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IMMOBILIZATION OF LIPASE ON SEPABEADS AND ITS APPLICATION IN PENTYL OCTANOATE SYNTHESIS IN A LOW AQUEOUS SYSTEM

Zorica D. Knežević-Jugović, Svetlana V. Šaponjić, Dejan I. Bezbradica and Dušan Ž. Mijin

The object of the study was to investigate the process conditions relevant for the pentyl octanoate production with the lipase from Candida rugosa immobilized on Sepabeads EC-EP carrier. This is an epoxide-containing commercial polymethacrylic carrier with suitable characteristics for enzyme immobilization. The immobilized lipase suitable for pentyl octanoate synthesis has been prepared by a direct lipase binding to polymers via their epoxide groups. The enzymatic activity was determined by both hydrolysis of olive oil in an aqueous system and esterification of n-pentanol with octanoic acid in a low aqueous system. The influence of several important reaction parameters such as temperature, initial water content, initial substrate molar ratio, enzyme loading and time of adding of molecular sieves in the system is carefully analyzed by means of an experimental design. Production of the ester was optimized and an ester production response equation was obtained, making it possible to predict ester yields from known values of the five main factors. Almost complete conversion (>99%) of the substrate to ester could be realized, using lipase loading as low as 37 mg/g dry support and in a relatively short time (24 h) at 45 ^{o}C, when high initial substrate molar ratio of 2.2 is used.

KEYWORDS: Lipases, covalent immobilization, Sepabeads, esterification, response surface methodology, pentyl octanoate

INTRODUCTION

In the drive towards green, sustainable methodologies for ester synthesis, the use of lipases has much to offer: mild reaction conditions, high catalytic activity, high specificity, and economic viability. More importantly, the product quality of such enzyme-synthesized esters normally is better than the chemical-derived product due to the lower reaction temperature and avoidance of strong acid-catalyzed degradation products. Con-

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sequently, numerous attempts have been made to develop an efficient lipase system for the synthesis of food acceptable esters (1,2). However, industrial application is often hampered by a lack of long-term operational stability, specially in the presence of organic solvents, and difficult recovery and re-use of the enzyme.

Immobilization of lipases may make them more attractive for industrial applications, enabling easy separation and the possibility of operation in a packed-bed or fluidized-bed reactor even under extreme conditions of temperature and pH, as well as in the presence of organic solvents (3-5). However, design of an efficient lipase immobilized system possessing high loading capacity and activity retention as well as improved stability for the esters synthesis in non-conventional media still has many unresolved issues. One common problem is the reduction of the enzymatic activity, because the active sites can be involved in the bonding between the enzyme and the support. Another problem is the lack of active sites on the polymer. Consequently, most previous studies used glutaraldehyde as a non-specific cross-linking agent to fix the enzyme on the polymeric matrix, but the results were often unsatisfactory-with low immobilization yield and low final enzyme activity (6). Therefore, development of new techniques for lipase immobilization on inexpensive and industrially applicable carriers is of economical significance.

The synthetic polymethacrylic carriers, as Sepabeads, deserve special attention as carriers for lipases immobilization because they have excellent chemical and thermal stability, resistance to attrition and osmotic shock, high protein binding capacity, low swelling tendency in high molar solution and in common solvents, excellent performance in stirred batch reactors, etc. Among them, Sepabeds EC-EP, an epoxy-activated carrier, is almost-ideal ones to perform very easy immobilization of enzymes at both laboratory and industrial scale (7). Epoxide groups are convenient for the covalent binding of enzymes since they are able to directly react with amino, hydroxyl, or sulfhydryl groups of enzymes depending on pH of the buffer used. Thus, it is not necessary to activate the carrier or enzyme to achieve covalent immobilization. Moreover, the N-C, O-C or S-C bonds formed by the epoxide groups are extremely stable, so that the epoxide-containing commercial polymers such as Eupergit or Sepabeads can be successfully used for the immobilization of enzymes and proteins (8,9). Although a number of studies have shown that Sepabeads are good carriers for enzyme immobilization, their potential for lipase binding for the purpose of ester synthesis in non-aqueous system was not fully explored.

