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Application of lipase encapsulated in silica aerogels to a transesterification reaction in hydrophobic and hydrophilic solvents: Bi-Bi Ping-Pong kinetics

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Abstract

Biocatalysts were prepared by encapsulation of the *Burkholderia cepacia* lipase in hydrophilic or hydrophobic silica aerogels dried under supercritical CO_2. The hydrophobicity of the aerogels was modified by changing the ratio of two silicon precursors, the tetramethoxysilane and the methyltrimethoxysilane. The catalytic activities of the industrial lipase powder and entrapped lipase were compared in the transesterification reaction of 1-octanol with vinyl laurate in various hydrophilic and hydrophobic solvents containing a low water ratio. This made it possible to study the influence of the entrapment, of the composition of the catalytic support and of the solvent mixture on the catalytic activity of the enzyme.

This study showed that the aerogel network and the nature of liquid solvent had two significant and independent effects. That of the solvent is known but still not understood. As for the aerogel, it primarily offers a medium of immobilization allowing to dry the enzyme by supercritical CO_2, therefore without compressing it. Also, it maintains the enzyme dispersed at the quasi-molecular level as if it were in solution even when using it in a liquid organic solvent where it would not be soluble. Moreover, the aerogel does not modify the nature of the kinetic mechanism proposed which is of the Bi-Bi Ping-Pong type with inhibition by the 1-octanol. Nevertheless, it increases considerably the maximum transesterification rate since it multiplies it of a factor approximately 80 compared to the industrial lipase powder, because of a better enzyme dispersion.

Keywords: Biocatalysis; Aerogel; Lipase; Encapsulation; Bi-Bi Ping-Pong

1. Introduction

The synthesis of biocatalysts by entrapment of enzymes in inorganic polymer matrix has received substantial attention in recent years and begins to present some interest in fine chemistry. This method pioneered by Avnir et al. [1] is based on the sol–gel process [2], a chemical synthesis technique principally used for preparing gels, glasses and ceramic powders [3–5].

The solid network is formed via the hydrolysis and condensation of molecular precursors in solution. Two types of routes are usually selected, depending on the chemical nature of the precursors [4]. The aqueous route is based on inorganic salts dissolved in water, whereas alkoxides ($M(OR)_n$ where M is Al, Si, Ti, ... and R is an organic group) dissolved in organic solvents are used in the metal-organic route [5]. The formation of silica gels, for example, is achieved by hydrolysis of $Si(OR)_4$ followed by condensation to yield a polymeric oxo-bridged SiO_2 network. Hydrolysis converts the Si–OR bonds to Si–OH bonds which condense together to form the oxo-bridged Si–O–Si structure. These reactions occur in a localized region leading to the formation of sol particles. As polycondensation proceeds, the degree of cross-linking between particles increases and sol viscosity increases. This viscous material solidifies and leads to the formation of a porous gel [6].

While silica xerogels are dried by evaporation, aerogels are dried by the supercritical method. Bringing the liquid

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in the gel beyond its supercritical point before drying, has the effect to largely attenuate (in theory suppress) the capillary stresses which compress the gel network. This usually has a minor effect on the gel specific surface area which is not much different in aerogels and in xerogels. On the other hand, this has a drastic effect on the specific pore volume which is much higher in aerogels, $\geq 90\%$ of a monolith gel volume [7] than in xerogels (typically 60–70 vol.%). Silica aerogels are extremely porous materials with larger pore sizes in the mesoporous range than in xerogels, high specific surface areas (500–1000 $m^2\,g^{-1}$), low bulk densities (0.003–0.35 $g\,cm^{-3}$), low thermal conductivities (0.014 $W\,m^{-1}\,K^{-1}$), and refractive indexes between 1.008 and 1.4 [8].

Because of their low temperature process, high porosity, large surface area and low density, aerogels and xerogels have been applied in heterogeneous catalysis and specially biocatalysis. Recently, they have been successfully developed as encapsulation media of lipases and applied to fatty component esterification, transesterification, or hydrolysis [9–17]. These biocatalysts are known, in the best cases, to operate more efficiently than the corresponding free enzymes [13]. However, their operating mechanism remains unknown.

Lipases (E.C. 3.1.1.3) have been used as versatile and very efficient biocatalysts for a wide variety of chemical reactions. Because of their high activity and selectivity, they have a great potential for the use as biocatalysts in industrial applications [18]. These enzymes are structurally characterized by a so-called lid. When hydrophobic substrates interact with the lipase, the lid opens and thus exposes the active site in a process called interfacial activation [19].

It is also known that the nature of the liquid solvent in which a lipase is dispersed, in particular its hydrophilic or hydrophobic character, is very important to control the catalytic activity of the lipase [20,21]. Another solvent parameter of particular importance is water and a number of studies have addressed the effect of water thermodynamic activity a_w on reactions with lipases [22–26].

In this work, four types of silica aerogels having a specific surface area ranging from 388 to 837 $m^2\,g^{-1}$, and a pore size between 2 and 13 nm [27] were synthesized, with the enzyme encapsulated inside the gel before supercritical drying, as explained in the experimental section. One type of aerogel was made from only tetramethoxysilane (TMOS) as the silicon precursor, and three other types from TMOS plus an increasing proportion of methyltrimethoxysilane (MTMS).

A transesterification reaction was chosen for the aim of easily comparing the activity of the enzyme inside the aerogel and without the gel. One possibility to characterize the action of the aerogel, was to compare its effect on transesterification kinetics to that of solvents with different hydrophobicity. Also, it was possible to combine such solvents with the aerogel, to examine how the influence of the solvent was displaced by the aerogels. The enzyme tested was the lipase from *Burkholderia cepacia*. The kinetics with the industrial enzyme powder was first studied. Then the lipase was encapsulated in a series of silica aerogels with different hydrophilic characteristics and the same catalysis experiments were made. The catalytic behaviour of the aerogels was analyzed as a function of their content in hydrophobic methyl groups, and compared with those of mixtures of hydrophobic and hydrophilic organic solvents. The final part of this work was the determination of a kinetic mechanism for the transesterification reaction of the industrial enzyme powder and for the encapsulated lipase.

2. Experimental

2.1. Materials and methods

The materials used in this study were tetramethoxysilane (TMOS, Aldrich, 98%), methyltrimethoxysilane (MTMS, Aldrich, 98%), aqueous ammonia solution (R.P. Normapurs-Prolabo, 0.1 M), methanol (R.P. Normapur-Prolabo, for analysis 99.8%, M.W. 32.04, d 0.791), polyvinyl alcohol (PVA, Fluka, M.W. 15000), metal salts (Fluka), hydrochloric acid (Prolabo, 37%), tris(hydroxymethyl)aminomethane (Fluka, 99.8%) noted Tris, ammonium sulfate (Fluka, 99.8%), albumin solution and bicinchoninic reagents A and B (commercial test BCA-200 Protein Assay Kit from Pierce), technical grade acetone, deionized and ultra pure water prepared by an ELGA PURELAB UHQ water purification system. The enzyme provided by Amano was the lipase of *B. cepacia* (40 $U\,mg^{-1}$) noted BCL, previously known as lipase of *Pseudomonas cepacia*.

