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Electrospun polyacrylonitrile nanofibrous membranes for lipase immobilization

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Abstract

Polyacrylonitrile (PAN) nanofibers could be fabricated by electrospinning with fiber diameter in the range of 150–300 nm, providing huge surface area for enzyme immobilization and catalytic reactions. Lipase from *Candida rugosa* was covalently immobilized onto PAN nanofibers by amidination reaction. Aggregates of enzyme molecules were found on nanofiber surface from field emission scanning electron microscopy and covalent bond formation between enzyme molecule and the nanofiber was confirmed from FTIR measurements. After 5 min activation and 60 min reaction with enzyme-containing solution, the protein loading efficiency was quantitative and the activity retention of the immobilized lipase was 81% that of free enzyme. The mechanical strength of the NFM improved after lipase immobilization where tensile stress at break and Young's modulus were almost doubled. The immobilized lipase retained >95% of its initial activity when stored in buffer at 30 °C for 20 days, whereas free lipase lost 80% of its initial activity. The immobilized lipase still retained 70% of its specific activity after 10 repeated batches of reaction. This lipase immobilization method shows the best performance among various immobilized lipase systems using the same source of lipase and substrate when considering protein loading, activity retention, and kinetic parameters.
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1. Introduction

Enzyme immobilization has been a popular strategy for most large-scale applications due to the ease in catalyst recycling, continuous operation, and product purification. Furthermore, immobilizing enzymes onto various insoluble or solid supports is a useful tool to increase their thermal and operational stabilities [1]. Poor biocatalytic efficiency of immobilized enzymes, however, often limits the development of large-scale bioprocessing to compete with traditional chemical processes. Improvements of biocatalytic efficiency can be achieved by manipulating the structure of carrier materials for enzyme immobilization [2]. Non-porous materials, to which enzymes are attached to the surfaces, are subject to minimum diffusion limitation but enzyme loading per unit mass of support is usually low [3]. On the other hand, porous materials can afford high enzyme loading, but suffer a much greater diffusional limitation of substrate [4].

Reduction in the size of enzyme-carrying materials can generally improve the efficiency of immobilized enzymes. Nanomaterials provide many key factors that the structure of carrier materials for better catalytic activity will need, including surface area, mass transfer resistance, and effective enzyme loading. Various nanomaterials, such as nanoparticles, nanofibers, and nanotubes have been shown to be potential candidates for preparing nanoscale biocatalysts [5,6]. The high surface area to volume ratios of nanomaterials has been the principal driving force for developing nanoscale biocatalysts, in addition to other unique behaviors that distinguish them from traditional immobilized systems.

Nanoparticles, such as silica, magnetite and gold provide an ideal solution to the usually contradictory issues encountered in the optimization of immobilized enzymes, as high surface area and enzyme loading are usually accompanied by high mass transfer resistance within the supports [7,8]. In the cases of nanoparticles, nevertheless, their dispersion in reaction solution

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and the subsequent recovery for reuse are often difficult. The handling of dry powders of nanoparticles also presents certain health and environmental concerns [9]. Similarly, carbon nanotubes, which exist in powder form when in a pure state, also share the same concerns when used as enzyme supports [9]. Nanofibers, however, have the benefit of being much easier to produce and handle without sacrificing the high surface area to volume ratio, which is two-thirds that of nanoparticles.

Electrospinning (ES) is a simple and versatile method for producing nanofibers from a variety of materials with fiber diameters ranging from several micrometers down to tens of nanometers [10–12]. During the ES process, nanofibers are processed under a high-voltage electrostatic field operated between a metallic nozzle of a syringe and a metallic collector. The changed polymer solution jetted from the metal needle to the grounded collector. In the working distance, the polymer jet elongates, solidifies, and deposits on the collector. The fibers are deposited in the form of a non-woven fabric onto the target collector through a random deposition process of projected jet of polymer solution [13]. The most attractive feature of this approach lies in its ability to generate non-woven mats or uniaxially aligned arrays of nanofibers with well-controlled compositions and diameters. The electrospun nanofibrous membrane (NFM) has high specific area and porous structure, so there are excellent candidates for filtration, drug delivery carrier, tissue engineering, wound dressing, nano-sensors and enzyme immobilization [14–16]. NFM offers many attractive features when used as supports for enzyme immobilization, such as the large surface area for the attachment of enzymes, the nanofibrous morphology for improvement of the mass-transfer rate of substrate, and the membrane-like structure for easy recover from reaction media and continuous operations in a bioreactor.

