed enzyme solution and the residual activity in periodically withdrawn samples of this solution. [36 – 38] Once the residual activity became constant, the enzyme solution was removed, and the system was finally rinsed with deionized water and dried with nitrogen overnight. No permeate was obtained during the immobilization because of the low pressure applied.

This procedure usually ensures unchanged substrate specificity, and regeneration is possible after treatments with strong denaturants. The performance has been found to be better when a hydrophobic support was used, as in the present case, in terms of loses of enzyme and activity enhancement [1, 39]

2.3. Enzyme-catalyzed reaction

The reaction mixture included ethyl octanoate dissolved in tert-butanol. Tert-butanol cannot participate in the reaction, because of steric hindrance [3]. For the SIR membrane, the mixture volume was kept constant at 250 mL, but the proportions of each compound varied from 12.5 mL ester in 237.5 mL alcohol (0.25 mol/L of ethyl octanoate) to 125 mL ester in 125 mL alcohol (2.5 mol/L of ethyl octanoate). For the PEEK membrane, a volume of 100 mL (20 mL ester in 80 mL alcohol) was employed. During the reaction, each mixture was pumped to the shell side of the hollow fiber module, whilst ammonia gas was fed to the inside of the fibers in a dead-end mode.

The system experimental setup (Fig. 2) consists of an Ismatec variable speed gear pump, and stainless steel pressure gauges located before and after the module in the fiber side. A security pressure-relief valve is located at the outlet of the module in the ammonia side.

The humidity intake in the 250/ 100 mL of mixture substrate-solvent was limited by placing it in a closed jacketed feed tank instead of in an open one. In order to measure the ammonia gas consumption, a known-volume stainless steel container was installed between the ammonia pressurized cylinder and the module. By measuring the pressure in the reservoir it is possible to determine the usage of the ammonia gas. A flask containing a weak acid solution was placed at the end of the ammonia gas line for collecting the gas when the experiments finished (which avoids the atmospheric release). Stainless steel and polypropylene tubing were used for the ammonia gas line and viton tubing for the reaction mixture line.

After the reaction, the system was rinsed with small volumes of tert-butanol (to eliminate both the products and the remaining reactants) and dried by flowing nitrogen.

It has to be pointed out that the ammonia gas consumption could be control only to a certain extent since some overpressure in the ammonia side of the membrane was needed to ensure ammonia gas flow thought it and to prevent the reaction mixture flowing though the membrane. In this study, the pressure was set to 0.05 bar in the liquid side and 0.1 bar in the ammonia side, which led to an usage of 1 equivalent of ammonia gas every 4 h, approximately, and kept the organic phase saturated. However, it could be possible to further optimize this parameter if an automated control of the transmembrane pressure would be set, keeping a transmembrane pressure as low as 0.01-0.02 and, therefore, reducing significantly the ammonia gas usage.

2.4. Analysis

Samples of the reaction mixture were analyzed on a HRGC 5300 Megaseries Carlo Erba equipped with a FID detector, an A200S autosampler and a CP Sil 5B column (Chrompack, NL). Hexane (at which few drops of ethyl alcohol were added in order to dissolve the

octanamide) was used as solvent and 1-hexadecanol was employed as internal standard. The temperature program was set as follows: 1 min at 60°C (base line) before sampling, from 60 to 100°C at 4°C/min, from 100 to 300°C at 30 °C/min, stay at 300°C for 3 min and then the temperature was decreased to baseline again. Temperature in both injector and detector was set to 320°C. In order to improve the detection of the octanoic acid, samples were methylated with diazomethane prior to analysis. Commercial octanoic acid and octanamide isolated from a bulk reaction were used to determine the retention time of the reaction products.

2.5. Mathematical modeling of the reaction

A modification

A Michaelis-Menten equation, extended for simple cases of competitive and uncompetitive reversible inhibitions of the enzyme, has been adapted from the work of Staniszewki [40]. The scheme is represented in Fig. 3, with the typically employed nomenclature: *E* for enzyme, *S* for substrate and *P* for products (being P_1 the octanamide and P_2 the octanoic acid). *ES* is the active enzyme-substrate complex, whereas EP_1, EP_2, *ESS*, ESP_1 and ESP_2 are the inactive complexes. By using the quasi-stationary approximation, the following expression for the rate of substrate consumption was obtained:

$$-\frac{d[S]}{dt} = \frac{v_m[S]}{K_M\left(1+\frac{[P_1]}{K_{IP1}}+\frac{[P_2]}{K_{IP2}}\right)+\left(1+\frac{[P_1]}{K^*_{IP1}}+\frac{[P_2]}{K^*_{IP2}}+\frac{[S]}{K^*_{IS}}\right)[S]} \quad (1)$$