2.2. Purification of the industrial lipase powder

The preparation of the aqueous lipase solutions was carried as follows. The industrial enzyme powder of the BCL is only partially water-soluble. That is why it was purified before its use. For this purpose, 1.6 g of the industrial BCL powder were dispersed in 20 ml of pH 7.5 buffer solution prepared by mixture of 50 ml of 12.114 $g\,l^{-1}$ Tris solution, 40.3 ml of 0.1 M HCl solution and 9.7 ml of ultra pure water. In order to eliminate the insoluble particles, a centrifugation at 3000 rpm was carried out for 10 min producing a supernatant liquid floating above a pellet. The supernatant solution was noted further on “protein solution” and was purified by adding 6 g of ammonium sulfate $(NH_4)_2SO_4$ which precipitated the proteins according to a technique described by Secundo et al. [28]. This operation was carried out under mixing at 4 °C during 2 h in order to induce precipitation of the existent proteins. Further on, the supernatant was separated from the precipitate by centrifugation at 3000 rpm during 20 min. The pellet obtained was dissolved in 10 ml of the buffer solution in order to obtain a solution which was dialyzed against ultra pure water for 48 h at 4 °C through a dialysis cell (MWCutOff 10000). In the text, this dialyzed solution is termed enzyme solution.

Table 1
Molar proportions of chemical products used in the synthesis of the aerogels

	Methanol (mmol)	TMOS (mmol)	MTMS (mmol)	NH_4OH (mmol)	Water (mmol)	PVA (mmol)
GBCO	5.43	2.78	–	–	10.67	4×10^{-4}
GBC20	5.43	2.22	0.57	–	10.67	4×10^{-4}
GBC40	5.43	1.67	1.15	10^{-3}	10.11	4×10^{-4}
GBC60	5.43	1.11	1.72	2×10^{-3}	9.56	4×10^{-4}

GBC0, GBC20, GBC40, GBC60 (*B. cepacia* lipase entrapped in aerogels with 0, 20, 40 and 60% MTMS, respectively).

The protein concentration of this solution was determined by UV spectrometry using the commercial test "BCA-200 Protein Assay Kit". It was determined that the BCL industrial powder contains ≈10% of protein and ≈1% of enzyme.

2.3. Silica aerogels synthesis

The silica aerogels containing water or the aqueous enzyme solution were prepared with different proportions of the reactants according to the procedure described in a previous paper [29]. The different quantities of the used reactants were as follows: $n_{CH_3OH} = 5.43$ mmol, $n_{TMOS} =$ 2.78, 2.22, 1.67 and 1.11 mmol, $n_{MTMS} = 0$, 0.57, 1.15 and 1.72 mmol, $n_{NH_4OH} = 0$, 1 and 2 μmol, $n_{PVA} = 0.4$ μmol, $n_{H_2O} = 10.67$, 10.11 and 9.56 mmol. Table 1 gathers all the molar ratios of the used chemicals.

The synthesis of the aerogels containing the industrial enzyme powder is very close to that of the aerogel with the aqueous enzyme solution. The enzyme powder is added to the emulsion instead of the aqueous enzyme solution. Nevertheless in this case, dispersion of the powder in water is only partial. For this reason a strong agitation was found essential throughout the synthesis in order to avoid precipitation of the insoluble part of the powder and ensuring a homogeneous dispersion of the particles in the medium. No enzyme is lost during the gelation step because a silica sol completely transforms to a single gel monolith without expelling any liquid. On the other hand, some enzyme can be lost during acetone dialysis, then during CO_2 supercritical drying, in particular during the first step when the acetone is replaced inside the gel by liquid CO_2. Analysis of the acetone after dialysis with the BCA assay kit showed that no detectable amount of enzyme was lost during this step. To estimate the loss during the supercritical step, an experiment was designed to gather the liquid expelled from the autoclave in an Erlenmeyer flask [30]. As the liquid CO_2 gradually transformed to a gas to only leave the acetone with the expelled enzymes, analysis with the BCA assay kit then showed that ≈20% of the lipase initially mixed in the silica sol was lost. However, because this is only an estimate, all kinetics data are presented in this paper per mg of the initially added pure enzyme, itself proportional to the initially added enzyme commercial powder. That is to say, it can be estimated that the true activities are all under evaluated by a value of the order of 20%.

2.4. Transesterification reaction

The biocatalysts prepared by encapsulation in aerogels of the BCL lipase, were tested in catalytic transesterification of 1-octanol with vinyl laurate producing octyl laurate and acetaldehyde (Fig. 1).

The *B. cepacia* lipase was used in several former studies [12,16,31] where it had been tested as an industrial powder or encapsulated in xerogels and aerogels in esterification of lauric acid with 1-octanol. In the same time, a secondary reaction was observed between the vinyl laurate and the water added to the medium in a small amount and essential for the activation of the enzyme according to previous work [32]. This reaction is the hydrolysis of vinyl laurate, which produces lauric acid and acetaldehyde. The synthesized lau-

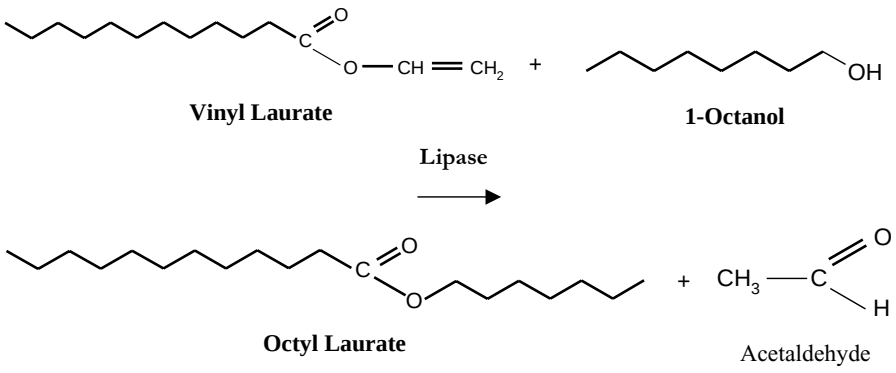

Fig. 1. Transesterification reaction of 1-octanol with vinyl laurate catalyzed by the BCL lipase.

ric acid has been esterified with the 1-octanol present in the medium to produce octyl laurate.

In accordance with former work [29,33], this paper contributes to the study of the influence of the encapsulation medium and of the solvent on the catalytic activity of the lipase and a comparison of reaction kinetics, when reactions are catalyzed by the industrial enzyme powder or by the encapsulated enzyme in aerogels. Also, several mixtures of solvents followed by pre-equilibration at different water thermodynamic activities were compared. The operating conditions were selected by referring to former work, to avoid problems of diffusion. The substrates for the catalytic tests were vinyl laurate and 1-octanol. These substrates were dissolved in solvent (10 ml) to which either industrial enzyme powder or an aerogel (typical aerogel mass ≈200 mg) containing water-soluble BCL enzyme (0.07 mg) were added, inside a 30 ml glass bottle considered as a batch reactor. The solvents used were either 2-methyl-2-butanol containing water at different initial molar fractions, water-saturated isooctane, or a mixture of these two solvents. The glass bottle was placed in a shaking bath from Bioblock operating at 180 rpm and 30 °C. At various time intervals, aliquots of 50 μl were taken and analyzed after dilution in 700 μl of isooctane. Analysis was performed on a Shimadzu GC-14B gas chromatograph (GC) equipped with an SGE BP21 capillary column (polyethylene glycol, polar, 12 m long, 0.22 mm inside diameter) and a flame ionization detector (FID). A constant sample volume was injected into the GC. The injector and detector temperatures were at 200 and 220 °C, respectively. Nitrogen was used as the carrier gas. The temperature program used was: 100 °C for the initial 5 min, followed by heating to 190 °C at 10 °C min^{-1}, 1 min at 190 °C, and finally cooling to 100 °C.