Recently, both natural (silk fibroin, casein, dextran, and cellulose) and synthetic polymers (polystyrene, polyvinyl alcohol, poly caprolactone, polysulfone, and polyacrylonitrile (PAN)) have been electrospun into NFM for immobilization of lipase, chymotrypsin, cellulose, and lysozyme [17–26]. Electrospun NFM from natural polymers are suitable candidates as for enzyme immobilization as functional groups are available in the polymer backbone for covalent bindings of enzyme molecules. However, proteins or polysaccharides need to be electrospun in aqueous solution, which is more difficult than using organic solvents. Besides, NFMs from natural polymers are generally less stable chemically and mechanically than those from synthetic polymers. Polyacrylonitrile is a polymer with good stability and mechanical properties. For enzyme immobilization, a derivative of PAN has to be synthesized with an aim to introduce functional groups into the polymer backbone due to the inertness and hydrophobicity of acrylonitrile monomer. Thus, a PAN derivative, poly(acrylonitrile-*co*-maleic acid) (PANCMA) containing reactive carboxyl groups, was synthesized and fabricated into NFM for lipase immobilization [27,28]. However, to facilitate the preparation of immobilized biocatalyst, it will be desirable to have a method for direct conjugation of enzyme molecules onto the surfaces of PAN nanofibers without using a PAN derivative, whose synthesis demands tedious and complicated steps. In this study, PAN nanofibers were used directly for lipase immobilization by activation of the nitrile groups through amidination reaction [29,30], followed by reacting with lipase solution. Properties of the immobilized lipase were fully characterized and compared to those reported in previous studies employing the same lipase and assay substrate.

2. Materials and methods

2.1. Preparation of PAN nanofibrous membrane by electrospinning

Polyacrylonitrile with an average molecular weight of 1.5×10^5 Da was obtained from Scientific Polymer Products (Ontario, NY, USA). *N*,*N*′-dimethylformamide (DMF) (99.8%, TEDIA, USA) was used as a solvent to dissolve the polymer. The apparatus for ES includes a glass syringe, an 18 gauge stainless-steel needle, a syringe pump (KD Scientific Co.), a high-voltage power supply (Glassman, USA), and an aluminum foil as the collector. PAN solution was drawn vertically from the needle tip with the electrostatic force generated from the high voltage applied between the tip and the grounded collector. The PAN polymer solution formed Taylor cone and jetted through the tip of needle to the collector. The concentration of the polymer solution, the flow rate of the polymer solution, the applied voltage, and the distance between needle tip and collector were controlled at 8% (w/w), 1.5 ml/h, 20.0 kV, and 20.0 cm, respectively. After evaporation of the solvent, nanofibers deposited on the collector in the form of a non-woven mat. The electrospun NFM was dried under vacuum before it was detached. Membrane thickness was measured with a micrometer and the apparent density (ρ_a) of membrane is calculated by measuring the weight of a circular piece of 2 cm diameter. The porosity is calculated as $(1\text{-}\rho_a/\rho_p) \times 100$, where ρ_p is the density of PAN (1.18 g/cm^3).

2.2. Immobilization of lipase

The PAN nanofiber was activated through amidination reaction by activation of the nitrile groups, followed by reacting with lipase solution (type VII, from *Candida rugosa*, Sigma, St. Louis, MO, USA) in 0.05 M phosphate buffer solution (pH 7.0) (Fig. 1). The NFM was cut into 2 cm × 2 cm pieces, placed in absolute ethanol, and bubbled with hydrogen chloride to produce the corresponding imidoester derivatives. After activated for 5 min, the membrane was removed from the solution, washed with distilled water, and placed in 1 ml of 5 mg/ml lipase solution. The mixture was shaken at 100 rpm at 30 °C for 1 h. After

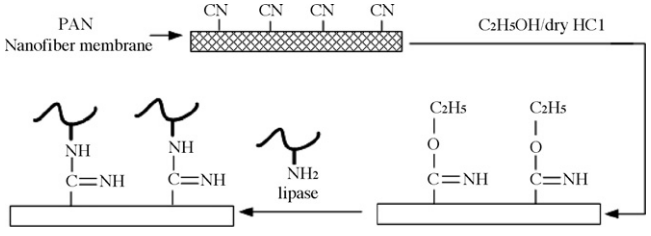

Fig. 1. Schematic illustration of lipase immobilization to PAN nanofibrous membrane by amidination reaction.

the immobilization reaction, the membrane was removed from the solution and washed with phosphate buffer solution several times to remove any unbound enzyme.

