Optimization of *Candida rugosa* Lipase Immobilization Parameters on Magnetic Silica Aerogel Using Adsorption Method

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ABSTRACT

Magnetic silica aerogel in hydrophobic and hydrophilic forms were used as support to immobilize Candida rugosa lipase by adsorption method. Response surface methodology (RSM) was employed to study the effects of the three most important immobilization parameters, namely enzyme/support ratio (0.3-0.5, w/w), immobilization time (60-120 min) and alcohol percentage (20-40, v/v%) on the specific activity of immobilized lipase on the hydrophobic supports. For hydrophilic supports, alcohol percentage was removed as there was no need for pre-wetting step in enzyme immobilization process. Second order regression models with high coefficient determination (R^2) values of higher than 0.98 were fitted to predict the response as function of immobilization parameters. The results indicated that for hydrophobic supports, optimum values for enzyme/support ratio, immobilization time and alcohol percentage were obtained at 0.45 (w/w), 94.00 min and 37.22%, respectively, in which specific activity were predicted at 15.34 U/mg-protein. For hydrophilic supports, the optimum enzyme/support ratio and immobilization time were predicted at 0.47 (w/w) and 83.47 min, respectively. Specific activity in these conditions was obtained 11.21 U/mg-protein. As the difference between the experimental and predicted values was shown as non-significant, the response surface models employed could be considered as adequate.

1. Introduction

Lipase (EC3.1.1.3) is one of the most famous enzymes among biocatalysts that have various applications in many different fields in industry like chemistry, food, pharmaceutical, agrochemical and biotechnology. This enzyme catalyzes both the hydrolysis and synthesis reactions [1-3].

Lipase can be produced and extracted from animals, plants and microorganisms. Among them, due to easy extraction, high yields and catalytic activities, ease of genetic manipulation and potential for regular supply, microbial lipases have advantages which make them suitable for industrial applications [4]. Among microorganisms, fungus is the main microbial source to produce lipase. Yeast *Candida rugosa* (formerly known as

Candida cylindracea) is a non-sporogenic, pseudofilamentous, unicellular yeast and imperfect hemiascomycete. It grows aerobically in nutritive media with different carbon and nitrogen source. Lipases from *Candida rugosa* are one of the most commonly used enzymes due to their high activity and non genotoxic or cancerogenic effects on human health [5].

Nevertheless, although soluble in aqueous solution like other enzymes, lipases could not be easily recovered and reused when implemented in industrial processes. Immobilization of enzyme on solid support is an effective method to produce insoluble and reusable biocatalysts. Furthermore, immobilization can improve their stability and activity in the case of lipase immobilization on hydrophobic supports by adsorption method [6,7]. Moreover, most lipases have a special catalytic mechanism. Catalytic site in these enzymes is covered by a lid that is opened in the presence of hydrophobic substrate or media. Thus with immobilization of lipase in hydrophobic carriers, these enzymes can be kept in open and active form [8,9]. In addition, the enzyme immobilization onto magnetic supports allows selective and easy enzyme separation from the reaction medium under the magnetic force with no need for expensive liquid chromatography systems, centrifuges, filters or other equipment [10].

There are several techniques to immobilize the enzymes such as encapsulation, covalent binding, ionic binding, cross linking and adsorption. Among these methods, adsorption is very simple, reversible and inexpensive. Silica aerogels, due to their uncommon properties such as high specific surface area (500–1200 m^2/g), high porosity (80–99.8%) and low density (~0.003 g/cm^3) can be used

as a suitable support in enzyme immobilization [11]. Several studies have been carried out on lipase immobilization on mesoporous structure of silica aerogels and their composites. These studies concentrated on different immobilization techniques, especially encapsulation and various lipases with different sources, and the effects of various parameters such as pore structure, aging in different solvents, hydrophobic or hydrophilic carriers and the type of drying process on the enzyme activity were evaluated [12-19]. A few studies have been done on lipase immobilization using adsorption on silica aerogel [11,20].

In this work *Candida rugosa* lipase was immobilized by adsorption method and the parameters that affect lipase activity were optimized by response surface methodology (RSM) and central composite design (CCD). Enzyme/support ratio (w/w), immobilization time (min) and alcohol percentage (v/v%) were optimized to design an efficient enzymatic immobilization system for hydrophobic supports. Since for hydrophilic supports there is no need for pre-wetting step, only two parameters, enzyme/support ratio (w/w) and immobilization time (min) were investigated.

