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Research Article

Immobilization of *Candida rugosa* Lipase on Aminated Polyvinyl Benzyl Chloride-Grafted Nylon-6 Microfibers

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Abstract

This paper demonstrates a simplified procedure for the preparation of a nylon-6 microfibers based support for the immobilization of *Candida rugosa* lipase via covalent attachment to enhance the stability and reusability of lipase. The preparation of the support was done by radiation induced graft copolymerization (RIGC) of vinyl benzyl chloride (VBC) onto nylon-6 microfibers followed by amination with ethanolamine to facilitate the immobilization of lipase. Fourier transfer infra red (FTIR) and scanning electron microscope (SEM) were used to study the chemical and physical changes following grafting, amination and immobilization. Response surface methodology (RSM) was applied for the optimization of lipase immobilization on the aminated microfibers. The optimization parameters were incubation time, pH, and lipase concentration. Moreover, this study investigated the effect of temperature, pH, and storage stability and reusability on the lipase in its immobilized and free forms. The developed model from RSM showed an R^2 value of 0.9823 and *P*-value < 0.001 indicating that the model is significant. The optimum temperatures for both immobilized and free lipases were 45 °C, whereas the best pH values for lipase activity were at pH 8 and pH 7, respectively. This study also identifies values for K_M and V_{max} for both immobilized and free lipase accordingly. Based on the results, immobilized lipase had significantly improved the stability and reusability of lipase compared to that in free forms.

Keywords: Lipase immobilization; PVBC-grafted nylon-6 microfiber; amination; response surface methodology; optimization; enzyme activity

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1. Introduction

An enzyme is a biocatalyst that exhibits many advantages over a chemical catalyst. The superiority of enzymes includes a high specificity towards specific substrates, catalytic efficiency and the ability to react in mild conditions [1]. Among enzyme classes, lipase (EC 3.1.1.3) is a favorable enzyme due to its biocatalytic potential in both aqueous and nonaqueous environments [2]. Furthermore, lipase has broader bio-

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logical functions such as triacylglycerol hydrolysis, esterification, and transesterification [3]. Lipase from *Candida rugosa* has gained attention due to its biotransformation properties that consist of broad specificity (enantioselectivity), high catalytic activity and regiospecificity [4,5].

Various applications, such as food, bioremediation and biodiesel, use lipase enzymes, however, applying such enzymes in free-form results in the loss of enzyme activity due to denaturation and its inability to be recycled. Also, in its free form, lipases are very sensitive to the changes in temperature and pH. Therefore, immobilization of lipase has been widely used to replace free lipase for the inherent advantages not only on the process of reusability but also on the stability of pH and temperature resistance as well as reusability [2,3].

One of the main elements affecting the performance of immobilized lipase is the physical and chemical properties of the support system. Thus, the interest in improving the characteristics of the support is ever growing. Features, such as: porosity, surface area over volume, and surface functionalization are most frequently investigated [6]. Non-porous support matrix is found to have high diffusion resistance and low enzyme loading. On the other hand, porous materials having high enzyme loading capabilities suffer from weak interaction between immobilized enzyme and substrates [7]. Thus, designing support materials for immobilization of enzyme is of utmost significance.

Nylon-6 is a synthetic commercially available nylon that provides a particularly exciting substrate for convenient immobilization when modified adequately. Besides being cheap, it is non-toxic and has excellent mechanical strength [8]. The modification of nylon-6 microfibers with various monomers applying various grafting methods such as redox initiated grafting of hydroxyethyl methacrylate [9], methacrylic acid [10], glycidyl methacrylate [11], photografting of maleic anhydride [12], and chemical grafting with acrylamide in the presence of dibenzoyl peroxide [13].

When considering all grafting methods, the radiation-induced grafting (RIG) technique is reported as being advantageous in terms of simplicity, due to the absence of initiators and its ability to control grafting distribution by offering a surface to bulk modifications [14]. In this regard, our research group early reported a modification of nylon-6 with vinyl benzyl chloride (VBC) using RIG in a solvent medium [15] and very recently in an emulsion [16]. The obtained grafted intermediates were used to develop highly selective adsorbent containing glutamine for boron removal [17]. Nevertheless, the obtained PVBC-grafted nylon-6 microfibers seem to be attractive support for enzyme immobilization.

In addition, there is no specific study on enzyme immobilization of such material in its current fibrous form. The use of PVBC- grafted nylon-6 microfibers for enzyme immobilization in general and *Candida rugosa* lipase in particular is likely to enhance the enzyme activity, stability, reusability and eventually improve the economy of biocatalytic reactions. More details on the various types of supports for enzyme immobilization and the advantages of fibrous supports can be found in the recent review by Zdarta *et al.* [18].

This article reports the preparation of aminated PVBC-grafted on nylon-6 using radiation-induced emulsion grafting and its application for covalent immobilization of lipase. The immobilization parameters such as incubation time, pH and concentration of lipase were optimized using response surface methodology (RSM) to maximize the lipase activity. The performance of the free and immobilized lipase with regards to the optimum temperature and pH, stability, reusability and kinetics study were investigated.

2. Material and Methods

2.1 Materials

The VBC (used as received without any treatment), *Candida rugosa* lipase powder (type VII, ≥ 700 unit/mg solid) and *p*-nitrophenyl palmitate (*p*-NPP) were procured from Sigma-Aldrich. Reliance Sdn Bhd (Malaysia) supplied the nylon-6 microfibers (average fiber diameter of 15 mm). Ethanolamine and polyoxyethylene sorbitan monolaurate (Tween-20) surfactant was obtained from Merck & Co. Solvents, such as: methanol, ethanol, and propanol, were purchased from HmbG, whereas Sigma-Aldrich supplied butanol and pentanol. All solvents were reagent grade and used as received. Deionised water or DI (18 mΩ) was produced using Millipore Direct-Q™ water purifier.