2.3. Assays of lipase activity and protein

Lipase activity was measured using 0.5% *p*-nitrophenyl palmitate (*p*-NPP) in ethanol as the substrate. 0.1 ml lipase or 4 cm^2 NWF containing immobilized lipase was added to a mixture of 1 ml of 0.5% (w/v) *p*-NPP solution and 1 ml of 0.05 M phosphate buffer solution (pH 7.0) at 30 °C. After incubating for 2 min, the reaction was terminated by adding 2 ml of 0.5N Na_2CO_3 and then centrifuged at 10,000 rpm for 10 min. The increase in absorbance at 410 nm from the release of *p*-nitrophenol during the enzymatic hydrolysis of *p*-NPP was measured at 410 nm with a UV/VIS spectrophotometer (UV-1240, Shimadzu, Japan). A molar extinction coefficient of 13,200 M^{-1} cm^{-1} for *p*-nitrophenol was used. One unit of lipase activity (U) was defined as the amount of enzyme to hydrolyze 1.0 μmol of *p*-nitrophenol per minute under the assay condition. Specific activity (SA) was defined as the number of enzyme unit per milligram of protein. The specific activity of free lipase was determined to be 36.80 ± 0.89 U/mg. Activity retention (or relative specific activity) was calculated as the ratio of specific activity of immobilized enzyme to that of free enzyme.

Protein content of immobilization was estimated by the method of Bradford using Bio-Rad protein dye reagent concentrate. Bovine serum albumin was used as the standard.

2.4. Properties of immobilized lipase

Lipase activity was measured over the temperature range of 20–50 °C and pH range of 5–9 to evaluate the effect of temperature and pH on the activities of free and immobilized lipase. To evaluate the reuse stability, the lipase-immobilized NFM, after each reaction with 0.5% *p*-NPP solution, was washed with phosphate buffer solution and reintroduced into fresh 0.5% *p*-NPP solution to start the next batch of reaction. This process was repeated up to 10 cycles. The storage stabilities of the free and immobilized Lipases were determined by incubating in 0.05 M phosphate buffer solution (pH 7.0) at 30 °C up to 20 days and assayed for residual activity at predetermined times.

2.5. Analytical methods

The electrospun NFMs were sputter coated with gold and analyzed for fiber diameters and surface structure by high resolution field emission scanning electron microscopy (JEOL JSM-6700F). The diameters of the electrospun nanofibers were calculated by measuring at least 12 fibers at random. Attenuated total reflectance (ATR) Fourier transform infrared spectroscopy (FTIR) (Horiba 730 FTIR spectrometer) was used for identifying the immobilized lipase on the surface of electrospun NFM. The uniaxial tensile property of the NFM was determined by a tensile tester (Materials Testing Machines, Tinius Olsen H1KT, UK). A 1 cm × 5 cm × 100 μm specimen was vertically mounted on two mechanical gripping units of the tensile tester at its ends, leaving a 3 cm gauge length for mechanical loading. Load-deformation data were recorded at a deforming rate of 1 mm/min and ultimate tensile strength and Young's modulus could be obtained from the stress-strain curves. Static water contact angle of NFMs were measured with a sessile drop method under an atmosphere of saturated water vapor at 25 °C by a self-made instrument with precision of ±1.25°. For this measurement, a droplet of DI water (5 μl) was placed onto the NWF surface from a fixed height (1 cm) and maintained for 5 s. Contact angles were measured on both sides of the droplet several times and averaged.

3. Results and discussion

3.1. Fabrication and characterization of NFMs

The morphology of electrospun NFM can be influenced by various parameters including polymer solution properties (surface tension, conductivity, and viscosity), spinning voltage, flow rate, and distance between needle tip and collector [31]. In fact, one of the most significant parameters influencing fiber morphology is the solution viscosity, which is determined by polymer concentration [32]. When the solution viscosity is low, the jets from the needle are unstable and likely to break up and form beads on fiber surface, and it will be difficult to get a continuous nanofibrous mat on the collector. For high viscosity solutions, the jets would not break up but rather travel and split into filaments and form fibers with increased diameter. By choosing 8% (w/w) PAN solution in DMF and other ES parameters, NFM with 88 ± 7 μm thickness, 0.23 ± 0.03 g/cm^3 apparent density, and 81.3 ± 7 porosity could be obtained.