where K^*_{IS} ,K^*_{IP1} and K^*_{IP2} are the uncompetitive inhibition constants for substrate S and products P_1 and P_2, respectively; whereas K_{IP1} and K_{IP2} are the competitive inhibition

constants for the products. $K_M = (k_{-\alpha} + k_{P1} + k_{P2})/k_\alpha$ and $v_m = (k_{P1} + k_{P2})[E_0]$. By defining the substrate conversion as $x_S = 1 - [S]/[S_0]$, and assuming that the concentrations of the intermediates *ES*, EP_1, EP_2, *ESS*, ESP_1 and ESP_2 are much lower than those of the products [41, 42], Eq.1 becomes:

$$\frac{dx_S}{dt} = \frac{1}{A + \frac{B}{(1 - x_S)} + C x_S} \tag{2}$$

$$A = \frac{[S_0]}{v_m}\left(1 - \frac{K_M \eta_1}{K_{IP1}} - \frac{K_M \eta_2}{K_{IP2}} + \frac{[S_0]}{K_{IS}^*}\right) \tag{3}$$

$$B = \frac{K_M}{v_m}\left(1 + \frac{[S_0]\eta_1}{K_{IP1}} + \frac{[S_0]\eta_2}{K_{IP2}}\right) \tag{4}$$

$$C = \frac{[S_0]^2}{v_m}\left(\frac{\eta_1}{K_{IP1}^*} + \frac{\eta_2}{K_{IP2}^*} - \frac{1}{K_{IS}^*}\right) \tag{5}$$

$$\eta_1 = \frac{k_{p1}}{k_{p2} + k_{p1}}, \qquad \eta_2 = \frac{k_{p2}}{k_{p2} + k_{p1}} \tag{6}$$

The mathematical development has been compiled in Appendix A. The integration of Eq.2 gives

$$t = A x_S - B\ln(1 - x_S) + \frac{1}{2} C x_S^2 \tag{7}$$

3. RESULTS AND DISCUSSION

3.1. Enzyme immobilization and preliminary screening

The amount of immobilized enzyme varied for each type of membrane used. This was estimated by measuring the remaining activity in the feed solution at the end of the

immobilization step and assuming that the difference (with the initial one) was the actual immobilized amount. In the PEEK fibers, with an area of 0.004 m^2, the immobilized enzyme leaded to an activity of 1920 μmol/min (equivalent to 16 mg of the freeze dried powder). However, in the SIR module (0.04 m^2), the estimated activity was around 15400 μmol/min (corresponding of 56 mg of lipase coming from the frozen solution). The immobilized activity of the PEEK membrane showed a value of 480,000 μmol/m^2min, whilst the SIR membrane had a specific activity of 385,000 μmol/m^2min, which indicates that a square meter of PEEK is more adsorptive than the same area of SIR. In both cases, the residual activity in the feed solution became constant after 20 hours of recycle.

For the preliminary screening, solutions containing 1.0 mol/L of ethyl octanoate were fed to the shell side of the hollow-fiber arrangements at 25ºC. Since the amount of enzyme adsorbed in each membrane was different, it was decided to maintain an enzyme/ substrate ratio around 1.0 (expressed as mg enzyme/ mL substrate) in order to allow a better comparison between the results of both membranes. Hence, 50 mL of ethyl octanoate were utilized (mixed with 200 mL of tert-butanol to achieve the concentration of 1.0 mol/L) when dealing with the PEEK membrane (because there were 56 mg CALB on it), and 20 mL of ethyl octanoate (mixed with 80 mL of tert-butanol) for the SIR membrane (where there were 16 mg CALB). Fig.4a displays the time-dependence of the overall ester conversion (i.e. molar ratio of products at a given time divided between the initial ester concentration) and Fig.4b shows the yield of the reactions. As expected, the highest reaction rate (and therefore, the fastest conversion) was seen in the SIR membrane, because a gram of the enzymatic preparation retained on this membrane is twice more active than a gram of the enzymatic preparation absorbed on the PEEK membrane. Nevertheless, the yield difference was surprising: the PEEK membrane reached a 36.4% after 150 hours and the SIR membrane

gave a value of 93% after 24 hours. These behaviors cannot be explained by the reaction rate alone, because in the PEEK membrane the amide yield tended to a plateau. Although further work would be necessary, we hypothesize that the hydrophobicity of the material could be a key parameter with respect to enzyme stability and the extent of a hydrolysis reaction. The contact angles found in the literature lied between 110º and 101º for the silicon rubber [43, 44] whilst the values of 91º and 88º were reported for the other polymer [45, 46]. A more hydrophilic surface, such that of the PEEK membrane, could favor the hydrolysis, as a higher water activity could be expected in the proximity of the catalytic sites immobilized onto the membrane surface

In view of these results, the module with the SIR membrane was selected for further experimentation.