2.2 Preparation of PVBC-grafted Nylon-6 Microfibers

Radiation-induced emulsion graft copolymerization was used for the preparation of PVBC-grafted nylon-6 microfibers as explained by Ting *et al.* [15]. In short, microfibers were

placed in a polyethylene zipped bag and purged using purified N_2 gas to eliminate the air and sealed. The sealed bag containing microfibers was irradiated using electron beam accelerator (EPS 3000) to a total dose of 300 kGy at 10 kGy/pass obtained at an acceleration voltage of 1 MeV and current throughput of 10 mA. The irradiated nylon-6 microfibers were removed and introduced to a grafting solution containing 5% of VBC and 0.5% Tween-20 in DI water. The grafting solution was bubbled with purified N_2 gas and transferred into an evacuated ampoule containing the irradiated microfibers. Then the ampoule was sealed and kept in a water bath at 50 ºC for 3 h to react. After the grafting reaction was completed, the grafted sample was removed and repeatedly washed with methanol to remove any homopolymer or unreacted monomer. Finally, the grafted sample was dried under vacuum at 40 ºC for 24 h [15].

2.3 Activation of PVBC-grafted Nylon-6 Microfibers

PVBC-grafted nylon-6 microfibers were treated with 20% ethanolamine in methanol solution for 4 h in an incubated shaker at a shaking speed of 140 rpm and a temperature of 55 ºC. The aminated microfibers were subsequently rinsed 3 times in methanol to eliminate the residual amine reagent. The treated microfibers were dried for 16 h in an oven at a temperature of 60 ºC before enzyme immobilization. [19]

2.4 Immobilization of Lipase on Aminated PVBC-grafted Nylon-6 Microfibers

The enzyme was immobilized on aminated PVBC-grafted nylon-6 microfibers by adding 1.0 mg/mL of lipase powder to 20 mg of microfibers in a 3.0 mL phosphate buffer solution, PBS (0.1 M, pH 7.0) following the method described by Huang *et al.* [20]. The microfibers sample was then placed in an incubator shaker for 5 h at a temperature of 37 ºC at a shaking speed of 150 rpm. The microfibers with immobilized lipase were subsequently rinsed 3 times in PBS (0.1 M, pH 7.0) to eliminate residual lipase.

The amount of lipase immobilized on the microfibers was determined with Coomassie brilliant blue reagent as stated in Bradford method [21]. Bovine serum albumin (BSA) was used to establish the calibration curve. Applying equation (1), accordingly calculates the amounts of protein immobilized on the microfibers. Finally, the lipase activity was determined and assayed.

$$Immobilized\ lipase\left(\frac{mg}{ml}\right) = Initial\ lipase\left(\frac{mg}{ml}\right) - Residual\ lipase\left(\frac{mg}{ml}\right) \quad (1)$$

2.5 Lipase Activity Assay

The activities of the free and immobilized lipase were assayed using *p*-nitrophenyl palmitate substrate (*p*-NPP) using the method reported by [22]. Hydrolysis of *p*-NPP by lipase produced *p*-nitrophenol palmitate that was observed by the solution color change from colorless to yellow. Typically, a 0.02 mL of free lipase or 20 mg immobilized lipase microfibers was added to a mixture of 0.1 mL of substrate solution (prepared by dissolving 10.6 mM of *p*-NPP in 10 mL of obtain a final volume of 2 mL. The reaction continued for 15 minutes at 37 ºC before termination by immersing the mixture flask in boiling water (100 ºC). The collected supernatant was extracted and subsequently measured by UV-vis microplate spectrophotometer (Thermo Fisher Scientific Multiskan Go Microplate) at 410 nm wavelength. The amount of the enzyme required to produce 1 μmol of *p*-nitrophenol (*p*-NP) per minute defines one unit (U) of the lipase activity.

2.6 Design of Experiment for Lipase Immobilization

Response surface methodology (RSM) established the optimal values for the variables, by considering the significant effects that maximised residual enzyme activity. The investigated independent variables were incubation time (3-7 h), pH (7-9) and enzyme concentration (0.4-1.0 mg/mL). Faced centred central composite design (FCCCD) from Design Expert software version 7.0 was used to design the experiments.

2.7 Effect of Temperature and pH on Lipase Activity

By observing the enzyme function at temperatures ranging from 25 ºC to 65 ºC at pH 8.0, the optimal temperature was identified for both free and immobilized lipases. Lipase activity was identified according to standard lipase activity assay in PBS (0.1 M, pH 8.0) for 15 minutes time before the absorbance reading was taken [16,17].

The identified optimal pH for free and immobilized lipase was within a pH range of 6 to 11 at optimum temperatures. The measured activity of lipase followed the standard lipase

activity assay in 0.1 M PBS (pH 8) for 15 minutes. The effects of temperature and pH were observed in terms of relative activity [23].

2.8 Temperature and pH Stability of Lipase Activity

Essential indicators for enzyme operational performance are the enzyme's thermal, pH and storage stabilities and its reusability. To determine the thermal stability of free and immobilized lipase test conditions were conducted within a temperature range of 25-65 ºC at pH 8.0 and observed at 10 ºC incremental steps. At the observed optimum temperature, the determination of pH stability is conducted within a pH range of 6-11 at 1 pH incremental steps. In both tests, a control lipase sample without substrate was kept at the predetermined conditions for 30 minutes [24].

2.9 Storage Stability and Reusability

To determine the storage stability of both free and immobilized lipases samples are stored in PBS (0.1 M, pH 7.0) at 4 ºC for up to 30 days. The reusability of immobilized lipase was determined by washing used lipase immobilized microfibers 3 times with PBS (0.1 M, pH 8.0) to remove any residuals before using it again in the subsequent assay up to 8 cycles.

2.10 Kinetic Study

Kinetic investigations of catalytic behaviors of free and immobilized lipases was done by comparing Lineweaver-Burk plots and consequently determined the Michaelis-Menten kinetic parameters: K_M and V_{max}. The concentrations of the substrate and *p*-NPP was varied in the range of 0.1-3 mM at optimum temperatures and pH.