As shown in Fig. 2, the morphology of the nanofiber is very uniform with diameter ranging from 150 to 300 nm. After lipase immobilization, the diameter and the morphology of the nanofiber did not change substantially. Covalently immobilized enzyme molecules can be seen at higher magnification (Fig. 2d) as enzyme aggregates attaching to the surface of the nanofiber. Previously, similar enzyme aggregates of chymotrypsin were also observed on polystyrene/poly(styrene-*co*-maleic anhydride) NFM after covalent binding [22]. In order to further investigate the binding of lipase on the nanofiber, FTIR spectra of both original and lipase-immobilized NFM were measured as shown in Fig. 3. It can be seen that compared with original NFM, the lipase-immobilized NFM shows new adsorption bands at 1530 and 1650 cm^{-1}, which correspond to amide I and amide II from the vibration of the C=O bonds and a combination of C–N stretching and N–H vibration in protein backbone. Also, a new peak at 1062 cm^{-1} appears, representing C–N bonds formed between the enzyme and PAN after lipase immobilization (Fig. 1). The FTIR spectra are rather noisy in the present case presumably due to the uneven thickness and poor transparency of the NFM.

With the intrinsic hydrophobicity of PAN, it would be important to have a more hydrophilic fiber surface after lipase immobilization for reaction in aqueous solution. From static contact angle measurements, the water contact angle of original PAN nanofiber is about $105 \pm 3°$ and the value decreased sharply to $10 \pm 5°$ after lipase immobilization, indicating that

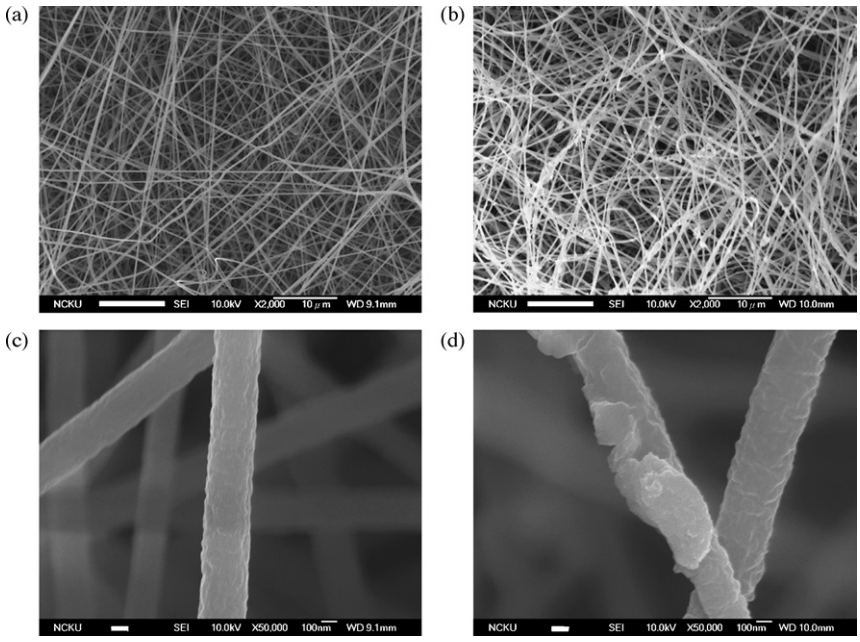

Fig. 2. Scanning electron micrographs of PAN nanofibrous membrane, (a, c) original NFM; (b, d) lipase-immobilized NFM. Magnification: (a, b) 2000×; (c, d) 50,000×.

lipase immobilization has changed the surface property of PAN nanofiber to a more hydrophilic one, which will be beneficial for carrying out the hydrolysis reaction. It should be noted here that, unlike film substrate, the water contact angle could eventually reach zero for a hydrophilic NFM due to its fibrous and porous nature [33]. Therefore, the experiment data could not be compared to that with a flat film. Nonetheless, by following the protocol outlined in the experimental section those data could be used as a reference for comparing the hydrophilicity of nanofiber surface after enzyme immobilization.

Table 1 compares the mechanical properties of different PAN NFMs. The ultimate tensile stress and Young's modulus increased by 123 and 88%, respectively, after enzyme immobilization, but the strain at break decreased by 44%. It can be inferred from the decrease in the tensile strain of activated NFM that chemical treatment with hydrogen chloride in ethanol apparently makes the membrane more brittle and less elastic. Nonetheless, enzyme molecules showed positive influence on mechanical properties of the membrane by improving the tensile stress and Young's modulus of the activated NFM to give a more robust and mechanically stable immobilized biocatalyst preparation.