The degree of advancement ξ of the reaction at any given time was determined from the relative magnitudes of the integrated signals from the FID corresponding to each product and substrate, using previously established calibration curves. Moreover, the selectivity to a given product, defined as the ratio between the quantity of this product to the total quantity of all products, was found to be independent of the advancement of the reaction, in the present study. Hence, ξ could be used to calculate the conversion rate of each substrate, as well as the formation rate of each product, with the same precision. In the present study, it was chosen to report the rate data as the initial conversion rate of each substrate, because the further aim was to determine the kinetics mechanism as a function of the molar concentration of these two substrates. The formation rate of each product could be readily obtained by multiplying these rates by the appropriate selectivity coefficient, reported separately.

3. Results and discussion

3.1. Catalytic activity of the lipase in different organic solvents

The catalytic activity of the lipase industrial powder was measured in various polar and non-polar organic solvents, including 2-methyl-2-butanol (2M2B), isooctane, dioxane, 2-pentanone, pyridine and dimethylsulfoxide (DMSO). 10 ml of each of these solvents were added to an equimolar mixture of the substrates (1 mmol) at 30 °C. Initial rates, defined as explained previously, are represented in Fig. 2. These results show that only 2M2B and isooctane allowed a high reaction rate between the substrates, whereas for 2-pentanone and dioxane, these initial rates represented only ≈1/10 of those measured for the first two solvents. In pyridine and DMSO, no reaction took place. With regard to the formed products, it was noticed that the octyl laurate was the only product obtained after 5 h (100% selectivity) except when 2M2B was the solvent. In this last case, a second product appeared with 6% selectivity. This product characterized by gas chromatography coupled to mass spectrometry (GC–MS), proved to be methyl laurate coming

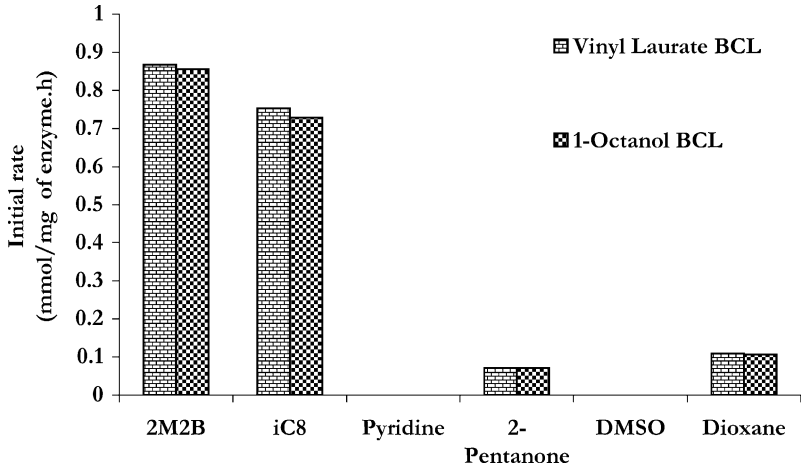

Fig. 2. Initial conversion rates of the transesterification reaction between of 1-octanol with vinyl laurate in different organic solvents, catalyzed by the BCL lipase.

from the transesterification reaction of methanol with vinyl laurate, present as an impurity in the 2M2B. After these observations, 2M2B and isooctane were chosen to be used in this work.

3.2. Blank tests

Five blank tests were carried out. In the first test, an equimolar solution of vinyl laurate and 1-octanol (1 mmol) was added to 10 ml of 2M2B-300w (10 ml of 2M2B with 300 μl of water) without any enzyme. No reaction was observed after 8 days. In the second test, a similar mixture of vinyl laurate and 1-octanol, without any enzyme, was added to 2 ml of 2M2B-300w and 8 ml of isooctane saturated with water. No reaction took place after 3 days. In the third test, a mixture similar to the first test, but without any 1-octanol, was examined. No reaction occurred after 8 days. In the fourth blank test, a similar solution to the first test, but containing in addition 1 ml of lauric acid, was tested. No reaction was observed even after 3 days. In the last test, a GBC20 aerogel without enzyme was added to a solution similar to that in the first test, in order to examine whether the aerogel was able to catalyze by itself this reaction. A conversion not exceeding 1% of the two substrates was observed after 24 h, which is negligible compared to the conversion due to the enzyme.

3.3. Influence of water in 2M2B on the catalytic activity of the enzyme

This section was published in a previous paper [29]. In summary, it was found that the reaction of vinyl laurate with 1-octanol in 10 ml of 2M2B with 300 μl of water was faster than in other solvent mixtures. It was also observed that the selectivity to any product was independent of the vinyl laurate conversion.

The conversion of vinyl laurate to octyl laurate was consistent with a competition between two main reactions: the transesterification of 1-octanol with vinyl laurate and the hydrolysis of vinyl laurate. Beside these main reactions, the transesterification of methanol with the methyl laurate present as an impurity in the 2M2B was observed.

3.4. Catalytic tests in isooctane either dry or saturated with water

Transesterification reactions of 1-octanol with vinyl laurate catalyzed by the enzyme industrial powder in dry isooctane or isooctane saturated with water, was tested during 24 h. The isooctane saturated with water is a mixture of isooctane and water agitated for 5 min and then left to rest. As isooctane and water are not miscible, they form at rest two superimposed phases where isooctane occupies the higher position since it is the least dense. The organic phase obtained is supposed to be thermodynamically saturated with water. The results obtained, reported in Fig. 3, where the conversion is presented as a function of time, show a quasi-identity of the results obtained with dry isooctane or isooctane saturated with water. According to these results, the use of isooctane dry or saturated with water does not present any significance influence on the rate of this reaction.

3.5. Esterification reaction of 1-octanol with lauric acid

This reaction catalyzed by the industrial BCL powder was studied in different solvents. The first series of solvents includes 2M2B–water mixtures containing 20, 100, 200 or 300 μl water dissolved in 10 ml of 2M2B. The results showed that the BCL catalyzes this reaction in the isooctane saturated with water [12]. Consequently, it appears that the octyl laurate produced in 2M2B comes from a direct transes-

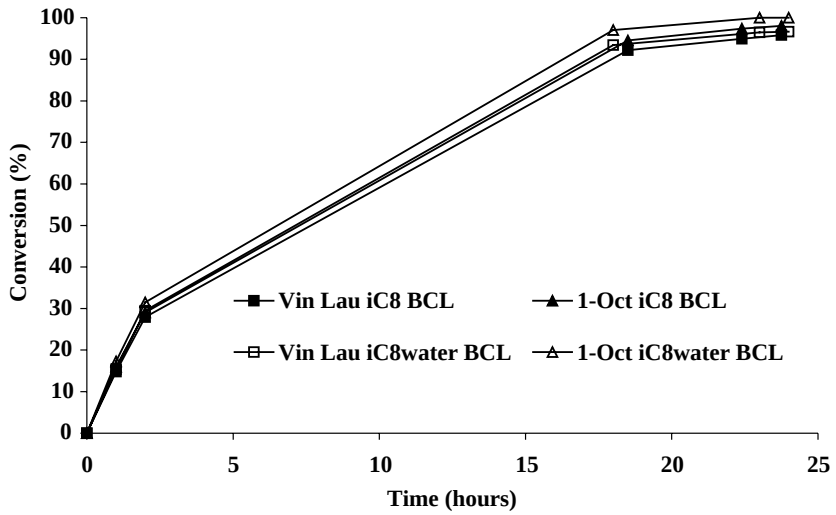

Fig. 3. Conversion of the two substrates vinyl laurate and 1-octanol in the reactions catalyzed by the BCL lipase in isooctane, dry or saturated with water.

terification of 1-octanol with vinyl laurate. As for the lauric acid, it is the product of the hydrolysis of vinyl laurate.

