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Comparison of *Yarrowia lipolytica* Lipase Immobilization Yield of Entrapment, Adsorption, and Covalent Bond Techniques

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Abstract The purpose of this study was to immobilize lipase from *Yarrowia lipolytica* using three methods including inclusion, adsorption, and covalent bond to study enzyme leaching, storage, and catalytic properties. Sodium alginate and chitosan were the polymers selected to immobilize lipase by inclusion. The beads of each polymer were dried by freeze drying and fluidization. The results show that chitosan was more adapted to the inclusion of lipase. Even though freeze dried, bead activity was low compared to that of fluidized beads. The freeze-drying process seems to produce suitable beads for storage at 4 and 20 °C. The immobilization by adsorption was carried out on both celite and silica gel. Maximum immobilization yield of 76% was obtained with celite followed by 43% in silica gel. The enzyme adsorbed on the two supports exhibited greater stability at a certain temperature (50 °C) and in no polar solvents (Isooctane, *n*-heptane, and *n*-hexane). In addition, the lipase immobilized by covalent bond retained residual activity equitable to 70%. It was demonstrated that the enzyme immobilized by covalent bond showed greater activity (80%) after 5 months of storage.

Keywords *Yarrowia lipolytica* lipase · Enzymatic activity · Immobilization · Reusability

Introduction

Enzymes present numerous potentialities of applications in many sectors: food technology, environmental, and pharmaceutical. Lipases or triacylglycerol acylhydrolases (EC 3.1.1.3)

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are particular catalysts able to catalyze the hydrolysis reactions of triacylglycerol esters in aqueous medium and ester syntheses in nonaqueous medium and are able to preserve their catalytic activity in organic solvents, biphasic systems, and micelles solutions. Many interesting applications have been found for lipases [1, 2]; however, these applications in many cases are limited for economic reasons. To use them more economically and efficiently, their stability and biological activity can be improved by immobilization [3–6]. Many methods have been developed for lipase immobilization, and a lot of effort is still being devoted to the search for new support materials and novel techniques. The use of a judicious immobilization method can reduce significantly process costs involving lipases. Various techniques have been proposed including chemical and physical attachment to solid surfaces [7–9]. Inclusion in polyanionic or polycationic polymers by addition of multivalent covalent counterions is a simple and common method of enzyme immobilization. Gels prepared from natural and synthetic polymers have been successfully employed, and these offer one of the simplest methods because relatively milder conditions are required for enzyme immobilization. The adsorption shows to be the easy and less expensive method. However, the adsorption is generally not very strong, and some of the adsorbed protein will be desorbed during washing. Different supports have already been exploited to immobilize the enzyme as the silica gel, the beads of porous glass, alumina, and the celite [10]. The immobilization by covalent bond has been developed to get very strong links between enzymes and supports but have the disadvantage of denaturizing the native enzyme during the binding process. Before realization of the covalent bond, a previous activation is necessary. The activation of the adsorption supports is the subject of numerous studies because the activation of the functional groups of the enzyme can denature the enzyme. The present paper deals with immobilization of *Yarrowia lipolytica* lipase by three different methods: inclusion, adsorption, and covalent bond. Catalytic activities of the immobilized and free enzymes in the three systems were compared, and the stability of the immobilized enzyme was tested in several hydrolysis cycles.

Materials and Methods

Materials

Lipase from *Y. lipolytica* LgX6481 was produced in a 2,000 l bioreactor (LSL Biolafitte, Poissy, France) under the same conditions as described by [11, 12]. The broth produced was centrifuged on a BTPX205 continuous centrifuge (Alfa Laval, Sweden) at 12,000×*g*, at a flow rate of 500 l h^{-1}, and the supernatant was freeze dried for further studies. Sodium alginate, celite 535, and silica gel were obtained from Fluka Biochemika (Switzerland). Chitosan and Trizma acid were purchased from Sigma Aldrich (Belgium), column HiTrap™ *N*-hydroxysuccinimide (NHS)-activated HP from Pharmacia Wikstroms (Sweden), calcium chloride, sodium tripolyphosphate, and all other chemicals used were from Merck (Germany) and were of analytical grade.

