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Application of silica aerogel encapsulated lipases in the synthesis of biodiesel by transesterification reactions

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Abstract

Two types of commercial lipases preparations, one from *Burkholderia cepacia*, the other one from *Candida antartica*, were encapsulated in silica aerogels reinforced with silica quartz fibre felt and dried by the CO_2 supercritical technique. These immobilized biocatalysts were applied in biodiesel synthesis by transesterification of sunflower seed oil with methyl acetate. They were found to be efficient even with mixtures of both substrates without any solvent addition. The aerogel encapsulation technique made it possible to maintain the enzymes in a dispersion state similar to the dispersion prevailing in an aqueous solution, even for further use in organic hydrophobic media. In transesterification in excess *iso*-octane, the two lipases encapsulated in aerogels made from 40% MTMS, were found to have activities relatively close to each other and comparable with commercial Novozyme 435. On the other in transesterification with mixture of oil and methyl acetate without any solvent, the kinetics were severely limited by substrate diffusion inside the aerogels. This was particularly true with the *C. antartica*, so that the corresponding aerogel encapsulated enzyme was much less active than commercial Novozyme 435, although it improved after a few tests.

Keywords: Lipase; Biodiesel; Transesterification; Immobilization; Silica; Aerogel

1. Introduction

The partial replacement of fossil fuels by fuels synthesized from plants, is a subject of great practical importance. At present, it is possible to synthesize alcohols such as ethanol or biodiesel compounds from alcohol producing plants (beats, corn, wheat), oleaginous plants (colza, soybean, sunflower seed) or ligneous cellulose plants (wood, straw).

The fermentation of alcohol producing plants produces ethanol, which can further be converted to classical hydrocarbons. From oleaginous plants, some viscous natural oil composed mainly of triglycerides is first extracted. By esterification with either methanol or ethanol, this oil is then transformed to glycerol and a mixture of esters termed either fatty acids methyl esters (FAME) when obtained with methanol or "biodiesel". The catalyst used in this transformation can be either a traditional "chemical" (i.e. not biochemical) catalyst, or an enzyme. In traditional chemical transformation, the reaction is usually catalyzed by a strong inorganic base such NaOH, KOH or $NaOCH_3$ in proportions from 0.5% to 1% of the total mass [1]. In optimal conditions, a conversion from 95% to 99% can be achieved, at a temperature ≈80 °C.

With the enzymatic method, a lipase is used in place of a strong base. The reaction temperature is lower (50 °C or lower) than with a chemical catalyst. The main biological function of lipases is to catalyze the hydrolysis of triacylglycerols. However, these enzymes have also been extensively studied in biocatalytic reactions of esterification, transesterification and inter-esterification, in organic medium. A direct transesterification of oil with methanol in a solvent free medium, with a good conversion to FAME (>83%), was achieved by Köse et al. [2]. These authors used a high ratio of immobilized Novozyme 435® (30% of substrate by weight). Nevertheless, other publications indicated that lipases were generally inactivated in such a direct transesterification when a high molar ratio of methanol to oil was applied, unless an organic solvent was also added in high proportion [3,4]. To avoid such a possible inactivation, an alternate route was reported [4]. It used a transesterification

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of oil with methyl acetate, a substrate which could be used in excess with lipases. Because of this potential problem it was decided, in the present study, to first investigate the application of sol–gel encapsulated lipase along this alternate route. Application of the best sol–gel materials according to the present study, can be applied to the direct transesterification with methanol, as well as ethanol in further work.

It is important to immobilize enzymes for practical applications, in order to be able to recover and use them repeatedly. Actually, the most successful immobilization techniques of lipases have been achieved in sol–gel materials, for applications in organic solvent [5–7]. The reason is that a gel maintains the lipase in a dispersed state, while agglomeration of the lipase occurs if this enzyme were used directly in an organic solvent. Hence sol–gel immobilized lipase should be directly applicable to the synthesis of biodiesel from vegetal oils. This was the aim of the present study.

2. Experimental part

2.1. Materials

Two types of lipases (E.C. 3.1.1.3) were used in the present study: the lipase of *Burkholderia cepacia* (termed BCL) from Amano and an aqueous solution of lipase from *Candida antartica* (termed CALB) referenced as Lipozyme CALB L®, gracefully provided by Novozyme France. The reference for BCL was Lipase PS Amano of commercial activity: 30 U mg^{-1}_{powder}, with 1 U defined as the quantity of enzyme which liberates 1 μmol fatty acid from an emulsion of 5 mL olive oil in 4 mL 0.1 M Phosphate buffer at pH 7 and 37 °C in 20 min. Previous analysis showed that 1 g of such BCL powder contained ≈10% (by mass) of protein and ≤1% (by mass) of enzyme [8]. Lipozyme CALB L was gracefully provided by Novozyme with a commercial activity of 600 U $mg^{-1}_{protein}$, where 1 U is the amount of enzyme that will release 1 μmol butyric acid from tributyrin under standard conditions (pH 8, 50 °C). Analysis by the BCA-200 Protein Assay Kit from Pierce showed that this CALB L solution contained a mass of protein ≈ 17.6 $mg_{protein}$ mL^{-1}.