The aim of this work was to improve the performance of lipase from *C. rugosa* for catalysis in a low aqueous system by immobilizing it covalently on an inexpensive and industrially applicable carrier such as Sepabeads. The immobilized enzyme was characterized by evaluating the potential effects of immobilization on its activity in both hydrolysis and synthesis, especially in comparison with free enzyme. The synthesis of pentyl octanoate in isooctane was chosen as a model reaction in low aqueous system. Optimization of the ester synthesis was performed by application of the factorial design and response surface methodology. The initial water content, reaction temperature, enzyme loading, initial acid/alcohol molar ratio and time of adding of molecular sieves were the variables investigated.

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EXPERIMENTAL

Materials

Commercial *C. rugosa* (EC 3.1.1.3) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Octanoic acid and *n*-pentanol were purchased from Merck (Darmstadt, Germany). A Sigma lipase Substrate (St. Louis, MO, USA) was used to determine lipolytic activity in aqueous medium. Isooctane of p.a. grade purchased from Merck (Darmstadt, Germany) was dried over 3-Å (0.3 nm) molecular sieves for at least 24 h prior to use, and as such was used for esterification activity in non-aqueous medium. Solvents used in analytical procedures, and other reagents were reagent grade and were purchased either from Aldrich Chemical Co. (St Louis, MO, USA) or Sigma Chemical Co. (St Louis, MO, USA).

Lipase immobilization

The method for lipase immobilization on Sepabeads EC-EP support involves the direct enzyme binding on polymers via epoxide groups (Fig. 1). Unmodified Sepabeads EC-EP (500 mg of wet carrier) was incubated with 10 cm^3 of native enzyme attachment solutions in a shaking water bath (130 strokes per min) at 25 ^oC. Enzyme attachment solutions containing 0.5-3.0 mg/cm^3 of enzyme were prepared in a 1.25 M potassium phosphate buffer at pH 8.0. After incubation for 48 h, the beads were collected by vacuum filtration using a glass filter (Whatman), washed with 1 M NaCl (3x20 cm^3), afterwards with potassium phosphate buffer, pH 8.0 (3x20 cm^3) and stored in it at 4 ^oC until use. Samples of the filtrate and enzyme solution before immobilization, together with the washing solutions, were taken for protein content and enzyme activity assay. The amount of bound enzyme was determined indirectly from the difference between the amount of enzyme introduced into the coupling reaction mixture and the amount of enzyme in the filtrate and in the washing solutions.

Enzyme hydrolytic activity assay

Hydrolytic activities of free and immobilized lipase were assayed by the standard olive oil emulsion method, see (8). Activities are expressed as international units (IU), where 1 IU is defined as the amount of enzyme required to produce 1 μmol of free fatty acid per minute under the assay conditions (37 ^oC, pH 7.7). It was determined that 1 mg of free lipase Sigma had an activity of 0.55±0.09 IU.

Catalytic properties of the biocatalysts in non-aqueous medium

Esterification reactions were performed with the immobilized lipase in screw-capped 100 cm^3 flasks in isooctane. *n*-Pentanol and octanoic acid were added at different molar ratios followed by different amounts of water, according to the experimental design. The reaction mixture was then diluted up to the volume of 10 cm^3 with isooctane and incubated on a shaker at 150 rpm and at different temperatures prior to the addition of the immobilized lipase. The immobilized enzyme (0.5 g) with varying enzyme loadings was added in the reaction mixture only after the correct temperature was attained and samples were taken for analysis after 24 h. Control experiments were also conducted without lipase under similar conditions.

Analysis

Esterification reactions were monitored by determination of the residual acid content by titration against standard sodium hydroxide using phenolphthalein as an indicator and methanol as a quenching agent. The molar conversion was determined from the values obtained for the blank and the test samples. Reactions were also monitored by measuring products concentrations by gas chromatography (model: Varian 3400) equipped with a Carbowax 20-M column (3 m length, 3.175 mm internal diameter) and flame ionization detector (FID). Nitrogen was used as a carrier gas with a flow rate of 30 cm^3 min^{-1}. Column oven, injection part, and detector temperatures were at 100, 200, and 250 ^oC, respectively. The reported percentage ester yield was defined as the amount of ester produced to initial substrate in defect (mol ester/mol initial substrate in defect x 100). The percentage of esterification determined by both GC analysis and titration were found to be in good agreement.