Methods

Immobilization by Inclusion

Alginate Beads Alginate, $CaCl_2$, and lipase solutions were prepared in Tris–HCl buffer pH 7.2, 0.05 mM. Alginate solution with 4% (*w*/*v*) was prepared by dissolving sodium

alginate powder in buffer and stirring continuously into complete dissolution. Lipase powder (1.2 g) was dissolved in 20 ml buffer. The two preparations were mixed and stirred with a magnetic bar to ensure complete mixing. As soon as the mixed solution (alginate and lipase) was added dropwise into 400 ml of $CaCl_2$ solution (0.3 M) at a rate of 70 ml h^{-1}, accomplished using a 10-ml plastic syringe and peristaltic pump (101U/R Watson-Marlow Cornwall, England), Ca-alginate beads were formed. After 20 min of hardening, the beads were separated from the calcium chloride solution by simple filtration. They were washed two times with 30 ml of Tris–HCl buffer, and the beads were used as such or freeze dried and fluidized for further studies. Beads have been dried by freeze drying with the freezer Koeltechniek (Belgium). The freeze-drying protocol was previously described [13]. Beads were also dried by fluidization. The bed fluidizer is a device of Niro Aeromatic (Denmark). The product is introduced in the vat of the bed and is dried by barbotage. The temperature of air entry that crosses the layer of the product is 50 °C, and the temperature inside the product is 37°C at the end of drying. After drying, beads were stored at 4 and 20 °C. The decanted $CaCl_2$ solution and the two washings were collected for lipase activity determination.

Chitosan Beads Chitosan solution with concentration of 3% (*w*/*v*) was prepared by dissolving chitosan powder in 1% (*v*/*v*) acetic acid. To 80 ml of the chitosan preparation, 20 ml of lipase solution, prepared previously by dissolving 1.2 g of lipase powder in acetic acid (0.5% *v*/*v*), was added. The contents were stirred gently to ensure complete homogenization. This solution was then extruded dropwise through a syringe and peristaltic pump into a beaker containing 200 ml of 0.136 mM sodium tripolyphosphate ($Na_5P_3O_{10}$) solution, which was prepared in 0.05 mM Tris–HCl (pH 7.2). After 75 min, the formed beads were recuperated by simple filtration and washed two times using 30 ml of 0.05 mM Tris–HCl (pH 7.2). Afterward, they were used as such or freeze dried and fluidized for further studies. After drying, beads were stored at 4 and 20 °C. The decanted $Na_5P_3O_{10}$ solution and the two washings were collected for lipase loss measurements.

Beads Morphology The surface and cross-sectional morphology of the freeze-dried and fluidized beads were examined using electron microscopy. All samples were sputter coated with gold prior to observation. Micrographs were obtained using a scanning electron microscope (JSM-7500-F, JEOL, Belgium). Bead size measurements were determined by using the same electron microscopy. Measurements were carried out for 15 beads.

Immobilization by Adsorption

Immobilization of Lipase on Celite 535 Y. lipolytica lipase was immobilized on celite by adsorption following the method described by [14]. An appropriate amount of support (20 g) was mixed thoroughly with 1.2 g of lipase in 100 ml phosphate buffer (pH 7.5), and the mixture was kept stirring for 4 h. The immobilized lipase was freeze dried and stored at 4 °C for further studies.

Immobilization on Silica Gel 60 G The same protocol described earlier for immobilization on celite 535 was applied for silica gel. However in this case, the quantity of support was different. Six grams of support was mixed thoroughly with 1.2 g of lipase in 100 ml phosphate buffer (pH 7.5), and the mixture was kept stirring for 4 h followed by freeze drying. The freeze-dried preparation was stored at 4 °C for further use.

Immobilization by Covalent Bond

The immobilization by covalent bond was achieved on the HiTrap™ NHS-activated HP column (Pharmacia Wikstroms). This column is constituted from sepharose linked to the matrix by the epichlorhydrin. Before establishing the suitable method of lipase immobilization, the column was activated by the NHS according the method described in the notice. Lipase solution with a concentration of 10 mg ml^{-1} was prepared by dissolving lipase powder in $NaHCO_3$ (0.2 M) and NaCl (0.5 M). Before introducing lipase preparation (1 ml) to the column with a peristaltic pump, the isopropanol contained in the column was eliminated with 6 ml of HCl (1 mM). After 30 min, the column was washed to eliminate the excess of lipase that was not immobilized. After immobilization, the column was stored at 4 °C.

Assay of Lipase Activity

The hydrolytic activities of free and immobilization lipase were measured by a titrimetric method previously described by [11] using olive oil (Extra Vierge Bertolli, Italy) as the substrate. Activities are expressed in international units, where 1 U of lipase is the amount of enzyme able to catalyze the release of 1 μmol of fatty acid per minute at pH 7 and at 37 °C. For lipase immobilized by covalent bond, activity was measured by spectrophotometer using *para*-nitrophenyl acetate (PNPA) as substrate to avoid the colmatage problem. All the essay of activities was repeated three times.