3.6. Industrial lipase powder in binary and ternary mixtures of solvents

After measuring the catalytic activities of the industrial lipase powder in transesterification reactions in 2M2B containing different water proportions, then in dry isooctane or saturated with water, these activities were determined in binary solvents including various proportions of isooctane and 2M2B, and in ternary solvents containing isooctane, 2M2B and water. These ternary solvents were prepared by mixing different volumes of two main solutions: the first containing 300 μl of water in 10 ml of 2M2B, noted 2M2B-300w, the second is isooctane saturated with water, noted iC8-water.

The initial rates measured in isooctane–2M2B–water, represented in the Fig. 4a, show that the presence of water in the medium accelerates the transformation of the vinyl laurate by promoting the hydrolysis of the vinyl laurate compared to transesterification. This difference is marked in solvents having percentages between 10 and 30% of 2M2B-300w, the mixture with 10% of 2M2B-300w shows a maximum rate of transformation of the vinyl laurate. The mixture with 5% 2M2B-300w presents the maximum rate of conversion of the 1-octanol which corresponds to the maximum formation rate of the octyl laurate in the medium. The esterification reaction which takes place between the lauric acid resulting from the hydrolysis and the 1-octanol present in the medium produces octyl laurate. Consequently, the selectivity to octyl laurate increases while that of lauric acid decreases. It can be noted that the selectivity to methyl laurate remained negligible (<1%), so the rate of formation of the latter ester is very low compared to those of other reactions taking place.

For the reactions in binary mixtures, isooctane–2M2B (Fig. 4b), it appears that the conversion showed a maximum for mixtures between 10 and 40% of 2M2B. For all binary mixtures isooctane–2M2B, the conversion rates of vinyl laurate and 1-octanol were the same.

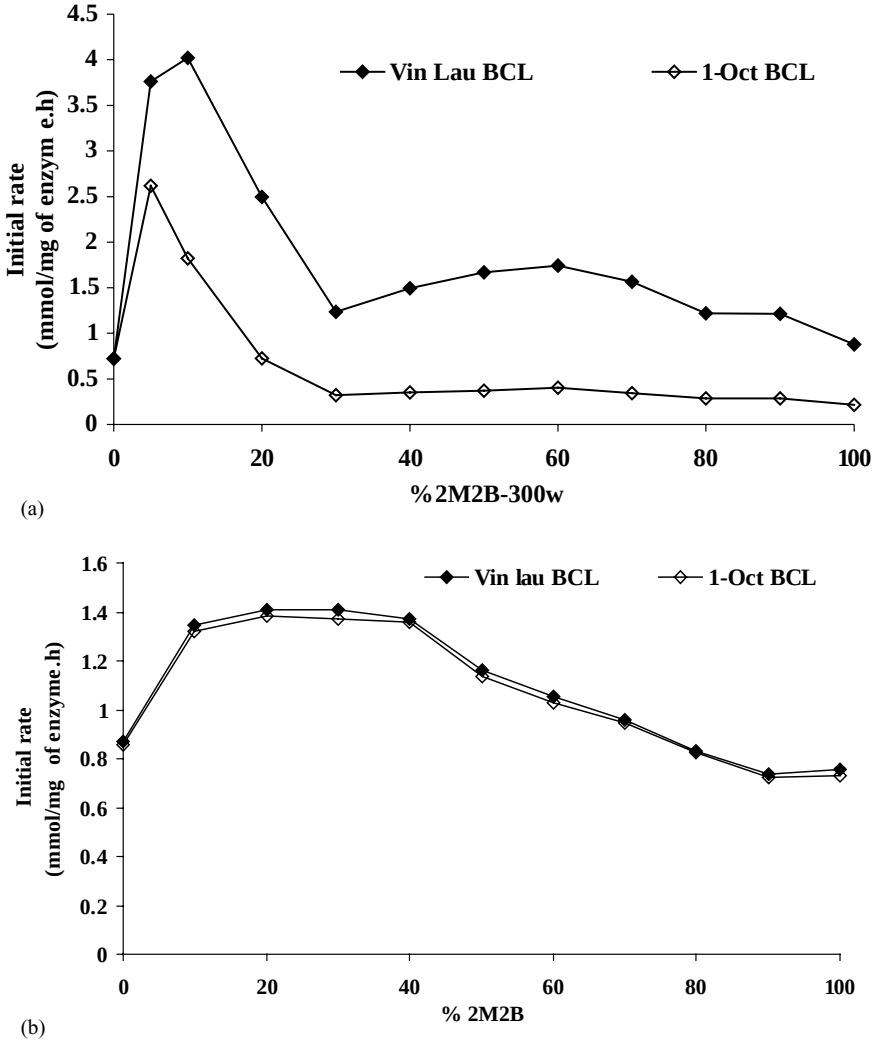

Fig. 4. Initial conversion rates of vinyl laurate and 1-octanol in the reactions catalyzed by the BCL lipase in ternary (a) and binary (b) solvents.

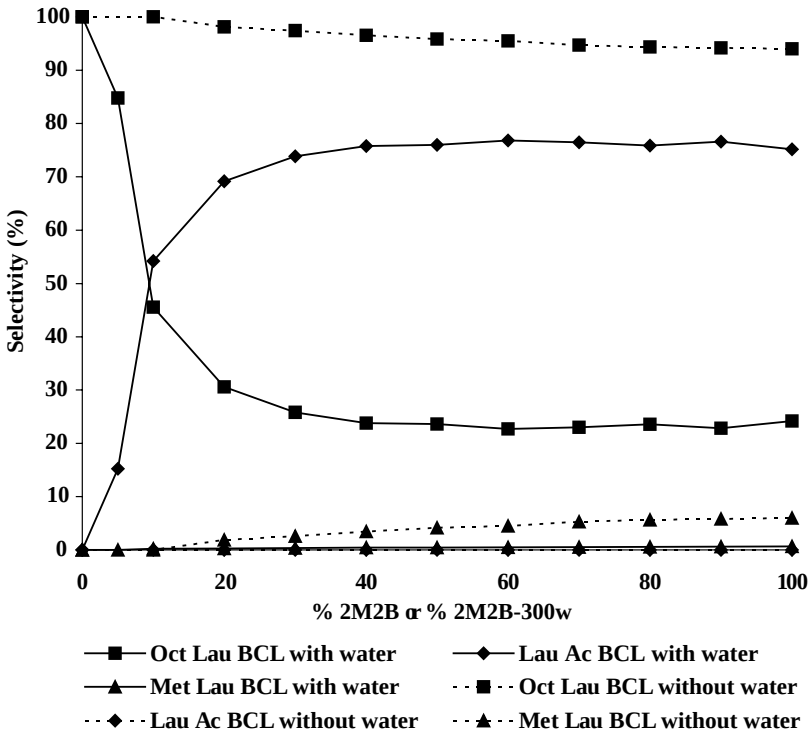

Fig. 5. Selectivities to the products formed by transesterification of 1-octanol with vinyl laurate, catalyzed by the industrial BCL lipase powder, in binary and ternary solvents. (Oct Lau: octyl laurate; Met Lau: methyl laurate; Lau Ac: lauric acid.)

By comparing the selectivities obtained in these binary solvents (Fig. 5), it appears that the selectivity to octyl laurate decreased when the percentage of 2M2B increased between 0 and 10%, whereas for higher percentages it remained stable at 100%. This decrease between 0 and 10% of 2M2B coincides with the lauric acid formation while no methyl laurate is formed. For the BCL, a formation of methyl laurate was observed, but it did not exceed ≈6%.