3.2. Kinetic study

Reactions with initial substrate concentrations of 2.5, 0.5 and 0.25 mol/L were performed, and the corresponding ester conversions are displayed in Fig. 5a. Results obtained in the previous section for 1.0 mol/L have also been included. As can be seen, the amount of ethyl octanoate at zero-time had an important effect on the kinetics of the ammoniolysis, being faster when less substrate was used. In Fig. 5b, it can be seen that the influence of the initial concentration in the amide yield is only considerable in the first hours of reaction, and that the plateau obtained beyond 20 hours is independent of the ester amount at the beginning of the reaction.

Experimental times were fitted to Eq. 7. The values of the seven kinetic parameters were determined by the least squares regression method, minimizing the following objective function (OF) with the help of the mathematical routine UMPOL, implemented in the software Fortran Power Station 4.0:

$$OF = \sum_{i=1}^{NDP} \left(t_i^{exp} - t_i^{calc}\right)^2 \tag{11}$$

where *NDP* is the number of data points and superscripts "*calc*" and "*exp*" mean "calculated" and "experimental", respectively. The obtained values of v_m, K_M, K_{IS}^*, K_{IP1}, K_{IP2}, K_{IP1}^* and K_{IP2}^* were introduced Eq. 2, which was solved numerically by the finite differences approach, and the goodness of the fitting was evaluated by means of the mean absolute percentage error (MAPE) and the coefficient of determination (R^2) with regards to x_S.

$$MAPE = \frac{100}{NDP} \sum_{i=1}^{NDP} \left| \frac{x_S^{calc}(t_i) - x_S^{exp}(t_i)}{x_{Si}^{exp}(t_i)} \right| \tag{12}$$

$$R^2 = 1 - \frac{\sum\left(x_S^{exp}(t_i) - x_S^{calc}(t_i)\right)^2}{\sum\left(x_S^{exp}(t_i) - \langle x_S^{exp} \rangle\right)^2} \tag{13}$$

being $\langle x_S^{exp} \rangle$ the average experimental conversion. The best fitting parameters are listed in the first column of Table 1.The high values of the equilibrium constants K_{IS}^*, K_{IP1}^* and K_{IP2}^* indicate that the uncompetitive inhibition of the enzyme is negligible and that the dominant mechanism is the competitive inhibition by the two products because the low value of K_{IP1} and K_{IP2}. Therefore, the model was refitted with only four kinetic parameters (v_m, K_M, K_{IP1}, K_{IP2})

$$A = \frac{[S_0]}{v_m}\left(1 - \frac{K_M \eta_1}{K_{IP1}} - \frac{K_M \eta_2}{K_{IP2}}\right) \qquad B = \frac{K_M}{v_m}\left(1 + \frac{[S_0]\eta_1}{K_{IP1}} + \frac{[S_0]\eta_2}{K_{IP2}}\right) \qquad C = 0.0 \tag{14}$$

and the values obtained are those reported in the second column of Table 1. The corresponding MAPE and R^2 are almost the same than those of the Eqs. 2 – 5. Moroever, since K_{IP2} was higher than $K_{IP1,}$ we have further simplified the model by assuming that only the octanamide causes inhibition

$$A = \frac{[S_0]}{v_m}\left(1 - \frac{K_M \eta_1}{K_{IP1}}\right) \qquad B = \frac{K_M}{v_m}\left(1 + \frac{[S_0]\eta_1}{K_{IP1}}\right) \qquad C = 0.0 \qquad (15)$$

As can be seen in the third column of Table 1, the quality of the fitting is as good as in the two previous cases. Additional experiments are needed in order to know with security if both products inhibit the reaction or only the major one does. Calculated conversions for the three-parameter model are also shown in Fig. 5a.

The amide yield was between 93.4 and 89.9%, depending on the initial ester concentration.

3.3. Effect of Temperature

As shown in Fig.6, temperature had an effect of the initial reaction rate of the mixture of 12.5 mL ethyl octanoate in 237.5 mL tert-butanol, but its influence on the final conversion or the selectivity was marginal The rate increased as temperature rose from 25 to 40 °C, but a further increase up to 55 °C did not show the same trend. Amide yields after 10 hours also increased from 91.65% at 25ºC to 95% at 40ºC, but decreased again to 92% at 55ºC. The experimentally determined value of 95% at 40ºC is identical to that reported by Zoete et al. [3, 8] for the same reaction time (24 hours), but in this work it is obtained with a much lower enzyme/substrate ratio (4.48 mg/mL), although the ester conversion is not complete (92.4%).

The poorer performance of the system at the highest temperature could be the result of the enzyme being partially deactivated.

3.4. Enzyme stability

Since the enzyme was immobilize only once in the SIR membrane and the whole set of experiments were then run, the first reaction studied was repeated after every three experiments, to check the enzyme stability and eventually, enzyme deactivation. During the whole set of experiments (on a period over 3 weeks) the enzyme was shown to remain active as it was at the beginning. Only after the last experiment at the highest temperature (55 °C), some deactivation occurred. This great stability of the immobilized enzyme, which allowed its reusability for a long period of time, is of capital importance in industrial applications due to the resulting savings in operating costs.

