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Immobilization and Stability of Lipase from *Mucor racemosus* NRRL 3631

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The lipase from *Mucor racemosus* NRRL 3631was partially purified by fractional precipitation using 60% ammonium sulfate, which resulted in a 8.33-fold purification. The partially purified lipase was then immobilized using different immobilization techniques: physical adsorption, ionic binding, and entrapment. Entrapment in a 4% agar proved to be the most suitable technique (82% yield), as the immobilized lipase was more stable at acidic and alkaline pHs than the free enzyme, plus 100% of the original activity was retained owing to the thermal stability of the immobilized enzyme after heat treatment for 60 min at 45°C. The calculated half-lives (472.5, 433.12, and 268.5 min at 50, 55, and 60°C, respectively) and the activation energy (9.85 kcal/mol) for the immobilized enzyme were higher than those for the free enzyme. Under the selected conditions, the immobilized enzyme had a higher K_m (11.11 mM) and lower V_{max} (105.26 U/mg protein) when compared with the free enzyme (8.33 mM and 125.0 U/mg protein, respectively). The operational stability of the biocatalyst was tested for both the hydrolysis of triglycerides and esterification of fatty acids with glycerol. After 4 cycles, the immobilized lipase retained approximately 50% and 80% of its original activity in the hydrolysis and esterification reactions, respectively.

Keywords: Lipase, enzyme immobilization, agar, stabilization, *Mucor racemosus*

Lipases are hydrolytic enzymes that can catalyze a wide range of reactions such as hydrolysis, alcoholysis, esterifications, aminolysis, and enantiomer resolution [8, 22, 27, 33, 34]. Therefore, these ubiquitous enzymes are used in numerous commercial applications, including additives (flavor modification), fine chemistry (synthesis of ester), detergents (hydrolysis of fats), waste water treatment, leather (removal of fats from animal skins), pharmaceuticals, and cosmetics (removal of lipids, medicines, and enzymes for diagnosis) [11].

However, lipase catalysis involves dramatic conformational changes of the enzyme molecule. Lipases exist in two basic structural forms; in one, the active site of the lipase is secluded from the reaction medium by a helical oligopeptide chain, called the "lid", making it the inactive (closed) form, whereas in the other, the lid is displaced and the active side is exposed to the reaction medium, making it the active (open) form of the lipase. In homogeneous aqueous media, the lipase molecule is in equilibrium between these two structures, with the closed form predominating. However, in the case of exposure to a hydrophobic substrate, such as lipid droplets, only the open form of the lipase is able to interact, and thus the equilibrium shifts towards the open form (interfacial activation) [9, 37]. This equilibrium between two very dissimilar molecular forms also exists in most immobilized lipases.

Immobilization into solid carriers is perhaps the most common strategy used to improve the operational stability of biocatalysts, including better operation control, easier product recovery without catalyst contamination, and flexibility of the reactor design. In addition, decreased inhibition by reaction products, selectivity towards non-natural substrates, and better functional properties compared with the corresponding soluble enzymes, all make immobilization the preferred methods for improving enzyme stability [24]. The ideal immobilization processes should also limit the use of toxic or highly unstable reagents.

Several chemical or physical techniques can be applied to immobilize an enzyme onto a solid support. In the case of chemical immobilization, where covalent bonds are formed with an enzyme, the methods include enzyme attachment to a matrix by covalent bonds, cross-linking between the enzyme and a matrix, and enzyme cross-linking by multifunctional substances [4, 23]. Meanwhile, in the case of physical immobilization, where there is a weak interaction between the support and the enzyme, the methods include entrapment of the enzyme molecules, microencapsulation with a solid membrane, and adsorption

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in a water-insoluble matrix [16, 36]. Among all these methods, multipoint covalent attachment is the most effective in terms of thermal stabilization [15]. However, thermal stability has also been reported for gel-entrapped enzymes [13].

The immobilization of protein has several general drawbacks, including some distortion on the active side and a reduction in the overall mobility of the protein groups. Moreover, the significant conformational changes experienced by some enzymes during catalysis can be further distorted during immobilization, resulting in immobilized enzymes with fully altered catalytic properties [24].