3.7. Catalytic activity of the encapsulated enzyme

The influence of the aerogel particle size on the reaction was studied. A mixture of 8 ml of isooctane and 2 ml of 2M2B was used to study the catalytic activity of the BCL encapsulated in the same type of silica aerogels having different particle size (complete monolith, pieces of a few mm or powder). The initial rates as well as selectivities did not show any significant difference between the three aspects of the biocatalyst and consequently the particle size of the catalytic support did not induce any diffusion limitation. Actually, broken monoliths were used in this study.

The BCL encapsulated in the four types of silica aerogels with increasing MTMS content was tested in binary and ternary solvents. The binary solvents consisted of isooctane and 2M2B whereas the ternary solvents were made up of various mixtures of 2M2B, isooctane and water. The 2M2B and water were introduced as a 2M2B-300w mixture as described previously. The results of the catalytic reactions carried out in binary solvents show that the encapsulation of the BCL improved its catalytic activity. The initial conversion rates of vinyl laurate and 1-octanol passed through a maximum between 10 and 30% of 2M2B, for all aerogels except GBC60 (Fig. 6). The most active biocatalyst was GBC40. Regarding the reactions in ternary solvents with the encapsulated BCL, the results (Fig. 7) show that the BCL encapsulated in silica aerogels presented a better catalytic activity than the industrial powder. Solvent mixtures containing between 10 and 40% of 2M2B-300w resulted in optimal catalytic activities. Moreover, it was noticed that the BCL encapsulated in GBC0 presents the weakest catalytic activity compared to the other biocatalysts. In general, GBC40 displayed the most important catalytic activity.

The selectivities are reported as a function of the proportion of 2M2B-300w in the ternary mixture of solvents (Fig. 8). It appears that the selectivity to octyl laurate decreased when the quantity of 2M2B-300w increased, while the selectivity to lauric acid increased. Moreover, this selectivity to octyl laurate slightly decreased when the proportion of MTMS in the aerogel increased (difference between 5 and 10% from GBC0 to GBC60). A slight increase of the selectivity to octyl laurate was observed for the encapsulated BCL compared to the industrial BCL powder.

The selectivity to methyl laurate produced during the reactions did not exceed 3.5%. Moreover, the latter selectivity also decreased when the proportion of 2M2B-300w increased, which might seem contrary with the forecasts

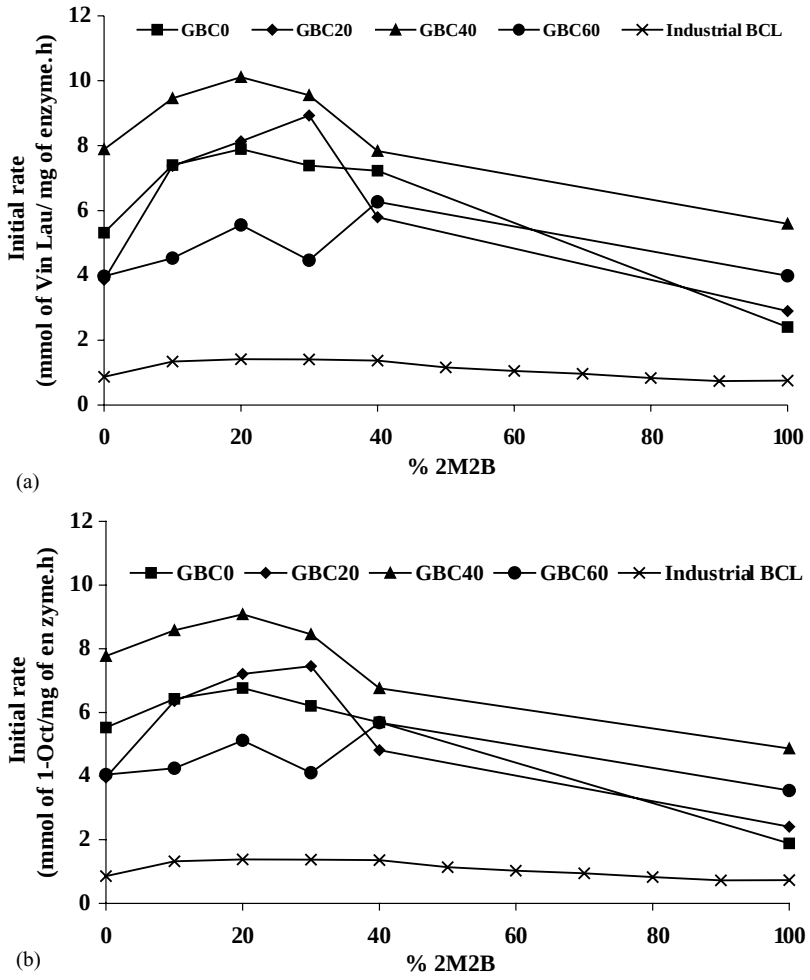

Fig. 6. Initial conversion rates of vinyl laurate (a) and 1-octanol (b) as a function of the molar proportion of 2M2B in 2M2B–isooctane binary solvents, for reactions catalyzed by encapsulated BCL.

since the methyl laurate was mentioned to come from an impurity present in the 2M2B, in biocatalysis with the free enzyme. Nevertheless, a second possible source of methyl comes from the silica aerogels, in biocatalysis with the encapsulated enzyme. The vinyl laurate can be transesterified with the methoxy groups which are present on the surface of the solid, essentially as adsorbed methanol. In fact, the quantity of methyl laurate which was produced decreased when the proportion of TMOS used to synthesize the aerogels decreased, which attenuated the formation of residual methoxy groups OCH_3.

3.8. Influence of the thermodynamic activity of water on the catalytic activity

In order to find the optimal water activity for the operation of the biocatalysts GBC40, biocatalysts and solvents were pre-equilibrated during 48 h with different metal salt solutions having different water thermodynamic activities a_w from 0.117 to 0.893. The solvent was a mixture of 20% 2M2B and 80% isooctane and the substrates were 1 mmol of vinyl laurate and 1 mmol of 1-octanol. These reactions were carried out at 30 °C in a batch reactor. This study showed that the best water activity $a_w \approx 0.506$, among the set of tested activities, with regard to the initial rate of conversion of vinyl laurate (Fig. 9). On the other hand, the selectivity to octyl laurate decreased when the water activity increased, whereas the selectivity to lauric acid increased and that of methyl laurate decreased. At $a_w = 0.117$, the selectivity to octyl laurate was ≈95% while at $a_w = 0.893$ it was ≈45%.

3.9. Enzyme kinetics

After studying the influence of the encapsulation medium and the solvent on the catalytic activity of the lipase, catalytic tests were carried out in order to determine the kinetic mechanism of this reaction. For this reason, a range of initial substrate concentrations was examined and the initial rates of conversion were determined. These kinetics were