5. CONCLUSIONS

The immobilized enzyme remains active for a long period of time (over 3 weeks), being affected only by exposure to high temperature. During that period, high conversions were achieved, with a high yield of the desired product, without the need of purifying the reagents or the solvent. The enzyme/substrate ratio is nearly 4.5 times lower than that reported previously in the literature, and taking into account the reuse of the enzyme, that ratio could be further reduced. The ammonia gas dosage through the membrane directly reached the catalyst and therefore was more effective than that in the multiphasic system with free enzyme, which could lead to a decrease of the ammonia gas consumption if an automated control of the transmembrane pressure would be set.

The reaction was influenced by the purity of the reagents (traces of water limit the selectivity of the ammoniolysis) and membrane material. The immobilized enzymes were stable up to 40ºC. At this temperature, initial rates of reaction were the highest and the final conversion and selectivity were the same as those obtained in the free enzyme system.

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APPENDIX A

The mass balance to the substrate gave

$$[S_0] = [S] + [ES] + [EP_1] + [EP_2] + [ESS] + [ESP_1] + [ESP_2] + [P_1] + [P_2] \quad \text{(A1)}$$

By assuming that the concentrations of the intermediates are low when compared with those of the products, the balance can be written as

$$[S_0] \approx [S] + [P_1] + [P_2] \quad \text{(A2)}$$

Introducing the conversion

$$x_S[S_0] \approx [P_1] + [P_2] \quad \text{(A3)}$$

and taking into account that the instantaneous selectivity can be defined as the ratio between the kinetic constants

$$x_S[S_0] \approx \left(1 + \frac{k_{p2}}{k_{p1}}\right)[P_1] = \left(1 + \frac{k_{p1}}{k_{p2}}\right)[P_2] \quad \text{(A4)}$$

$$x_S[S_0] \approx \left(1 + \frac{k_{p2}}{k_{p1}}\right)[P_1] = \frac{[P_1]}{\eta_1}, \qquad x_S[S_0] \approx \left(1 + \frac{k_{p1}}{k_{p2}}\right)[P_2] = \frac{[P_2]}{\eta_2} \quad \text{(A5)}$$

Finally, substitution into Eq. 1:

$$-\frac{d(1-x_S)}{dt} = \frac{v_m(1-x_S)}{K_M\left(1 + \frac{x_S[S_0]\eta_1}{K_{IP1}} + \frac{x_S[S_0]\eta_2}{K_{IP2}}\right) + \left(1 + \frac{x_S[S_0]\eta_1}{K_{IP1}^h} + \frac{x_S[S_0]\eta_2}{K_{IP2}^h} + \frac{[S_0](1-x_S)}{K_{IS}^h}\right)[S_0](1-x_S)} \quad \text{(A6)}$$

A slight manipulation of Eq. A6 gave Eqs. 2 – 5.

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TABLES

Table 1.

Parameters in the kinetic models

FIGURE CAPTIONS

Figure 1. CALB – catalyzed reaction of ethyl octanoate (I) with ammonia to produce octanamide (II) and with water to generate octanoic acid (III).

Figure 2. Scheme of the enzymatic membrane reactor

Figure 3. Schematic illustration of the kinetic model with competitive and uncompetitive reversible inhibition.

Figure 4. Influence of the membrane on the ammoniolysis of ethyl octanoate catalyzed by immobilized CALB at 25ºC and 1 mol/L of substrate. (a) Ester conversion; (b) yield of the amide.

Figure 5. Influence of initial substrate concentration on the ammoniolysis of ethyl octanoate catalyzed by CALB immobilized on a SIR membrane at 25ºC. (a) Substrate conversion; (b) yield of the amide. Solid lines are the values calculated with Eq.8.

