Catalytic Potential of a Nitrocellulose Membrane-Immobilized Lipase in Aqueous and Organic Media

Ashok Kumar, Shamsher S. Kanwar

Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla 171 005, India

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ABSTRACT: Artificial membranes are ideal supports for enzyme immobilization and biocatalysis. The binding of a commercial lipase, Lipolase T20 on to nitrocellulose (NC) membrane was achieved by physical adsorption followed by cross-linking with formalin (2%). The NC-immobilized lipase was characterized for its catalytic activity and operational stability, using *p*-nitrophenyl palmitate (*p*-NPP) hydrolysis as a model reaction system. Functionally membrane bound lipase was more stable than free lipase (55°C) at higher temperature (65 and 75°C) and at pH 9.0 to 9.5. The hydrolytic properties of NC-bound lipase were studied consecutively as a function of physicochemical parameters. The NC-bound lipase was highly hydrolytic toward relatively longer C-chain length esters. This indicated a preferential selectivity of NC-bound lipase toward the *p*-NPP with an activity of 6.03 + 0.30 IU/cm^2. The nonionic detergents like Tween-20, 40, and 60 promoted the hydrolytic activity of membrane-bound lipase, while Tween-80 prompted a decline in the activity of NC-membrane bound lipase. Among various salt ions, Ca^{2+}, Al^{3+}, Zn^{2+}, and Cu^{2+} showed a stimulatory effect while Fe^{2+}, NH^{4+}, Cd^{2+}, and Zn^{2+} antagonized the hydrolytic activity of the bound lipase. The immobilized lipase was studied for its reusability and was found to retain >50% of hydrolytic activity after fifth repetitive cycle. In DMSO, the synthesis of octyl ferulate at 55°C under shaking (150 rpm) using ferulic acid (75 m*M*) and 1-octanol (100 m*M*) was achieved with a yield of 61 m*M* of ester (1-octyl ferulate) as analyzed by gas liquid chromatography. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: E37–E44, 2012

Key words: nitrocellulose membrane; immobilization; formalin; octyl ferulate

INTRODUCTION

Lipases, an extremely versatile class of enzymes are widely used in dairy, food, leather, detergent, cosmetics, pharmaceuticals, and organic syntheses industries to perform reactions in nonaqueous media.[1,2] The applications of enzymes in organic media rather than aqueous media have several important advantages such as the shift in thermodynamic equilibrium in favor of the product over the hydrolytic reaction, an increased solubility of nonpolar substrates, elimination of side reactions, and an increased thermal stability of the enzyme in harsh conditions.[3] For industrial applications, like most enzymes, lipases have often been immobilized onto insoluble or solid supports. Immobilization of lipase is regarded as a useful tool/ approach to increase its operational stability, easy recovery, and to improve the enzymatic activity in organic media.[4,5] In particular, nonsoluble as well as solid supports prevent the thermal inactivation of bound enzyme.[6] Artificial membranes are ideal supports for enzyme immobilization and easy separation of reaction products in a chemical reaction.[7] The fact that lipases are activated in the presence of aqueous/ hydrophobic interfaces has made scientists to find polymeric membrane as an efficient carrier for the immobilization of enzyme.[8–10]

Membrane reactors constitute an attempt to incorporate catalytic conversion, product separation and/ or concentration, and convenient recovery of biocatalyst. Furthermore, for enzymatic processes in anhydrous organic media, the maintenance of an appropriate hydrophilic microenvironment around the enzyme structure results in a clear improvement in the stability against water-stripping phenomena produced by the organic solvents.[11,12] It appears that in the hydrophilic membranes unlike in hydrophobic membranes, a large part of the immobilized lipase is active.[13] The immobilization of the lipase (Lipolase T20) on to nitrocellulose (NC) membrane has offered excellent opportunities for continuous enzymatic processes to be achieved in organic media. The post-treatment of immobilized enzyme with cross-linking agents like glutaraldehyde or formaldehyde may enhance the activity and operational stability of immobilized biocatalyst. In the

Correspondence to: S. S. Kanwar (kanwarss2000@yahoo.com).

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present study, the immobilization process (activation of dynamic membrane, cross-linking with formalin, and enzyme attachment) was studied to achieve optimal adsorption/binding of the lipase on to the NC membrane. The NC-immobilized biocatalyst was used in the continuous synthesis of octyl ferulate from ferulic acid and 1-octanol in an organic medium. Previously, polyacrylonitrile-based membranes were successfully applied as supports for the immobilization of a series of enzymes, such as urease,[14] glucose oxidase,[15,16] cellulase,[17] and amyloglucosidase.[18] The glutaraldehyde has been used by a number of researchers as a coupling agent in the immobilization of enzymes onto chitosan powders.[19]

Ferulic acid is an antioxidant compound readily isolated from maize waste, where it comprises up to 3% (w/w).[20] Alkyl esters of ferulic acid, such as octyl ferulate, have been observed to have higher antioxidant activity than the acid itself and the activity of octyl ferulate was reported to be comparable to butyl hydroxyl toluene.[21] Similarly, triterpene alcohol monoesters such as 24-methylene cycloartenyl ferulate and cycloartenyl ferulate also display antioxidant activities[22] and were shown to inhibit oxidation more effectively than ferulic acid.[21] The main highlight of our study is the use of a cheap, easily available nitrocellulose membrane to perform immobilization of a commercial lipase to study the hydrolytic properties of bound lipase as well as possibility of synthesis of octyl ferulate in an organic medium.