Experimental design and analysis

A five-level-five-factor central composite design was employed in this study, requiring 32 experiments, which consisted of 16 factorial points, 10 axial points and 6 central points (10). The variables and their levels selected for the study of ester synthesis were: water content [0.1-0.9% (w/v)]; temperature [25-65 ^oC]; enzyme loading [16-60 mg/g dry support]; acid/alcohol molar ratio [1:2–5:2] and time of adding of molecular sieves [0-20 h]. These variables were chosen based on the results obtained in a preliminary study and are the most commonly used for modeling esterification reactions. In a preliminary study the effects of reactants concentration on the initial rate of the ester production were investigated with the reactants added in stoichiometric proportion. It seems that the alcohol above 0.5 M in isooctane caused inactivation of the immobilized lipase while the acid did not inactivate the enzyme in concentrations up to 1.5 M. Therefore, the effect of acid/alcohol molar ratio was investigated fixing the initial alcohol concentration at a lower value (500 $mmol/dm^3$) at different concentrations of acid.

The actual and coded settings of each of the five experimental factors are given in Table 1. To avoid bias, 32 runs were performed in a totally random order. The ester yield was taken as the response variable. The design of experiments employed is presented in Table 2. The data obtained were fitted to a second-order polynomial equation:

$$Y = \beta_{k0} + \sum_{i=1}^{5} \beta_{ki} X_i + \sum_{i=1}^{5} \beta_{kii} X_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta_{kij} X_i X_j \qquad [1]$$

where Y is the response (ester yield in mol%), β_{k0}, β_{ki}, β_{kii} and β_{kij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively and X_i and X_j are independent variables. The coefficients of the response function and their statistical significance were evaluated by the method of least squares by using the MATLAB software (version 6.5, Release 13, The MathWorks, Juc, Matick, MA, USA). Only the significant terms ($p \leq 0.05$) were considered for the final reduced model. The lack-of-fit test was used to determine whether the constructed model was adequate to describe the obtai-

ned data. The goodness of fit of the model was evaluated by the determination (R^2) and r coefficients complemented by the graphic plot of predicted values by the model *vs.* observed experimental values. High values of both R^2 and r suggest a good fit of the model to the experimental data. Response surfaces and contour plots were obtained using the fitted model, by keeping independent variables at a constant value while changing the other two variables.

Table 1. Coded and actual values of variables for design of experiment

Variables	Coded levels of variables				
	-2	-1	0	1	2
Water content, X_1 (%, w/v)	0.1	0.3	0.5	0.7	0.9
Temperature, X_2 (ºC)	25	35	45	55	65
Enzyme loadings, X_3 (mg/g of dry support)	16	27	38	49	60
Substrate molar ratio, X_4	1:2	1:1	3:2	2:1	5:2
Time of adding of molecular sieves, X_5 (h)	0	5	10	15	20

Table 2. Experimental setup for five-level, five-factor central composite rotatable design in terms of coded, actual values of variables and experimental data

Run no.	Water content	Temperature	Enzyme loadings	Acid/alcohol molar ratio	Molecular sieves	Yield
	X_1/%	X_2/ ºC	X_3/mg g^{-1}	X_4	X_5/ h	Y /%
1	1	1	1	1	1	87.43
2	1	1	1	-1	-1	48.18
3	1	1	-1	1	-1	49.84
4	1	1	-1	-1	1	21.51
5	1	-1	1	1	-1	93.69
6	1	-1	1	-1	1	80.18
7	1	-1	-1	1	1	98.16
8	1	-1	-1	-1	-1	76.18
9	-1	1	1	1	-1	82.95
10	-1	1	1	-1	1	57.51
11	-1	1	-1	1	1	71.77
12	-1	1	-1	-1	-1	23.29
13	-1	-1	1	1	1	92.35
14	-1	-1	1	-1	-1	77.07
15	-1	-1	-1	1	-1	88.32
16	-1	-1	-1	-1	1	70.84
17	2	0	0	0	0	90.32
18	-2	0	0	0	0	78.70
19	0	2	0	0	0	36.56
20	0	-2	0	0	0	81.97
21	0	0	2	0	0	92.75
22	0	0	-2	0	0	17.30