2. Materials and methods
2.1. Materials
Lipase from *Candida rugosa* (type VII) was purchased from Sigma Aldrich (Saint Louis, MO, USA). Olive oil with high purity was purchased from a local market. Ethanol, sodium hydroxide, acetone, phenolphthalein, sodium phosphate dibasic and sodium phosphate monobasic were obtained from Merck Company (Darmstadt, Germany). Gum Arabic was prepared from Sigma Aldrich. Sodium carbonate, copper

sulphate pentahydrate and Potassium sodium tartrate tetrahydrate were purchased from Merck Company to prepare Lowry reagent for protein determination. Albumin from bovine serum was used as the standard and prepared from Sigma Aldrich. For preparation of silica aerogels, sodium silicate (waterglass) with 1.35 specific gravity, ammonium hydroxide, n-Hexane, isopropyl alcohol (IPA) and Hexamethyldisilazane (HMDZ) from Merck Company were prepared. For ion exchange of sodium silicate, Amberlite IR-120 H^+ was also provided by Merck Company. Iron oxide nanoparticles were prepared from US Research Nanomaterials Company (Fe_3O_4, high purity, 99.5+ %).

2.2. Preparation of magnetic silica aerogel

In this work hydrophobic magnetic silica aerogel was used to immobilize *Candida rugosa* lipase. Magnetic silica aerogel was synthesized with iron oxide particles using ambient pressure drying method and modification with Hexamethyldisilazane as previously described [21]. The resulting nanocomposites had superhydrophobic property. After heating the composites at 500°C for 2 h, the -CH_3 groups changed to -OH groups and the supports became hydrophilic [22]. For comparison between hydrophobic and hydrophilic supports, both supports were used as lipase immobilization.

2.3. Characterization of magnetic silica aerogel

The bulk density of the nanocomposites was calculated using a microbalance scale (10^{-5} g precision, Mettler Toledo, AT261 DeltaRange, Greifensee, Switzerland) and coulisse. The BET surface area was measured using instruments by nitrogen adsorption-desorption method (Belsorp mini II, Japan

and Quantachrome Instruments CHEMBET -3000, USA). The pore structure and particle morphology were characterized by transmission electron microscopy, TEM, (Philips EM208, Eindhoven, Netherlands) and Field Emission Scanning Electron Microscopy (MIRA3 FE-SEM, Tescan, Czech). The hydrophobicity of the structures was tested by measuring the contact angle (θ) using sessile method with dynamic contact angle instrument (OCA15 plus, Data-Physics Instrument GmbH, Filderstadt, Germany).

2.4. Immobilization of lipase

To prepare enzyme solutions, the appropriate amount of enzyme powder (0.018-0.03 g) was added to 20 mL phosphate buffer solution (pH 7). For pre-wetting hydrophobic supports, 5 mL of ethanol solution (20-40, v/v%) was added to magnetic silica aerogel and then left in a closed conical flask for 3 hours. Next, the alcohol solution was discharged and the pre-wetted supports (0.06 g) were suspended into enzyme solution (with ratio 0.3-0.5, w/w) and mixture was stirred for various immobilization times (60-120 min) at ambient temperature to complete the adsorption process. After that, the suspension was filtrated and the supports with immobilized lipase were separated, washed with phosphate buffer to remove the unabsorbed enzyme and dried in the air. The filtrate and washing solutions were collected for protein determination. For lipase immobilization on hydrophilic supports, the pre-wetting step was removed.

2.5. Lipase activity assay

Immobilized lipase activity was measured using the method based on olive oil hydrolysis. In this method, 100 mL olive oil

emulsion was prepared by mixing 50 mL olive oil and 50 mL gum arabic solution (7 w/v%). 3.0 mL phosphate buffer (0.2 M, pH 7.0) and immobilized enzymes were mixed at 37°C using shaking in water bath at 100 rpm. After increasing the temperature of the enzyme solutions to 37°C, 5.0 mL substrate (olive oil) emulsion was added to it and after 15 min the reaction was stopped by the addition of 20 mL of acetone- ethanol solution (1:1 v/v). The liberated fatty acid in the medium was determined by titration with NaOH solution (0.05 N). One lipase unit corresponded to the release of 1 µmol of fatty acid per minute under the assay conditions. The specific activity is the number of lipase units per mg-protein [23,24].