EXPERIMENTAL

Chemicals

Ferulic acid was procured from Merck Schuchardt, Germany; dimethylsulfoxide (DMSO from Sigma-Aldrich); *p*-nitrophenyl formate (*p*-NPF), *p*-nitrophenyl acetate (*p*-NPA), *p*-nitrophenyl benzoate (*p*-NPB), *p*-nitrophenyl caprylate (*p*-NPC), *p*-nitrophenyl palmitate (*p*-NPP) and *p*-nitrophenyl myristate (*p*-NPM) were purchased from Lancaster Synthesis, Eastgate White Lund, Morecambe, England; bovine serum albumin was procured from Sisco Research Laboratory, Mumbai, India; and 1-octanol from HiMedia Laboratories Mumbai, India. All other chemicals were of analytical grade and were used as received. NC membrane was purchased from Millipore Corporation, Bedford, MA. A commercial lipase, Lipolase-T20 was procured from Novo Nordisk S.A. (Denmark).

Activation of NC membrane and immobilization of lipase

NC membrane pieces (1 cm^2) were cut and dipped into 95% (v/v) ethanol. The membranes rinsed with HPLC grade water and equilibrated with Tris buffer (pH 7.5) were incubated at 4°C overnight.

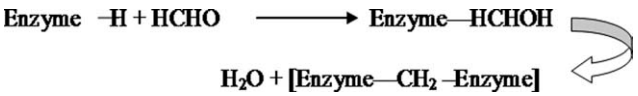

Figure 1 Reactions of formaldehyde with enzyme.

Immobilization of lipase on NC membranes

Lipase was immobilized onto NC membranes by physical adsorption. The Tris buffer (pH 7.5) equilibrated NC membranes (1 cm^2 pieces) were submerged in the lipase solution (10 mg/mL in Tris buffer 0.05*M*, pH 7.5) and the same were kept at 4°C for 12 h. The membranes were taken out and thoroughly rinsed with Tris buffer 0.05*M*, pH 7.5. The activity of NC membrane bound lipase was checked by a colorimetric method.[23] NC membrane bound protein was determined[24] indirectly using bovine serum albumin as a reference protein. After incubation of membrane with lipase, the supernatant was decanted to record its volume and unbound protein concentration. The protein loading onto NC membrane was determined by subtracting the protein content of the unbound enzyme (lipase) from the total lipase used for immobilization. The NC membrane bound lipase was cross-linked using 2% (v/v) formaldehyde (12 h at 8°C). The formaldehyde exposed membranes were thoroughly washed in 0.05*M* Tris buffer, pH 7.5. Two hundred NC membrane pieces (1 cm^2) were prepared for subsequent use to study the hydrolytic properties of the NC-bound biocatalyst as well as their esterification potential in organic medium.

Chemistry of cross-linking of NC-bound lipase with formaldehyde

Formaldehyde in water combines chemically to form methylene hydrate, HO-CH_2-OH (Fig. 1). Methylene hydrate molecules react with one another, to form polymer(s). The aldehyde group can combine with nitrogen and some other atoms of amino acids in proteins or with two such atoms if they are very close together, forming a cross-link "-CH_2-" called a methylene bridge. A lysine side-chain cross-links to a peptide nitrogen atom by formaldehyde reaction (Fig. 2). The binding of enzyme to NO_2 group on the NC membrane forms a polymeric network (Fig. 3).

Determination of hydrolytic activity of NC-bound lipase

The NC membrane-immobilized biocatalyst was used to perform the hydrolytic reactions by using *p*-NPP[23] unless stated otherwise. The reaction mixture