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Table 2. Continuation

Run no.	Water content	Temperature	Enzyme loadings	Acid/alcohol molar ratio	Molecular sieves	Yield
	X_1/%	X_2/ °C	X_3/mg g^{-1}	X_4	X_5/ h	Y /%
23	0	0	0	2	0	100.00
24	0	0	0	-2	0	32.60
25	0	0	0	0	2	90.33
26	0	0	0	0	-2	76.47
27[a]	0	0	0	0	0	73.21
28[a]	0	0	0	0	0	89.16
29[a]	0	0	0	0	0	84.51
30[a]	0	0	0	0	0	92.65
31[a]	0	0	0	0	0	79.16
32[a]	0	0	0	0	0	93.15

RESULTS AND DISCUSSION

Lipase immobilization

The method for lipase immobilization on Sepabeads EC-EP involves the direct enzyme binding on polymer via epoxy groups (Fig. 1). We studied the influence of the lipase concentration in the attachment solution in the range of 0.5-3.0 mg/cm^3 on the total enzyme loading on Sepabeads as well as activity of the immobilized enzyme. The results are shown in Fig. 2. In each experiment, 0.5 g of polymer particles was immersed in a certain volume of enzyme solution. The aim was to determine an efficient relationship between the enzyme and support.

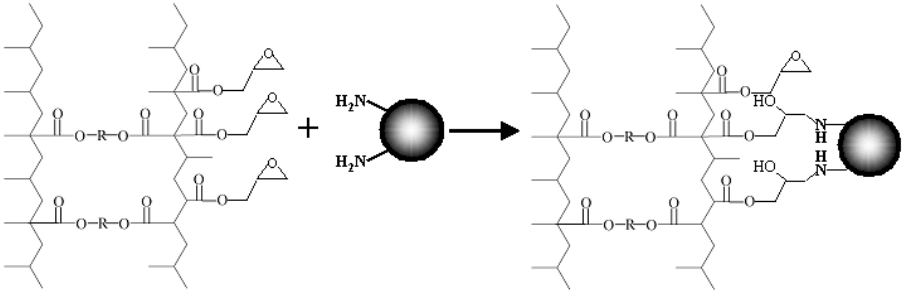

Figure 1. Immobilization of lipase on Sepabeads EC-EP carrier via epoxide groups

The hydrolitic activity of the immobilized lipase increased from 9.6 to 23.2 IU/mg of dry support as more lipase was loaded onto the support (8.18-42.23 mg/g). However, lower specific activities were obtained at higher lipase loadings, possibly due to close packing of the enzyme on the support surface which could limit the access of substrate needed in the reaction. Thus, high lipase concentration seems to result in high enzyme activity per unite of support surface but also in rather low specific activity, due to the diffusional limitations. The activity coupling yield has also been shown to depend on the

enzyme concentration in the attachment solution and the maximum yield of 95.3% was achieved working at low enzyme concentration. These results are favorable compared with those reported in the literature in terms of both specific activities of the immobilized enzyme and activity yields (11). Such yields are probably owing to the immobilization methodology used; i.e., the use of epoxyde groups for direct enzyme binding on the polymer. Similar results have been described by several researchers, indicating a trend in the use of epoxide groups as functional groups for lipase immobilization on different supports (9,12).