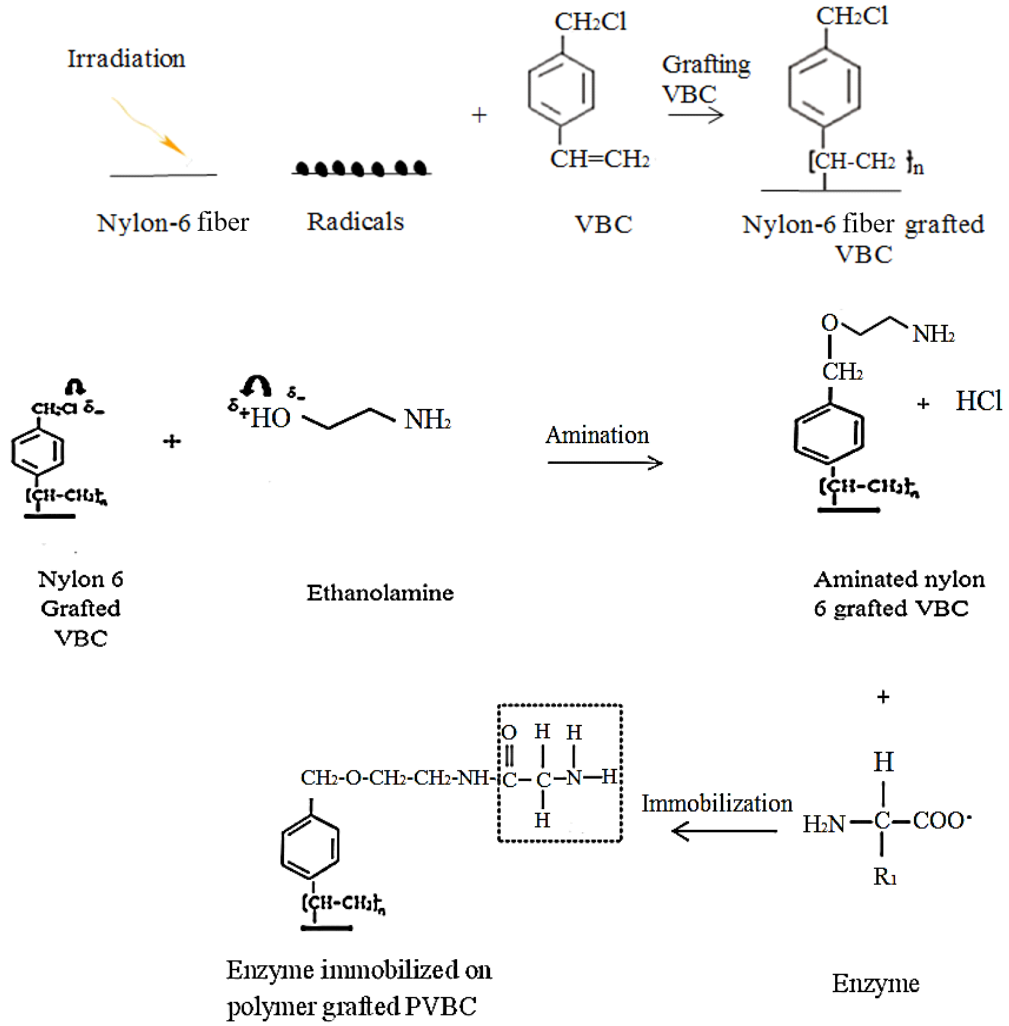

Figure 1. Schematic representation for preparation of grafted VBC on nylon-6, its chemical activation and subsequent immobilization with lipase

3. Results and Discussion

3.1 Preparation of Aminated PVBC-grafted Nylon-6 Support

Samples of PVBC-grafted nylon-6 with degree of grafting of 100% were obtained using the applied conditions during radiation-induced emulsion grafting. Figure 1 shows a schematic representation for the preparation of PVBC-grafted nylon-6 by radiation-induced graft (RIG) technique, its activation with ethanolamine and subsequent enzyme immobilization.

3.2 Evidences of Grafting VBC on Nylon-6 Microfibers, Amination, and Enzyme Immobilization

Figure 2 shows the FTIR of original nylon-6, PVBC-grafted nylon-6, aminated PVBC-grafted nylon-6, and the corresponding lipase immobilized microfibers. The intensities of peak at 1645 cm^{-1} is attributed to (C-N) and 1544 cm^{-1} is attributed to (C=O) and other peaks from original nylon-6 were slightly changed due to the effects of grafting of PVBC. The disappearance of the peak at 706 cm^{-1} is attributed to chloromethyl group (C-Cl) which indicates the change in microfibers functional groups after amination. The disappearance of this peak confirms the replacement of Cl with an amine group, alluding to the successful activation by ethanolamine. Activation of the fiber also resulted in the formation of peak at 1050 cm^{-1}