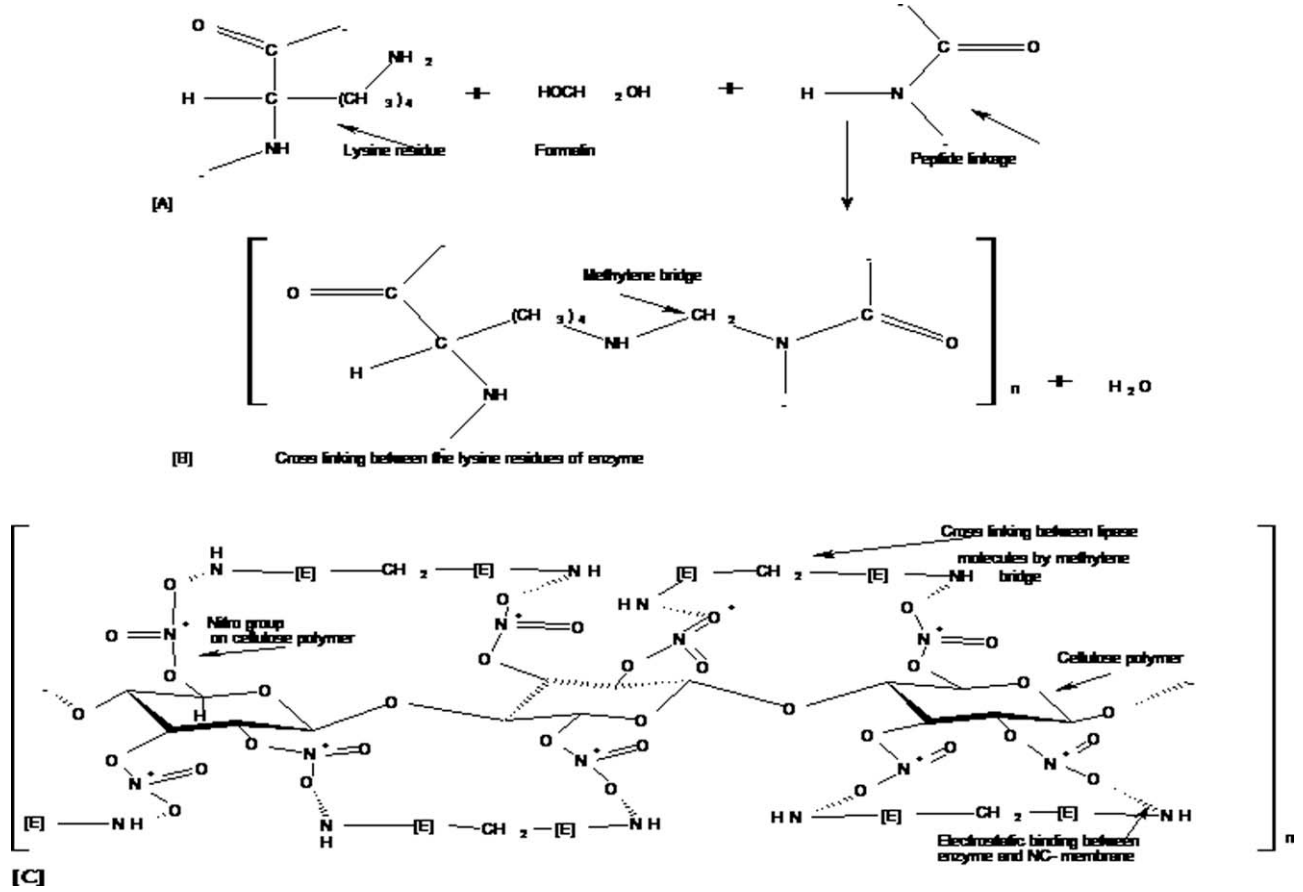

Figure 2 (A) Addition of a formaldehyde molecule(s) to lipase and formation of methylene bridge. (B) The formation of methylene bridge between the lipase molecules by formaldehyde results in a cross-linked network. (C) A detailed diagrammatic representation of the cross-linking of a lysine side-chain to a peptide nitrogen atom by formaldehyde reaction. [E] The enzyme molecule binds electrostatically to NO_2 group on nitrocellulose membrane and is also involved in covalent bonding to CH_2 group.

contained 100 μL of *p*-NPP stock solution (20 m*M* *p*-NPP prepared in isopropyl alcohol), 1 cm^2 membrane loaded with enzyme and Tris buffer (0.05*M*, pH 8.5 unless stated otherwise) to make final volume to 3 mL. The reaction cocktail was incubated at 55°C for 10 min in a water bath. The reaction was stopped by keeping the reaction mixture at −20°C for 10 min. An appropriate control with a native NC membrane (1 cm^2) was included with each assay. The absorbance of *p*-nitrophenol released was measured at A_{410}. Each of the assays was performed in triplicate, unless otherwise stated, and the mean values as well as standard deviations were recorded. One unit (IU) of lipase activity was defined as the micromoles of *p*-nitrophenol released per minute by the hydrolysis of *p*-NPP by 1 cm^2 membrane bound enzyme or 1 mL of free enzyme at 55°C under assay conditions. All the additives including buffer were preincubated at 55°C for a short period (3 min) before the enzyme was added to initiate the reaction.