Lipases have a specific immobilization protocol, consisting of interfacial activation in a hydrophobic support, which is a basic lipase mechanism and yields the "open state" of the enzyme [12, 25, 31].

In a previous report by the current authors [26], 29 different fungal species were tested for lipase production, and *Mucor racemosus* NRRL 3631 was shown to be the most promising organisms. Moreover, in a toxicity test, negative results were obtained for aflatoxin detection, thereby justifying the selection of *Mucor racemosus* NRRL 3631 for the current study.

Accordingly, this study made different immobilized lipase preparations and investigated the relative performances, such as the reusability. The properties of the free and immobilized enzymes were also compared.

MATERIALS AND METHODS

Microorganism

The *Mucor racemosus* NRRL 3631 was kindly obtained from the collection of the North Regional Research Laboratory (NRRL), U.S.A.

Lipase production

The *Mucor racemosus* NRRL 3631 was grown in 250-ml flasks containing 50 ml of a modified complex medium containing 3% peptone, 0.2% KH_2PO_4, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 1% glucose; 1% olive oil, and 0.01 M barium acetate as an additive; the pH was adjusted to 7 using a 0.2 M phosphate buffer [26]. The medium was sterilized by autoclaving for 15 min at a pressure of 1.5 lb/inch^2. The culture was incubated at 35°C on a reciprocal shaker (200 rpm) for 3 days.

Lipase Activity

The lipase activity was determined according to Parry *et al.* [29]. An emulsion was prepared by treating a mixture of olive oil and a gum Arabic solution in a top-drive homogenizer for 10 min. The reaction mixture contained 3 ml of the substrate (emulsion), 2.5 ml of deionized water, 1 ml of a 0.2 M Tris-HCl buffer (pH 7.5), and 1.0 ml of the lipase sample being tested. The reaction was carried out at 37°C for 2 h in a shaking water bath, and the reaction mixture was then supplemented with 10 ml of ethanol. The amount of oleic acid was determined by titrating the hydrolysis products with 0.05 N NaoH using a thymolphthalein indicator. A control sample was prepared and treated similarly using boiled enzyme samples. The lipase activity was measured under standard conditions (unless otherwise stated) and the values calculated as the average of three parallel determinations displaying a variation coefficient lower than 5%. The amount of enzyme catalyzing the formation of one micro equivalent (micromole) of oleic acid in 2 h at 37°C and pH 7.5 was taken as one unit of lipase activity.

1 U/ml=formation of U mol free fatty acids/1 ml enzyme solution.

The protein determination was carried out using the method of Lowry *et al.* [23].

Partial Purification of Lipase

The culture supernatant (900 ml) was fractionally precipitated using 60% ammonium sulfate and kept standing overnight at 4°C. The precipitate was then collected by centrifugation at 12,000 rpm for 30 min at 4°C and dialyzed against distilled water. The protein and enzyme activity was then determined in the dialyzed enzymatic fraction.

Immobilization of Partially Purified *Mucor racemosus* Lipase

Physical adsorption. This was carried out according to the method of Woodward [39], using 1 g of different carriers (alumina, polyvinyl alcohol, or silica gel) and 1 ml of the partially purified enzyme solution (containing 725 U/ml).

Ionic binding. This was also carried according to the method of Woodward [39], where 1 g of each wetted carrier or each cation or anion exchanger (Sephadex LH-20; Dowex LXB particle, size 0.075–15 mesh; Sephadex G75; DEAE-Sephadex A-50) was incubated with 1 ml of the enzyme and washed with a Tris-HCl buffer (pH 7.5) three times.

Entrapment. The partially purified enzyme solution was entrapped with 2%, 3%, 4%, or 5% calcium alginate gel beads according to the method of Bickerstaff [5]. Different concentrations of an agar or agarose were prepared to give a final concentration of 2%, 3%, 4%, and 5%, and then 1 ml of the partially purified enzyme was added to each of these concentrations. After solidification, the mixture was cut into 1-mm^3 fragments and washed with a Tris-HCl buffer (0.2 M, pH 7.5) to remove the unbound enzyme.

Hydrolytic Properties of Free and Immobilized Partially Purified Lipases

pH stability. The pH stability of the free and immobilized enzymes was examined by preincubating the enzyme samples at 30°C for 1 h at different pH values (4.0–9.0) and measuring the residual activity.