2.6. Protein assay

Protein content before and after immobilization was determined by the Lowry method using bovine serum albumin as the standard [25].

2.7. Experimental design, optimization and statistical analysis

For lipase immobilization on hydrophobic supports, a 2^3 factorial central composite design with six axial (α=1.682) points, eight cube points and six central points resulting in a total of 20 experimental points was used. A 2^2 factorial central composite design with four axial (α=1.414) points, four cube points and five central points resulting in a total of 13 experimental points was applied to lipase immobilized on hydrophilic supports. Response surface methodology (RSM) was used to evaluate the effects of independent variables on the response variable, namely specific activity of immobilized enzyme. The independent variables and their levels for hydrophobic and hydrophilic supports are shown in Table1. Two second order polynomial equations (1 and 2) were used to express the specific activity of immobilized enzyme on hydrophobic (Y) and hydrophilic (Y^*) supports as a function of the studied variables respectively.

$$Y= a_0+a_1x_1+ a_2x_2+ a_3x_3+a_{11}x_1^2+ a_{22}x_2^2+ a_{33}x_3^2+ a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 \tag{1}$$

$$Y^*= b_0+ b_1x_1^*+ b_2x_2^*+ b_{11}x_1^{*2}+ b_{22}x_2^{*2}+ b_{12}x_1^*x_2^* \tag{2}$$

Where, Y_i (Y_i^*) and x_i (x_i^*) represent the response and independent variables, respectively, a_0 (b_0) is a constant, a_i (b_i), a_{ii} (b_{ii}) and a_{ij} (b_{ij}) are the linear, quadratic and interaction coefficients, respectively. The analysis was done using decoded factors.

Table 1.

The main independent variables for hydrophobic and hydrophilic supports and their levels used in central composite design.

Factor	Variables	Unit	High axial (+α)	High factorial (+1)	Center (0)	Low factorial (-1)	Low axial (-α)
					Levels		
				Hydrophobic supports			
x_1	Enzyme/support ratio	(w/w)	0.57	0.5	0.4	0.3	0.23
x_2	Time	min	140.45	120	90	60	39.55
x_3	Alcohol percentage	v/v%	46.82	40	30	20	13.18
				Hydrophilic supports			
x_1^*	Enzyme/support ratio	(w/w)	0.57	0.5	0.4	0.3	0.23
x_2^*	Time	min	140.45	120	90	60	39.55

Adequacy of the models was examined taking into account the coefficient of determination (R^2) and adjusting the coefficient of determination (R^2-adj) besides. To fit the second order polynomial equations, analysis of variance (ANOVA) was used [26]. A small P-value ($p<0.05$) represents significance of each term in the models on the response variable. For graphical explanation of the independent variable interactions, three dimensional surface plots of the model were used. This is useful to visualize the relationship between the responses and the experimental levels of each factor. Numerical optimization was performed by the response optimizer for determining the exact optimum concentrations of independent variables in both hydrophilic and hydrophobic supports leading to maximum specific enzyme activity. The experimental design, data analysis and optimization procedure were performed using the Design Expert version7 statistical software (Stat-Ease Inc., NY, USA).

3. Results and discussion

3.1. Morphology and structure of magnetic silica aerogel

TEM image of magnetic silica aerogels (Figs. 1(a) and (b)) showed that the porous silica aerogel surrounded the iron oxide particles. It can also be explained by the fact that during dispersion of the magnetic particles in silica sol, the inherent affinity between the silica and iron molecules caused the formation of silica gel around the iron oxide particle [27]. After other solvent exchange and modification steps, the silica aerogel-iron oxide composite was synthesized. The morphology of pure magnetite was observed to be cubic with

particle size ranging from 100 nm to 250 nm. FESEM image of samples confirmed these results (Fig. 2).

Density and specific surface area of the prepared composite were 0.42 g/cm^3 and 499 m^2/g, respectively. The mean pore size of magnetic silica aerogel was 8.35 nm based on BET analysis. This mesoporous structure provides enough space for *Candida rugosa* lipase immobilization.