The structure of both enzymes is known [9,10]. They both have a molar mass close to 33 kg mol^{-1} and an active site comprising the three aminoacid residus Aspartate, Histidine and Serine. Nevertheless they differ by the localization of the active site. The latter one is inside a cavity for the BCL, while CALB does not have such a cavity [10].

The substrates used in the biodiesel formation were sunflower seed oil (Fluka) and methyl acetate (Acros). In some reactions, *iso*-octane (from Fluka) was used as a solvent. The sol–gel synthesis chemicals were tetramethoxyilane (TMOS, 98% from Fluka), methyltrimethoxysilane (MTMS, 98% from Aldrich), polyvinylalcohol (PVA, MW 15,000 from Fluka). Commercial methyl palmitate (99%), methyl linoleate (puriss.), methyl oleate (99%), methyl stearate (99%) from Fluka were used to determine the response coefficients of a gas chromatograph to these products. The silica gels were reinforced with silica fibre felt Quartzel® gracefully provided by the St. Gobain Company.

2.2. Aerogel encapsulation

The silica gel encapsulation procedure applied in the present study was described in previous reports and applied to the esterification of lauric acid by 1-octanol [7]. In practice, the present gels were made by hydrolysis and condensation of the silica precursors TMOS, and MTMS, in various molar proportions. The wet gels were dried to aerogels by the CO_2 supercritical method.

In a first step, methanol, MTMS and TMOS were mixed for 15 min. Then a 4% (by weight) PVA aqueous solution was prepared and mixed for 15 min to a given mass of either free BCL powder or CALB solution to which an aqueous ammonia solution was added. The BCL powder was homogeneously dispersed as a fine suspension in water, in which the true enzymatic component, which is water soluble, was actually dissolved. No obvious sedimentation was observed in the silica sol in the time before gelation. Overall, both the true BCl protein component and the CALB one, were homogeneously dispersed in solution in the silica sol before gelation, hence also in the final silica gel. There was no significant difference between using either the commercial BCL powder or the Lipozyme CALB-L solution regarding the encapsulation procedure. Indeed, using directly the BCl powder for encapsulation, instead of purifying it by a succession of centrifugation, ammonium sulphate precipitation and dialysis as this was done in previous work [8], makes it possible to minimize enzyme loss from the initial powder, while the other components were catalytically inert and largely dialyzed out during further use.

In a second step, the two previous solutions (silica and enzyme) were mixed with each other. Gelation occurred after a time which depended of the mole fraction of MTMS to MTMS + TMOS. The gelation time increased from 90 min for 20% MTMS, to 150 min for 60% MTMS. After homogenization but before gelation, the solution of enzyme in the silica sol which was obtained was distributed in some small Teflon® tubes of internal diameter 5 mm. In each tube, a small piece of the silica fibre felt Quartzel® was previously introduced. The latter step was necessary to mechanically reinforce the silica aerogels, as previously published [11]. Gelation then occurred while the silica sol was impregnating the fibre felt. After gelation, wet reinforced gels with encapsulated lipase was extruded out of the Teflon tubes and dried by the CO_2 supercritical method (CO_2 critical point $T_c = 31.1$ °C, $P_c = 74$ bars) according to a protocol previously described [11]. This protocol included an intermediate dialysis step in acetone for 24 h, acetone being an intermediate exchange fluid between polar aqueous solutions and liquid CO_2. The immobilized enzyme obtained could be recycled several times without any apparent mechanical deterioration by wear. Typically, any aerogel sample tested in the present report comprised 10 small quartz felt reinforced cylinders of dimension ≈5 mm (diameter) by ≈5 mm (height) and total dry weight ≈0.5 g (±5%). The quantity of enzyme it contained was the enzyme contained in the mass of commercial BCL powder, or Lipozyme solution, initially added to the silica sol before gelation, and further reported in the text.

Table 1
Physico-chemical characteristics of the solvent, substrates and methyl esters produced by transesterification of sunflower seed oil with methyl acetate

Products, substrates and solvent	Chemical formula	Retention time (1) (min)	Molar mass ($g\,mol^{-1}$)	Specific volume ($g\,mL^{-1}$)	Selectivity (mol%) (1) (Sunflower seed oil)
Methyl palmitate	$CH_3(CH_2)_{14}COOCH_3$	≈1.7	270.45		8.7 ± 2.2
Methyl oleate	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOCH_3$	≈2.5	296.48	0.87	86.7 ± 3.7
Methyl linoleate	$CH_3(CH_2)_3(CH_2CHCH)_2(CH_2)_7COOCH_3$	≈2.5	292.46	0.886	
Methyl stearate	$CH_3(CH_2)_{16}COOCH_3$	≈2.7	298.50		4.7 ± 1.9
Sunflower seed oil			861.4 (2)	0.919	
Methyl acetate	CH_3COOCH_3		74.08	0.93	
iso-Octane	C_8H_{14}		114.23	0.692	

(1) With the gas chromatograph conditions stated in Section 2.3. (2) Value estimated from the selectivity in each fatty methyl ester in the right hand column.