Thermal stability. The enzyme samples were exposed to different temperatures (30–60°C) at pH 7.5, with different incubation periods (15, 30, 60 min) for each temperature, and the residual activity was then measured.

Activation energy (Ea). This was determined by plotting the log of the relative activity of the assayed temperature against 1/T (Kelvin) of the free and immobilized samples.

Ea=slope×2.303 R (gas constant=1.976)

Half-life and deactivation constant rate. This was determined by plotting the log of the relative activity against time, according to the following equation

Half-life=0.693/slope

Deactivation energy=slope of the straight line

Test of lipase leakage from immobilized biocatalyst. The biocatalyst was suspended in 30 ml of Tris buffer (0.2 M at pH 7.5) under shaking conditions, and samples from the supernatant were periodically (2, 4, 6,....20 h) assayed for their lipase activity.

Reusability of Immobilized *Mucor racemosus* Lipase

Operational stability in hydrolysis reaction. The immobilized biocatalyst was incubated with the substrate in Tris buffer (0.2 M at pH 7.5) at 37°C for 2 h while being shaken. The immobilized enzyme was then collected by filtration through Whatman paper No. 1, washed with the buffer, and resuspended in a freshly prepared substrate to start a new run. For each run, the supernatant was assayed for lipase activity.

Operationd stability in esterification reaction. The previously prepared immobilized lipase was incubated with esterification reaction mixtures containing 0.002 M (0.6 ml) oleic acid and 0.12 M (11 ml) glycerol for 24 h, at 30°C and 200 rpm. using a rotating shaker. The immobilized enzyme was then washed with 0.2 M Tris buffer (pH 7.5) and resuspended in a freshly prepared esterification reaction mixture to start a new run. The previous reaction was stopped by adding 10 ml of chloroform and extracted with other volumes. The chloroform solution was evaporated using a rotary evaporator under reduced pressure. The resulting weighed product (100 mg) was applied as bands (alongside standard compounds) on a prepared silica gel plate (300 microns). The plate was then developed using a solvent system [diethyl ether:hexane:glacial acetic acid; 70:30:1 (v/v/v)]. The fractions corresponding to monoolein, diolein, non-reacted oleic acid, and triolein were visualized by iodine vapor and then scraped off the plate. The scraped fractions were extracted twice with moistened diethyl ether and the filtrate was then taken using a Pasteur pipette and evaporated gently to obtain a residue that was weighed accurately to determine the weight of each fraction. This was repeated for each cycle and the relative activity calculated for every run [38].

Immobilization parameters. The immobilization yield (IY) was calculated according to Eq. (1):

$$IY\% = [I/A - B] \times 100 \quad (1)$$

where A is the activity of the enzyme added to the immobilization solution, B is the activity of the unbound enzyme, and I is the activity of the immobilized enzyme

Relative activity %=activity of sample/activity of control×100

Specific enzyme activity (SEA)=activity of sample/protein content

Results and Discussion

A partially purified preparation was obtained by filtration of the broth to obtain a crude extract (specific activity 13.23 U/mg protein). The fraction obtained at 60% ammonium sulfate saturation showed the highest specific enzyme activity (110.27 U/mg protein) with 8.33-fold purification.

The partially purified lipase was immobilized with different carriers using different methods of immobilization. The efficiency of the enzyme immobilization was evaluated according to different parameters, including the retained enzyme activity, specific enzyme activity (SEA) of the immobilized enzyme, and loading efficiency (immobilized enzyme/g carrier). The immobilization yield is also a key

Table 1. Immobilization of partially purified *Mucor racemosus* lipases.