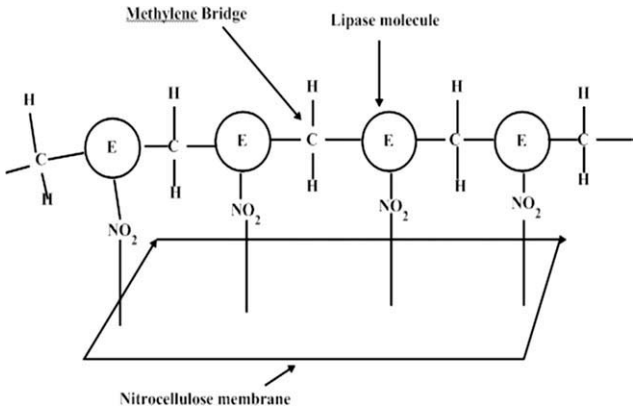

Figure 3 Schematic presentation of cross-linking of nitrocellulose membrane-bound lipase with formaldehyde.

Analysis of hydrolytic properties of NC-bound lipase

Effect of reaction temperature on the activity of the free and immobilized lipase was studied in a temperature range of 25 to 75°C under shaking (150 rpm). An optimum temperature was recorded for maximal hydrolytic activity of the free and the

bound lipase. To study the effect of pH on the stability of NC-bound lipase, the reactions were performed at selected pH (6–10) at 55°C for 1 h under shaking and the residual activity was measured. The optimum pH value at which NC-bound biocatalyst gave the optimum activity was selected to study the effect of specificity of the bound lipase toward various *p*-nitrophenyl esters prepared in 0.05*M* Tris buffer (pH 9.0) by performing hydrolytic reactions for 10 min at 55°C under shaking. The *p*-nitrophenyl acyl ester that yielded the maximum activity was selected to study the effect of various detergents/ surfactants, salt ions, and chelating agents under optimized conditions. The NC-bound lipase (1 cm^2) was preincubated with 1% (v/v) of selected detergent (Tween 20, Tween 40, Tween 60, Tween 80, and Triton X-100) or chelating agent (EDTA and sodium citrate, 1 m*M*) one at a time at 55°C for 1 h and the observed hydrolytic activity was recorded, thereof. The effect of various salt ions (Al^{3+}, NH_4^+, Mg^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Ca^{2+}, and Zn^{2+} as chloride salt) on the hydrolytic activity of immobilized lipase was also studied by preincubating each of the selected salt ions for 1 h at 1 m*M* final concentration in the reaction buffer (pH 9.0; 0.05*M* Tris). The reusability of the NC-bound lipase to repetitively catalyze the fresh hydrolytic reactions under optimized reaction conditions was also explored. Each fresh batch of hydrolytic reaction comprised *p*-NPP (prepared in 0.05*M* Tris buffer, pH 9.0) and the reaction was performed 55°C for 10 min under shaking. After first use, the biocatalyst was recovered by decantation of reaction mixture after the completion of the reaction, washed twice with 0.05*M* Tris buffer, pH 9.0, and used to catalyze the fresh reaction. The hydrolytic activity of NC-bound lipase was recorded after each use up to five cycles. All the above hydrolytic reactions were performed under shaking (150 rpm) in triplicate using a final reaction volume of 3 mL. The mean values ± standard deviation (SD) about the mean were recorded and presented.

Analysis of ester synthesized by NC-membrane immobilized lipase

The enzymatic esterification of ferulic acid (75 m*M*) and 1-octanol (100 m*M*) in DMSO was carried out with NC membrane-bound biocatalyst (1 cm^2) in 5 mL capacity capped Teflon-coated glass vials. Each of the reactions (2 mL reaction volume) was performed in triplicate and average values ± SD were calculated. Samples (100 μL) were withdrawn at specified intervals and analyzed by gas liquid chromatography (GLC). The octyl ferulate produced in the samples was analyzed by GLC equipped with a flame ionization detector and a packed type column (15% SE-30 Chrom WHP, 2 m length, mesh size 80–100, internal diameter 1/8 inches, maximum temperature limit 300°C; Netel Chromatograph, Thane, India). Nitrogen was used as a carrier gas (30 cm^3/ min). The injector was set at 260°C, detector at 270°C and the column/oven temperature was set at 250°C. The sample size for the GLC analysis was 2 μL.

RESULTS AND DISCUSSION

The effects of various physicochemical parameters on the hydrolysis of *p*-NPP and esterification reaction by the NC-bound lipase were studied systematically. In living cells, lipases catalyze the hydrolysis of long-chain fatty acid triacyl-glycerols (natural substrates) in aqueous media, and the similar model reactions involving hydrolysis of many natural and synthetic esters has also been achieved.[25] In organic media, enzyme does not work efficiently so we need some kind of modification to increase its stability/ activity to promote hydrolytic as well as esterification reactions. The immobilization of an enzyme on a suitable support often improves its stability, and thus immobilized lipase derivative can be characterized by analyzing changes in its "natural" enzymatic behavior/ hydrolytic activity by using a long-chain fatty acid synthetic substrate (i.e. *p*-NPP). The ability of the immobilized derivative to perform or not perform a synthetic reaction can be tested in organic medium by choosing another reaction model of industrial interest (i.e. 1-octyl ferulate synthesis).