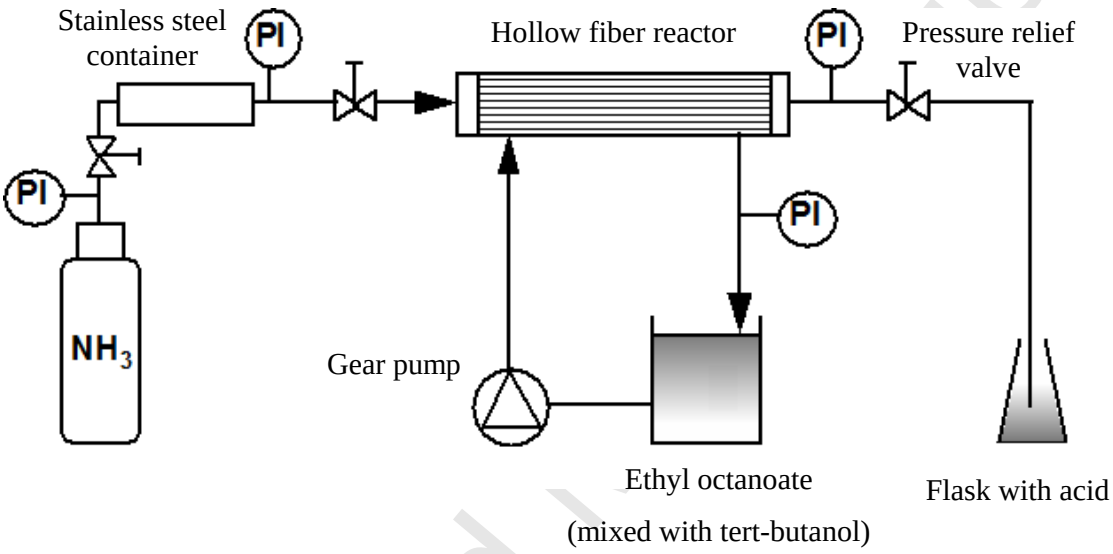
Figure 6. Influence of temperature on the conversion of 0.25 mol/L of ethyl octanoate catalyzed by CALB immobilized on a SIR membrane.

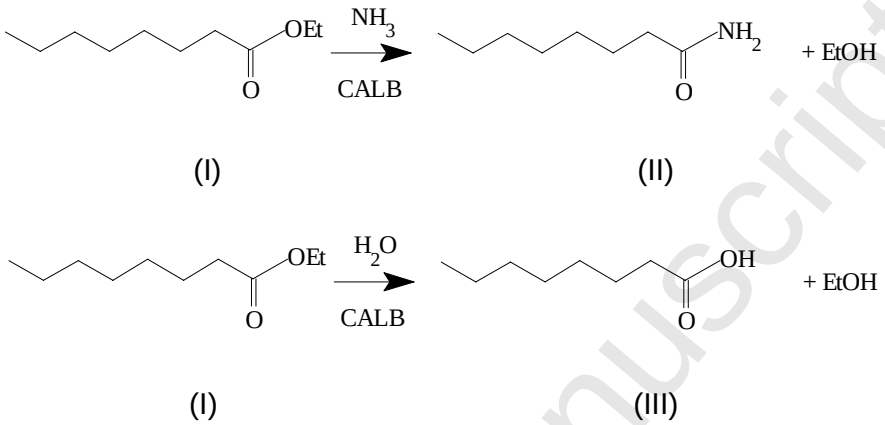
Table 1.

Parameters in the kinetic models

	Eqs. 2 – 5	Eqs. 2 and 14	Eqs. 2 and 15
v_m (mol/L/h)	0.09139	0.09152	0.09149
K_M (mol/L)	0.08936	0.08969	0.08937
K_{IS}^* (mol/L)	393618		
K_{IP1} (mol/L)	0.01850	0.01912	0.01795
K_{IP2} (mol/L)	0.05828	0.02849	
K_{IP1}^* (mol/L)	1821.26		
K_{IP2}^* (mol/L)	1184.42		
MAPE (%)	9.363	9.361	9.363
R^2	0.988	0.988	0.988

GRAPHICAL ABSTRACT





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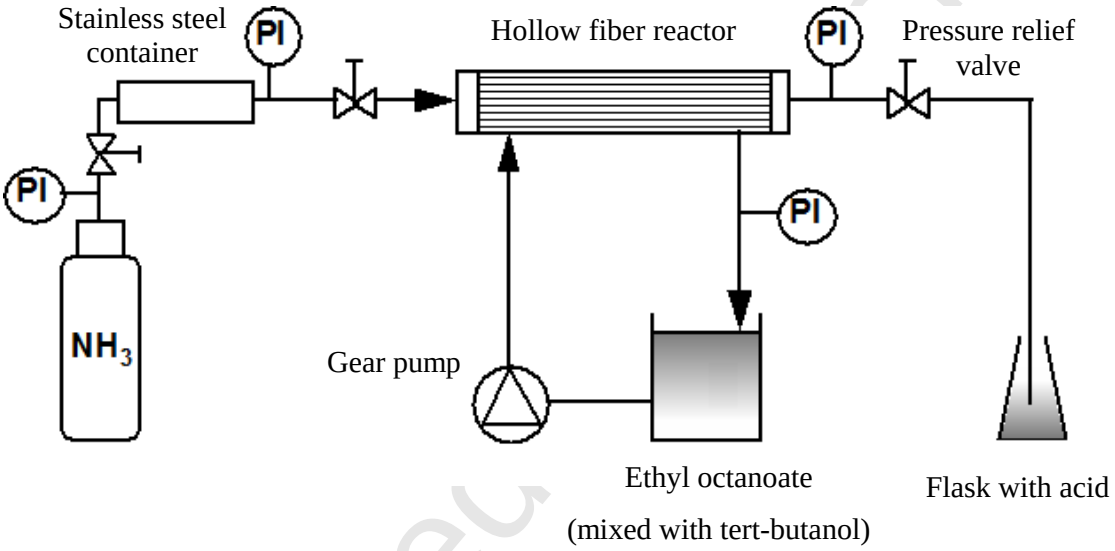