Method of immobilization	Carrier		Unbound enzyme (U/g carrier) B	Immobilized enzyme (U/g carrier) I	Immobilization yield (I/A-B)×100
Physical adsorption	Alumina		75.2±2	125.0±3.6	19.23
	Polyvinyl alcohol (PVA)		25.0±1.9	10.0±0.6	1.39
	Cellulose		15.0±3	10.0±0.2	14.41
	Silica gel		50.0±1.9	0.00±0.0	0.00
Ionic binding	Sephadex LH-20		125±3.2	115.0±4.1	19.16
	Dowex		115.0±2.9	115.0±3.9	18.85
	Sephadex G-75		120.0±4.1	75.0±3.6	12.39
	DEAE-Sephadex A-50		10.0±0.2	0.00±0.0	0.00
Entrapment	Agar	2%	95.0±4.2	325.0±6.6	50.79
		3%	115.0±3.3	375.0±6.8	61.47
		4%	125.0±2.6	495.0±7.0	82.50
		5%	106.0±3.4	331.6±5.8	53.63
	Agarose	2%	50.0±2.7	120.0±5.3	17.77
		3%	65.0±3.0	100.0±5.2	15.15
		4%	70.0±2.2	95.0±1.3	14.50
		5%	45.0±4.3	80.0±1.4	11.76
	Sodium alginate	2%	25.0±1.3	75.0±4.1	10.71
		3%	66.0±3.0	110.0±5.4	16.69
		4%	39.5±2.6	55.0±1.2	8.02
		5%	0.00±0.0	10.0±0.7	1.38

A =725 U/g carrier (added enzyme).

parameter, since it represents the general output and efficiency of the immobilization process. As such, agar proved to be the most suitable carrier (Table 1), as it gave the highest immobilization yield (82.5%). For currently available porous carriers, the activity retention at the maximum enzyme loading is often below 50%, owing to diffusion constraints [20]. The good results from just trapping the biocatalyst in a 4% agar may have been related to the formation of intermolecular aggregates. Palomo *et al.* [28] previously reported that lipase has a tendency of forming bimolecular aggregates, even at very low enzyme concentrations. A bimolecular structure of lipases can be formed by two open lipase molecules (interfacially activating each other) in very close contact, resulting in a very altered active center.

pH Stability

The pH stability was investigated after preincubating the enzyme at different pHs (4.0–9.0) for 1 h. At pH 7.5, the enzyme retained 100% of its activity in both forms. The immobilized lipase remained nearly stable at a pH range of 7.0–5.0 (Fig. 1), whereas the free enzyme showed a sharp decrease (77.11–46.73%). Both the free and immobilized enzymes lost about 50% of their activity at pH 8. The present results were very similar to those reported by Bagi *et al.* [2]. pH is known to promote changes in the partial configuration and activity of an enzyme [19]. However, lipases (agar biocatalysts) tend to form bimolecular aggregates [28], and changes to the aggregated state may not be possible at a pH range of 5.0–7.0, which in turn results in the stability of the immobilized enzyme.

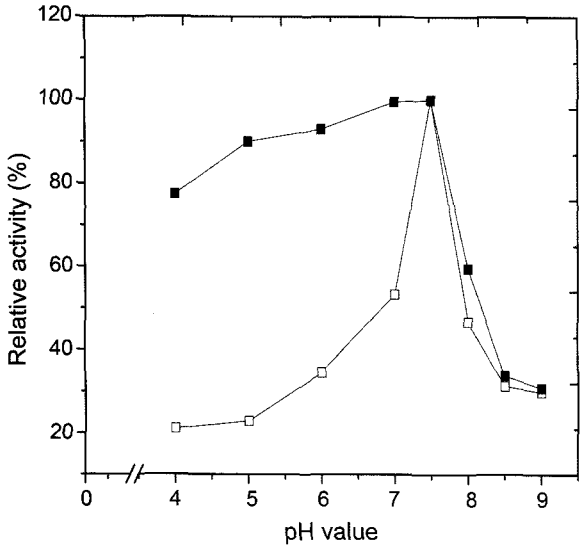

Fig. 1. pH stability of free and immobilized *Mucor racemosus* NRRL 3631 lipases.
Free (□) and immobilized (■) lipase samples were preincubated for 1 h at different pH values at 30°C, and then the residual activity was measured and the relative activity calculated. The activity of a sample without preincubation was taken as 100%.