Optimization of the enzyme immobilization process on nitrocellulose membrane

NC-membranes with immobilized commercial lipase "Lipolase" were prepared by adsorption followed by cross-linking of lipase. Formaldehyde was used as a cross-linking agent to produce the NC membranes with approximate 16% binding of protein (5.4 IU/cm^2). The NC membrane- bound lipase could be modified to enhance its enzymatic activity in two ways; either by increasing the potentially reactive centers for covalent linkage or by improving the attachment conditions[26] that could be possibly be achieved with formaldehyde or glutarayldehyde. The fixative action of formaldehyde is probably due entirely to its reactions with proteins. The reaction of lipase-bound formaldehyde with another enzyme molecule results in the condensation and removal of water molecule (Fig. 1). Most of the functional groups of proteins, such as amine, thiol, phenol, imidazole, and amino acid side chains are nucleophiles which can easily bind to aldehyde group.[27,28] The lysine residues, usually located on the surface of the lipase are otherwise not involved in the catalytic site, which allow the cross-linking by electrostatic interaction with formaldehyde to preserve the

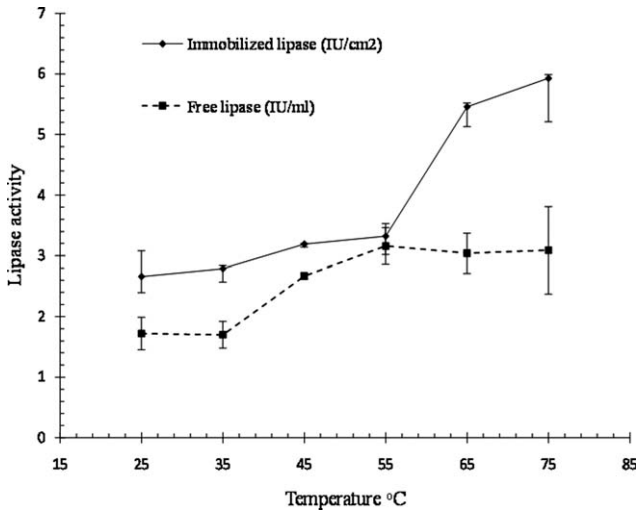

Figure 4 Effect of temperature on the activity of free and NC membrane-immobilized lipase.

protein conformation and its catalytic activity, too (Fig. 2). The surface modification with a cross-linking agent often traps the enzyme molecules within the pores of matrix that still allows reactant and product molecules to diffuse in and out of the pores. In a previous study functionalized hydrophobic polypropylene chloride membrane (PPC) aminated with chlorinated polypropylene with hexamethylene diamine was used for covalent immobilization of *Candida rugosa* lipase via glutaraldehyde coupling. The retained activity of the immobilized lipase was 76%.[29] In a recent study chitosan pretreated with glutaraldehyde, was used as support material preserving the catalytic activity almost intact and offering maximum immobilization capacity (76% and 91%, respectively). The chitosan-immobilized lipase could be repeatedly used for 10 cycles with more than 80% of its initial hydrolytic activity. A shift in the optimal temperature from 50 to 60°C and in the pH from 9.5 to 10, were observed for the immobilized lipase when compared with the free enzyme.[30] Also *Candida antarctica* lipase immobilized on Eupergit C (EC) was used for kinetic resolution of (R/S) 4-[2-hydroxy-3-[(1-methylethyl)amino] propoxy]benzeneacetamide (rac-atenolol) in an organic medium. Enantiomeric resolution of atenolol was carried out by a *trans*-esterification reaction using vinyl acetate as acylant and organic solvent as a reaction medium.[31]

Analysis of hydrolytic properties of NC membrane-immobilized lipase

Effect of temperature on the lipase activity

Effect of temperature on the activity of the free and immobilized lipase was studied in the temperature range of 25 to 75°C (Fig. 4). The membrane bound lipase was quite stable even at enhanced temperatures of 65 to 75°C as compared with free lipase (55°C). Unfortunately, the *p*-NPP undergoes autodegradation at a temperature of 65°C and higher. Hence subsequent hydrolytic reactions were performed at a temperature of 55°C. Often, enzymatic reaction becomes faster at an increased temperature, however, free enzyme may become denatured at higher temperature. The decline in the lipase activity of free lipase at temperature >55°C indicated the denaturation of the enzyme that did not happen in case of NC-membrane bound lipase even at temperature ≥65°C. The hydrophobic interaction between lipase and nitrocellulose that has increased the rigidity of membrane-lipase complex possibly avoided the distortion of lipase protein at high temperature thereby preventing enzyme from being leached away/and or any alteration in its tertiary structure accounting for retention of its hydrolytic activity.