2.3. Assay of catalytic activity

The catalytic activity was studied in the transesterification of vegetal sunflower seed oil with methyl acetate catalyzed either with either BCL or CALB. This reaction was carried out either in *iso*-octane, or without any solvent (only the vegetal oil + methyl acetate). The products of this reaction were a mixture of Fatty acid Methyl esters (FAME) and 1,2,3-triacetoxypropane (triacetin). These products were analyzed by injecting aliquots of the reaction in a gas chromatograph (GC) Shimadzu GC-14B, equipped with a nonpolar capillary column Zebron® ZB-5 from Phenomex of 15 m in length, 0.25 mm internal diameter and 0.10 μm thickness. The temperature heating schedule comprised a hold at 180 °C for 30 s, followed by heating to 300 °C at a rate of 10 °C min^{-1} and terminated with a hold at 300 °C for 10 min. The injector temperature was 245 °C and detection was made with a flame ionization detector (FID) at 305 °C. The detection signal area "*S*" (a number without a dimension) was a linear function of the mass *m* of ester dissolved in 10 mL *iso*-octane with a linear coefficient *k* determined experimentally by injection of pure products.

The sunflower seed oil studied was actually a mixture of triglycerides. It produced four different FAME listed in Table 1, by transesterification with methyl acetate. Their retention times in the GC, determined separately for each pure commercial chemical compound are also listed in this table. It appeared that the retention times for the methyl oleate and the methyl linoleate were similar, so that these two esters could not be distinguished in the GC. Moreover the response coefficient *k* was found to be the same for all esters, $k \approx 2.10^{11}\ g^{-1}$, with an excellent linearity ($R^2 > 0.996$), in a range of 0–15 mg ester dissolved in 700 μL aliquot.

The results of the catalytic tests were expressed either as yield (*Y*%) defined as the percent of initial methyl acetate converted to FAME, or as the initial transesterification rate ν_0 expressed in U, where 1 U = 1 μmol min^{-1} of FAME formed at the beginning of the reaction.

2.4. Gels characterization

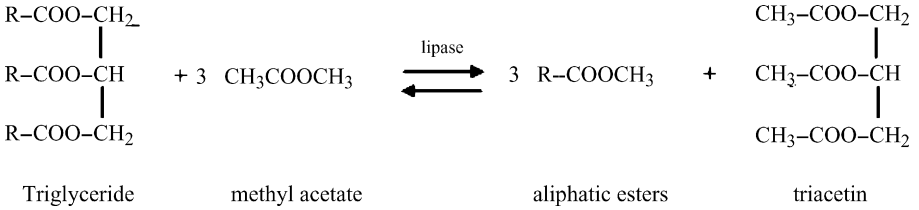
The textural characteristics of gels were analyzed by nitrogen adsorption isotherms on a custom made equipment, after desorption under vacuum for 6 h at 200 °C. The structural characteristics were analyzed by ^{29}Si-CP MAS-NMR, on a Bruker DSX-400 spectrometer at 400 MHz. The samples were spun at the magic angle at ca. 5 kHz with a contact time of 5 ms and a relaxation time of 5 s. The chemical shifts were measured using $Si(CH_3)_4$ as a reference. In order to provide information on the connectivity of the gel network, the integrated peaks were fitted with Gauss–Lorenz curves using WINNMR and XPLOT software.

3. Results and discussion

3.1. Activity of free BCL

Transesterification reactions with the as provided BCL powder were made in 30 mL flasks, containing 10 mL *iso*-octane to which 1 mmol sunflower seed oil and 3 mmol methyl acetate were added. These relative substrate mole numbers corresponded to the stoichiometric coefficients of the transesterification reaction. Each catalytic test was carried out at 30 °C in a bath under thermal agitation by shaking at a frequency of 180 rpm. Previously to each test, the enzyme, solvent and substrates were separately pre-equilibrated at a water thermodynamic activity $a_w = 0.81$. Previous studies had shown that the latter step provides the best and reproducible activity to the BCL [12]. Each reaction was followed by taking 100 μL aliquots, diluted in 600 μL *iso*-octane and analyzed in the GC by injection of 1 μL aliquots.

In the transesterification reaction (1) below, R represents unsaturated aliphatic chains comprising from 12 to 22 carbon atoms. Hence several types of FAME could form.



(1)

To discriminate between these esters, a first series of tests was made with as provided BCL powder. Fig. 1 shows the mole

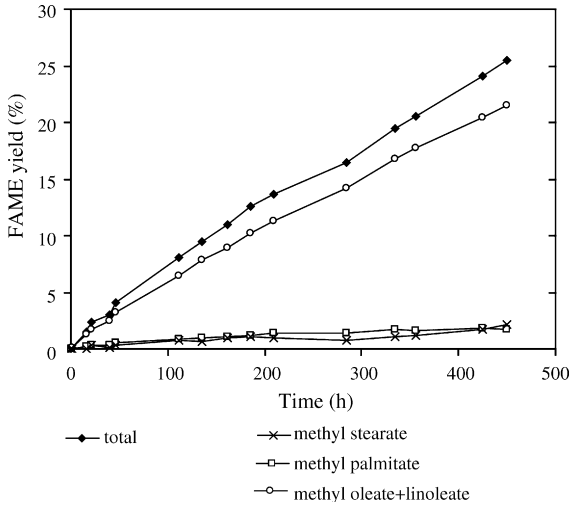


Fig. 1. Conversion kinetics of methyl acetate to the various fatty acids methyl esters (FAME) by transesterification of rapeseed oil with methyl acetate by 38 mg free BCL powder (*initial conditions*: 1 mmol oil, 3 mmol methyl acetate and 10 mL *iso*-octane).