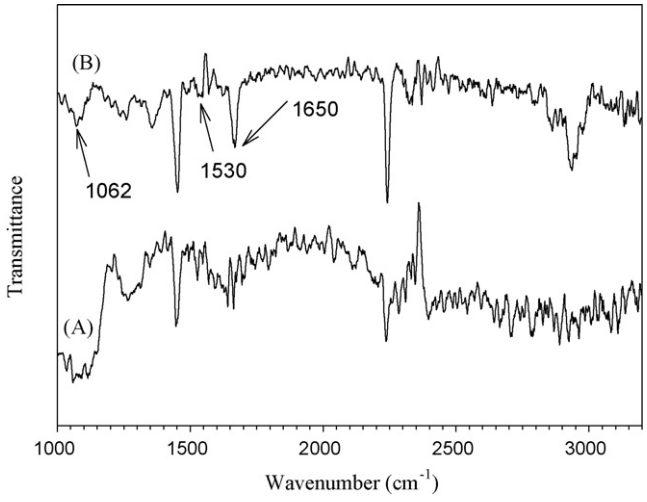

Fig. 3. FTIR spectra of (A) original nanofibrous membrane, and (B) lipase-immobilized nanofibrous membrane.

3.2. Activities and properties of Immobilized lipase

For enzyme immobilization by amidination reaction, the nitrile groups of PAN was first activated in ethanol by hydrogen chloride in ethanol to produce an imidoester derivative, followed by reacting with enzyme-containing solution for conjugation with the amino groups of lipase (Fig. 1) [29,30]. This method provide a simple yet effective method for covalently attaching enzyme to inert PAN with the immobilized enzyme showing good stability during repeated use [29,30]. However, the specific activity of the immobilized enzyme is low (*ca*. 19%) compared to that of free enzyme [29].

The efficiency of protein loading was studied by varying the activation time and the enzyme immobilization time and the results are reported in Fig. 4. The amount of protein immobilized reached the highest value close to 100% at 5 min activation

Table 1
The mechanical properties of PAN nanofibrous membrane (NFM)

	Tensile stress at break (MPa)	Tensile strain at break (%)	Young's modulus (MPa)
Original NFM	1.09	27	19.6
Activated NFM	1.28	15.4	11.6
Lipase-immobilized NFM	2.43	15	36.8

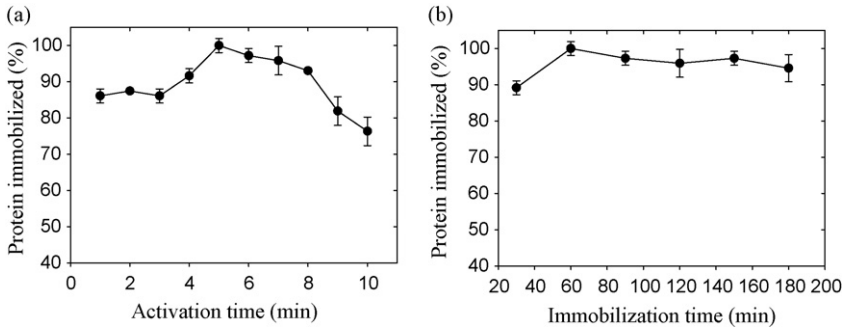

Fig. 4. The effect of (a) activation time, and (b) enzyme immobilization time on protein immobilization efficiency. The amount of protein added during the immobilization step was taken as 100%.

time and decreased gradually thereafter. It can be concluded that activation is a rather efficient process where conversion of nitrile group to the reactive imindoester derivative could reach equilibrium in a short time. Prolonged activation time may damage fiber surface and decrease the available site for protein conjugation. For the subsequent enzyme immobilization step, the amount of protein immobilized increased during the first hour and reached a constant value thereafter. Presumably, all available sites for enzyme binding were saturated after just 1 h of reaction. It should be noted that in the previous enzyme immobilization study using PAN as the matrix material, the authors used 1 h for activation and 6 h for enzyme reaction without investigating the effect of activation and enzyme immobilization time [34]. Overall, the present immobilization procedure provides an easy yet highly effective enzyme immobilization method where immobilization step could be completed in less than 70 min. It should be noted that the amount of lipase adsorbed to nascent PAN NFM was found to be *ca*. 7% of that immobilized by covalent bindings.