Immobilization Yield Immobilization yield was defined as follows:

$$\text{Immobilization yield } \% = \left(\frac{a_{\text{imm}}}{a_{\text{free}}}\right) \times 100 \quad [15]$$

where a_{imm} is the total activity of immobilized enzyme (U/g) and a_{free} is the total activity of the initial enzyme preparation (U/g).

pH Activity Profile The effect on the enzyme activity of free and immobilized preparations was checked in hydrolysis of olive oil in phosphate (0.1 M) or Tris–HCl (0.05 M) buffer at pH ranging from 2.0 to 12.0. Lipases activities were measured by incubating the samples at 37 °C for 15 min.

Reusability of Immobilized Lipase To evaluate the reusability of the immobilized lipase, beads were washed with water and buffer and were resuspended in a fresh reaction mixture to measure the enzymatic activity. The procedure was repeated until the enzyme lost 50% of its total activity.

For Column The study of reusability of immobilized lipase by adsorption was achieved using a glass column (P1 NS 12.5×2.5 mm, Merck, Belgium). The column contained 10 g of lipase powder immobilized on celite and silica gel. Initially, the powder was washed and hydrated by distilled water (30 ml). The esterase test was carried out for the enzyme activity according to the following protocol: A solution containing 10 ml of distilled water, 4 ml of PNPA (0.1 M) prepared in methanol and diluted 100 times, and 4 ml of phosphate buffer

was prepared. The solution rate was three drops per minute on the column using a peristaltic pump. The solution was analyzed with the spectrophotometer (Spectronic 20 Genesys Unican, USA) at 400 nm.

Thermal Stability Free and immobilized enzymes were placed in the buffer solution of optimum pH and incubated at 50 °C for different time intervals (0–7 h). Enzyme activity was determined as described earlier [11].

Effect of the Solvents on Immobilized Lipase by Adsorption Three solvents were used: *n*-heptane, *n*-hexane, and isooctane. Free (1 g) and immobilized lipases on the celite and the silica gel were dissolved in these 20 ml of three solvents. Afterward, the solutions were maintained in room temperature (20 °C), and then lipase activity was measured out at intervals of 1 h for 4 h.

Results and Discussion

Entrapment Enzyme by Inclusion

To investigate effects of support amount on loading efficiency, inclusion of enzyme in alginate was carried out using two quantities of alginate (3.2 and 6.4 g). The immobilization yield and lipase loss in $CaCl_2$ solution and the two washings were compared in Table 1. The results suggest that the loading yield of enzyme in sodium alginate beads increases significantly with the amount of the polymer, which is in agreement with earlier reports [16]. Indeed, percent of enzyme entrapped was increased from 38.8% to 66.2% for 3.2 and 6.4 g, respectively. It has also been demonstrated that the enzyme losses in $CaCl_2$ and washings solutions were limited considerably by increases in alginate amount. For further studies, an alginate amount of 6.4 g was retained. In addition, the percent of lipase entrapped in chitosan was examined. In this case, only one amount of support was tested. The results were summarized in Table 1. As can be seen from the data in Table 1, lipase entrapped in chitosan gave an immobilization yield of 23%. However, the loss in $Na_5P_3O_{10}$, used for chitosan beads preparation, seems to be inferior (19.7%) to those noted during manufacturing process in alginate beads (29.2%), even though the curing time of chitosan beads was 75 min, which was almost four times the 20-min curing time for alginate beads.

Table 1 Immobilization yield of entrapment *Y. lipolytica* lipase in alginate and chitosan.

	Fresh alginate beads		Fresh chitosan beads	Washing solution, Tris–HCl			$CaCl_2$		$Na_5P_3O_{10}$
	M1	M2		M1	M2	C	M1	M2	
Enzymatic activities (U g^{-1} of beads)	150±23	152±35	167±12	42±24	42±12	63±12	102±12	66±12	92±24
Immobilization yield (%)	38.8±5.2	66.2±2.4	23±5	8±2	2.7±2.1	43±3	52.2±6.2	29.2±4.6	19.7±2.7

In washing (Tris–HCl) and polymerization ($CaCl_2$ or $Na_5P_3O_{10}$) solution, activity is an U ml^{-1}.