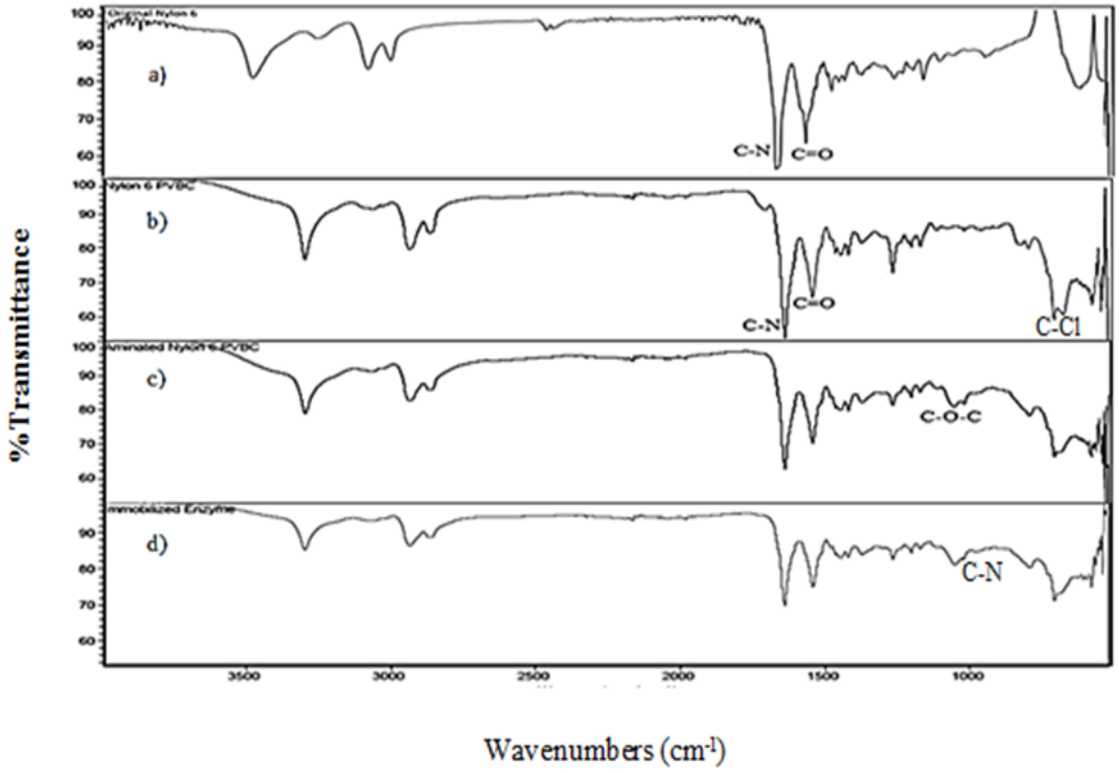

Figure 2. FTIR spectra of (a) Original nylon-6, (b) PVBC-grafted nylon-6, (c) PVBC-grafted nylon-6 after amination, (d) immobilized enzyme on PVBC-grafted nylon-6

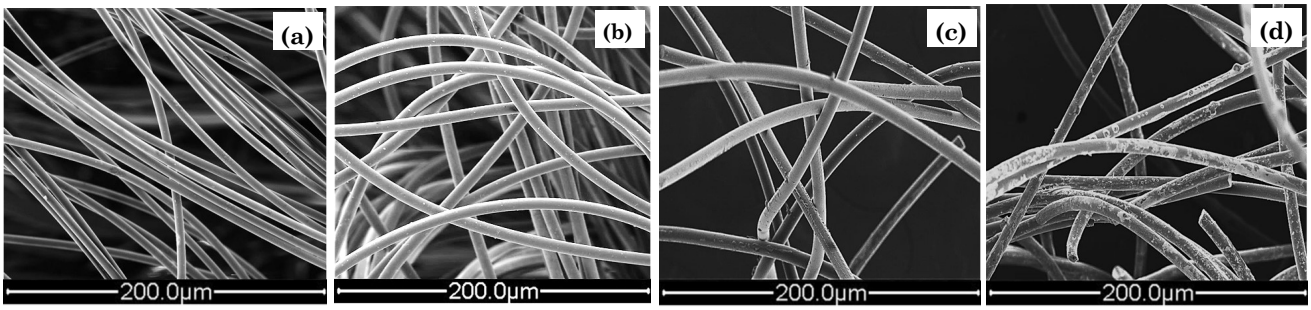

Figure 3. SEM of (a) original nylon-6 fiber, (b) grafted nylon-6, (c) aminated nylon-6 grafted VBC, (d) immobilized enzyme on nylon-6 grafted VBC

which is attributed to a C–O–C stretch. The chloromethyl group on the fiber underwent nucleophilic substitution after reacting with ethanolamine to form vinyl alkyl ether. The presence of (-NH_2) on the fiber is proposed to facilitate the peptide bond formation with (COO^-) on the enzyme during immobilization process [25]. FTIR of fiber after immobilization (Figure 2d) shows a peak at 1020 cm^{-1} attributed to the formation of a C–O stretch, which agrees to the proposed formation of peptide bond whereby COO^- is bonded to NH_2 forming (N–C=O) as shown in Figure 1.

Using scanning electron microscopy (SEM) allowed further observation and morphological analysis of the original nylon-6, grafted nylon-6, aminated and immobilized grafted nylon-6. The grafted nylon-6 shows an increase in the average diameter from 15 μm to 23 μm compared to the original nylon-6 due to the integration of PVBC as shown in Figure 3(a-b) [15]. The relative diameter sizes were similar between PVBC-grafted nylon-6 and aminated nylon-6 VBC, which indicates that amination did not affect the fiber diameter (Figure 3(c)). An increase in randomly distributed pores could be observed on the surface of aminated microfibers. Figure 3(d) shows white color deposits on the microfibers surface which could be attributed to the immobilization of lipase. Additionally, the microfibers diameters increased from an average of 23 μm to 26 μm following immobilization.

3.3 Optimization of Lipase Immobilization

Immobilization of lipase required certain conditions to effectively bound to the fiber**.** The critical parameters, which affect lipase immobilization, are incubation time, pH and enzyme concentration. These parameters were investi-

Table 1. Optimization of lipase immobilization by RSM

Run	Factors			Enzyme Activity (U/mg)
	Incubation time (h)	pH	Enzyme Conc. (mg/mL)	
1	0(5)	1(9)	0(0.7)	1.620
2	-1(3)	-1(7)	1(1.0)	0.785
3	0(5)	0(8)	0(0.7)	2.226
4	0(5)	0(8)	-1(0.4)	1.933
5	1(7)	-1(7)	-1(0.4)	1.655
6	0(5)	-1(7)	0(0.7)	1.748
7	-1(3)	-1(7)	-1(0.4)	0.661
8	0(5)	0(8)	0(0.7)	2.199
9	1(7)	1(9)	-1(0.4)	1.791
10	-1(3)	-1(9)	-1(0.4)	1.197
11	-1(3)	0(8)	0(0.7)	1.549
12	1(7)	1(9)	1(1.0)	1.439
13	0(5)	0(8)	0(0.7)	2.076
14	0(5)	0(8)	0(0.7)	2.268
15	1(7)	-1(7)	1(1.0)	1.518
16	0(5)	0(8)	1(1.0)	1.631
17	-1(3)	1(9)	1(1.0)	0.628
18	0(5)	0(8)	0(0.7)	2.281
19	1(7)	0(8)	0(0.7)	2.110
20	0(5)	0(8)	0(0.7)	2.069

gated using experimental design technique. Each variable is represented at three levels, i.e., a high (1), center (0) and low (-1). The design resulted with 20 experimental runs at various parametric combinations. The outcome of the design is obtained and presented in Table 1. As can be seen, the center points of FCCCD design (run no. 18) at which a combination of parameters of 5 h incubation time, 8 pH and 0.7 mg/mL enzyme concentration, attained a maximum lipase activity of 2.28 U/mg and therefore, there were selected as the optimum parameters for the immobilization of lipase for the subsequent experiments.