On the other hand, modification of the gels with HMDZ produces the -CH$_3$ groups and hydrophobic nature in composites that are suitable for lipase adsorption because of their
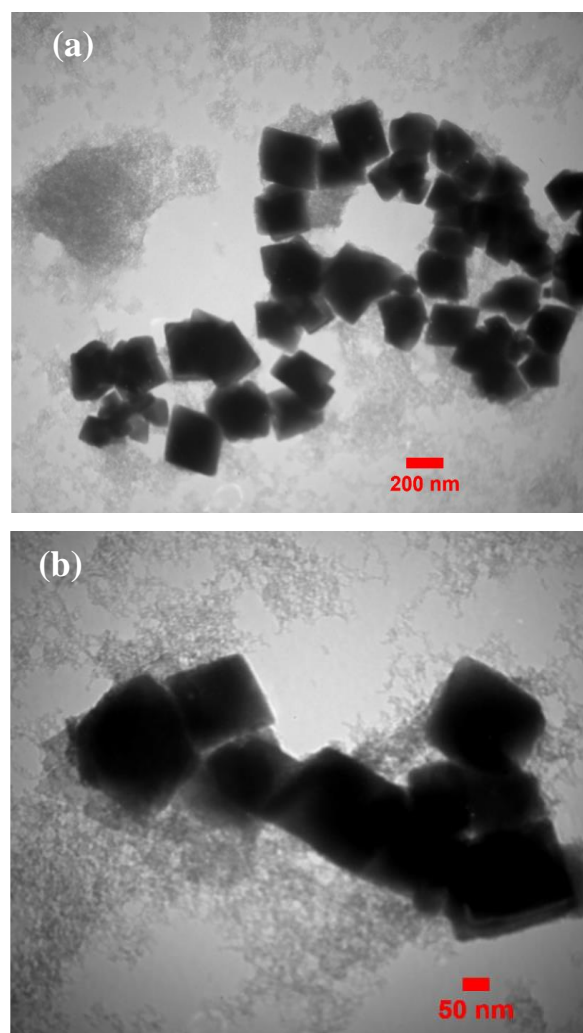
Figure 1. TEM images of iron oxide- silica aerogel composite at different magnifications (a) 200 nm (b) 50 nm.
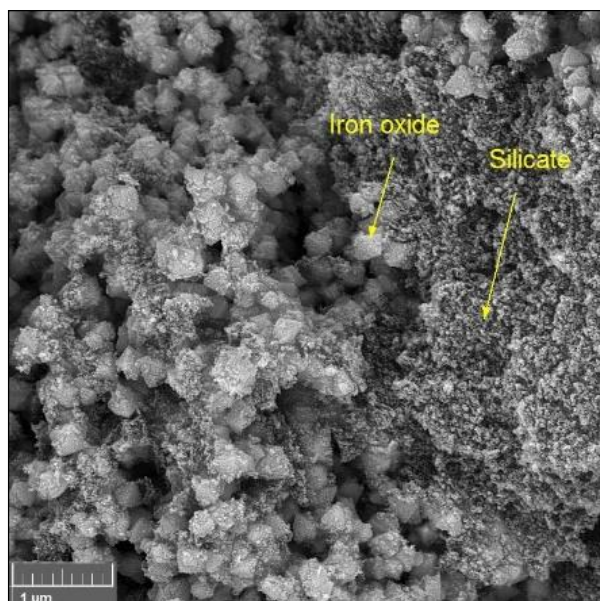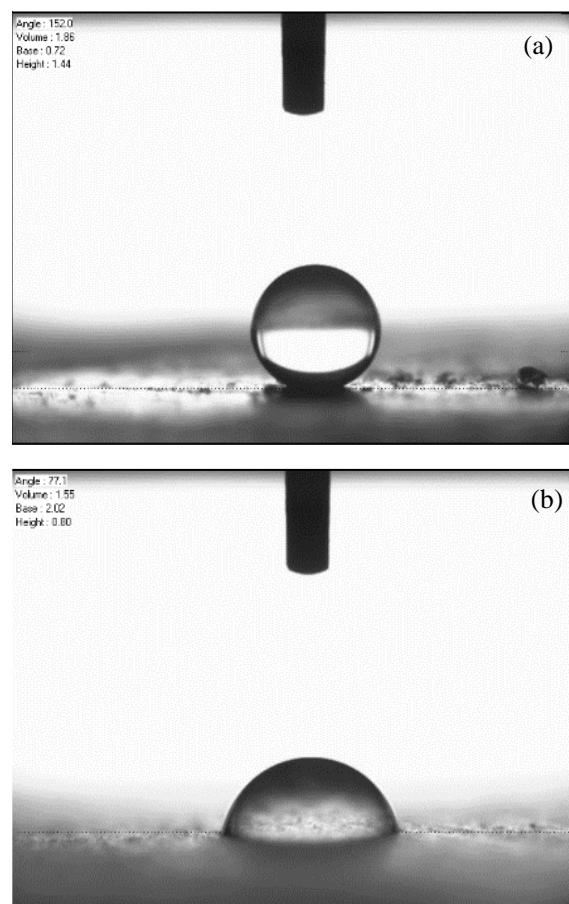
Figure 2. FE-SEM micrograph of magnetic silica aerogel composite.