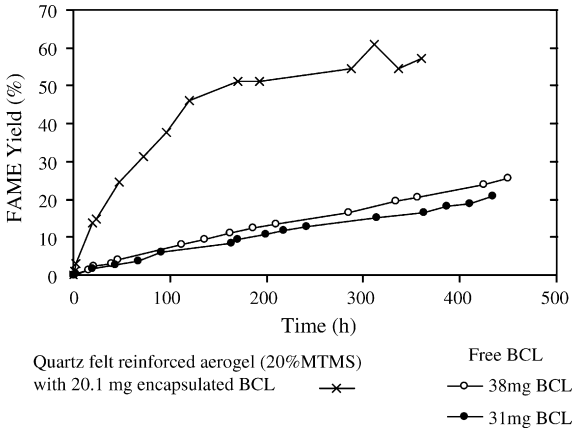


Fig. 2. Comparison of the conversion kinetics of methyl acetate to FAME for free BCL (31 and 38 mg powder), and a quartz felt reinforced silica aerogel made from 20% MTMS (20.1 mg BCL powder encapsulated) (*initial conditions*: 1 mmol oil, 3 mmol methyl acetate and 10 mL *iso*-octane).

number of the various FAME formed with 38 mg BCL powder, as a function of time. The selectivity in each group of esters was independent of time. These selectivities are listed in Table 1. Obviously, the largest contribution was provided by the two esters methyl olealate and methyl linoleate. On the other hand, some important random variations in the total transesterification kinetics could be observed in some tests with free BCL powder. These variations could be attributed to fluctuations dispersion of the enzyme powder dispersion in *iso*-octane. They were never observed with aerogel encapsulated powder.

3.2. Activity of encapsulated BCL

In a first step, the activity of free BCL lipase was compared with that of BCL encapsulated in small quartz felt reinforced silica aerogel cylinders of diameter ≈5 mm and length ≈5 mm. Fig. 2 shows that the transesterification reaction was much faster with reinforced aerogel encapsulated BCL than with free BCL, as already published [13].

The initial conversion kinetics for encapsulated BCL, encapsulated CALB L (Lipozyme) and commercial Novozyme 435®, up to 25% FAME yield, are compared in Fig. 3. These aerogels were made from 40% MTMS. The initial apparent activity ν_0 and specific apparent activity $\nu_{0,sp}$ derived from these data are reported in Table 2. In a reaction medium comprising 1 mmol oil, 3 mmol methyl acetate in 10 mL *iso*-octane, the conversion kinetics were slightly faster with 20 mg BCL than with 20.7 mg Novozyme 435. An aerogel made from 40% MTMS with 20 mg BCL powder showed a total activity $\nu_0 = 0.43$ U (Table 2). Considering a protein content ≈10% in BCL powder as previously mentioned, this corresponds to a specific activity per mg of protein $\nu_{0,sp}$ (BCL) = 0.21 U $mg^{-1}_{protein}$ (Table 2). On the other hand, in a reaction medium with 9 mmol oil and 27 mmol methyl acetate (no solvent), Table 2 shows that the relative efficiencies of both catalysts were reversed. A likely explanation is that the oil substrate had to diffuse inside an aerogel, which is not the

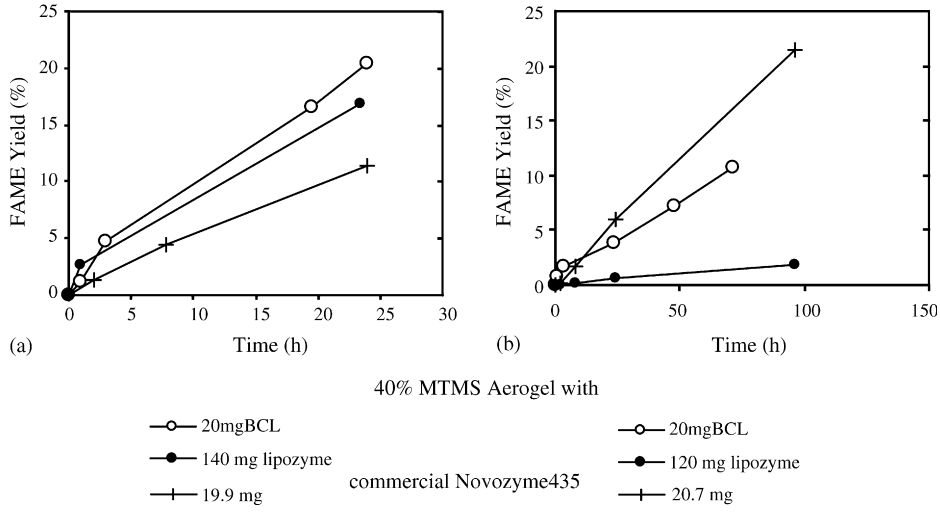


Fig. 3. Compared initial transesterification kinetics with encapsulated BCL, encapsulated CALB L and commercial Novozyme 435, with: (a) dilute substrate concentrations (1 mmol oil, 3 mmol methyl ester in 10 mL *iso*-octane); (b) high substrate concentrations (9 mmol oil, 27 mmol methyl acetate, no solvent).

