J Ind Microbiol Biotechnol (2009) 36:1149–1155
DOI 10.1007/s10295-009-0596-x

ORIGINAL PAPER

Optimized lipase-catalyzed synthesis of adipate ester in a solvent-free system

Naz Chaibakhsh · Mohd Basyaruddin Abdul Rahman · Suraini Abd-Aziz · Mahiran Basri · Abu Bakar Salleh · Raja Noor Zaliha Raja Abdul Rahman

Received: 10 December 2008 / Accepted: 11 May 2009 / Published online: 29 May 2009

Abstract Immobilized *Candida antarctica* lipase-catalyzed esterification of adipic acid and oleyl alcohol was investigated in a solvent-free system (SFS). Optimum conditions for adipate ester synthesis in a stirred-tank reactor were determined by the response surface methodology (RSM) approach with respect to important reaction parameters including time, temperature, agitation speed, and amount of enzyme. A high conversion yield was achieved using low enzyme amounts of 2.5% w/w at 60°C, reaction time of 438 min, and agitation speed of 500 rpm. The good correlation between predicted value (96.0%) and actual value (95.5%) implies that the model derived from RSM allows better understanding of the effect of important reaction parameters on the lipase-catalyzed synthesis of adipate ester in an organic solvent-free system. Higher volumetric productivity compared to a solvent-based system was also offered by SFS. The results demonstrate that the solvent-free system is efficient for enzymatic synthesis of adipate ester.

N. Chaibakhsh · M. B. Abdul Rahman (✉) · M. Basri
Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
e-mail: basya@science.upm.edu.my

M. B. Abdul Rahman
Structural Biology Research Center, Malaysia Genome Institute, MTDC-UKM, Smart Technology Centre, 43600 UKM Bangi, Selangor, Malaysia

S. Abd-Aziz
Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

A. B. Salleh · R. N. Z. R. A. Rahman
Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Keywords Lipase · Adipate ester · Solvent-free system · Optimization · Response surface methodology

Introduction

Synthetic oleochemical esters are artificially developed substitutes for petroleum-based lubricants. It is estimated that synthetic lubricants represent around 10% of the global lubricating oil production [10]. Esters of adipic acid are a broad and diverse family of synthetic lubricant basestocks that can be custom-designed to meet specific physical and performance properties. They are most widely used in the lubricants industry due to their excellent tribological properties, low volatility, high flash point, and low toxicity [7]. Adipates are also used in other applications such as paint strippers, food packaging, plasticizers, fragrances, perfumes, cosmetics, and coatings [1]. In addition to their excellent oxidative and thermal stability, adipate esters are highly biodegradable and thus more environmentally friendly [7].

Adipates are presently synthesized by esterification of adipic acid and monohydric alcohols using chemical catalysts. Several homogeneous and heterogeneous catalysts, such as methanesulfonic acid, cation-exchange resins, composite solid acid, and modified heteropoly acids, are used for this purpose [23]. However, the chemical method involves some problems such as the high reaction temperature, corrosive acid catalysts, complex and expensive reaction setup, large amounts of raw materials due to the unselectiveness of the process, and high waste generation. The use of enzymes as "green" alternatives to produce these high-value-added esters may offer significant improvements because of milder reaction conditions, higher selectivity and specificity, lower energy requirement, and much purer products [14].

Enzyme-catalyzed esterification reactions can be performed in different media [5]. Recently, several researchers have reported enzymatic synthesis of esters in organic solvent-free systems in an attempt to make the processes commercially feasible [3, 16, 22]. These systems offer several advantages including minimizing environmental impact by avoiding the use of toxic and flammable organic solvents, significant cost-savings due to the absence of down-stream processing, and easier and fewer purification steps.

Most of the work on enzyme-catalyzed reactions in solvent-free media has been done in systems in which substrates and products are completely miscible liquids at the reaction temperature [17, 18]. The present study deals with the esterification of adipic acid, a solid acid, with a fatty alcohol using immobilized *Candida antarctica* lipase B (CALB). Enzymatic synthesis of adipate ester in the presence of organic solvent has been previously reported by the authors [1]. Considering the extremely low solubility of adipic acid in organic solvent and fatty alcohol, it would be interesting to study the esterification behavior of CALB in a solvent-free system.

All enzymatic reactions are influenced by experimental conditions. Response surface methodology is an efficient statistical tool for optimizing multiple variables to predict best performance conditions at the lowest cost and with the fewest experiments [8]. RSM has successfully been applied to study and optimize the enzymatic synthesis of various esters [11, 13].

The aim of this work was to investigate the possibility of lipase-catalyzed synthesis of adipate ester without adding any organic solvent. The study also helps to understand relationships between the important process parameters and the reaction yield and to determine the optimum conditions for synthesis of dioleyl adipate in a solvent-free system.