Figure 3. Contact angle of magnetic silica aerogel with water (a) before annealing (b) after annealing in 500˚C for 2 h.

special structure [20]. The contact angle of the prepared composites with water before and after annealing, is shown in Figs. 3(a) and (b) respectively. The contact angle of these composites with water before heating was 152˚ which indicated the superhydrophobic nature of them. After heating the contact angle decreased to 77˚ due to formation of the hydroxyl groups.

3.2. Fitting the response surface models
The activities of immobilized enzyme on hydrophobic and hydrophilic supports obtained from experimental runs are shown in Tables 2 and 3, respectively. Equations 1 and 2 were used to fit the data. The estimated regression coefficients for final reduced models as well as the corresponding significance of regressions were given in Tables 4 and 5. It should be considered that lower p value and higher F ratio corresponds to higher significance of a term on studied response variations. The reduced models were obtained after removing the non-significant terms. The results indicated that in hydrophobic supports, the coefficient of

determination (R^2) of model for the response of the enzyme activity was 0.9910 (Table 4) and in hydrophilic supports, the R^2 value of response was 0.9891 (Table 5). The obtained high R^2 values confirmed the suitability of the suggested models. Furthermore, the attained non-significant lack of fits and high F values for the suggested models ensured the models were significant (Tables 4 and 5).

3.3. Analysis of response surfaces
As shown in Tables 2 and 3, enzyme activity varied from 13.5 to 15.3 U/mg-protein for hydrophobic and 9.4 to 11.2 U/mg-protein for hydrophilic supports, respectively. As clearly observed in Table 4, the linear and quadratic terms of all independent variables had significant ($p < 0.05$) effects on enzyme

Table 2.
Three factor central composite design and the experimental and predicted values of response for hydrophobic supports.

Run	Enzyme/support ratio (w/w)	Time (min)	Alcohol percentage (v/v%)	Specific activity (U/mg-protein) Experimental	Predicted
1	0.3	60	20	13.8	13.78
2	0.5	60	20	14.1	14.13
3	0.3	120	20	14	14.06
4	0.5	120	20	14.6	14.56
5	0.3	60	40	13.9	13.98
6	0.5	60	40	14.6	14.58
7	0.3	120	40	14.6	14.61
8	0.5	120	40	15.3	15.36
9	0.23	90	30	14.4	14.35
10	0.57	90	30	15.3	15.29
11	0.4	39.55	30	13.5	13.48
12	0.4	140.45	30	14.4	14.37
13	0.4	90	13.18	14.1	14.10
14	0.4	90	46.82	15	14.94
15	0.4	90	30	15.2	15.08
16	0.4	90	30	15.05	15.08
17	0.4	90	30	15	15.08
18	0.4	90	30	15.1	15.08
19	0.4	90	30	15	15.08
20	0.4	90	30	15.1	15.08

Table 3.
Two factor central composite design and the experimental and predicted values of response for hydrophilic supports.