Table 2
Compared initial apparent activity of encapsulated BCl, encapsulated CALB L and commercial Novozyme 435 (data derived by best square fit analysis of Fig. 3)

Biocatalyst	Activity	1 mmol oil, 3 mmol methyl acetate, 10 mL *iso*-octane	9 mmol oil, 27 mmol methyl acetate, no solvent
20 mg BCL powder	v_0 (U)	0.43	0.68
	$v_{0,sp}$ (U $mg^{-1}_{protein}$)	0.21	0.342
120 μL Lipozyme (2.15 mg protein)	v_0 (U)	0.361	0.09[a]
	$v_{0,sp}$ (U $mg^{-1}_{protein}$)	0.17	0.050[a]
20.7 mg Novozyme 435	v_0 (U)	0.24	1.0
	$v_{0,sp}$ (U mg^{-1}_{powder})	0.012	0.049

[a] 100 μL: 1.76 mg protein.

case when the enzyme is immobilized on the external surface of spherical powder as in Novozyme 435. Hence a more sever kinetics limitation can be expected with an aerogel, when the substrate concentration is high.

The influence of the hydrophilic character of a gel on the BCL activity was investigated in another series of aerogels. For this purpose, aerogels were made from mixtures of TMOS and MTMS with MTMS proportions of 20%, 40% and 60% (molar). Moreover, increasing substrates concentrations in *iso*-octane with a constant molar ratio methyl acetate/sunflower oil ≈ 3 were applied, including the same molar ratio of substrates without any solvent. The initial apparent activities v_0 per mg of BCL protein, determined as previously, are gathered in Fig. 4. These specific activities showed a maximum for a methyl acetate concentration ≈12 mol L^{-1}, although they remained important without any solvent. The behaviour differences were minor between the gels made from 20%, 40% or 60% MTMS, in a reaction medium comprising 1 mmol oil and 3 mmol methyl acetate in 10 mL *iso*-octane (Fig. 5). However, at higher substrate concentration, the gel made from 60% MTMS was significantly less active.

With encapsulated BCL, the final yield in FAME reached a plateau at $Y \approx 56 \pm 3\%$, independent of the type of aerogel and substrate concentration (in the molar ratio of methyl acetate:oil = 3:1). This constant final conversion is well illustrated in Fig. 5, for two extreme dilution concentrations of the substrates. It was moreover consistent with the results of Du et al. who used higher initial methyl acetate:oil ratio and obtained a final yield which tended to increase with this initial molar ratio [4].

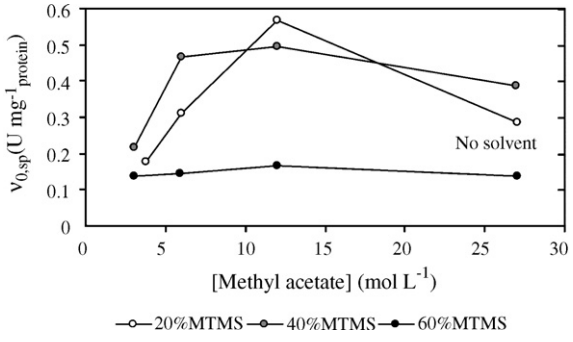


Fig. 4. Variation of the specific activity (per mg of protein) of encapsulated BCL, as a function of the proportion of MTMS used to synthesize the gel, and the molar concentration of substrates in the reaction medium (molar ratio methyl acetate/oil ≈ 3).

3.3. Activity of CALB

Transesterification tests of 1 mmol sunflower oil by 3 mmol methyl acetate in *iso*-octane catalyzed with an increasing mass of commercial CALB solution, showed very erratic results. This behaviour was due to a bad dispersion of such an aqueous solution in *iso*-octane. However, tests were made with commercially immobilized CALB on a polymer (Novozyme 435®). CALB L from the Lipozyme solution could also be encapsulated inside aerogels, because such materials were synthesized in aqueous media. Fig. 6 illustrates the conversion kinetics with 74 and 140 mg (≈120 μL) Lipozyme solution loading, in a 40% MTMS aerogel, as well as with 70 and 20 mg free commercial Novozyme 435 powder. Within the precision of gas chromatograph, these kinetics were consistent with those previously reported for Novozyme 435® and encapsulated BCL [4]. Overall, Table 2 shows that 140 mg encapsulated Lipozyme solution resulted in kinetics very close to those of 20 mg encapsulated BCL powder and of 70 mg commercial Novozyme 435®. The yield also tended towards a similar upper value after 100 h. On the other hand, with a lower mass of free commercial Novozyme 435® (20 mg) or a lower encapsulated Lipozyme solution loading (74 mg), the conversion appeared to tend towards a lower value after a long time (Fig. 6). Considering that the Lipozyme