The activity of residual lipase was modelled by RSM using the three parameters, namely, pH, incubation time and lipase concentration. A quadratic polynomial model was selected to fit the experimental data to predict the lipase activity as shown in Equation (2).

$$\text{Lipase activity} = 2.14 + 0.37\,A + 0.031\,B - 0.12\,C - 0.040\,AB - 5.390\times10^{-003}\,AC - 0.11\,BC - 0.25\,A^2 - 0.40\,B^2 - 0.30\,C^2 \quad (2)$$

where A is incubation time, B is pH and C is lipase concentration. The positive terms indicate a proportional effect on lipase activity, while the negative terms indicate the opposite effect.

Analysis of variance (ANOVA) for the model shown in Table 2 demonstrating a probability > F from the model is <0.0001, which is less than 0.05 indicating a significant model. Prob > F of

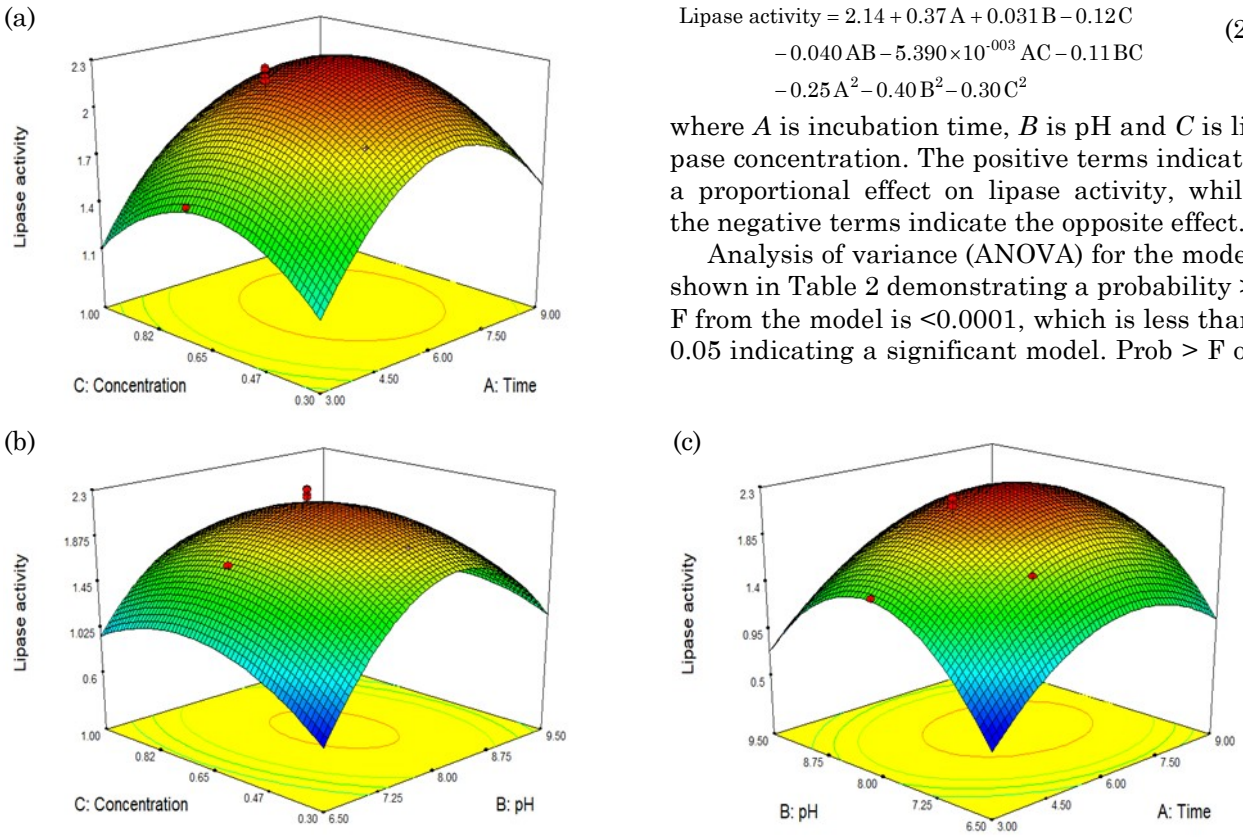

Figure 4. 3D surface plots for the effect of interaction between (a) incubation time (h) and enzyme concentration (mg/mL), (b) incubation time (h) and pH, (c) pH and lipase concentration (mg/mL) on lipase activity

Table 2. Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Source	F-Value	p-value Prob > F	Remarks
Model	36.61	< 0.0001	significant
A-Incubation time	90.17	< 0.0001	
B-pH	0.63	0.4453	
C-Concentration	10.14	0.0098	
AB	0.85	0.3775	
AC	0.015	0.9038	
BC	6.82	0.0260	
Lack of Fit	2.51	0.1675	not significant
R^2	0.9705		
Adjusted R^2	0.9440		

0.1675 indicates a required insignificant "lack of fit", which denotes that the model fits the experimental data. The R^2 and adjusted R^2 were 0.9705 and 0.9440, respectively. A value of R^2 close to 1.0 indicates that more data points fall within the regression line [26]. The model showed that R^2 agrees with the predicted R^2 value of 0.9663 for lipase activity. The independent variables used in optimizing immobilization process that had significant effects were incubation time and lipase concentration. Incubation time is highly significant compared to concentration. pH with probability > F of 0.4453 had an insignificant effect on immobilization according to ANOVA.

3-D surface plots in Figure 4 show the relationship between the enzyme activity and two of the independent variables. In other words, it facilitates the study of interaction between two independent variables during the immobilization process. Figure 4 shows that lipase activity increases with lipase concentration until the center point, after which the activity decreased. Observations of pH show similar behavior, while for the incubation time, lipase activity increased beyond the center point. It reached a maximum at about 7 h after which it decreased, which implies that maximum lipase activity requires sufficient incubation time.