Effect of reaction buffer pH

To investigate the effect of pH of reaction buffer on the stability of NC-bound lipase, the reactions were performed at selected pH (6–10) at 55°C for 60 min and the residual lipase activity was measured. The pH variations in the reaction medium can affect the stability of enzyme to consequently alter its activity (Fig. 5). It appeared that NC-bound lipase became more stable at high pH (pH 8–9.5) as it displayed better hydrolytic activity whereas the free lipase showed a sharp decline in its activity at pH >8.5. These observations indicated that the free or NC-bound enzyme was broadly alkaliphilic in nature. However, the activity of NC membrane-bound lipase improved even at raised alkaline pH. The lower hydrolytic activity of the NC-bound lipase at acidic pH may be associated with a change in enzyme's tertiary-structure that had been altered by

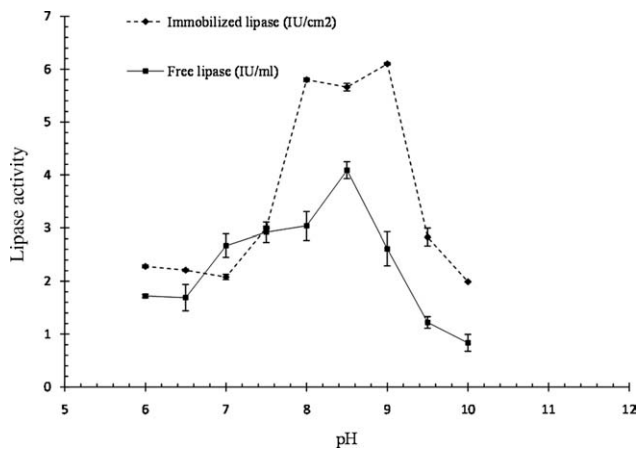

Figure 5 Effect of pH on the activity of free and NC membrane-immobilized lipase.

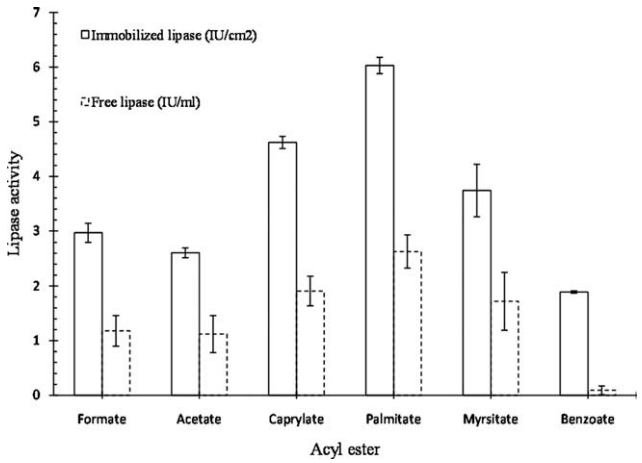

Figure 6 Effect of C-chain length of acyl ester (substrate) on the activity of free and immobilized lipase.

the absorption of excessive H^+ ions leading to irreversible enzyme denaturation. Alternatively, the pH effects could also be manifested due to partitioning of hydrogen ions between the solution and the support surface.[32]

Effect of C-chain length of acyl ester (substrate) on immobilized lipase

Both free as well as NC membrane-bound commercial lipase preparations were highly hydrolytic toward the relatively longer C-chain length esters (Fig. 6). This indicated a preferential specificity of "Lipolase" toward the *p*-NPP (bound lipase: 6.03 ± 0.30 IU/cm^2; as compared with free lipase: 2.63 ± 0.3; Fig. 5). NC-bound lipase also showed good hydrolytic potential toward *p*-NPC (4.62 ± 0.71 IU/cm^2) and *p*-NPM (3.74 ± 0.53 IU/cm^2). The specificity toward hydrolysis of *p*-nitrophenyl acyl derivatives is an individual specific property of any of the lipases obtained from diverse sources of microbes.

Effect of surfactants on the activity of NC membrane immobilized lipase

The influence of various nonionic detergents on the stability and catalytic activity of a membrane bound lipase were recorded. The NC membrane-bound lipase preincubated with 1% (v/v) Tween-20, Tween-40, Tween-60, and Triton X-100 at 55°C had a positive effect on bound-lipase activity (Fig. 7). However, Tween-80 (polyoxyethylene sorbitan monooleate) caused a decline in the hydrolytic activity of the NC-bound lipase. Tween-80 with C18 chain length appeared to prompt a sharp decline in the activity of the bound enzyme possibly because of strong surfactant effect and or its action as a competitive substrate that is preferred for hydrolysis by the NC-bound enzyme than *p*-NPP. In a previous study Tween 80 was found to increase the lipase activity of *Burkholderia glumae* in the production medium due to involvement of fatty acyl ester bond that functions as an inducer of lipase operon.[33] However, following, exposure to Tween-60, the NC-bound lipase displayed (5.68 ± 0.05 IU/cm^2) 40% higher lipase activity at 55°C than the control.