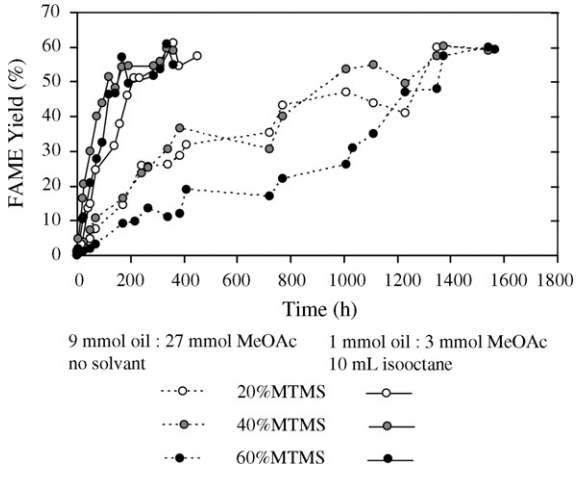


Fig. 5. Compared conversion kinetics of methyl acetate to FAME for 20 mg BCL powder in aerogels made from 20%, 40% and 60% MTMS, in reaction media initially comprising either 1 mmol oil, 3 mmol methyl acetate in 10 mL *iso*-octane, or 9 mmol oil, 27 mmol methyl acetate and no solvent.

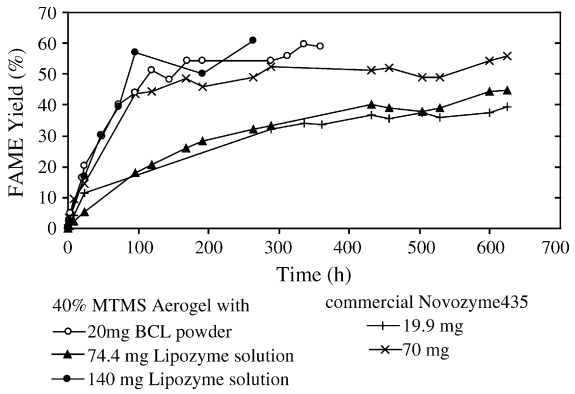


Fig. 6. Compared conversion kinetics of methyl acetate to FAME for 70 and 19.9 mg commercially immobilized CALB (Novozyme 435), 74.4 and 140 mg Lipozyme solution in a 40% MTMS aerogel and 20 mg BCL powder in a 40% MTMS aerogel (*initial conditions*: 1 mmol oil, 3 mmol methyl acetate and 10 mL *iso*-octane).

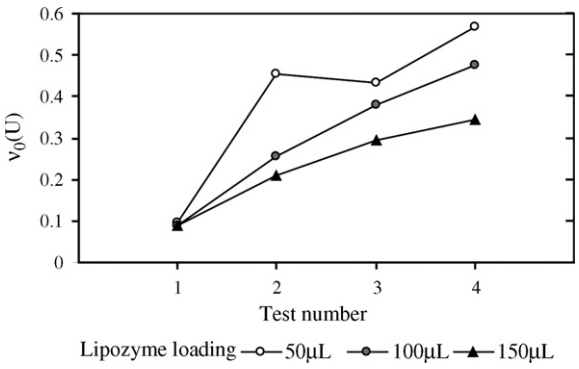


Fig. 7. Total activity in U (µmol min^{-1} methyl ester formed) after recyling, of quartz felt reinforced aerogels made from 40% MTMS with increasing Lipozyme loading. The reactions were carried out in a mixture of 9 mmol oil, 27 mmol methyl acetate and no solvent. The samples were abundantly washed in *iso*-octane after each test.

CALB L solution contained ≈17.6 mg protein mL^{-1}, as mentioned before and that its density was ≈1.2 g mL^{-1}, an aerogel made from 40% MTMS with 140 mg Lipozyme solution was found to have a protein specific activity $\nu_{0,sp}$ (CALB L protein) = 0.17 U $mg^{-1}_{protein}$, in transesterification in dilute solution in *iso*-octane (1 mmol oil and 3 mmol methyl acetate in 10 mL *iso*-octane). This is a value quite comparable with the one determined for the BCL protein. However, this specific activity of encapsulated Lipozyme decreased considerably when transesterification was performed without solvent (Table 2 and Fig. 3), while that of Novozyme 435® increased. Such results were consistent with a strong limitation by diffusion of the substrate towards the encapsulated enzyme, when the substrate concentration was high. The much stronger diffusion limitation with encapsulated CALB L (Lipozyme) than with encapsulated BCL, could possibly be explained by a high protein concentration, other than the lipase, in the commercial Lipozyme solution. Indeed, the Lipozyme solution density was high (≈1.2 g mL^{-1}). Hence it was quite possible that proteins other than the enzyme partially plugged the aerogel pores and made the limitation by diffusion worse.