M1 Method 1, which corresponds to ratio 1.2 lipase/3.2 alginate, *M2* method 2, which corresponds to ratio 1.2 lipase/6.4 alginate, *C* chitosan

It should be noted that these losses in the manufacturing process have been reported by several other workers [17].

Drying of Beads and Storage

The results of drying of fresh alginate and chitosan beads by freeze drying and fluidization were summarized in Table 2. The immobilization yield of chitosan beads was higher compared to the alginate beads dried under the same conditions, which is in agreement with earlier reports [13]. Thus, as compared to alginate beads, chitosan beads were more adapted to the inclusion of lipase produced by *Y. lipolytica*. According to this author, some ionic polymers including sodium alginate decreased the activity of lipase. The freeze-dried beads show a lower immobilization yield compared to fluidized beads, which is probably the result of the amount of enzyme lost to the bead surface during the freeze-drying process (no significant loss of activity for alginate and chitosan beads). Other studies suggested that the initial process of freezing might be the process where the major part of the lipase was lost [13]. This is because the freezing of water might have led to lipase remaining on the surface of the beads while the water froze. In addition, the immobilization yield in the case of alginate beads was found to decline after drying even though it was higher in fresh alginate beads compared to fresh chitosan beads. This can be possibly explained by the fact that alginate is more hydrophilic than chitosan, and therefore, water could be drawn in more rapidly, which causes a leaching of the enzyme from the beads. Determination of dry matter of dried beads (Table 2) suggested that the freeze-drying process allowed producing suitable beads for storage at 4 or 20 °C. Indeed, the results (Table 2) showed that the freeze-drying process allowed the retention of more than 80% of lipase activity after 4 months of storage at 4 and 20 °C while the activity does not exceed 34% in fluidized beads. Thus, the freeze-drying process was retained for further studies.

Beads Morphology

The results of Figs. 1 and 2 relating to the morphology of the beads show a significant difference between the microscopic aspects of two polymers. The drying process has an effect on the beads morphology. Comparatively to fluidization, drying by freeze drying of the beads makes them more friable, which could be the cause of enzyme leakage and could limit their reuse. In addition, the average bead size measured was 1.41±0.11, 2.43±0.01,

Table 2 Residual activity of immobilized *Y. lipolytica* lipase storage at 4 and 20 °C.

Polymers	Drying method (I.Y. %)	Dry matter (%)	*T* (°C)	0	1 month	2 months	3 months	4 months
Chitosan	Freeze dried (54)	94.88±0.02	4	100	98±4	94±6.1	90±5	88±6
			20	100	96±6	90±5.2	90±6	84±4
	Fluidized (57)	85.59±0.05	4	100	71±9	54±4	48±4.2	34±6
			20	100	57±5	38±4	28±3	22±4.1
Alginate	Freeze dried (36.1)	93.03±0.04	4	100	98±6	94±3	90±5	85±5
			20	100	96±6.2	88±6	86±5	80±2
	Fluidized (39.2)	86.69±0.02	4	100	71±7.1	58±5	44±4	33±6.2
			20	100	63±4.5	30±7	14±2	14±2.1