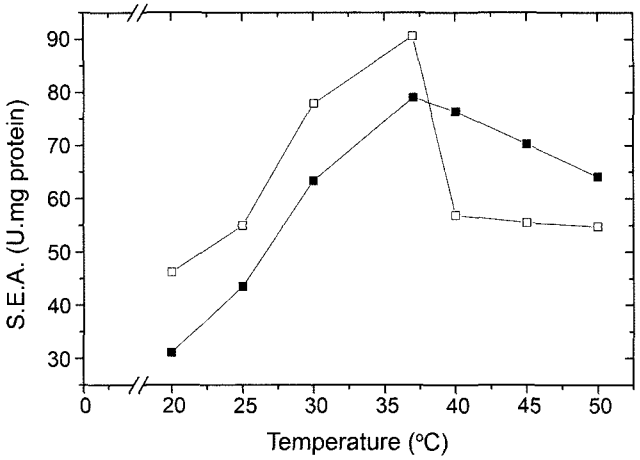

Fig. 2. Effect of assay temperature on free and immobilized *Mucor racemosus* NRRL 3631 lipase activity.
Free (□) and immobilized (■) lipase samples were added to a reaction mixture containing 3 ml of an emulsion, 2.5 ml of deionized water, and 1 ml of a 0.2 M Tris-HCl buffer. The reaction mixture was incubated at different temperatures for 2 h while shaking, and then the lipase activity was determined and the specific enzyme activity calculated.

Effect of Temperature on Activity of Free and Immobilized *Mucor racemosus* Lipases

The maximal specific enzyme activities of both forms of the enzyme were obtained at 37°C (Fig. 2). The immobilized enzyme exhibited higher values for the specific enzyme activity at temperatures from 40–50°C. The temperature data were reported in the form of Arrhenius plots (Fig. 3).

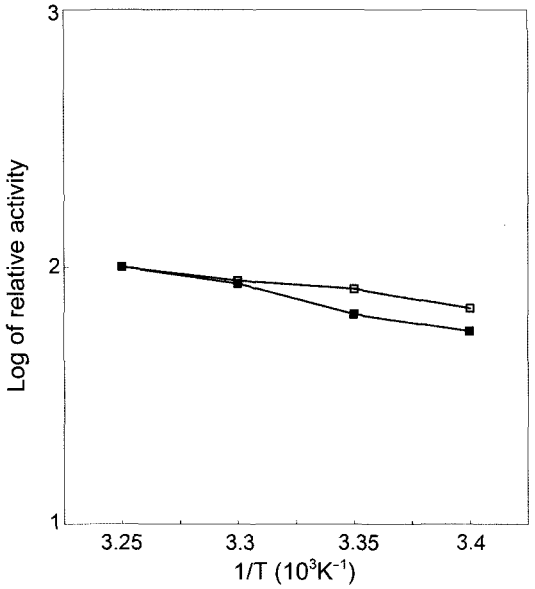

Fig. 3. Temperature data in the form of Arrhenius plots to calculated values of activation energies for free (□) and immobilized (■) *Mucor racemosus* NRRL 3631 lipases.
TK: Temperature in kelvin units.