Figure 2. Scheme of the enzymatic membrane reactor

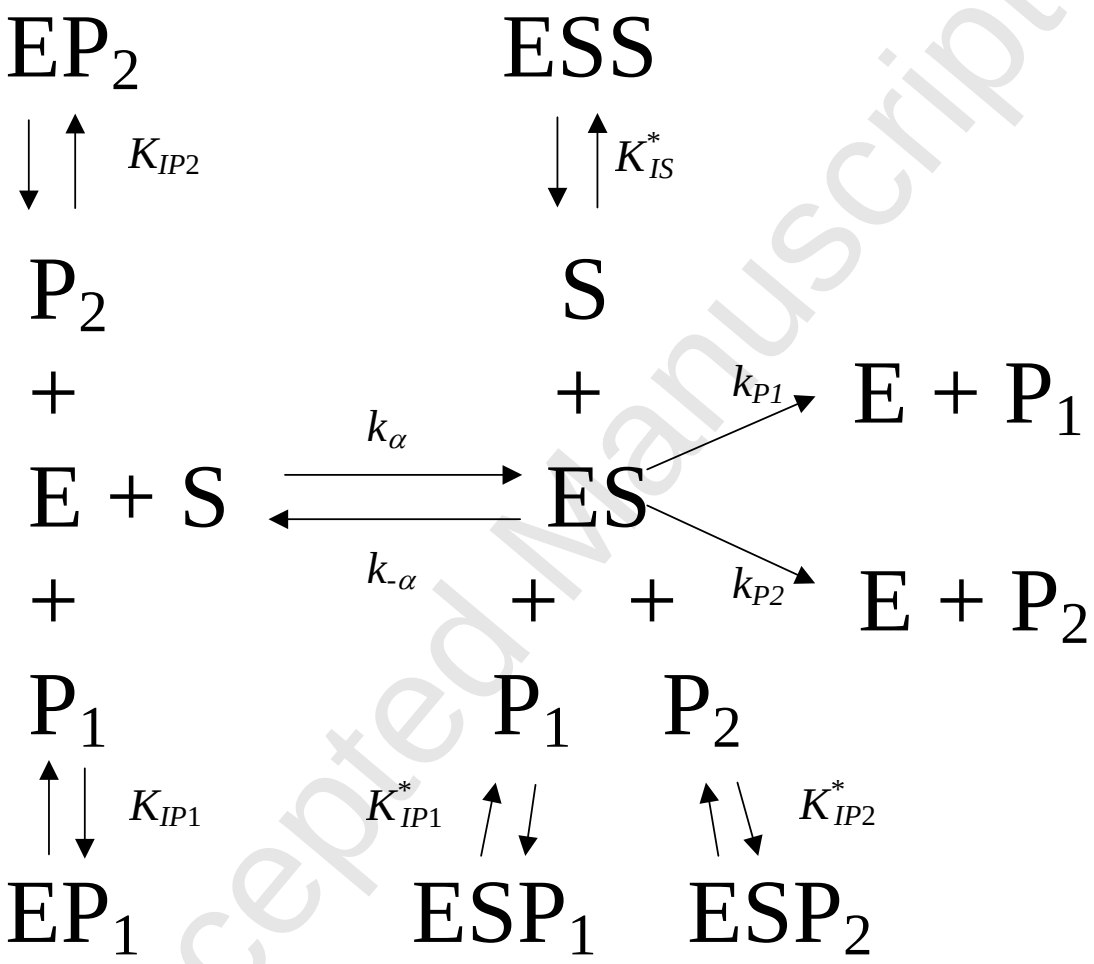
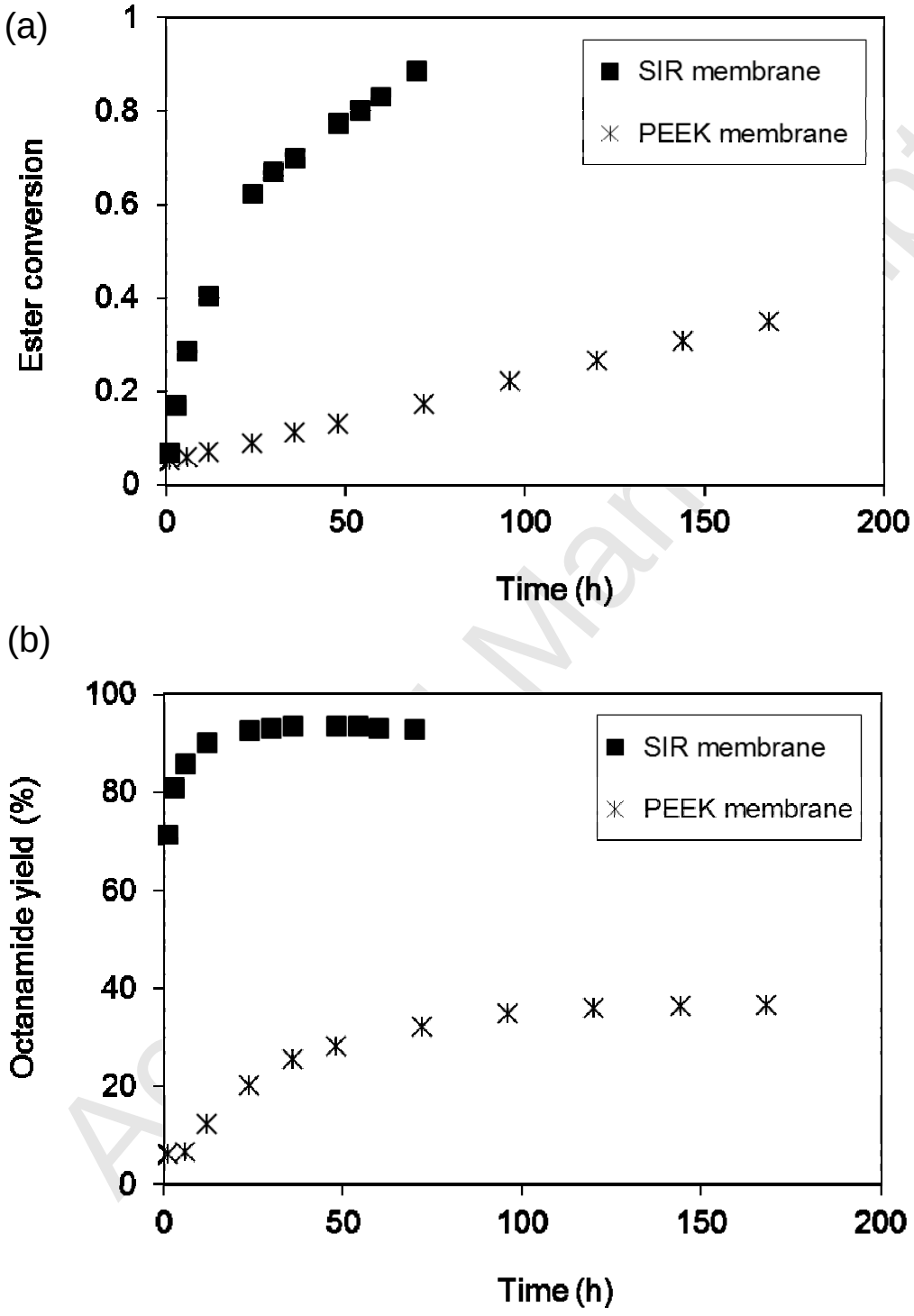