I.Y. Immobilization yield

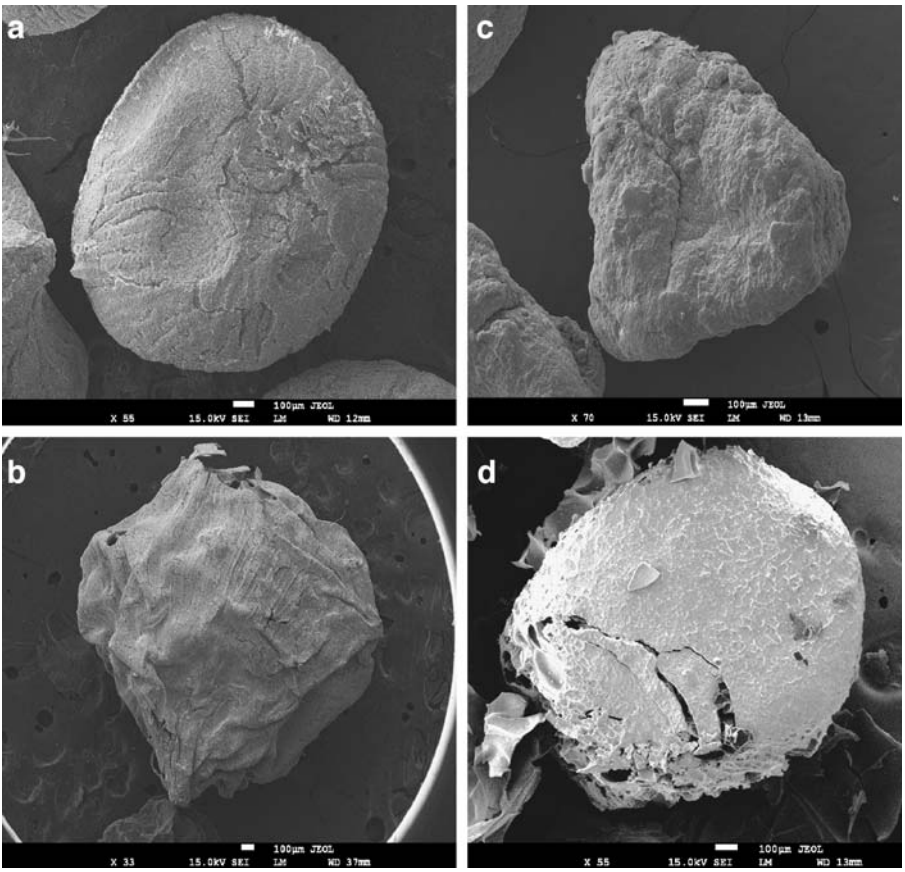

Fig. 1 Microphotographs of the beads obtained from: **a** alginate fluidized, **b** alginate freeze dried, **c** chitosan fluidized, and **d** chitosan freeze dried

1.19±0.01, and 1.50±0.05 mm for alginate fluidized beads, alginate freeze-dried beads, chitosan-fluidized beads, and chitosan freeze-dried beads, respectively. Immobilization yield of lipase immobilized in fluidized beads was higher than freeze-dried beads (Table 2). As expected, activities of lipase entrapped in the beads decreased as the bead size increased. Other workers have also reported that the activity of immobilized enzyme decreases with increasing bead size due to mass transfer resistance [18, 19]. The chitosan shows relatively irregular pores of an approximate size of 0.70 μm. This could explain partially brittleness of the chitosan. Certain authors [20] stipulate that the formation of the pores is due to the viscosity of the polymer, which prevents a complete interaction with the complexion agent. Alginate on the other hand seems to have a strong structure and does not have any macroporous structure.

Optimum pH and Thermal Stability of Lipase Immobilized by Inclusion

Lipase activity of various enzyme preparations (free and immobilized) was measured at 37 °C at different pH values ranging from 2 to 12. The results are shown in Fig. 3. The pH