Effect of temperature on the relative activity of free and immobilized lipase is shown in Fig. 5. The immobilized lipase showed an optimum reaction temperature at about 35 °C, whereas free lipase had an optimum temperature about 30 °C. This result could be ascribed to the restriction of conformational mobility of the immobilized enzyme as a result of covalent bond formation between the enzyme and the matrix, which requires higher activation energy for reacting with the substrate. Alternatively, a restriction in the diffusion of the substrate at high temperature may also be responsible.

The pH-dependent activities of free and immobilized lipase were evaluated and shown in Fig. 6. The charges on the support materials could influence the pH enzyme activity profile. Binding of enzymes to polycationic supports, such as chitosan would result in an acidic shift of the pH optimum [35]. PAN is a non-ionic polymer and neutral amidine bonds are formed after reacting amino groups of lipase with imidoester derivative. The optimum pH did not shift when compared to free enzyme as expected. The immobilized lipase was less sensitive to pH changes at acidic pHs than alkaline pHs compared to that of free lipase.

For immobilized enzyme, the reusability and enhanced storage stability are important advantages. Fig. 7 shows the effect of repeated use on activity of the immobilized lipase. After 10 repeated use, the immobilized lipase retained about 70% of its original specific activity, which is better than previous studies using NFM as the matrix (see Table 2 and Section 3.3). The storage stability of the immobilized lipase is shown in Fig. 8. From the figure, the activity of free lipase decreased

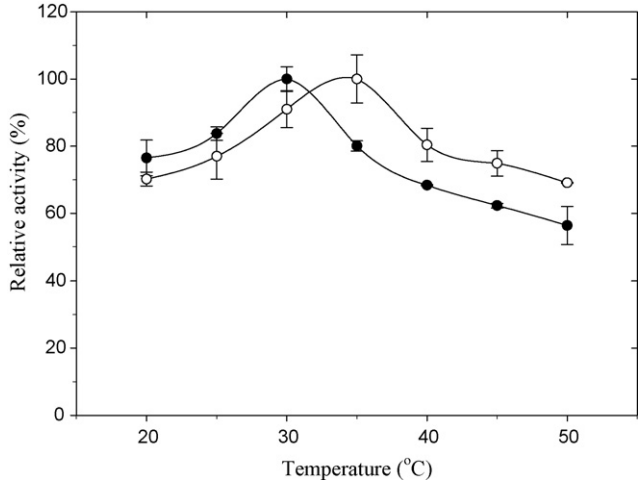

Fig. 5. Effect of temperature on lipase activity: (●) free lipase; (◯) immobilized lipase. The relative activities at the optimum temperature were taken as 100% for free and immobilized lipase, respectively.

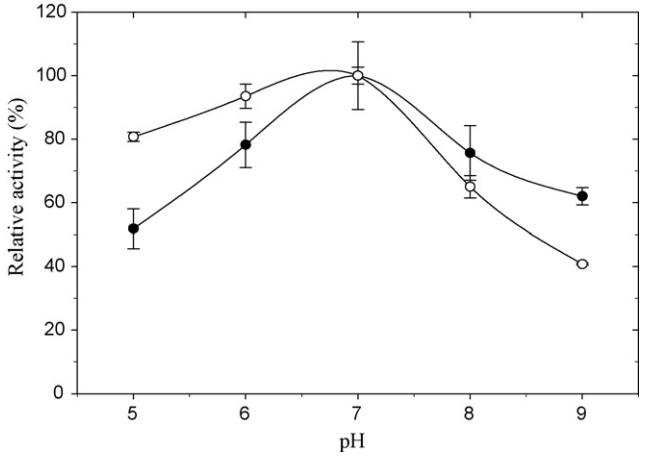

Fig. 6. Effect of pH on lipase activity: (●) free lipase; (◯) immobilized lipase. The relative activities at the optimum pH were taken as 100% for free and immobilized lipase, respectively.