3.4 Effect of Temperature and pH on Lipase Activity

Figure 5(a) shows the results of experimentation looking at the effects of temperature on both free and immobilized lipase activity conducted in the range of 25-65 °C. The optimum temperature for both free and immobilized lipase were at 45 °C. The activity of free lipase rapidly decreased when temperature increased above 45 °C. However, immobilized lipase retained more than 80% of its activity up to 65 °C. The higher activity of immobilized lipase compared to free lipase could be related to the lipase-fiber covalent bonding which will require high energy for conformational denaturation to take place [27]. Considering the lipase activity at pH range 6-11 (Figure 5b), the opti-

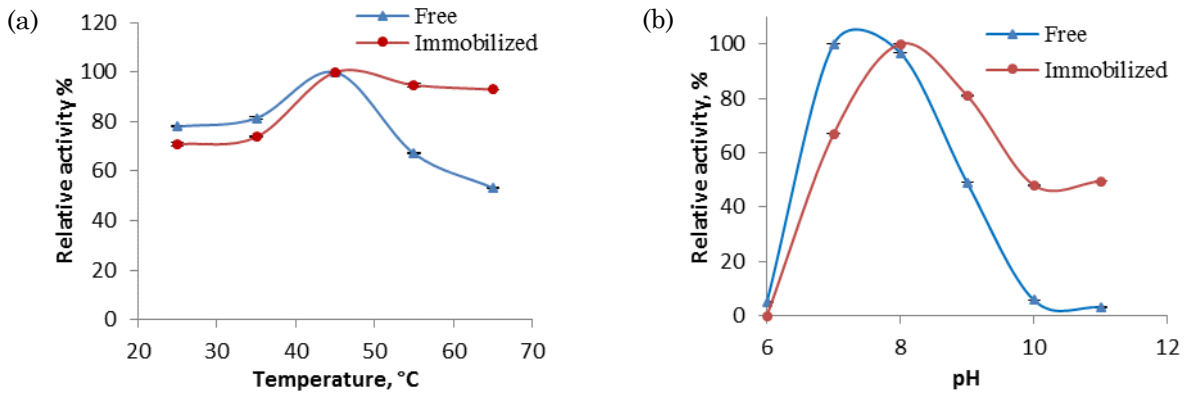

Figure 5. Effect of (a) temperature and (b) pH on immobilized (●) and free (▲) lipase activity

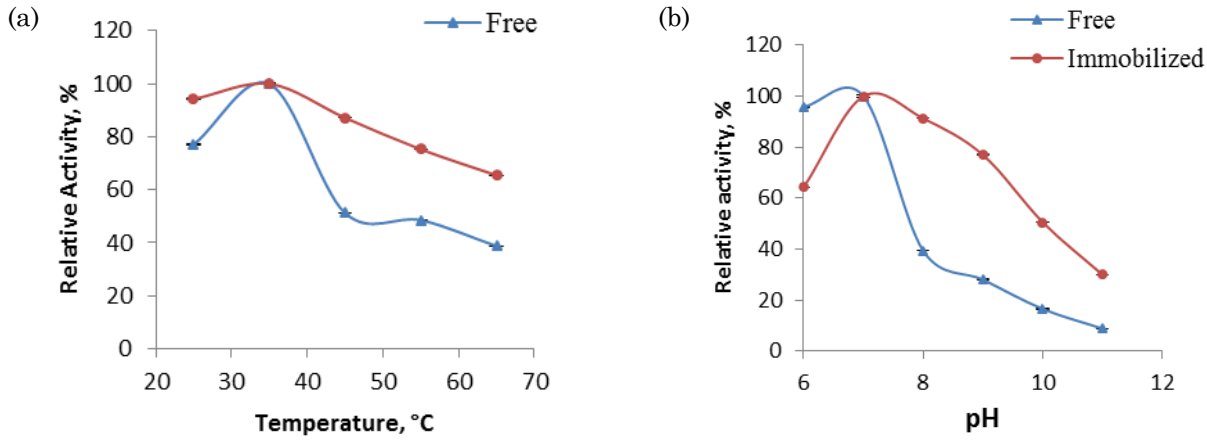

Figure 6. Effect of: (a) temperature and (b) pH on stability for both immobilized (●) and free (▲) lipases

mum pH for free lipase was 7 while for immobilized lipase is shifted to pH 8. The shift to slightly alkaline pH could be an effect from the alteration in the microenvironment of enzyme caused by immobilization on the support system [19,20].

In general, within the specified temperature range and the pH peaks for immobilized lipase, which retain higher lipase activity indicates higher stability compared to free lipase. This agrees with previous work where lipase was immobilized on electro spun cellulose nanofiber membrane [20].

3.5 Stability of Lipase Immobilization

The stability evaluation was within a temperature range of 25 ºC to 65 ºC. Based on Figure 6 (a), the relative activity for immobilized lipase was highest at 35 ºC and the activity retained up to 65% after 65 ºC. On the contrary, the relative activity of free lipase was 100% at 35 ºC and decreased rapidly to 38% as the temperature increased to 65 ºC. The ability of immobilized lipase to retain its catalytic activity at various temperatures could be due to the diffusional limitation effect and multi covalent attachment of lipase onto the support system that inhibits the changes of conformational binding site and denaturation of enzyme structure at higher temperature [5,12].

The activity of free and immobilized lipase at a range of pH from 6 to 11 is shown in Figure 6(b). Free lipase retained the catalytic activity at pH 6 to 7 and abruptly lost its activity as pH was increased to an alkaline condition. However, immobilized lipase retained up to 50 % of its relative activity at pH 10, indicating more resistance of immobilized lipase to an alkaline environment. In agreement with previous studies, the multipoint effect of a covalent bond from immobilized lipase provided stability at wider pH range, hence retaining the catalytic activity [20,21].