Run	Enzyme/support ratio (w/w)	Time (min)	Specific activity (U/mg-protein) Experimental	Predicted
1	0.30	60.00	9.4	9.36
2	0.50	60.00	10.6	10.65
3	0.30	120.00	10.1	9.98
4	0.50	120.00	10.8	10.77
5	0.26	90.00	9.4	9.50
6	0.54	90.00	11	10.97
7	0.40	47.57	9.9	9.88
8	0.40	132.43	10.3	10.4
9	0.40	90.00	11.1	11.1
10	0.40	90.00	11.05	11.1
11	0.40	90.00	11.1	11.1
12	0.40	90.00	11.2	11.1
13	0.40	90.00	11.06	11.1

activity of the immobilized enzyme on hydrophobic supports. The results indicated that enzyme/support ratio, time and alcohol percentage had more significant effects on enzyme activity of the immobilized enzyme, respectively, due to their high F ratio values (Table 4). The resulted regression cofficients in hydrophobic supports showed that, all the main terms had a positive effect on enzyme

activity. It means that at low enzyme/support ratio, time and alcohol percentage, the enzyme activity increased by an increase in all the mentioned independent variables and vice versa. The opposite results were obtained for effects of all the independent variables at their high level on the activity of immobilized enzyme on the hydrophobic support (Table 4).

Table 4.

The significance probability (p value, F ratio) of regression coefficients and Lack-of-fit, regression coefficients, R^2 and R^2 adjusted for the final reduced model of hydrophobic supports.

Parameters	Variables	Enzyme activity (Y)		Regression coefficient Y	
		F ratio	P value		
				a_0	7.5246
Main	x_1	203.81	< 0.0001	a_1	6.9936
	x_2	182.99	< 0.0001	a_2	0.0767
	x_3	163.30	< 0.0001	a_3	0.0914
Quadratic	x_1^2	22.35	0.0008	a_{11}	-9.0014
	x_2^2	459.60	< 0.0001	a_{22}	-0.0005
	x_3^2	106.04	< 0.0001	a_{33}	-0.002
Interaction	x_{12}	2.15	0.1730	a_{12}	-
	x_{13}	5.98	0.0345	a_{13}	0.0625
	x_{23}	11.72	0.0065	a_{23}	0.0003
Regression		122.28	< 0.0001		
Lack-of-fit		0.82	0.5848		
R^2		0.9910			
R^2 adjusted		0.9829			

Table 5.

The significance probability (p value, F ratio) of regression coefficients and Lack-of-fit, regression coefficients, R^2 and R^2 adjusted for the final reduced models of hydrophilic supports.

Parameters	Variables	Enzyme activity (Y^*)		Regression coefficient Y^*	
		F ratio	P-value		
				b_0	-4.2853
Main	x^*_1	271.98	< 0.0001	b_1	43.5334
	x^*_2	33.72	0.0007	b_2	0.1192
Quadratic	x^{*2}_1	163.20	< 0.0001	b_{11}	-43.2250
	x^{*2}_2	203.14	< 0.0001	b_{22}	-0.0005
Interaction	x^*_{12}	7.85	0.0265	b_{12}	-0.0417
Regression		127.58	< 0.0001		
Lack-of-fit		3.95	0.1090		
R^2		0.9891			
R^2 adjusted		0.9814			

As clearly observed in Table 4, the interaction of enzyme/support ratio and time had non-significant effect on enzyme activity. While other interactions had significant effects on activity of lipase immobilized on hydrophobic supports. Figs. 4(a) and (b) show the interactive effect of parameters on lipase activity in hydrophobic supports.

The effect of different alcohol percentages and enzyme/support ratio on specific activity of immobilized lipase on hydrophobic supports (at a constant time of 90 min) is shown in Fig. 4(a). As clearly observed in this figure, at constant alcohol percentage, by increasing the enzyme/support ratio the enzyme activity was effectively increased. This increase in high alcohol percentage was more significant. The amount of lipase immobilized on carrier was limited because the porous sites were saturated [24]. Thus the various amounts of enzyme/support ratio were examined to find the best ratio. Based on the obtained results in Fig. 4(a), the

(a)