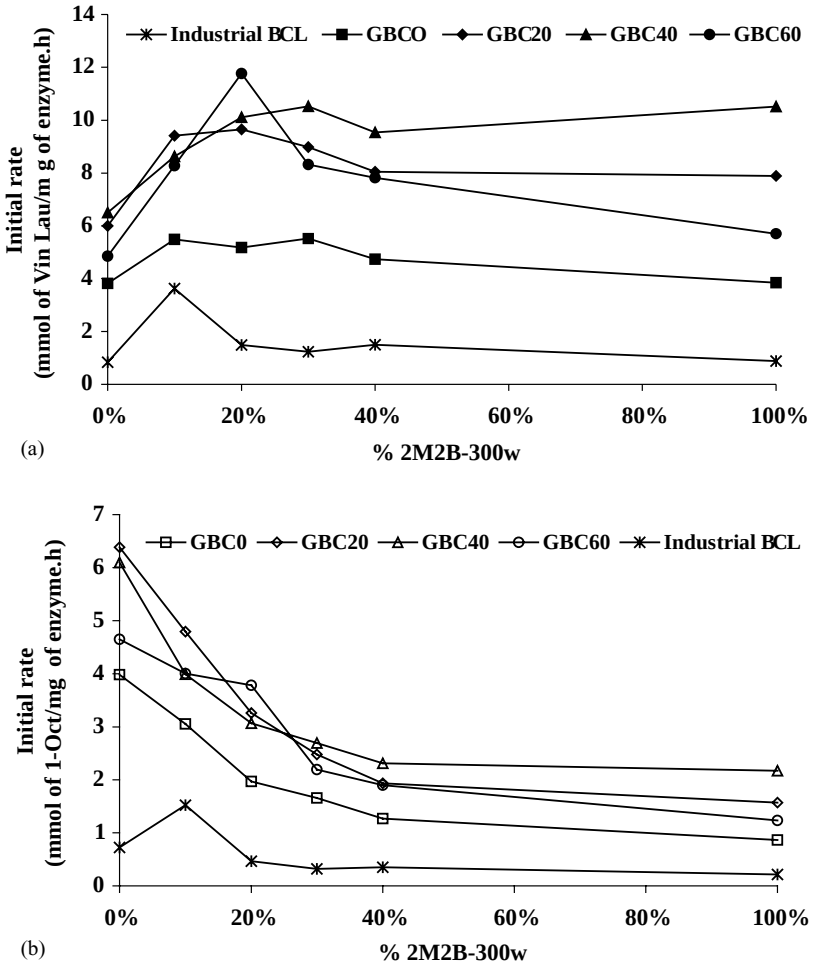

Fig. 7. Initial conversion rates of vinyl laurate (a) and 1-octanol (b) as a function of the composition of ternary 2M2B–isooctane–water solvent mixtures, in reactions catalyzed by encapsulated BCL.

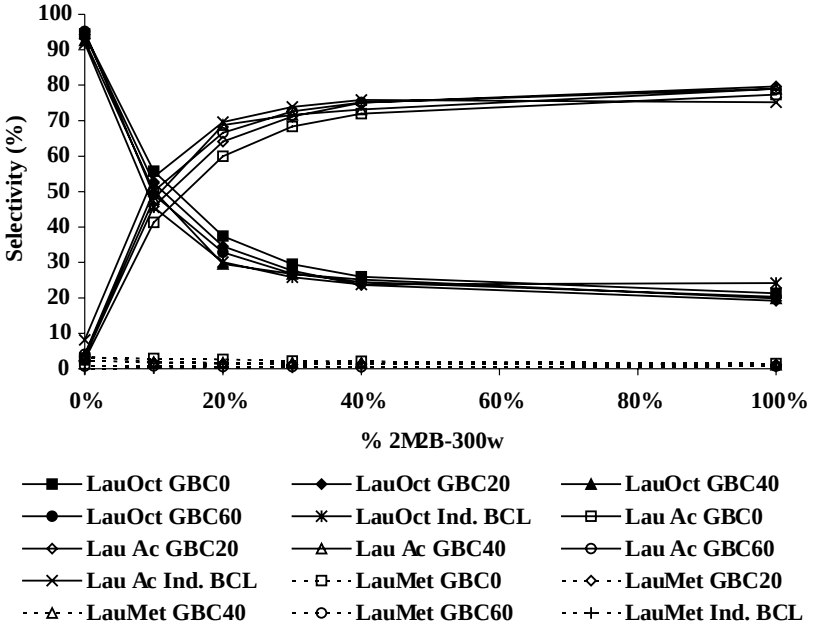

Fig. 8. Selectivities to the products formed as a function of the composition of ternary 2M2B–isooctane–water solvent mixtures, in reactions catalyzed by encapsulated BCL.

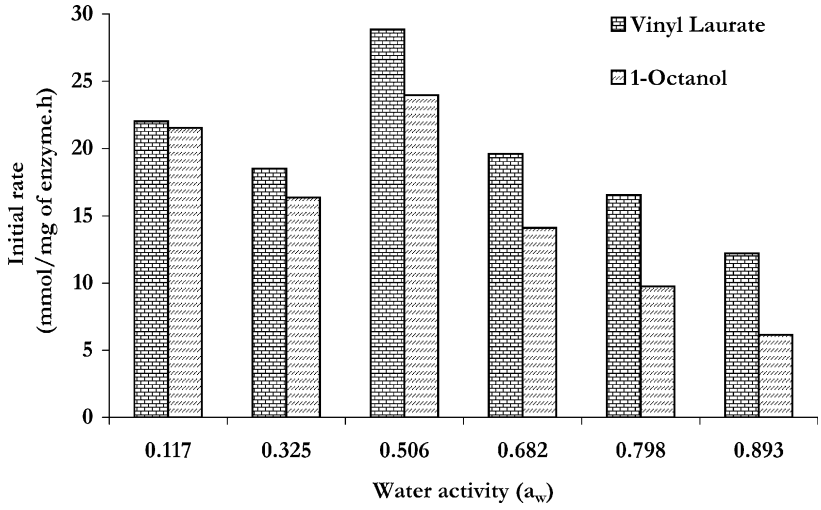

Fig. 9. Initial conversion rates of the two substrates, obtained with the BCL lipase encapsulated in aerogels GBC40 pre-equilibrated at different thermodynamic water activities a_w.

determined for the industrial BCL powder and for the BCL encapsulated in a GBC40 aerogel.

3.10. Kinetics mechanism of the industrial BCL powder

The kinetics of the industrial BCL powder was studied at 30 °C in a binary solvent containing 8 ml of isooctane and 2 ml of 2M2B in a batch reactor with vinyl laurate and 1-octanol as substrates.

Hence, for the study on the transesterification kinetics mechanism, approximately 20 mg of industrial BCL powder was used (therefore containing ≈0.2 mg of pure enzyme). This mass of enzyme powder was selected because it was within the range of enzyme content where the total conversion rate of the substrates was found to be proportional to the mass of enzyme. The mole number of the substrates laurate of vinyl and 1-octanol were 0.25, 0.5, 1, 1.5, 2 and 5 mmol., and all possible combinations between these concentrations for the two substrates were examined. In practice, the concentration of one of the two substrates was maintained constant while the full series of concentration for the second one was investigated. The results on the initial conversion rates of 1-octanol as a function of the mole number of vinyl laurate or 1-octanol are presented in Fig. 10. The shape of the graphs in this figure were found to qualitatively correspond to a Bi-Bi Ping-Pong model with inhibition by 1-octanol, according to the various kinetics models detailed by Segel [34]. This mechanism with competition between the substrates and inhibition by one of them is characterized by the existence of a maximum in the conversion rate (of vinyl laurate and 1-octanol) as a function of the mole number of 1-octanol (Fig. 10b). This maximum, observed for all concentrations of vinyl laurate, increases with the concentration of vinyl laurate. It occurs at 1-octanol concentrations of ≈1 and ≈2 mmol, for respectively the weakest and the highest concentrations of vinyl laurate.