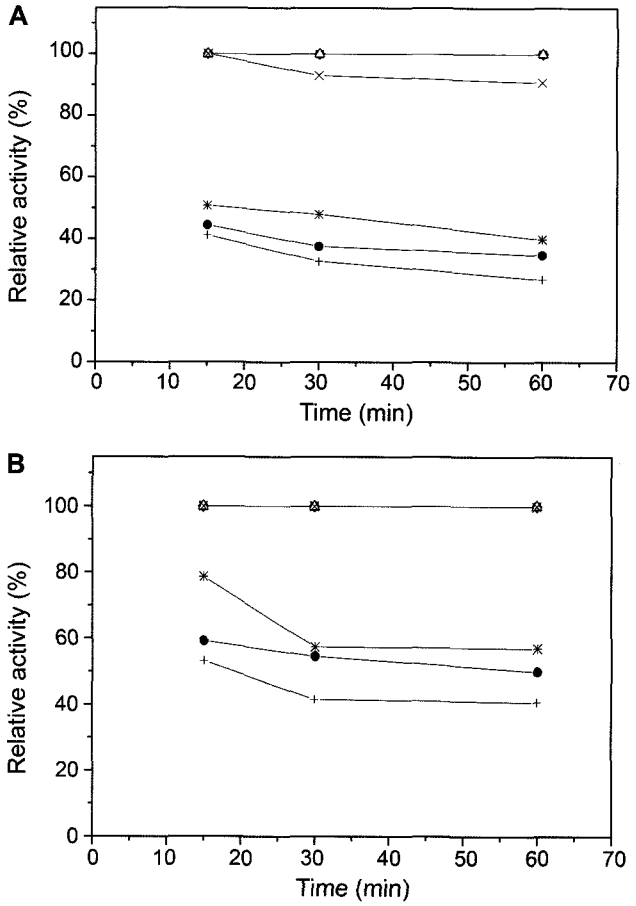

Fig. 4. Thermal stability of free and immobilized *Mucor racemosus* NRRL 3631 lipases.
Free (**A**) and immobilized (**B**) lipase samples were preincubated at 30°C (◆), 35°C (■), 40°C (△), 45°C (x), 50°C (*), 55°C (●), and 60°C (+) for 15, 30, and 60 min. Note: preincubation of the free enzyme at 30–40°C gave 100% activity after 15, 30, and 60 h, so 100% curves are overlapped. Moreover, using the immobilized enzyme, the 100% overlapped curves represent preincubation at 30–45°C.

The plots for the immobilized and free enzymes were linear, and the calculated values for the activation energies (Ea) were 9.85 and 4.55 kcal/mol, respectively.

Thermal Stability

The immobilized enzyme retained 100% of its activity at 45°C during all the preincubation periods tested (Fig. 4B). After 1 h at 50°C and 55°C, the immobilized enzyme retained 68.74% and 50% of its original activity, respectively, whereas the free one retained only 39.9% and 34.8%, respectively (Fig. 4A). At 60°C, the agar-immobilized lipase preserved about 40.74% of its original activity. Gomes *et al.* [14] found that the *Candida rugosa* lipase preserved about 40% of its original activity after preincubation for 1 h at 40–60°C. Thermal stabilization has previously been reported for gel-entrapped enzymes [16]. The better stability of an immobilized enzyme compared with the free form could be explained by the lipase location inside the micropores of the support (molecular confinement, which often occurs during the entrapment process), where the enzyme is protected against alterations of the microenvironment [6]. In the current study, the free and immobilized enzymes remained completely stable for 1 h at 30–40°C and 30–45°C, respectively.

Table 2. Activation energy, half-life, and deactivation rate constant for free and immobilized partially purified *Mucor racemosus* lipases.

Kinetic parameter	Partially purified free enzyme	Partially purified immobilized enzyme
Activation energy (kcal/mol)	4.55	9.85
Half-life (min) at		
50°C	311.8	472.50
55°C	280.0	433.12
60°C	173.25	268.50
Deactivation rate constant (min^{-1}) at		
50°C	2.22×10^{-3}	1.46×10^{-3}
55°C	2.46×10^{-3}	1.60×10^{-3}
60°C	4.00×10^{-3}	2.57×10^{-3}

When the log of the relative activity was plotted against time, at temperatures causing inactivation (50, 55, and 60°C), both the free and immobilized enzymes gave a straight line, meaning that the thermal inactivation process for both corresponded to the theoretical curves of a first-order reaction.

The calculated values for the half-life of the free and immobilized enzymes at different temperatures are represented in Table 2. The results showed that the immobilized enzyme was more thermostable than the free one; for example, the calculated half-lives of the free enzyme at 50, 55, and 60°C were 311.8, 280.0, and 173.25 min, respectively, which were all lower than those of the immobilized enzyme.

Effects of Different Substrate (Olive Oil) Concentrations on Lipase Activity

The investigation of the effect of the substrate concentration on the free and immobilized enzymes registered a low specific activity for the immobilized enzyme when compared with that for the free enzyme (Fig. 5). This drop in the specific activity (12.8–36%) after immobilization is a common phenomenon and could be attributed to a diffusion limitation of the substrate as regards the movement of products to and from the immobilization matrix, thereby causing problems not found with the free enzyme [19]. These diffusion processes often result in lower concentrations of the substrate at the enzyme active site than in a bulk solution. Consequently, the maximum reaction rate of the reaction