Table 2
Comparison of the performance of different immobilized *Candida rugosa* lipase systems with *p*-nitrophenyl palmitate as the substrate[a]

Matrix	Protein loading (mg/g matrix)	Activity retention (%)	V_{max} (Imm/Free) (U/mg)	K_m (Imm/Free) (mM)	Activity after 10 reuse	Reference
Chitosan bead	0.287	91.5	96.1/92.5	18.1/1.67	74%	[36]
Chitosan bead	0.199	111.32	NA	NA	60%	[37]
PANCMA hollow fiber membrane[b]	2.36 ± 0.06	33.9 ± 1.6	16.1/46.4	1.36/0.45	62%	[27]
PANCMA NFM	21.2 ± 0.71	37.6 ± 1.8	16.5/46.4	0.98/0.45	NA[c]	[27]
Chitosan-modified PANCMA NFM	22.5 ± 0.75	45.6 ± 1.8	22.1/46.4	1.22/0.45	55%	[28]
Gelatin-modified PANCMA NFM	20.7 ± 0.75	49.7 ± 1.8	23.3/46.4	1.05/0.45	60%	[28]
PANCMPC NFM[d]	22.9 ± 1.5	76.8 ± 0.6	34.0/44.6	0.86/0.44	NA	[38]
Polysulfone NFM	0.8	NA	8.88/46.4	0.691/0.450	NA	[39]
PAN sheet membrane	2.1 ± 0.15	50.6 ± 0.9	23.8/44.6	1.64/0.44	NA	[38]
PAN NFM	21.2 ± 1.3	81.3 ± 1.1	31.2/39.7	0.548/0.456	70%	This work

[a] Immobilization are by covalent bindings except for PANCMPC NFM, polysulfone NFM, and PAN sheet membrane, which are by adsorption.
[b] PANCMA NFM: poly(acrylonitrile-*co*-maleic acid) nanofibrous membrane.
[c] NA: not available.
[d] PANCMPC NFM: poly[acrylonitrile-*co*-(2-methacryloyloxyethyl phosphorylcholine)] nanofibrous membrane.

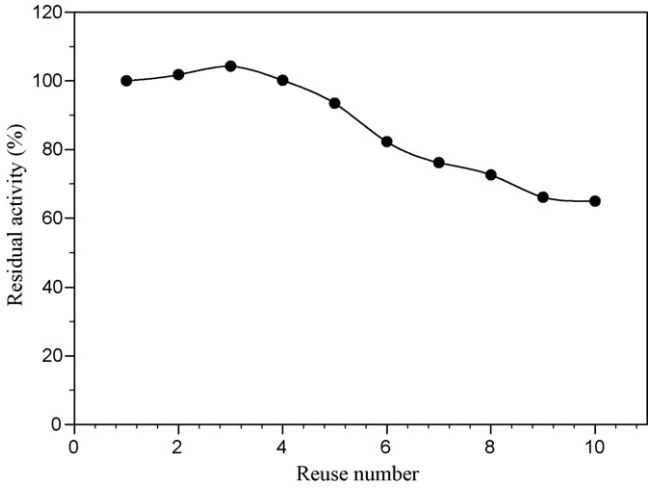

Fig. 7. Effect of repeated use on residual activity of immobilized lipase at 30 °C and pH 7.0.

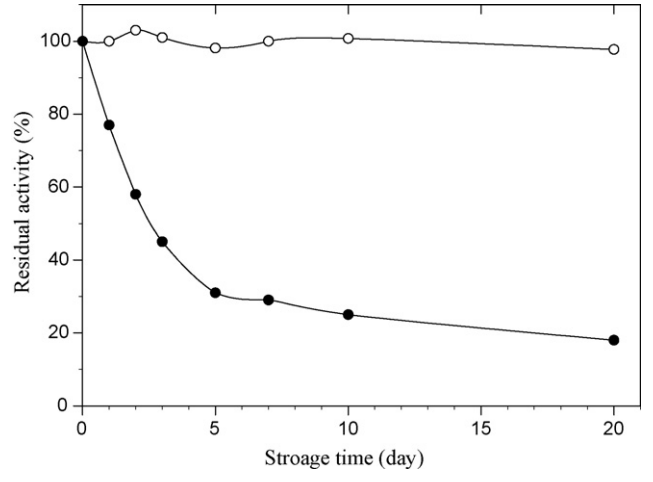

Fig. 8. Storage stability of lipase at 30 °C and pH 7.0: (●) free lipase; (○) immobilized lipase.

rapidly in the first 5 days and showed only 20% residual activity after 20 days. In contrast, the residual activity of immobilized lipase was 95% after 20 days, indicating that enzyme immobilization has considerably enhance the storage stability of lipase.