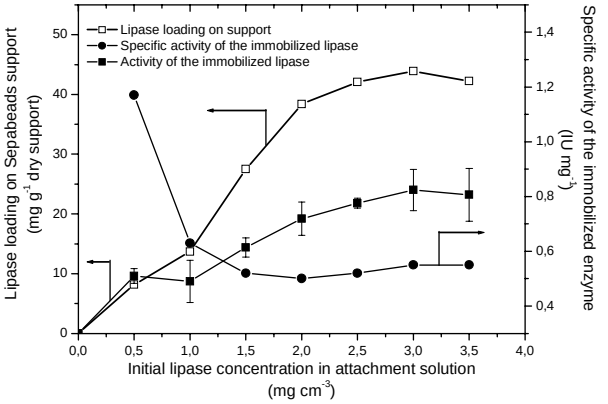

Figure 2. Effects of initial lipase concentration in attachment solution on the lipase loading as well as activity and specific activity of the immobilized lipase

Response surface analysis

The aim of this work was to evaluate the performance of the immobilized lipase from *C. rugosa* on Sepabeads in non-aqueous medium using a reaction system of interest from an industrial point of view. Synthesis of pentyl octanoate was chosen as the case study, for being an already known ester of current interest. Response surface methodology (RSM) and 5-level-5-factor central composite rotatable design (CCRD) were used to optimize and understand the relationship between the important reaction parameters. RSM is an optimization technique that determines optimum process condition by testing several variables at a time, uses special experimental designs to cut the number of required determinations. In addition, this technique allowed us to quantify the individual effect of each factor and to investigate their possible interactions.

The data showing the experimental yield of ester for the 32 experiments of the statistical design are presented in Table 2. The ester synthesis appears to show a wide variation in yield (17.3-100%) as can be seen in the table. Among the various treatments, the greatest molar conversion (≈100%) was achieved in run no. 23 (water content of 0.5%, 45 ^{o}C, enzyme loading of 38 mg g^{-1}, substrate molar ratio 5:2, 10 h), while the smallest conversion (only 17.3%) was achieved in run no. 22 (water content of 0.5%, 45 ^{o}C, enzyme loading of 16 mg g^{-1}, substrate molar ratio 3:2, 10 h).

A statistical analysis was carried out on the experimental, and the main effects and interaction effects of the variables were estimated. Both the *t*-test and *p*-value statistical parameters were used to confirm the significance of factor studied. It seems that the most relevant variables for the ester synthesis were initial substrate molar ratio with an estimated effects of 14.4. The effects of temperature, enzyme loading and temperature-enzyme loading interaction were also significant ($p< 0.05$). However, while substrate molar ratio and enzyme loading seem to have positive effect, temperature had a significant negative influence on ester yield (-13.6) which is in agreement with thermal stability data for this lipase in non-aqueous medium (13,14). Three quadratic terms were also significant ($p<0.05$). The final response equation obtained after eliminating the insignificant terms was as follows:

$$Y = 87.0 - 13.6X_2 + 11.3X_3 + 14.4X_4 + 6.11X_2^2 - 7.18X_3^2 - 4.35X_4^2 + 6.27X_2X_3 \qquad [2]$$

Interestingly, effect of water content on the conversion was not significant and could be neglected in the range of test. Perhaps, it is because water content in this range was sufficient to preserve the catalytic conformation of the enzyme and the lipase itself contains sufficient water to maintain its activity. In general, it is observed that only a very small amount of water is needed to successfully use enzymes in organic solvents, however the optimal level of water should be determined for particular reaction system (13). In our experimental setup, the water content did not significantly influence the ester yield. Therefore, added water was constant at 0 levels (0.5%) in the following discussion. The fit of the model was checked by the R^2, which was calculated to be 0.912, indicating that 91.2% of the variability in the response could be explained by the model. The model also showed statistically insignificant lack of fit, as is evident from the lower calculated F value than the theoretical F value at 5% level. The plot of experimental values of ester yield (%), versus those calculated from the above equation, indicated a good fit (Fig. 3) with a correlation coefficient, *r* of around 0.902. Overall, these results revealed good correspondence between predicted and experimental values, implying that the empirical model derived from RSM can be used to adequately describe the relationship between the factors and response in the lipase-catalyzed synthesis of the pentyl octanoate.