(b)
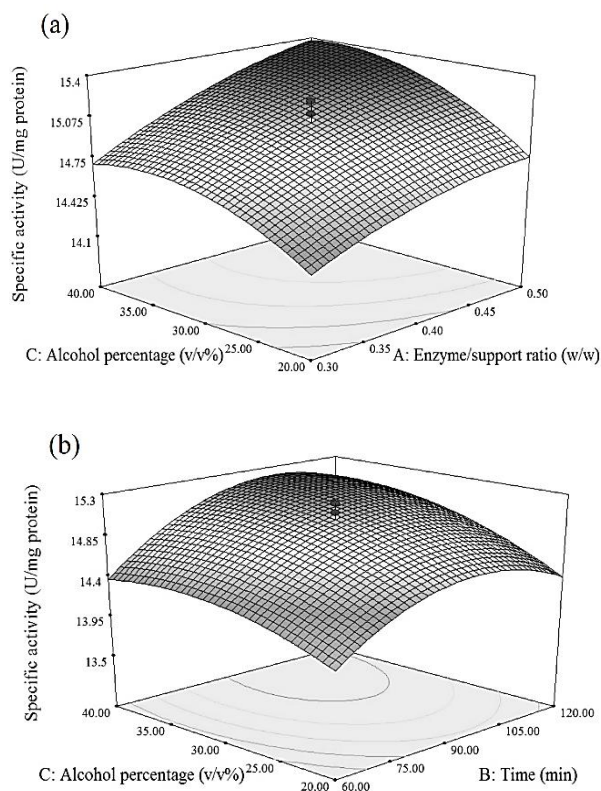
Figure 4. Response surface plot of specific activity versus (a) Enzyme/support ratio and alcohol percentage (time was fixed at 90 min) (b) Time and alcohol percentage (enzyme/support ratio was fixed at 0.4) in hydrophobic supports.

enzyme specific activity must increase when the enzyme/support ratio increases.

However, higher enzyme/support ratio might possibly lead to higher specific activity of immobilized lipase. To avoid higher enzyme cost, maximum value of enzyme/support ratio was set 0.5 in this study.

On the other hand, at constant enzyme/support ratio, by increasing the alcohol percentage the lipase activity was increased in low amount of alcohol percentage (< 35%) and then in high amount of alcohol percentage, the activity was decreased. In fact, as clearly observed in Fig. 4(a), the minimum immobilized lipase activity was obtained at low enzyme/support ratio and alcohol concentrations, which in

turn were in agreement with the obtained experimental values (Table 2). The results also indicated that at high enzyme/support ratio (0.5) and alcohol percentage of 38.4%, the enzyme activity had maximum value (15.39 U/mg-protein).

The superhydrophobic nature of supports did not allow free enzyme to penetrate in pores. It seems that with a considerable decrease in interfacial tension, the penetration of lipase into inner surface of the pores is possible. In fact, by pre-wetting the supports with alcohol the lipase penetrated easily into the supports and, after drying, when the alcohol left the pores lipase presented in active form in hydrophobic media and acted at its highest capacity. It is possible to improve the penetration of free enzyme and its activity by increasing alcohol percentage. On the other hand, the treatment supports with some polar solvents on immobilized lipases increased their activity and stability. Activity enhancement was explained by opening the lid of the enzyme that was caused by ethanol solution [28,29]. The results obtained in the present study were in agreement with the findings of Gao *et al.* [30]. They also found that alcohol treatment of hydrophobic supports increased the activity of immobilized lipase on silica aerogel.

On the other hand, in high ethanol concentration adsorption of lipase on supports was decreased because the hydrophobicity of the enzyme solution increased after the addition of alcohols. Therefore polarity difference between the support and the enzyme solution decreased, which in turn weakened the hydrophobic interactions between lipase molecule and the support. Also, some alcohols may cause rearrangement of the secondary structure of

lipases and may compress the molecule, so as to prevent its accessibility into the pores [30,31].

Fig. 4(b) shows the interactive effect of alcohol percentage and time on enzyme activity. Results indicated that by increasing enzyme/support ratio and time, specific activity was first increased and then limited. A condition with immobilization time (103 min) and alcohol percentage (37.3%) favored maximal activity. An increase in time up to 103 min resulted in less activity. The presence of curvature in the Fig. 4(b) confirmed that the variation of enzyme activity value could be explained as a nonlinear function of alcohol percentage and time.