The specific activity of the immobilized lipase is 21.2 ± 1.3 U/mg, which translates into 81.3% activity retention. The decrease in activity is usually observed after enzyme immobilization. This could be explained by the modification in the three-dimensional structure of the enzyme, which leads to conformation change of the active center. The presence of matrix which hindered the accessibility of substrate to the enzyme active site, and limitation of mass transfer of substrate and product to or from the active site of the enzyme may also be responsible.

3.3. Comparison of different immobilization matrix

Table 2 compares the performance of immobilized *C. rugosa* lipase when using *p*-NPP as the substrate. Although chitosan bead was reported to enhance the activity of lipase after immobilization (up to 111% activity retention) [37], the protein loading was extremely low (about two orders less) when compared to that on NFM. The high protein loading is due to the high specific surface area of NFM in contrast to porous chitosan bead. Compared to hollow fiber membrane [27], lipase immobilized on NFM also showed 10-fold increase in the amount of protein immobilized and higher activity retention with the reduction in fiber diameter and diffusion resistance.

Previously, the covalent immobilization of lipase to PANCMA NFM was carried out by EDC/NHS activation of the –COOH groups [27]. Alternatively, the –COOH containing matrix can be first derivatized with chitosan or gelatin and then coupled to lipase by crosslinking with glutaraldehyde [28]. By employing a different enzyme immobilization scheme, the activity retention in this study was found to be better than all other studies using NFM as the matrix and the protein loading capacity is comparable (Table 2).

The Michaelis-Menten kinetics of the free and immobilized Lipases was investigated using varying initial substrate concentrations. Kinetic parameters K_m and V_{max} as evaluated from the double reciprocal plot are included in Table 2 and compared with those obtained in other studies. The increase in the K_m values after enzyme immobilization is either due to the conformational changes of the enzyme resulting in a lower possibility to form the substrate-enzyme complex or due to the lower accessibility of the substrate to the active site of the immobilized enzyme caused by the increased diffusion limitation. From Table 2, it can be seen that K_m values of the immobilized lipases are lower for NFM than hollow fiber membrane, chitosan beads, and PAN sheet membrane. This result is largely again attributed to the presence of the large specific surface area of the NFM, which can create a more favorable interface for the mass transfer of substrate or product to or from the active site of the enzyme. The diffusion resistance was also remarkably reduced by the reduction of the geometric size of the enzyme support and the catalytic efficiency of the immobilized lipase was effectively improved. Furthermore, the percentage of increase in K_m value after enzyme immobilization is the lowest for the present study among all studies using NFM as the matrix, indicating a tighter binding between enzyme and substrate. The modified PAN surface contains both hydrophilic and hydrophobic groups, and the presence of the hydrophobic region on the NFM surface may enhance the affinity between the substrate molecule and the active site of enzyme through hydrophobic interaction, and hence influence K_m.

The V_{max} values decrease after enzyme immobilization, except for chitosan beads (Table 2). However, for chitosan beads the K_m value increase up to 10.8 times after immobilization and the protein loading is extremely low. Compared with other studies, the present study gives the highest percentage of retention of V_{max} value after enzyme immobilization. Electrospinning of the synthesized copolymer PANCMA [27], or subsequently coating the NFM with biomolecules (chitosan, gelatin) [28] does not result in a more efficient immobilized biocatalyst over the direct conjugation method used here. This result could be ascribed to the difference in conjugation chemistry and microenvironment. By introducing phospholipids moiety into the copolymer backbone, poly[acrylonitrile-*co*-(2-methacryloyloxyethyl phosphorylcholine)] (PANCMPC) NFM was also fabricated for immobilization of lipase by adsorption. However, the activity retention and kinetic parameters are still inferior to those from this study [38,39]. It is obvious that the present immobilization system provided the most efficient immobilized biocatalyst among all studies listed in Table 2.

4. Conclusions

PAN NFM with uniform fiber diameter below 300 nm could be fabricated by ES and used for lipase immobilization after activation by amidination reaction. Enzyme molecules could be covalently bound to the nanofiber and formed aggregates on the fiber surface, which also became more hydrophilic and robust after enzyme immobilization. With the huge specific surface area provided by the nanofiber, protein loading could reach as high as 2.1% (w/w) in the NFM while the immobilized enzyme still retains high activity at 81.3%. After enzyme immobilization, the storage stability was substantially improved over that of free enzyme. This simple but effective enzyme immobilization system shows improvements in enzyme properties over previous immobilized lipase systems using the same enzyme and substrate.

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