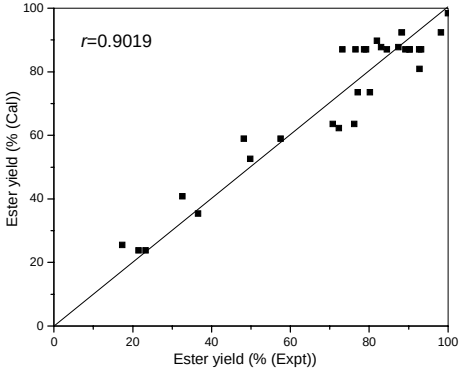

Figure 3. Correlation of calculated versus experimental values for pentyl octanoate synthesis catalyzed by the immobilized lipase from *C. rugosa*

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Influence of process conditions on the molar conversion

The influence of significant variables, reaction temperature, enzyme loading, and initial substrate molar ratio on the yield of ester is discussed using the statistical model shown in Eq. [2]. The model shows that the ester yield has a complex relationship with the independent variables that encompasses both first-order and second-order polynomials and may have more than one maximum point. Figs. 4 and 5 show the response surface plots and contour plots for the predicted values for the yield of ester versus of any two of the variables by holding the other one at its center point value.

The shape of the three-dimensional surface-representing yield of ester *versus* temperature and enzyme loading-is shown in Fig. 4a. The effect of temperature on yield was negative, probably due to thermal inactivation of enzyme (Figs. 4a and 4b). Temperature also showed a positive interactive effect with the enzyme loading, indicating that higher enzyme loadings could compensate for the lower reaction rates at higher temperatures. The maximum yield of ester could be obtained when working at low temperatures reaching a value close to 95% at 37 ^{o}C for enzyme loading of around 40 mg g^{-1}. The result suggests that the *C. rugosa* lipase, like some other lipases such as Novozym SP 435 from *Candida antarctica* (15,16) or porcine pancreatic lipase (17) was inactivated when it is subjected to a high temperature for a long period under non-aqueous conditions.

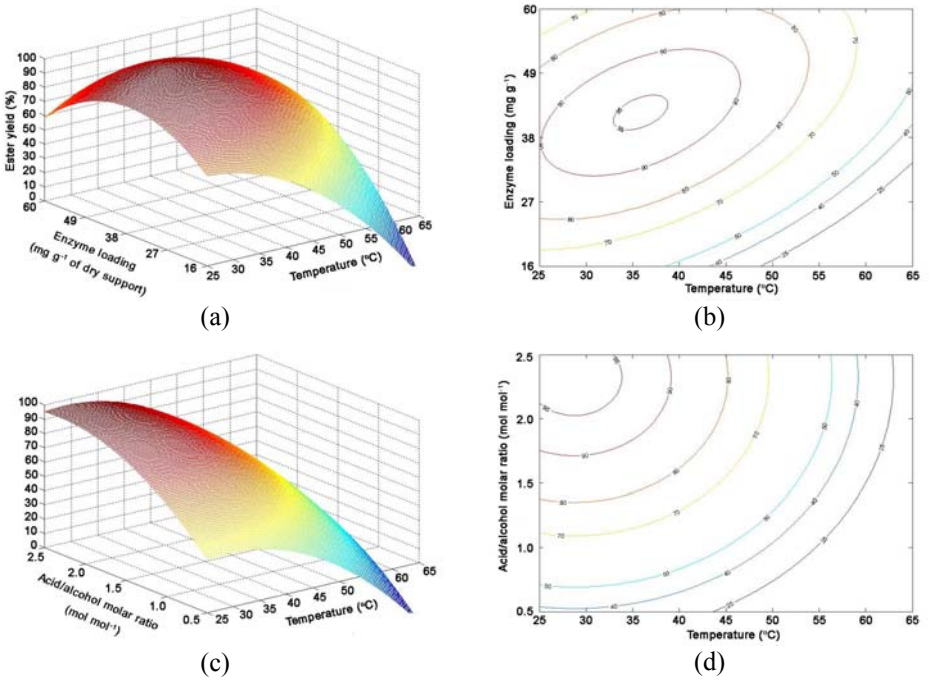

Figure 4. Response surface and contour plots for the ester yield as a function of temperature and enzyme loading (a) and (b), respectively; temperature and substrate molar ratio (c) and (d). Other synthesis parameters are constant at 0 level