3.6 Reusability and Storage Stability

Reusability of lipase was evaluated to observe the ability of immobilized lipase to retain its activity after several cycles of usage. The activity of lipase retained about 73% after three cycles and the activity decreased to 20% after 8 cycles (Figure 7a). The result shows that immobilized lipase enhances the reusability of lipase after several cycles. Similar study reported that the immobilized lipase retained a 30% activity after 8 cycles [19,29]. A gradual loss of activity might indicate the dissipation of lipase from the support surface after continuous use [19]. Figure 7(b) illustrates the storage stability for free and immobilized lipase. Based on the graph, immobilized and free lipase retained the activity at 78% and 69% respectively after 30 days of storage in phosphate buffer (pH 7.0). A previous study reported that the immobilized lipase retained more than 50% activity when stored for more than 30 days [29,30]. Thus, our results indicated immobiliza-

Table 3. K_M and V_{max} values of free and immobilized lipase

Polymer	Parameter	Free lipase	Immobilized lipase
PVBC-grafted Nylon 6	R^2	0.989	0.994
	V_{max}, mM/min	14.43	17.76
	K_M, mM	2.01	1.71

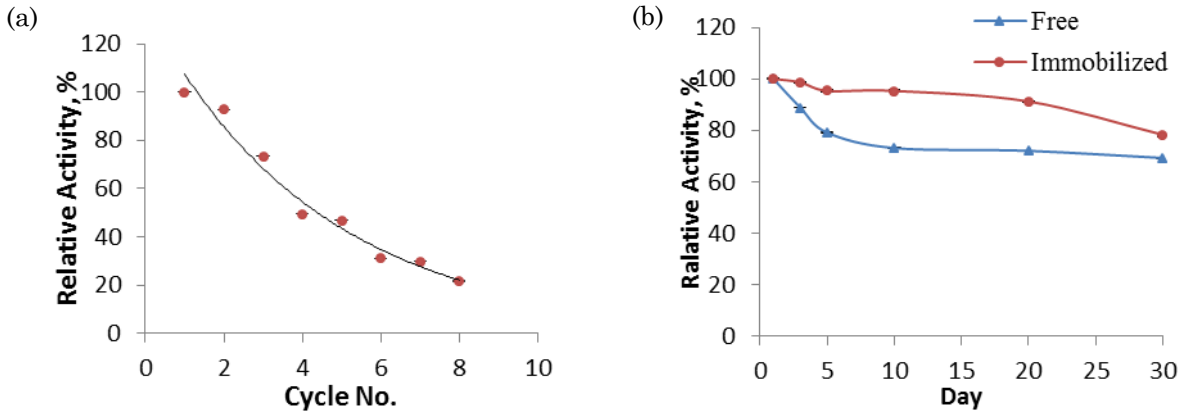

Figure 7. Relative activity versus: (a) reusability and (b) storage stability for both immobilized (●) and free (▲) lipases

tion enhances the performance of lipase activity in terms of reusability and storage stability.

3.7 Effect on Kinetic Study

Table 3 lists the results of studying the hydrolytic activity kinetics of free and immobilized lipase at different substrate concentrations. K_M values for immobilized and free lipases were 1.71 mM and 2.01 mM, respectively, which suggest that immobilized lipase has a lower affinity towards the substrates compared to free lipase. In other words, the immobilized lipase is more efficient as it required less substrate to perform a hydrolytic reaction. Furthermore, V_{max} value indicates the rate of enzyme ability to hydrolyse the substrates at saturation. The V_{max} value of immobilized lipase was found to be higher (17.76 mM/min) than free lipase (14.43 mM/min). It is worth mentioning that kinetic parameters trend relies highly among others on the enzymes used, substrate, conditions and procedure applied during the immobilization process [32].

4. Conclusions

Preparation of fibrous supports for lipase immobilization was successfully carried out by radiation-induced emulsion grafting of VBC on nylon-6 microfibers followed by treatment with ethanolamine and subsequent enzyme immobilization. Notably, a combination of optimum parameters of pH 8, 5 h incubation time and 0.7 mg/mL lipase concentration produced maximum lipase activity of 2.81 U/mg. The optimum temperature and pH for free and immobilized lipase at 45 ºC were pH 7 and pH 8 respectively. The immobilization of lipases on the fiber enhances the performance of lipase activity compared to free lipases regarding pH and temperature stability. Further, immobilized and free lipases retained 78% and 69% of catalytic activity respectively after storing up to 30 days at 4 ºC. Immobilized lipase retained 21% of its activity when recycled 8 times. The kinetic study identified K_M and V_{max} for immobilized and free lipases. PVBC-grafted nylon-6 microfibers showed to be promising support for lipase immobilization.

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References

[1] Garcia-Galan, C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., Rodrigues, R.C. (2011). Potential of Different Enzyme Immobilization Strategies to Improve Enzyme Performance. *Adv. Synth. Catal.,* 353: 2885–2904.

[2] Jegannathan, K.R., Abang, S., Poncelet, D., Chan, E.S., Ravindra, P. (2008). Production of biodiesel using immobilized lipase--a critical review. *Crit. Rev. Biotechnol.,* 28: 253–264.

[3] Miranda, M., Urioste, D., Andrade Souza, L.T., Mendes, A.A., de Castro, H.F. (2011), Assessment of the Morphological, Biochemical, and Kinetic Properties for *Candida rugosa* Lipase Immobilized on Hydrous Niobium Oxide to Be Used in the Biodiesel Synthesis. *Enzyme Res.,* 2011: 1–10.

[4] Kuo, C., Peng, L., Kan, S., Liu, Y., Shieh, C. (2013). Lipase-immobilized biocatalytic membranes for biodiesel production. *Bioresou. Technol.* 145: 229-232. doi:10.1016/j.biortech.2012.12.054

[5] Brena, B., Batista-Viera, F. (2013). Immobilization of enzymes: A literature survey. *Methods Mol. Biol.,* 1051: 15–31.

[6] Narwal, S.K., Saun, N.K., Dogra, P., Chauhan, G., Gupta, R. (2015). Production and Characterization of Biodiesel Using Nonedible Castor Oil by Immobilized Lipase from *Bacillus aerius*. *Biomed. Res. Int.,* 2015: 1: 1-6

[7] Xu, Z., Wan, L., Huang, X. (2009). Membrane with Biocatalytic Surface. *Adv. Top. Sci. Technol. China,* Springer. pp.263–305.