Figure 3. Schematic illustration of the kinetic model with competitive and uncompetitive reversible inhibition.



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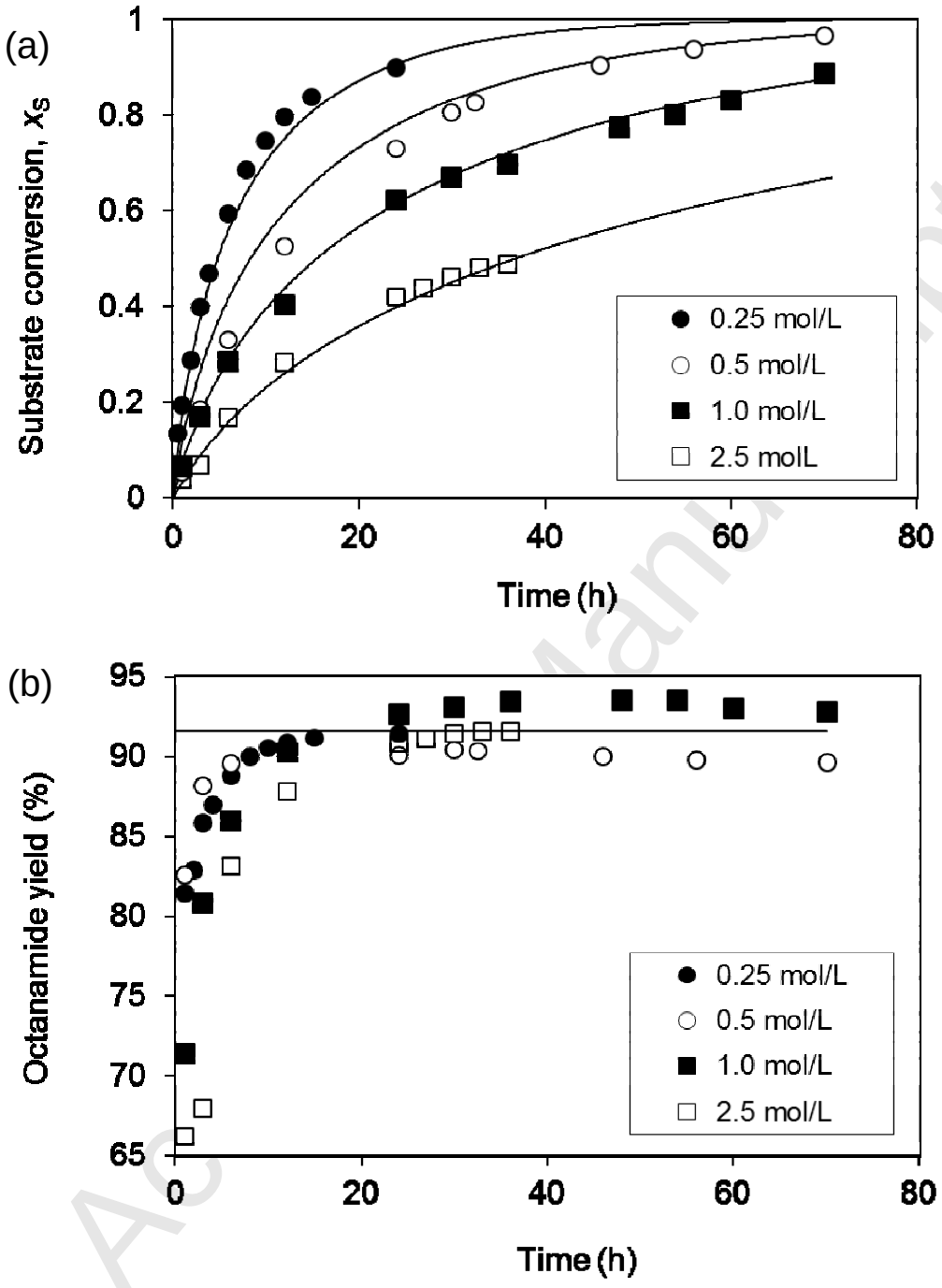

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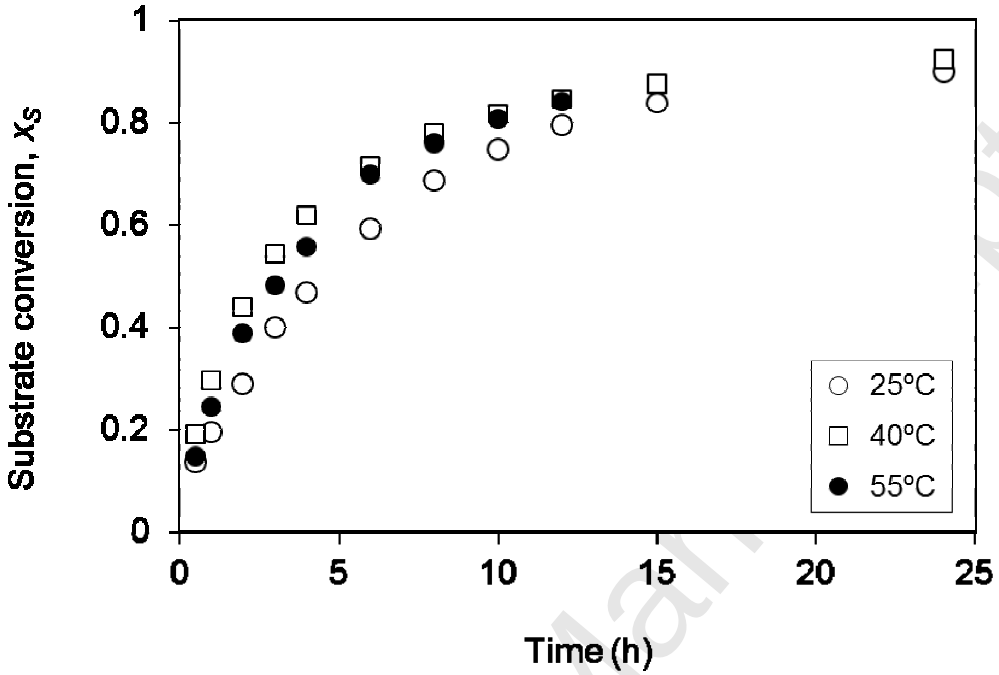

Figure 6. Influence of temperature on the conversion of 0.25 mol/L of ethyl octanoate catalyzed by CALB immobilized on a SIR membrane.