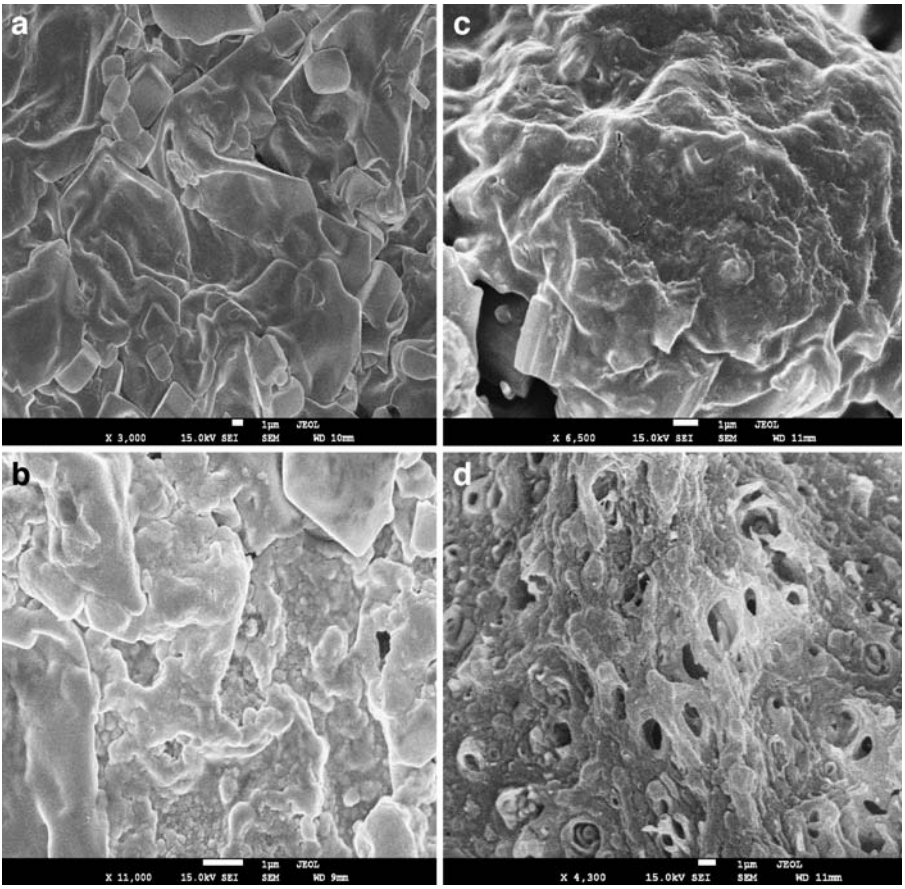

Fig. 2 Microphotographs of the beads surface obtained from: **a** alginate fluidized, **b** alginate freeze dried, **c** chitosan fluidized, and **d** chitosan freeze dried

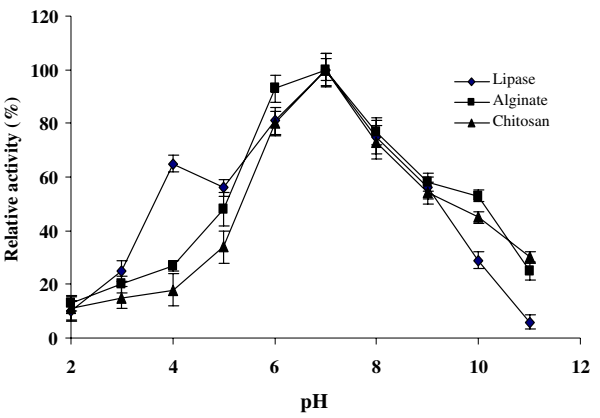

Fig. 3 Optimum pH of free and immobilized lipases by inclusion

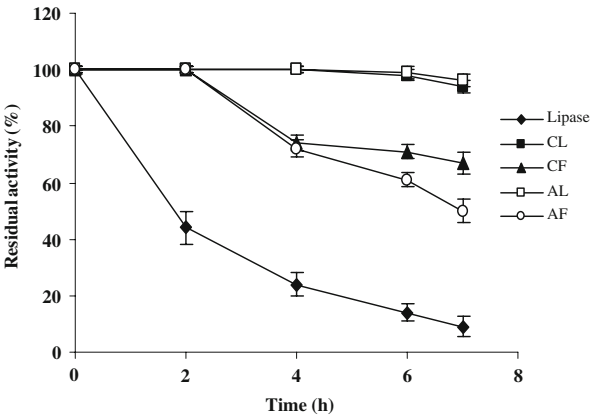

Fig. 4 Thermal stability (50 °C) of free and immobilized *Y. lipolytica* lipases by inclusion (*AL* Alginate freeze dried, *AF* alginate fluidized, *CL* chitosan freeze dried, *CF* chitosan fluidized)

activity profiles of lipase immobilized in chitosan and alginate gel were relatively similar to the soluble enzyme. The optimum pH of all preparations was 7. However, the immobilization seems to have a protective effect on the lipase at alkaline pH. In addition, thermal stability of the free and immobilized lipase was examined by preincubating these preparations at the specified temperature (50 °C) for 7 h in the absence of the substrate. These enzyme preparations were then equilibrated at the respective temperature and pH optimum for their activity before starting the assay. The results presented in Fig. 4 show that lipase activity was improved by immobilization. The free enzyme lost 80% to 90% of its activity at 50 °C for 7 h. In contrast, freeze-dried and fluidized entrapped enzyme conserves more than 80% and 60% of their activities, respectively. The greater stability of the immobilized enzyme may be ascribed to the stabilizing effects of immobilization [21, 22].

Repeated Use of Lipase-entrapped Beads

To test the stability of lipase entrapped in the alginate and chitosan beads, the freeze-dried and fluidized beads were used three times for the hydrolysis reaction. Each run lasted 15 min; after which, the beads were separated and washed with 50 mM Tris–HCl buffer (pH 7.2). The reaction medium was then replaced with fresh medium. The activity of freshly prepared beads in the first run was defined as 100%. Table 3 summarizes the results of repeated use of lipase-entrapped chitosan and alginate beads. Repeated use brought about activity loss (40% to 50%) in all cases. This might be mainly due to enzyme leakage from the beads. Another possible explanation is damage of the beads during repeated use [15].

Table 3 Repeated use of immobilized *Y. lipolytica* lipase.