[8] Isgrove, F.H., Williams, R.J.H., Niven, G.W., Andrews, A.T. (2001). Enzyme immobilization on nylon – optimization and the steps used to prevent enzyme leakage from the support. *Enzym. Microbial. Technol*. 28: 225–232.

[9] Shalaby, S., Al-Balakoey, N., Abo El-Ola, S. (2007). Surface Modification of Nylon-6 Fibres for Medical Applications. *Appl. Polym. Sci.,* 104: 3788–3796.

[10] Shalaby, S., Al-Balakoey, N., Abdel-Fatah, O., Elshafei, A. (2008). Antimicrobial Finishing of Regular and Modified Nylon-6 Fabrics. *Appl. Polym. Sci.,* 110(2): 738–746.

[11] Shalaby, S.E., Beliakova, M.K. (2015). Simple Method for Grafting of Glycidyl Methacrylate onto Nylon-6 Nonwoven Fabrics for Ion Exchange Applications. *Int. J. Sci. Res.,* 4: 689–696.

[12] Liu W, Zhang S, Yu Jl, Chen X, Li L, Feng Q, Zhu X (2011) Surface Photografting: New Application for Flame Retardant Finishing of Polyamide6.6 (PA6.6) Fabric. *Appl. Polym. Sci.*, 119: 66–72.

[13] Li, X., Gu, X., Zhang, S., Li, H., Feng, Q., Sun, J., Zhao, Q. (2013). Improving the fire performance of nylon 6,6 fabric by chemical grafting with acrylamide. *Ind. Eng. Chem. Res.,* 52: 2290–2296.

[14] Nasef, M.M., Hegazy, E.S.A. (2004). Preparation and applications of ion exchange membranes by radiation-induced graft copolymerization of polar monomers onto non-polar films. *Prog. Polym. Sci.,* 29: 499–561.

[15] Ting, T.M., Nasef, M.M., Hashim, K. (2015). Modification of nylon-6 fibres by radiation-induced graft polymerisation of vinylbenzyl chloride. *Radiat. Phys. Chem.,* 109: 54–62.

[16] Ting, T.M., Nasef, M.M., Sithambaranathan, P. (2017). Kinetic investigations of emulsion- and solvent-mediated radiation induced graft copolymerization of glycidyl methacrylate onto nylon-6 fibres. *J. Radioanal. Nucl. Chem.,* 311: 843–857.

[17] Ikeda, K., Umeno, D., Saito, K., Koide, F., Miyata, E., Sugo, T. (2011). Removal of boron using nylon-based chelating fibers. *Ind. Eng. Chem. Res.,* 50: 5727–5732.

[18] Zdarta, J., Meyer, A., Jesionowski, T., Pinelo, M. (2018). A General Overview of Support Materials for Enzyme Immobilization: Characteristics, Properties, Practical Utility. *Catalysts,* 8: 92.

[19] Nasef, M.M., Abbasi, A., Ting, T.M. (2014). New CO_2 adsorbent containing aminated poly(glycidyl methacrylate) grafted onto irradiated PE-PP nonwoven sheet. *Radiat. Phys. Chem.,* 103: 72–74.

[20] Huang, X.J., Chen, P.C., Huang, F., Ou, Y., Chen, M.R., Xu, Z.K. (2011). Immobilization of Candida rugosa lipase on electrospun cellulose nanofiber membrane. *J. Mol. Catal. B Enzym.,* 70: 95–100.

[21] Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72: 248–254.

[22] Chiou, S.H., Wu, W.T. (2004). Immobilization of Candida rugosa lipase on chitosan with activation of the hydroxyl groups. *Biomaterials,* 25: 197–204.

[23] Pahujani, S., Kanwar, S.S., Chauhan, G., Gupta, R. (2008). Glutaraldehyde activation of polymer Nylon-6 for lipase immobilization: Enzyme characteristics and stability. *Bioresour. Technol.,* 99: 2566–2570.

[24] Doğaç, Y.I., Teke, M. (2013). Immobilization of bovine catalase onto magnetic nanoparticles. *Prep. Biochem. Biotechnol.,* 43: 750–765.

[25] Li, G., Nandgaonkar, A.G., Lu, K., Krause, W.E., Lucia, L.A., Wei, Q. (2016). Laccase immobilized on PAN/O-MMT composite nanofibers support for substrate bioremediation: a de novo adsorption and biocatalytic synergy. *RSC Adv.,* 6: 41420–41427.

[26] Osho, M., Popoola, T., Adeleye, T., Adetunji, C. (2016). Response surface methodology for optimal immobilization of Aspergillus niger ATCC 1015 lipase by adsorption method. *Int. J. Biol. Res.,* 4: 56.

[27] Zhu, J., Zhang, J., Lai, Q., Jiang, B., Gong, R. (2013). Covalent immobilization of lipase onto citric acid-esterified loofah sponge. *BioResources,* 8: 3289–3298.

[28] Ibrahim, A.S.S., Al-Salamah, A.A., El-Toni, A.M., Almaary, K.S., El-Tayeb, M.A., Elbadawi, Y.B., Antranikian, G. (2016). Enhancement of alkaline protease activity and stability via covalent immobilization onto hollow core-mesoporous shell silica nanospheres. *Int. J. Mol. Sci.,* 17(2): 184.

[29] Su, R., Shi, P., Zhu, M., Hong, F., Li, D. (2012). Studies on the properties of graphene oxide-alkaline protease bio-composites. *Bioresour. Technol.,* 115: 136–140.

[30] Zhu, W., Zhang, Y., Hou, C., Pan, D., He, J., Zhu, H. (2016). Covalent immobilization of lipases on monodisperse magnetic microspheres modified with PAMAM-dendrimer. *J. Nanoparticle Res.,* 18: 1–13.

[31] Arıca, M.Y., Kaya, B., Bayramoglu, G. (2005). Food Chemistry Immobilization of Candida rugosa lipase onto spacer-arm attached poly (GMA-HEMA-EGDMA) microspheres. *Food Chem.,* 92: 261–268.

[32] Xu, D.Y., Yang, Y., Yang, Z. (2011). Activity and stability of cross-linked tyrosinase aggregates in aqueous and nonaqueous media. *J. Biotechnol.,* 152: 30–36.