Table 5 showed that all linear, quadratic and interaction effects of enzyme/support ratio and time had significant ($p<0.05$) effects on lipase activity immobilized on hydrophilic supports. Results indicated that at low levels, the enzyme/support ratio had a stronger effect than time on enzyme activity due to its higher F ratio value. The results also indicated that the quadratic effects of enzyme/support ratio were less than time because of its lower F ratio value.

The enzyme/support ratio and time interaction are shown in Fig. 5. This figure and the regression coefficients in Table 5 revealed that main terms of the independent variables had positive effects on specific activity, while their quadratic terms affected enzyme negatively. The maximum amount of enzyme activity was obtained in enzyme/support ratio of 0.46 and 93 min.

The results showed that specific activity in hydrophilic supports was less than that in the hydrophobic ones because interaction with hydrophobic supports can cause the lid to open, making the active site accessible. So
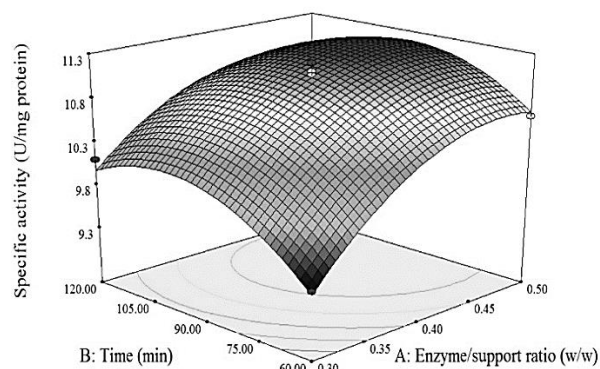
Figure 5. Response surface plot of specific activity versus enzyme/support ratio and time in hydrophilic supports.

immobilized lipase in hydrophobic carriers was in active form and performed with maximum capacity [9]. On the other hand, high porosity of supports and high density of hydrocarbon groups on the surface of hydrophobic supports were the main reasons of high specific activity in magnetic silica aerogels.

3.4. Optimization and verification of the immobilization conditions

The amount of enzyme/support ratio, time and alcohol percentage would be considered optimum if enzyme activity attained the largest possible values. Numerical optimization was used to find the exact optimum levels of the studied variables. The optimum immobilization conditions for hydrophobic supports were: 0.45 (w/w) enzyme/support ratio, 94.00 min and 37.22% alcohol percentage; for hydrophilic supports, 0.47 (w/w) enzyme/support ratio and 83.47 min. The highest specific activity in these conditions predicted by the equations is shown in Table 6.

The optimal conditions were verified using an experimental test and were in good agreement with the experimental result (Table 6), implying that the models derived

Table 6.
Experimental and predicted values obtained using optimum condition of immobilization in hydrophobic and hydrophilic supports.

Optimum conditions	Hydrophobic supports		Hydrophilic supports	
Response variables	Predicted	Experimental	Predicted	Experimental
Specific activity (U/mg-protein)	15.34	15.1±0.33	11.21	11.3±0.4

from RSM can be used to adequately describe the relationship between the factors and response in immobilization of *Candida rugosa* lipase in hydrophobic and hydrophilic magnetic silica aerogel supports.

4. Conclusions

Immobilization of *Candida rugosa* lipase on magnetic silica aerogel was done successfully by adsorption method. Response surface methodology (RSM) and central composite design (CCD) were employed to optimize the parameters that effect specific enzyme activity. The used composites were synthesized by ambient pressure drying method and modification with hexamethyldisilazane (HMDZ) and have a hydrophobic nature which, after heating at 500˚C for 2 h became hydrophilic. To compare the operation of hydrophobic and hydrophilic supports in lipase activity, both supports were used. The results demonstrated that the activity of immobilized enzyme in hydrophobic supports was considerably higher than those of the hydrophilic supports. Magnetic silica aerogel showed a promising future for immobilization enzymes as it allowed easy immobilization and separation from reaction media through a simple and inexpensive process.

Acknowledgements

Abbreviations

ANOVA	Analysis of Variance
BET	Brunauer–Emmett–Teller
CCD	Central Composite Design
FE-SEM	Field Emission Scanning Electron Microscopy
HMDZ	Hexamethyldisilazane
IPA	Isopropyl Alcohol
RSM	Response surface methodology
TEM	Transmission Electron Microscopy

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