3.4. Recycling aerogel encapsulated lipase

Recycling with BCL encapsulated in an aerogel was previously investigated in similar reactions performed in dilute solution in *iso*-octane [12]. It was found that the BCL activity was significantly higher (≈75% higher) in the second test than in the first one. Then the activity gradually decreased to reach ≈60% of the initial activity after 11 tests. However, these aerogels were not reinforced and a small mass of gel was lost after each test during washing.

In the present case the aerogels were reinforced with quartz fibre felt to practically eliminate gel loss during washing [13]. Hence, a limited series of four recycling were carried out with encapsulated Lipozyme, in a reaction medium without any solvent. This type of reaction medium was selected because it appeared the most interesting one for potential industrial applications. The data are reported in Fig. 7. The first test activity was roughly independent of the Lipozyme loading, clearly indicating that the rate limiting step was the diffusion of the substrate towards the enzyme. Moreover, as this was previously reported for the BCL [12], the activity of encapsulated Lipozyme increased after the first cycle. This activity tended to progressively level out after an increasing number of tests. A likely explanation is that debris and proteins smaller than the enzyme, present in the Lipozyme solution, were progressively evacuated from the gel during the successive tests. This evacuation could be viewed as a direct dialysis of the Lipozyme solution, after encapsulation in the gel. It was expected to take an increasing time as the Lipozyme loading was increased, in agreement with the results. As a consequence of such a dialysis phenomenon, the gel pores progressively un-plugged and limitation by diffusion of the substrate progressively attenuated. For future work, this also suggests to encapsulate a purified Lipozyme solution.

The limitation by diffusion of substrates in an aerogel also explains that the commercially immobilized Novozyme 435 was presently superior to the aerogel, at high substrate concentration (reaction without any solvent). Indeed, in such conditions, the substrate did not have to diffuse inside a porous network with the Novozyme 435, contrary to an aerogel. Nevertheless, it should be possible to shape an aerogel with encapsulated enzyme as thin coatings (less than a micrometer thick) on surface such as honeycomb surfaces, or the internal surface of small diameter tubes. This is likely to attenuate largely the limitation by diffusion, while allowing a very easy recycling of the catalyst,

Table 3
Textural characteristics of aerogels made with various lipase loading: specific surface area A_{sp}; specific pore volume V_{sp}; contribution of pores with a size larger than the Kelvin limit to the specific surface area $A_{sp,Kel}$ and the specific pore volume $V_{sp,Kel}$

Lipase loading	A_{sp} (m^2/g)	V_{sp} (cm^3/g)	$A_{sp,Kel}$ (%)	$V_{sp,Kel}$ (%)
None	593 ± 10	1.159	73.4	91.4
20 mg BCL	533 ± 16	0.652	58.7	82.2
35 mg CALB	618 ± 11	1.444	80.6	93.2
70 mg CALB	565 ± 12	1.086	75.0	92.8
140 mg CALB	531 ± 9	1.004	71.5	91.5
350 mg CALB	409 ± 9	0.757	71.4	91.2

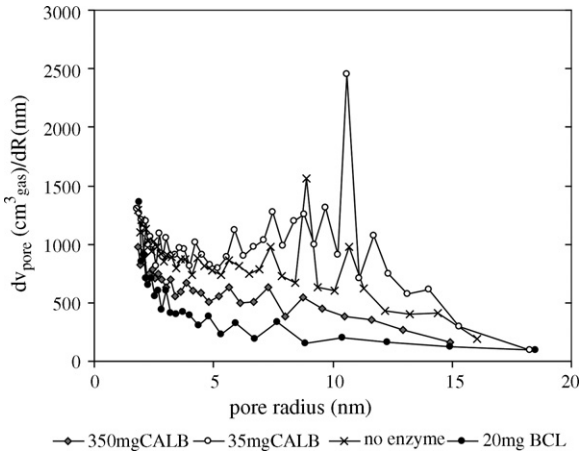


Fig. 8. Pore size distribution of aerogels made from 40% MTMS without enzyme, or with encapsulated BCL powder or CALB solution.

including continuous processing. Hence the sol–gel process is worth to keep investigating.

3.5. Aerogel characterization

The texture of aerogels made with various lipase loading was characterized by recording and analyzing their nitrogen adsorption isotherms. For all samples, these adsorption isotherms were of type IV according to the classification by Brunauer, Emmet and Teller [11], corresponding to mesoporous materials. The specific surface area was determined by the method of Brunauer, Emmett and Teller (BET) [14] and the pore size distribution by the Roberts' method applied to the desorption branch of the isotherms [15]. The results are gathered in Table 3 and in Fig. 8. Table 3 shows that the specific surface area A_{sp} and specific pore volume V_{sp} decreased as the mass of CALB solution increased, with is consistent with a plugging of the aerogel pores by components other than the enzyme, in the Lipozyme solution. The contributions of mesopores with a size larger than the Kelvin limit, to the specific surface area and specific pore volume, respectively, $A_{sp,Kel}$ and $V_{sp,Kel}$, were also larger with CALB than with the BCL. Fig. 8 shows that the introduction of a similar mass of CALB solution and BCL powder (respectively, ≈35 and ≈20 mg) resulted in a significantly different aerogel texture. With the BCL, the pore size distribution was very large, while with CALB a maximum near a pore radius ≈10 nm was observed, not very different from that observed in an aerogel without any enzyme.