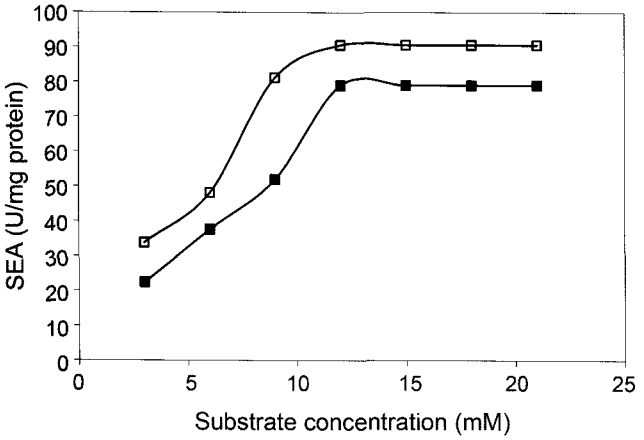

Fig. 5. Effects of different substrate concentrations on free and immobilized *Mucor racemosus* NRRL 3631 lipase activity.
Free (□) and immobilized (■) lipase samples were added to a reaction mixture containing different concentrations of olive oil (3–21 mM). The reaction mixture was incubated at 37°C for 2 h while shaking, and then the lipase activity was determined and the specific enzyme activity calculated.

catalyzed by the immobilized enzyme was lower than that catalyzed by the free enzyme [1]. However, no measurable changes were found to occur in the specific enzyme activities of the free and immobilized enzymes of *Mucor racemosus* with a substrate concentration of 12 mM. Possible explanations for this include the effect of the enzyme substrate concentration ratio, inhibition of the enzyme by the excess substrate concentration, or a change in the physicochemical characteristics [21].

The kinetic parameters for the Michaelis–Menten equation were determined from a double-reciprocal plot of the *Mucor racemosus* lipase activities for both the free and immobilized enzyme. The immobilized enzyme exhibited a higher K_m value (11.11 mM) than the free enzyme (8.33 mM), owing to the lower accessibility of the substrate to the active side of the immobilized enzyme. Meanwhile, the calculated maximal reaction rate (V_{max}) for the free enzyme (125.0 U/mg protein) was higher than that for the immobilized enzyme (105.26 U/mg protein). A decrease in the V_{max} after immobilization has already been reported by many authors [1]. This decrease may be due to the fixation of the enzyme within the immobilization carrier, thereby leading to a decrease in the flexibility of the enzyme molecule, which is commonly reflected by a decrease in the catalytic activity. Borkar *et al.* [7] determined the K_m and V_{max} values for the purified *Pseudomonas aeruginosa* SRT9 lipase in the case of the hydrolysis of triolein into triglyceride and pNP-palmitate and pNP-laurate into *p*-nitrophenyl esters. The K_m values were 1.11 mM for triolein as the substrate, and 0.11 and 0.037 for pNPL and p-NPP, respectively. Meanwhile, the V_{max} values were 0.055 mmol/l/min for triolein, and 161.3 and 188.6 mmol/l/min