Other important parameter affecting the economic feasibility of the process is the acid to alcohol molar ratio. Figs. 4c and 4d show the response surface plot and contour plot for the predicted values of ester yield as a function of the initial substrate molar ratio at different temperatures for an enzyme loading of 38 mg g^{-1} and an incubation period of 24 h. It seems that while temperature exerted a negative influence, acid excess had a significant positive effect on ester yield, indicating the importance of using an excess of acid compared with the stoichiometric amount for maximum conversion to the ester. Beneficial effect of excess acyl donor was also observed for the synthesis of short-chain esters using microbial lipases by several authors. For example, studying the ethyl butyrate synthesis with the same lipase as the catalyst, Chen verified that the molar ratio between ethanol and butyric acid was a critical factor for attaining a high yield of ethyl butyrate, requiring an amount of butyric acid on the order of 3.3 times that of ethanol (18). Yadav and Lathi also found that there was an increase in the reaction rate with an increase in quantity of isobutyric acid using Novozym SP 435 lipase and dealing with the synthesis of butyl isobutyrate (16). In an excess of fatty acid, most of the enzyme is found in the acylated form, preventing it from binding the product. In addition, a higher concentration of free acid in the reaction system was beneficial to the incorporation of acid from the view of reaction equilibrium, but excessive free fatty acid could also result in substrate inhibition (19). In our experimental setup, the substrate molar ratio had positive influence on the ester yield and we found that the acid did not inactivate catalyst in concentrations up to 1.5 mol dm^{-3}.

The most interesting result of the part of study focused on the statistically analyzed influence of enzyme loading and initial substrate molar ratio in a 3-dimensional graph (Fig. 5a). In addition, the contour plot also could indicate the desirable combination of variables that can be selected by the manufacturer because there were several optimal combinations available to obtain the highest ester yield (Fig. 5b). Ester production is represented by a convex surface described by a second order polynomial with a maximum at an enzyme loading of about 37 mg g^{-1} of dry support for a molar acid/alcohol ratio equal to about 2.2. The results indicate that high yields are possible with small amounts of enzyme when high substrate molar ratio levels are used, which is beneficial from the economic viewpoint since the cost of enzyme is usually higher than that of substrates. In general, for enzymatic esterification reactions, the lipase concentrations required to achieve higher yields of esters are often too high and reaction times relatively too long for industrial application. Welsh et al. have reported 75.8% conversion in 48 h with 2% native lipase from *C. cylindracea* at 0.05 M substrate (E/S ratio was 400 g mol^{-1}) (20). Chowadary et al. have reported 85% isovalerate acid conversion to isoamyl isovalerate at 0.5 M acid concentration with 1% enzyme concentration for 144 h incubation time in n-hexane (E/S ratio was 20), by using Lipozyme IM-20 lipase from *R. miehei* (21). In this study, it was shown that high conversion of around 99% could be achieved at 0.5 M alcohol concentration (substrate in defect), using amounts of enzyme as low as 40 mg g^{-1} ((E/S ratio of ~4 g mol^{-1}) and in a relatively short time (24 h) at 45 ^{o}C at fixed acid/alcohol molar ratio of 2.2. The feasibility of the ester synthesis by *C. rugosa* lipase immobilized on Sepabeads EC-EP under solvent-free condition was also explored, and a reasonably high yield of esters (72%) was achieved under optimal conditions (data not shown).