^{29}Si NMR spectra provided some information on the Si coordination species of the type $Si(OSi)_{4-n}(OX)_n$ or $(CH_3)Si(OSi)_{3-n}(OX)_n$, respectively, known as Q^n and T^n, where OX can be OH or OCH_3. Fig. 9 shows the spectra recorded with aerogels made from 40% MTMS, without enzyme loading or

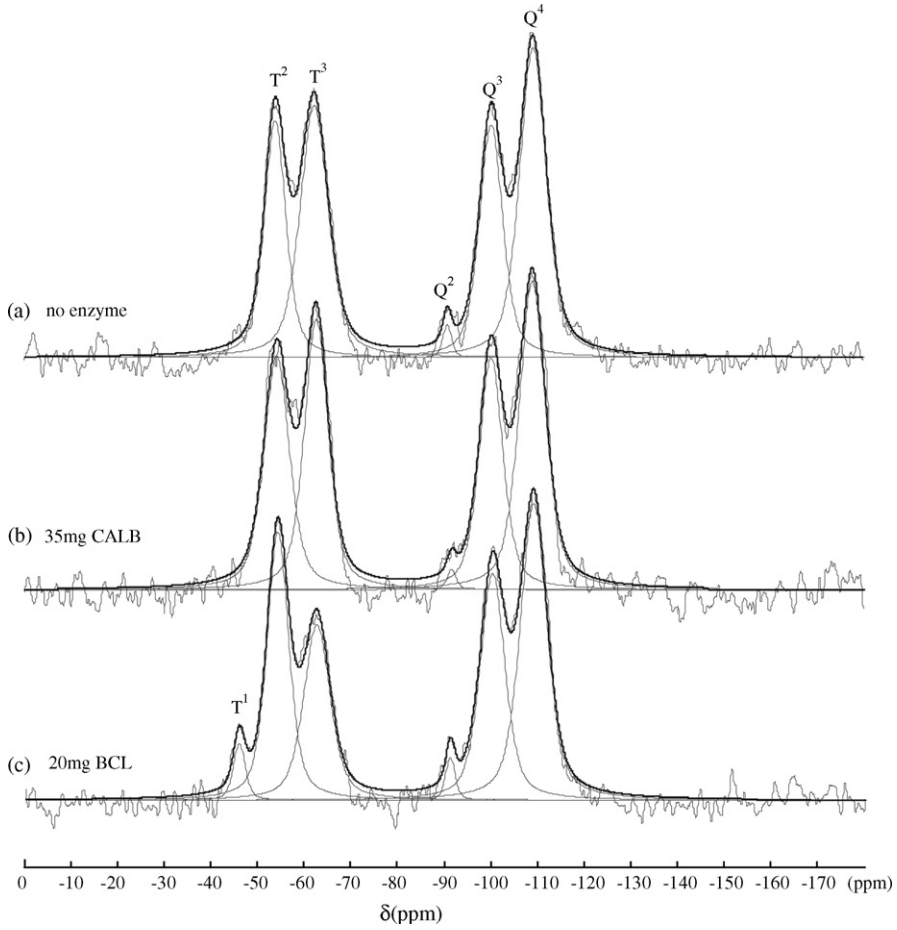


Fig. 9. ^{29}Si NMR spectra of aerogels made from 40% MTMS: (a) without enzyme; (b) with 35 mg CALB solution; and (c) with 20 mg BCL powder.

with encapsulated BCL or CALB. The Q signals were roughly similar in the three samples. Nevertheless, some significant difference could be observed regarding the T signals (coming from MTMS). The gel without enzyme and the gel with encapsulated CALB were very similar, showing only T^2 and T^3 signals. On the other hand the gel with BCL showed T^1, T^2 and T^3 signals, with a relatively less intense T^3 signal. These results seem to indicate that, while BCL had an effect of the silica gelation process, this was not the case with CALB.

4. Conclusions

Two types of lipases, one (BCL) from *B. cepacia*, the other (CALB) from *C. antartica*, could be encapsulated in silica aerogels and applied to the transesterification of sunflower seed oil with methyl acetate. With both encapsulated enzymes, the biocatalytic reaction occurred with dilute substrate concentrations in *iso*-octane, as well as with mixture of substrates without any solvent. For a molar methyl acetate:sunflower oil of 3:1, a final conversion of the order of 56 was achieved %, which was consistent with previous reports for this reaction. Besides, gels with hydrophobic groups made from 40% MTMS were found to be the most efficient ones. In such aerogels, quite similar protein specific activity were achieved with BCL and CALB, respectively, $\approx$0.39 and $\approx 0.30\,\mathrm{U\,mg^{-1}_{protein}}$. However, in a reaction medium with a high substrate concentration and without any solvent, sever limitation by diffusion of the substrates made the commercially immobilized Novozyme 435® superior. Textural and ^{29}Si NMR structural studies showed that the encapsulation of CALB did not modify significantly the silica aerogel, while the structure and the texture were significantly modified by encapsulation of BCL.

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