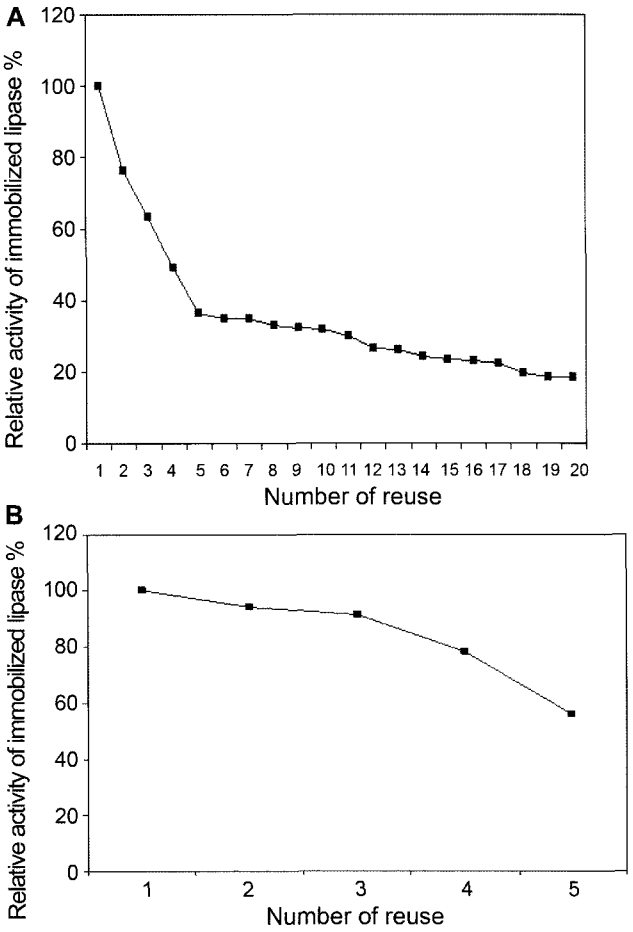

Fig. 6. Operational stability of immobilized *Mucor racemosus* NRRL 3631 lipase.
A. Reusability in the triglyceride hydrolysis process. **B.** Reusability in the esterification process using oleic acid and glycerol. See Materials and Methods section.

for pNPL and pNPP, respectively. Pimentel *et al.* [30] found that both soluble and immobilized *Penicillium citrinum* lipases exhibited Michaelis–Menten kinetics for the hydrolysis of 4-nitrophenylpalmitate, with a K_m value of 233 μM for the soluble enzyme and 276 μM for the immobilized derivative. Moreover, the *Candida cylindracea* lipase immobilized by adsorption to magnetic poly (VAC-DVB) microspheres showed a K_m value (4-nitrophenylacetate) of 3.6 mM at 20°C, whereas the K_m value for the native enzyme was 2.2 mM [17].

Operational Stability of Immobilized *Mucor racemosus* Lipase

The main advantage of immobilizing an enzyme is that it allows repeated use, which is important in the case of expensive enzymes. In this study, the stability of the immobilized system was assessed by reusing the immobilized *Mucor racemosus* lipase 4 times. After the fourth cycle, almost no

decrease in activity was observed (Fig. 6A). However, after the fifth cycle, only 36.6% of the initial activity was available.

Lipases in their natural environment catalyze the hydrolysis of esters formed from glycerol and long-chain fatty acids [35]. However, under appropriate experimental conditions, these enzymes are also very active biocatalysts for the esterification of fatty acids [3]. Thus, for a comparative study, the immobilized enzyme was used as a biocatalyst for the esterification of oleic acid and glycerol (see Materials and Methods). The results in Fig. 6B indicate that the biocatalyst could be used for 4 runs with a minimal loss of activity. In the second and third runs, the activity decreased by less than 10% when compared with the original activity; however, in the fifth run, there was only 55.63% residual activity. The activity loss in both reactions may have been related to inactivation of the enzyme caused by denaturation of the protein [18], or to the adsorption and accumulation of reaction products in the biocatalyst.

A decrease in activity due to enzyme leakage from the entrapment materials can be disregarded according to the results obtained from the leakage test, which indicated that the leakage during the first four cycles only increased from 0.2%–1%, whereas starting from cycles 5–10, the lipase leakage from the agar support decreased. Entrapment is a physical method for immobilizing or physically enclosing enzymes using the lattice structure of a gel. The pore size of the gel lattice can be controlled (using a 4% agar) so that the structure is tight enough to prevent enzyme leakage while allowing suitable movement of the substrate and products. The lower immobilization yield in the case of a lower concentration of agar as the carrier may have been due to the large pore size and consequently greater leakage of the enzyme from the matrix [10]. Using 4% agar as the support was sufficient to prevent enzyme leakage. The unique property of an agar, which allows it to be used as a mechanically stable support matrix, is its ability to form an aqueous colloid (sol) at a high temperature, which upon cooling is transformed into a gel network that does not revert back to the sol form under temperatures used for most enzymatic reactions.

For any application based on immobilized lipases, the feasibility of regenerating the lipase activity (and consequent reuse of the support) provides clear economic benefits for industrial use. Moreover, an agar is comparatively cheap and quite rigid in nature. Thus, it was demonstrated that immobilizing a lipase by entrapment in an agar gel was advantageous as regards the catalytic properties and stability.

The stability of the immobilized lipase was improved when compared with that of the free one. The additional advantage of the *Mucor racemosus* lipase was that the immobilized form exhibited an operational stability in both hydrolysis and esterification processes, and these two reactions are the most important applications of lipase for the production of certain high-value compounds.

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