Effect of chelating agents

The chelating agents such as EDTA and sodium citrate may influence the activity of NC-bound biocatalyst. Each of the selected chelating agents (1 m*M*) when preincubated separately with the NC-bound lipase enhanced the activity of immobilized lipase with respect to control (without chelating agent; Fig. 8). However, EDTA appeared to strongly modulate the hydrolytic activity of the NC-bound lipase (4.63 ± 0.02 IU/cm^2) while a decline in the activity of the free lipases was observed in the presence of EDTA. Previously, an increase in the activity of lipase from *Bacillus coagulans* MTCC-6375 has been reported by 20 m*M* EDTA.[34]

Effect of salt ions

The Ca^{2+}, Al^{3+}, Zn^{2+}, and Cu^{2+} ions showed a stimulatory effect on the NC-bound lipase while only Zn^{2+} had a positive effect. Moreover, Fe^{2+}, NH_4^+, Cd^{2+}, and Zn^{2+} ions had antagonistic effect on the activity of the bound lipase as well as free lipase. In a previous study on *Burkholderia multivorans* V2, the exposure to ions such as Ca^{2+}, Mg^{2+}, and Mn^{2+} stimulated the lipase activity while Cu^{2+}, Fe^{2+}, and Zn^{2+} ions antagonized the biocatalytic potential of lipase.[35] The inhibitory nature of transition metals has been thought to be due to interaction of ions with charged side chain groups of surface amino acids, thus influencing the conformation and stability of the enzyme.[36] However, in the present study interestingly, the activity of NC-bound lipase was

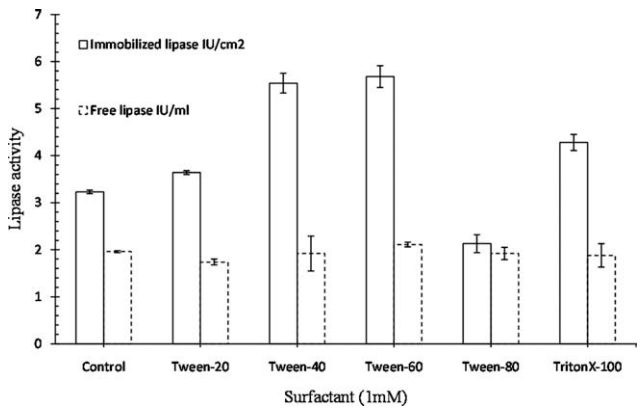

Figure 7 Effect of detergents on the activity of free and NC-immobilized lipase.

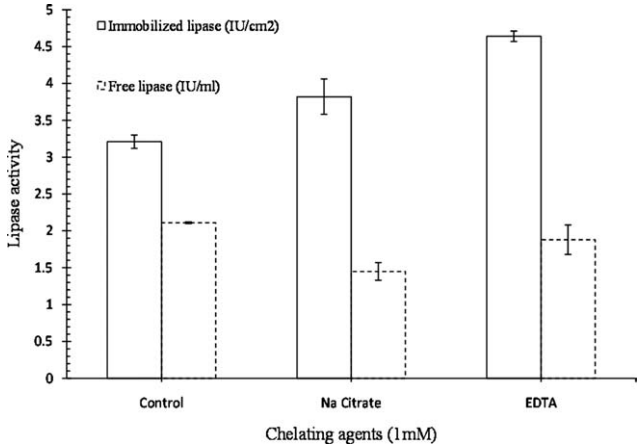

Figure 8 Effect of chelating agents on the activity of free and immobilized lipase.

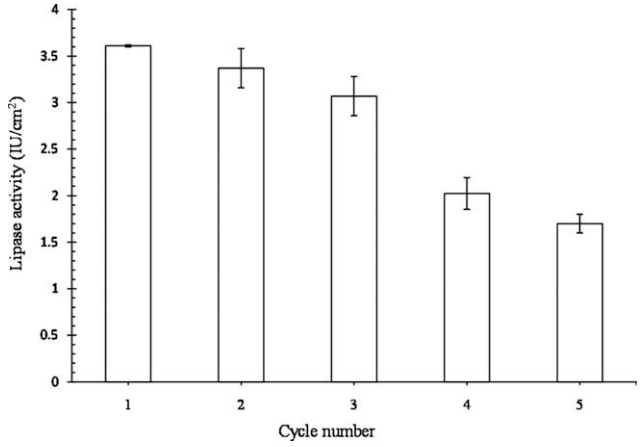

Figure 10 Repetitive use of NC membrane-immobilized lipase for hydrolysis of *p*-NPP. The hydrolytic reaction was carried out at 55°C.

increased by Al^{3+}, Zn^{2+}, Ca^{2+}, and Cu^{2+} ions in that order (Fig. 9).