The fitting of these experimental points with a theoretical model function were achieved with the data-processing software Sigmaplot 8.0 from SSPS. The equation of the function corresponding to the Bi-Bi Ping-Pong model with inhibition by one of the substrates [34] is the following:

$$V_i = \frac{V_{max} n_{Oct}}{K_{mOct} + n_{Oct}[1 + (K_{mLauVin}/n_{LauVin})}(1 + (n_{Oct}/K_i))]$$

The inverse initial conversion rate of 1-octanol is presented as a function of the inverse mole number of vinyl laurate or 1-octanol in Fig. 11. In this representation, the experimental points can be fitted with the following equations:

$$\frac{1}{V_i} = \frac{K_{mOct}}{V_{max}}\frac{1}{n_{Oct}} + \frac{K_{mLauVin} + n_{LauVin}}{V_{max} n_{LauVin}} + \frac{K_{mLauVin}}{n_{LauVin} V_{max} K_{i,Oct}}\frac{1}{(1/n_{Oct})}$$

$$\frac{1}{V_i} = \frac{1}{n_{LauVin}}\left(\frac{K_{mLauVin}}{V_{max}} + \frac{K_{mLauVin} n_{Oct}}{V_{max} K_{i,Oct}}\right) + \frac{1}{V_{max}}\left(\frac{K_{mOct}}{n_{Oct}} + 1\right)$$

These graphs show typical characteristics of the above equations, as described by Segel [34] when one substrate, in the present case 1-octanol, is an inhibitor. Indeed, Fig. 11a illustrates that, when the mole number of vinyl laurate increased from 0.25 to 5 mmol, all the plots of $1/V_i$ as a function of $1/n_{1\text{-octanol}}$ converged towards the same

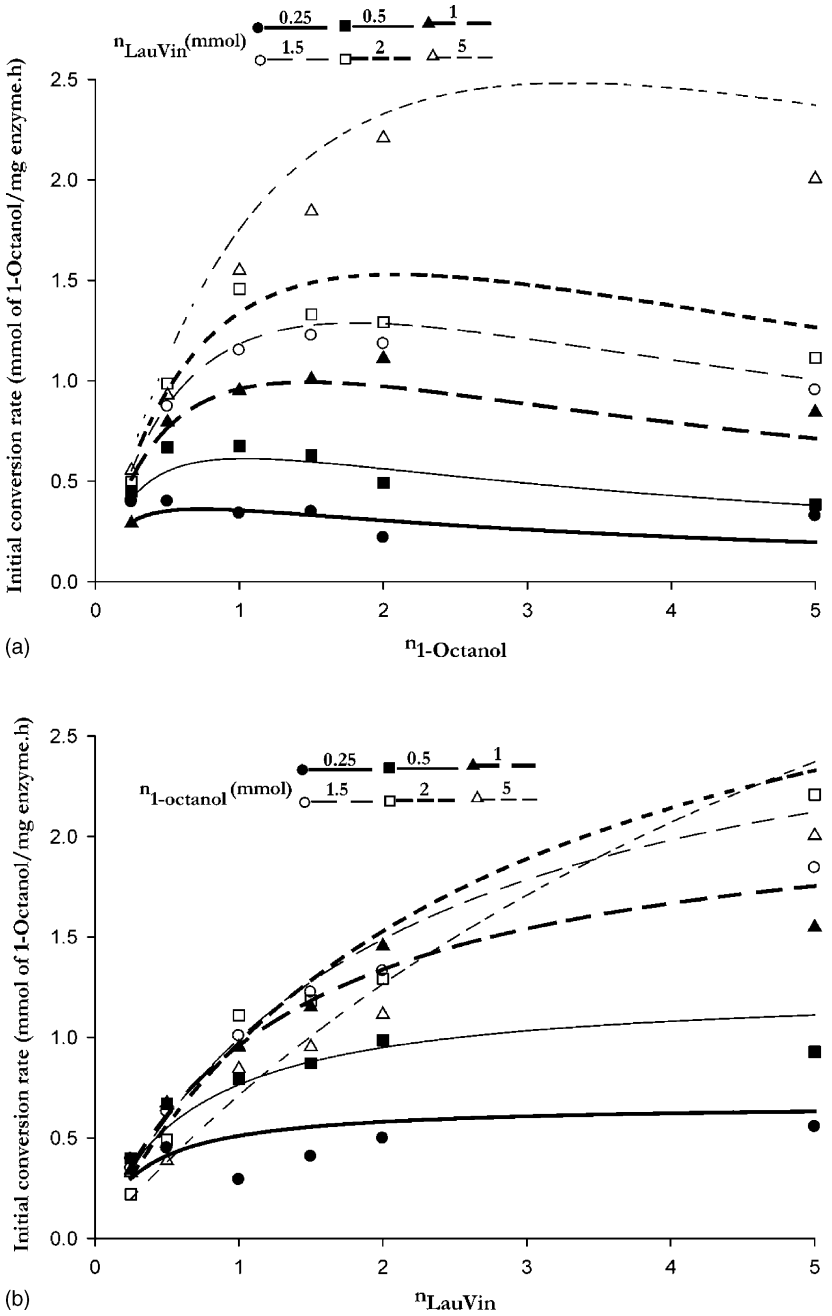

Fig. 10. Initial conversion rates of 1-octanol as a function of the mole number of vinyl laurate (a) and 1-octanol (b) for industrial BCL powder.

lower curve. On the other hand when $1/V_i$ was plotted as a function of $1/n_{LauVin}$ (Fig. 11b), the plots progressively transformed from quasi parallel straight lines at low $n_{1\text{-octanol}}$ to lines secant near the vertical axis at high $n_{1\text{-octanol}}$. Overall, it appeared that the theoretical continuous curves derived from this Bi-Bi Ping-Pong mechanism, were in reasonable agreement with the experimental data points. Both the experimental data points and the continuous theoretical curves are represented in Fig. 10. The kinetics constants determined by this curve fitting were the following:

V_{max} ≈9.4 mmol of 1-octanol mg^{-1} of enzyme h^{-1}	$K_{i,Oct}$ ≈2.7 mmol l^{-1}
$K_{mLauVin}$ ≈4 mmol l^{-1}	K_{mOct} ≈3.2 mmol l^{-1}

3.11. Kinetics mechanism of the GBC40

The kinetics of the same reaction catalyzed by the encapsulated BCL in GBC40 was followed in the same solvent mixture and at the same temperature.

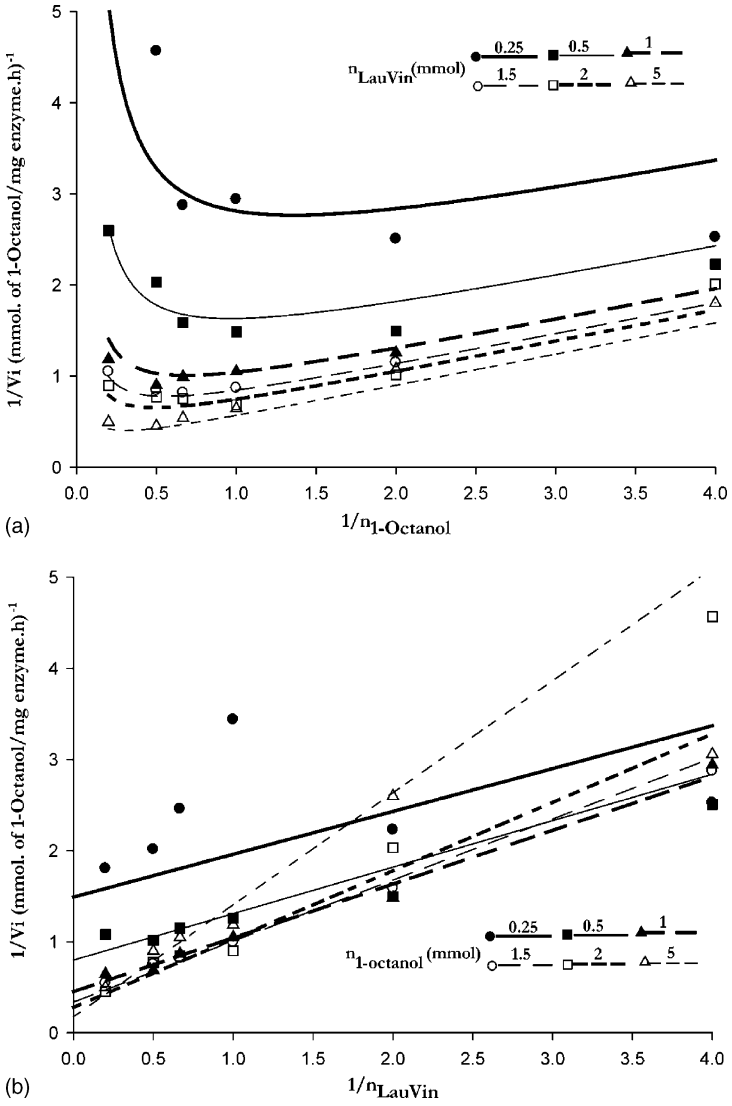

Fig. 11. $1/V_i$ as a function of $1/n_{1\text{-octanol}}$ (a) and $1/n_{\text{LauVin}}$ (b) for industrial BCL powder.