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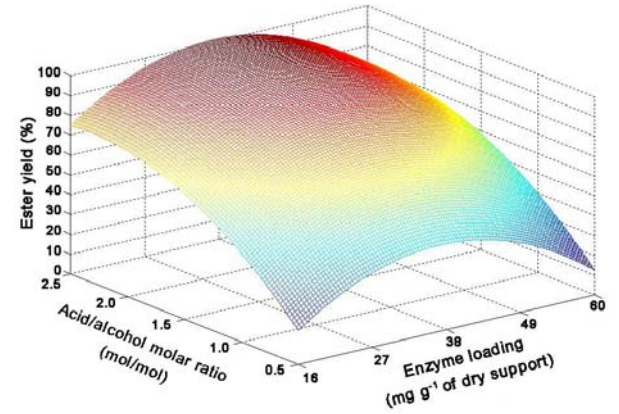
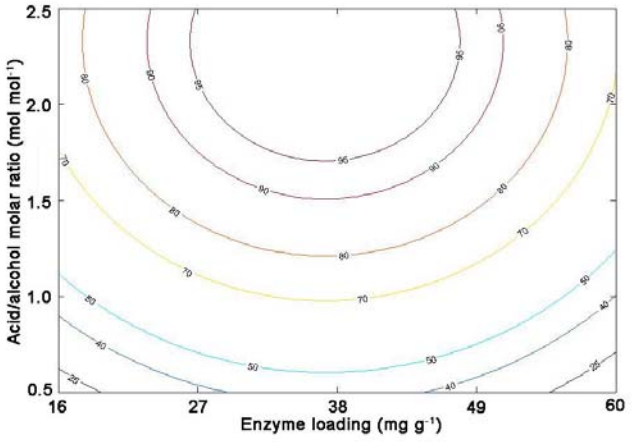


(a)



(b)

Figure 5. Response surface (a) and contour plots (b) for the ester yield as a function of lipase loading and substrate molar ratio after 24 h at 45 ^{o}C

CONCLUSIONS

Covalent immobilization of *Candida rugosa* lipase on Sepabeads EC-EP was studied in view of its possible application in pentyl octanoate synthesis in isooctane. The esterification reaction in non-conventional media was chosen for this assay because the ultimate aim of this study is to examine the feasibility of such reactions. Response surface methodology and 5-level-5-factor CCRD were performed to identify the factors that influence the ester production and to verify whether any changes should be made in their settings to improve this reaction. The initial water content, reaction temperature, enzyme

loading, acid/alcohol molar ratio and time of adding of molecular sieves were the variables investigated. Production of the ester was optimized and an ester production response equation was obtained, making it possible to predict ester yields from known values of the five main factors. The optimal conditions for the esterification were found to be: 37 ^{o}C, enzyme loading 37 mg g^{-1}, and acid/alcohol molar ratio 2.2, while initial water content and time of adding of molecular sieves did not significantly influence the ester yield. At these conditions, almost complete conversion (>99%) of the substrate to ester could be realized. The immobilized lipase is suitable for further studied for ester synthesis in a solvent-free system.

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ИМОБИЛИЗАЦИЈА ЛИПАЗЕ НА КОМЕРЦИЈАЛНИ ЕПОКСИДНИ НОСАЧ ЗА СИНТЕЗУ ПЕНТИЛ-ОКТАНОАТА У МИКРОВОДЕНОМ СИСТЕМУ

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У раду су испитани утицаји процесних параметара на синтезу пентил-октаноата помоћу липазе из *Candida rugosa* имобилисане на комерцијални полиметакрилатни носач (Sepabeads EC-EP). У раду је примењена метода имобилизације ензима која се заснива на директном везивању ензима за носач преко епоксидне групе полимера. Активност имобилисаног ензима испитана је у воденом систему на модел реакцији хидролизе маслиновог уља као и у неводеном систему на моделу синтезе пентил-октаноата у изооктану. Испитани су утицаји пет процесних фактора на ензимску синтезу датог естра применом методе планираних експеримената и методоло-

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гије одзивних површина и то садржаја воде, температуре, масе везаног ензима на носачу, почетног моларног односа реактаната и тренутка додавања молекулских сита у систем. Синтеза естара је оптимизована, извршена је оцена значајности параметара и утврђен је адекватан математички модел на основу кога се може предвидети понашање система (принос естара) у функцији наведених фактора.

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