Repeated use of NC membrane-immobilized lipase

In general, free enzymes are difficult to be recovered out of the reaction system and reused. The recovery and reusability of immobilized lipase on the matrices are therefore, important aspects that deserved investigation. In the present study, the NC-bound lipase was repeatedly used as a biocatalyst to perform hydrolytic reactions. The hydrolytic activity of NC membrane-bound lipase was recorded after each use up to five cycles at 55°C. The NC-bound lipase retained more than 50% of its original activity after fifth repetitive cycle of hydrolysis (Fig. 10). In a previous study, the hydrogel bound lipase was repetitively used six times for the synthesis of isopropyl myristate, and 38.2 ± 2 m*M* conversion was recorded after third esterification cycle.[37] It means that generally some leaching of biocatalyst from the nonsoluble supports at each successive cycle of hydrolysis/esterification is unavoidable possibly because of somewhat hydrophobic nature of the reactants and/or products. However, the rate of leaching of enzyme from the membrane is a significant factor for its application in different solvent systems at industrial scale.

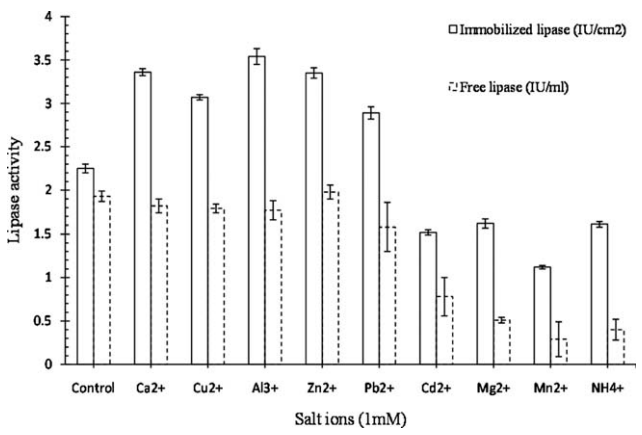

Figure 9 Effect of salt ions on the activity of free and immobilized lipase.

Esterification of ferulic acid and 1-octanol using NC membrane-bound lipase

The activity of the NC-immobilized lipase was also investigated by using a model reaction involving esterification of 1-octanol with ferulic acid to produce octyl ferulate. In kinetically controlled synthesis, the lipase involves hydrolysis to produce ester.[38–40] In order to improve the ester synthesis, it is necessary to optimize various parameters that affect the enzyme activity or solubility of reactants and/or products. Enzymes are in fact, very sensitive

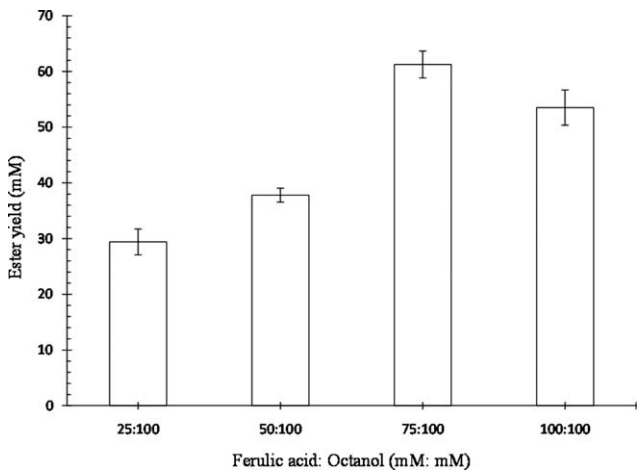

Figure 11 Synthesis of octyl ferulate using NC membrane-bound lipase. The reaction was carried out at 55°C for 3 h under shaking at 150 rpm.

to high temperature, because of their basic protein nature. However, the lipase immobilized on the NC membrane was stable at 65 to 75°C. Unfortunately, the ferulic acid used in the esterification reaction can easily undergo oxidation at high temperature above 60°C. Thus the reaction was carried out at 55°C with 75 m*M* ferulic acid and 100 m*M* 1-octanol. After 3 h of incubation, 61.02 ± 0.5 m*M* yield was obtained in DMSO (Fig. 11). These results showed that NC membrane-bound lipase possessed adequate synthetic activity, emphasizing its suitability to efficiently perform octyl ferulate synthesis in an organic medium, too.

CONCLUSION

The effectiveness of an immobilization process depends on the nature of the support used. The stability and retention of the lipase activity shown by membrane-bound lipase was favorable to perform hydrolytic as well esterifications reaction in aqueous and organic medium, respectively. The NC-membrane is an easily available support that can be efficiently used to bind enzymes such as lipases to perform repetitive reactions in most of the organic and aqueous solvents moreover the ease of recovery and easy handling makes it more attractive biocatalyst.

The cross-linking/stabilization of the NC membrane-adsorbed protein/ lipase molecules with formaldehyde play an important role in enzyme's stability. To achieve high enzyme loading and catalytic efficiency for large-scale operation and application, supports with high surface to volume ratio, are often desirable. Bioconversions, which require a stable catalyst delivery, could be optimally performed in a membrane reactor with the biocatalyst uniformly spread in the reaction system. Moreover the amount of biocatalyst can be increased by increasing the size of the membrane-bound biocatalyst.

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