Materials and methods

Materials

Novozym 435, *C. antarctica* lipase B (EC 3.1.1.3) immobilized on a macro porous acrylic resin (10,000 propyl laurate units/g) was purchased from NOVO Nordisk A/S (Bagsvaerd, Denmark). Adipic acid and oleyl alcohol were purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were of analytical grade.

Esterification reaction

Synthesis of dioleyl adipate was carried out in a 500-ml stirred-tank reactor with a working volume of 350 ml. The reactor was equipped with a flat-blade disk turbine agitator, temperature-control system (Julabo MB-13, Germany), and sampling ports. Oleyl alcohol and adipic acid were mixed in the reactor with a molar ratio of 5.3:1. According to our preliminary studies (unpublished results), this ratio was found to be optimal for a solvent-free system to achieve maximum yield. Different amounts of Novozym 435, which were generated by RSM, were subsequently added. Selection of Novozym 435 as catalyst was based on prior studies in which several lipases including Novozym 435, Lipozyme RM IM, Lipozyme TL IM, and layered double hydroxide (LDH) immobilized *C. rugosa* were screened for activity via lipase-catalyzed esterification of adipic acid and different alcohols [1]. The reaction was performed at various agitation speeds and for different time periods generated by RSM, as indicated in Table 1. The initial rates were calculated from the time profiles corresponding to the first minutes of reaction (for which the profiles were approximately linear) and expressed as the amount of acid converted per unit of time per unit of weight of enzyme [20].

Analysis and characterization

The reaction was terminated by dilution with ethanol:acetone (50:50 v/v), and the enzyme was removed by filtration. A 5-ml sample was taken, and the remaining free acid was determined by titration with 0.1 M NaOH using phenolphthalein as the indicator. The moles of acid reacted were calculated from the values obtained for the blank (without enzyme) and the test samples. The ester formed was expressed as equivalent to conversion of the acid [1]. Product was also monitored by thin-layer chromatography (TLC) using chloroform/dichloromethane (95:5 v/v) solvent system and gas chromatography/mass spectroscopy (GC/MS) on a Shimadzu (model GC 17A; model MS QP5050A; Shimadzu, Tokyo, Japan) instrument with a BPX5 column (0.25 mm × 30 m, 25 μm).

Experimental design, statistical analysis, and optimization

A four-factor, five-level central composite rotatable design (CCRD) was employed in this study, requiring 30

Table 1 Coded and actual levels of variables for the central composite rotatable design

Variable	Levels				
	−2	−1	0	+1	+2
Temperature, *A* (°C)	35.0	45.0	55.0	65.0	75.0
Reaction time, *B* (min)	30.0	142.5	255.0	367.5	480.0
Enzyme amount, *C* (%)	1.00	3.25	5.50	7.75	10.00
Agitation speed, *D* (rpm)	100	200	300	400	500

experiments. The variables and their levels selected for the adipate ester synthesis were temperature (35–75°C), time (30–480 min), amount of enzyme (1–10% w/w of substrates), and agitation speed (100–500 rpm) (Table 1). The experiments were produced in random order, and triplicate measurements of esterification percentage were run on each experiment.

A software package of Design Expert Version 6.0.6 (Stat-Ease, Statistics Made Easy, Minneapolis, MN, USA) was used to fit the second-order model to the independent variables using the following equation:

$$y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} {x_i}^2 + \sum_{i=j}^{3} \sum_{j=i+1}^{4} b_{ij} x_{ij} + e \qquad (1)$$

where y is the dependent variable (percentage of yield) to be modeled; x_i and x_j are the independent variables (factors); b_0, b_i, b_{ii}, and b_{ij} are the regression coefficients of the model; and e is the error of the model. An analysis of variance (ANOVA) was used to determine whether the constructed model was adequate to describe the observed data. Optimum conditions for lipase-catalyzed synthesis of adipate ester were generated using the software's numerical optimization function.

Results and discussion

Model fitting and analysis of variance (ANOVA)

To obtain a proper model for optimization of dioleyl adipate synthesis in a solvent-free system, the central composite rotatable design, which is generally the best design for response surface optimization [9], was selected with four factors and five levels including temperature, reaction time, amount of enzyme, and agitation speed. Experimental and predicted data are given in Table 2. The predicted values were obtained using a model-fitting technique and seen to be sufficiently correlated to the observed values. Fitting of the data to various models and their subsequent ANOVA showed that the reaction of oleyl alcohol and adipic acid was most suitably described with a quadratic polynomial model. Equation of the model is as follows:

$$\begin{aligned} \text{Yield}(\%) = {} & +93.57 + 1.63A + 7.39B + 5.79C + 1.60D \\ & - 1.08AB - 1.26AC - 5.22BC - 1.17A^2 \\ & - 4.25B^2 - 3.12C^2 \end{aligned} \qquad (2)$$

where A is the temperature, B the time, C the amount of enzyme, and D the agitation speed.