	Alginate beads freeze drying	Alginate beads fluidizing	Chitosan beads freeze drying	Chitosan beads fluidizing
Relative activity (%) First use	100	100	100	100
Relative activity (%) Second use	44±6	56±5.2	36±4	52±6.2
Relative activity (%) Third use	15±3.5	30±5	10±2	35±6

Table 4 Yield of *Y. lipolytica* lipase immobilization by adsorption and covalent bond.

Immobilization method	Support	Relative activity of free lipase (%)	Immobilization yield (%)	
Adsorption	Celite	100	Method 1	Method 2
			57±2	76±5
	Silica gel		43±5	35±4.5
Covalent bond	Sepharose	100	70±4	

Method 1=ratio (1.2/6), Method 2=ratio (1.2/20)

Those authors suggested the coating of beads surface with polymeric materials. According to them, lipase entrapped in the coated beads retained higher activity than lipase entrapped in the uncoated beads.

Immobilization by Adsorption

Lipase immobilization by adsorption was carried out using several supports (celite, amberlite, silica gel, and the active carbon; data not shown). Highest activities immobilization yield of 76% and 43% were obtained with celite and silica gel, respectively (Table 4). The superior activity with celite has been attributed to greater affinity of lipase for celite than for silica gel or to lipase deactivation upon adsorption on silica gel. Wang et al. [14] reported 22.3% of lipase activity immobilized on celite compared to 14.8% for silica gel.

Optimum pH and Thermal Stability of Lipase Immobilized by Adsorption

The activity of the free lipase and enzymes immobilized is measured at different pH in the phosphate buffer. The profiles of activity of free lipase and immobilized enzymes toward pH 2 to 7 (Fig. 5) are similar, where maximum activities were found. The immobilization improves lipase activity to the alkaline pH. To study the thermal stability, the free and immobilized enzyme preparations were incubated at 50 °C during 4 h, and residual activities were determined in the periodically withdrawn samples (1 h). Results obtained are

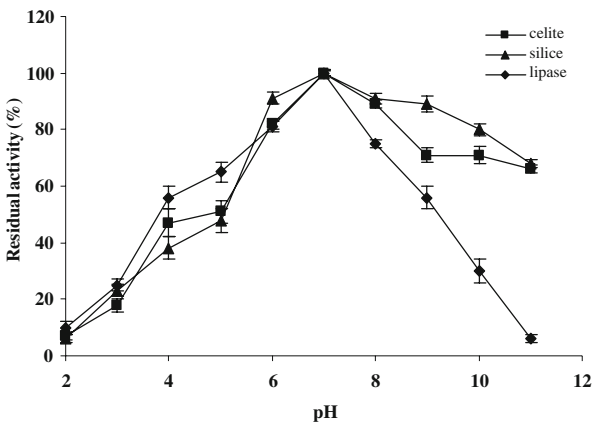

Fig. 5 Optimum pH of free and immobilized *Y. lipolytica* lipases by adsorption

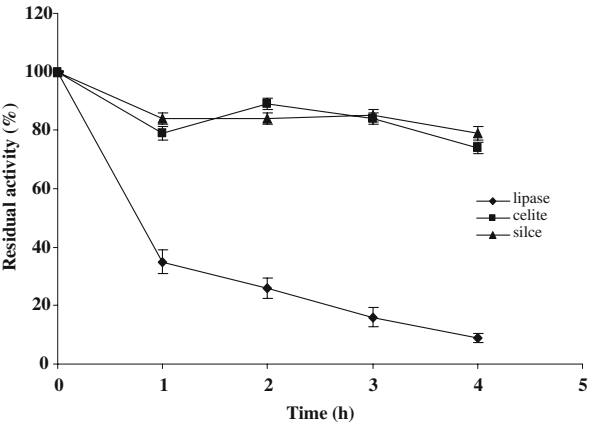

Fig. 6 Thermal stability of free and immobilized *Y. lipolytica* lipase by adsorption at 50 °C

illustrated in Fig. 6. A significant improvement in the thermal stability of immobilized enzyme over the native form was observed. Indeed, free lipase lost about 90% of its activity after 4 h, whereas immobilized enzymes retain more than 80% of their activity. According to the authors [23], the immobilization by adsorption seems to play an important role in the stabilization of the enzymatic protein conformation and its resistance to the thermal denaturation.

Effect of Solvents

The inclusion technique leads to enzyme leakage; for this reason, the lipase immobilized by adsorption was retained to study the effect of solvents.