The aerogels contained approximately 0.078 mg of purified enzyme which corresponds to approximately 7.8 mg of industrial BCL powder. This mass of purified enzyme was selected because it was within the range of enzyme content where the total conversion rate of the substrates was found to be proportional to the mass of encapsulated purified enzyme. The series of concentrations of both substrates investigated were 0.25, 0.5, 1, 1.5, 2 and 5 mmol. The initial rates were calculated from aliquots taken after 10 min of reaction. The inverse initial rate, plotted as a function of the inverse mole numbers of the two substrates, is presented in Fig. 12.

The shape of the graphs drawn with the experimental points, again suggested that the same kinetics mechanism was operating, that is to say the Bi-Bi Ping-Pong mechanism with competition between the substrates and inhibition by 1-octanol. Curve fitting with the theoretical kinetics function provided the following values for the kinetics constant.

$V_{\max}$ ≈769 mmol of 1-octanol mg^{-1} of enzyme h^{-1}	$K_{i,\text{Oct}}$ ≈1.8 mmol l^{-1}
K_{mLauVin} ≈10.8 mmol l^{-1}	K_{mOct} ≈18 8 mmol l^{-1}

It appears that all the kinetics constants were increased by the aerogel, except for the inhibition constant by 1-octanol which decreased. In particular, the maximum rate $V_{\max}$ was multiplied by a factor ≈80 mg^{-1} of enzyme. In turn, this means that there was an effect due to the aerogel network, of a nature different from the effect due its surface groups. The latter effect can be seen in Fig. 7 for instance, where the graphs which represent the rates obtained with different MTMS content, have a shape similar to each other, and also similar to the graph obtained with the industrial enzyme powder.

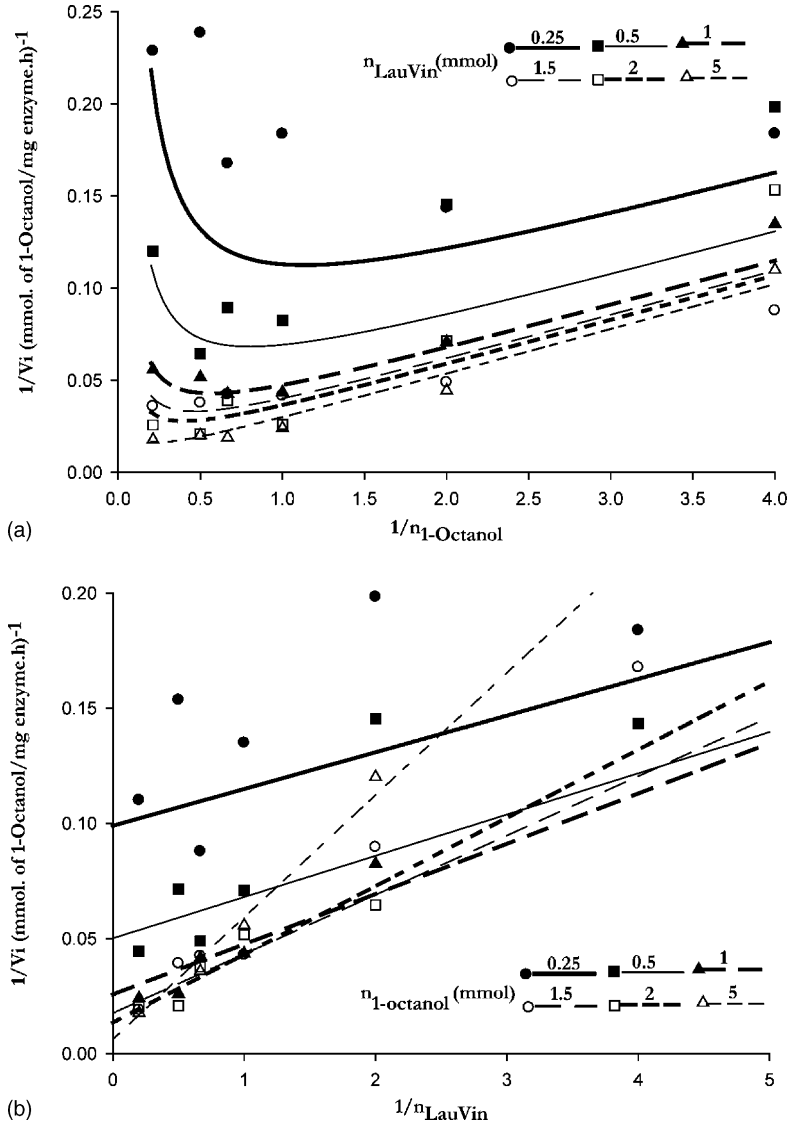

Fig. 12. $1/V_i$ as a function of $1/n_{1\text{-octanol}}$ (a) and $1/n_{\text{LauVin}}$ (b) for the encapsulated BCL in aerogels GBC40.

4. Conclusion

This study showed that the kinetic mechanism Bi-Bi Ping-Pong with inhibition by 1-octanol remained unchanged for the reactions catalyzed by the industrial powder of *B. cepacia* lipase or encapsulated in aerogels where the molar fraction of precursor MTMS = 40%. On the other hand, the values of the kinetic constants were modified, in particular V_{max} was multiplied by approximately 80 when the lipase was encapsulated in the aerogel.

The difference between the aerogels made with molar fraction in MTMS of 0, 20 or 40% remained moderate, whereas the difference was important by comparison with the industrial enzyme powder. This result proved to be true for a whole series of catalytic tests in mixtures of hydrophobic and hydrophilic solvents. Hence, it can be proposed that the aerogel network exerts an effect on the enzyme activity, distinct from the effect of the surface functionalities. This effect could be to maintain the enzyme dispersed at the quasi-molecular level, due to the addition of enzyme as a solution in water. This dispersed state is maintained by the aerogel network even during further use in an organic solvent, whereas under such conditions the enzyme agglomerates and loses partially its activity. This effect probably adds up to the reduction in the compression of the enzyme during drying, due to the use of a supercritical drying technique. This study also confirmed that the effect of the solvent hydrophilic/hydrophobic nature was very important. Nevertheless, this is an effect independent from that of the aerogel network since a low catalytic activity of the enzyme due to the solvent nature will not be improved by encapsulating the enzyme in an aerogel.

Concerning the aerogels surface groups, an effect difficult to quantify was observed. This effect is of a secondary importance compared to those due to the enzyme dispersion in the aerogel network and of the nature of the solvent. Overall,

it appeared that the aerogel made from 40% of MTMS was appreciably better than the others. Nevertheless, this effect could be explained by a lower wetting by the residual polar liquids (water, methanol) during the sol–gel process, inducing a better aerogel resistance to contraction during drying.

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