Figure 7 summarizes the effect of the three solvents on immobilized and free lipase. The lipase exhibits higher activities when it was immobilized on silica gel and celite compared

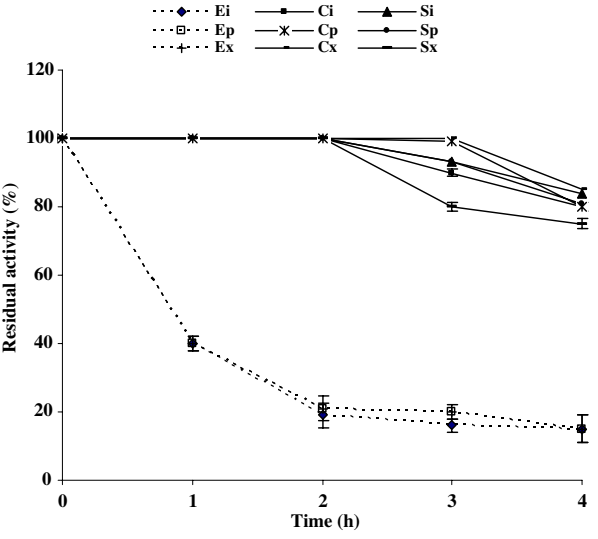

Fig. 7 Effect of three solvents on immobilized and free *Y. lipolytica* lipases; *Ei*, *Ep*, *Ex* free enzyme in isooctane, *n*-heptane, and *n*-hexane, respectively, *Ci*, *Cp*, *Cx* immobilized enzyme on celite in isooctane, *n*-heptane, and *n*-hexane, *Si*, *Sp*, *Sx* immobilized enzyme on silica gel in isooctane, *n*-heptane, and *n*-hexane

to the free form. Free lipase loses 80% of its activity in isooctane, *n*-hexane, and *n*-heptane, while immobilized enzymes retain 80% of their activities after 4 h in the solvents, which is in agreement with earlier studies [24]. In fact, it is well known that the polarity of the organic solvents employed for the esterification reaction affects negatively the enzyme activity [25]. It is generally recommended to use solvents with a log $P>4.0$ (nonpolar). The higher enzymatic activity in the nonpolar solvent may be attributed to the minimum distortion of the hydration layer around the enzyme by the solvent, whereas the polar solvent, due to its high affinity for water, might remove the essential hydration layer around the enzyme, thus decreasing enzyme activity.

Immobilized Enzyme Reactor

To study the use of the column in the synthesis reaction, esterase activity of the immobilized enzymes (celite and silica gel) has been measured in reactor column by the hydrolyze of a PNPA solution. The reusability of immobilized enzymes was examined by using the same enzyme preparation repeatedly, with a fresh amount of substrate (PNPA). Adsorbed enzymes showed a light gradual decrease in activity with an increasing number of cycles. Eighty percent of activity could be retained, even after five repeated cycles. Thus, a major drawback of free enzymes could be addressed by immobilization; however, storage of the column at ambient temperature seems to be difficult as immobilized enzymes loss about 50% of their activities after 4 days of use (data not shown).

Covalent Bond: Immobilized Lipase and Storage

From Table 4, it can be seen that the sepharose column can be employed as a support material for lipase produced by *Y. lipolytica*. An amount of 70% of free enzyme was immobilized on a proactive column. It is well known that chemical methods including covalent attachment are highly stable [26]. However, some authors suggest that these methods have the disadvantage of denaturing the native enzyme during the binding process.

In addition, after repeated use, the immobilized activity only slowly decreased. These catalyst was found to be stable (activity higher than 80% of the initial immobilized activity) during 5 months (data not shown). Similar results were obtained for immobilization of lipase from *Candida rugosa* in a previous work [27]. The establishment of the covalent bond between the support and the lipase attributes immobilized lipase good stability conformation that permits a long conservation.

Conclusion

Effects of *Y. lipolytica* lipase immobilization techniques on loading efficiency, thermostability, resistance to solvents, and ability to reuse were investigated. Three techniques were tested: (a) inclusion in alginate gel and chitosan, (b) adsorption in celite and silica gel, and (c) immobilization by covalent bond. Lipase activity at alkaline pH, thermostability, and resistance to solvents were enhanced by immobilization. In addition, lipase immobilization by inclusion does not allow multiple reuses of immobilized enzymes. This might be improved by coating the surface of beads with polymeric materials. However, adsorption and covalent bond allow a multiple reuse of the immobilized enzymes during a long period. These results are very promising for the use of the enzyme in interesterification or transesterification processes.

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