The analysis of variance for the model is shown in Table 3. In the ANOVA, the F value is the ratio of regression mean sum of squares and error mean sum of squares (the difference between the observed and predicted values). The P value is the probability value for the corresponding F value. If the P value is very small (less than 0.05) then the individual terms in the model have a significant effect on the response. In this study, the computed F value of the model (819.54) is very high compared to the tabular value of $F_{10,19} = 2.37$ even at $P = 0.05$, implying the model is significant. The very small P value (<0.0001) and a suitable coefficient of determination ($R^2 = 0.9977$) also show that 99% of the variability can be explained by the model, and it is sufficient to present the actual relationship between the response and the significant variables. According to ANOVA of factors, the "lack of fit F value" of 1.88 is lower than the tabular $F_{0.05(14,5)}$ value (4.63), implying the lack of fit is not significant relative to the pure error. This indicates that the model represents the relationships of reaction parameters well within the ranges selected [8].

Effect of parameters

The relationships between reaction parameters and response can be better understood by studying the planned series of contour plots generated from the predicted model. Contour plots in Fig. 1 represent the effect of varying temperature and reaction time on the synthesis of dioleyl adipate at 300 rpm and 5.5% w/w enzyme. The percentage of yields increased with increase in time up to 346 min and subsequently started to decrease. Prolonging the time will increase the volume of water produced by the reaction and thus hydrolysis of ester will occur [21]. According to the F value (153.06), temperature is not as significant as reaction time in the model (Table 3). As the temperature increased, ester formation increased only marginally at shorter reaction times. By increasing the time, at any given temperature, an increase in ester formation was observed. Higher temperatures may increase the solubility of the acid and also decrease the viscosity of the mixture at longer reaction times, resulting in enhancement of the yield up to a certain point and then decreasing thereafter. Denaturation of the enzyme at higher temperatures can also be observed at longer incubation periods.

The effect of varying enzyme amount and temperature on reaction yield is shown in Fig. 2. The agitation speed and reaction time were fixed at their center points. It was observed that temperature has less effect on the yield as compared to the amount of enzyme. Novozym 435 has been reported to have a high catalytic activity in the temperature range of 40–90°C [9, 12]. The model predicts that a high percentage of yield (>93%) could be achieved under a wide range of temperatures from 43 to 75°C, where it can be observed that the percentage of conversion increased with increased enzyme amount. In fact, greater amounts of

Table 2 Composition of the various runs of the central composite rotatable design, actual and predicted responses

Run no.	Variable					
	Temperature (°C)	Time (min)	Enzyme amount (% w/w)	Agitation speed (rpm)	Actual yield (%)	Predicted yield (%)
1	65.0	142.5	3.25	400	72.1	72.2
2	65.0	367.5	3.25	400	94.7	95.2
3	55.0	255.0	5.50	300	93.6	93.6
4	55.0	255.0	5.50	300	94.1	93.6
5	35.0	255.0	5.50	300	85.4	85.6
6	55.0	480.0	5.50	300	91.8	91.3
7	45.0	367.5	3.25	200	87.6	88.4
8	55.0	255.0	5.50	300	93.8	93.6
9	65.0	367.5	7.75	400	93.3	93.6
10	45.0	142.5	7.75	400	89.4	88.8
11	55.0	30.0	5.50	300	60.8	61.8
12	55.0	255.0	10.00	300	92.2	92.7
13	65.0	142.5	7.75	400	92.6	91.7
14	55.0	255.0	1.00	300	69.5	69.5
15	65.0	142.5	7.75	200	88.6	88.5
16	45.0	142.5	3.25	400	65.0	64.3
17	65.0	142.5	3.25	200	69.4	69.0
18	75.0	255.0	5.50	300	91.8	92.1
19	45.0	367.5	3.25	400	92.0	91.6
20	55.0	255.0	5.50	300	94.1	93.6
21	45.0	367.5	7.75	400	94.8	95.3
22	45.0	142.5	3.25	200	60.9	61.0
23	55.0	255.0	5.50	300	93.3	93.9
24	55.0	255.0	5.50	500	96.4	96.8
25	45.0	142.5	7.75	200	85.5	85.6
26	45.0	367.5	7.75	200	92.8	92.1
27	65.0	367.5	7.75	200	91.2	90.7
28	55.0	255.0	5.50	300	92.8	93.6
29	55.0	255.0	5.50	100	89.9	90.4
30	65.0	367.5	3.25	200	92.6	92.0

enzyme increased the formation of the acyl-enzyme complex to produce the ester. The highest yield was achieved at 7.7% w/w of enzyme. Above this point, extra enzyme molecules are only present inside the bulk of the reaction mixture without involvement. This may cause diffusion and mass transfer limitation [8, 15].

Figure 3 shows the effect of varying the enzyme amount and reaction time on the synthesis of adipate ester at 55°C and agitation speed of 300 rpm. According to the linear coefficients and F value of the parameters, time has the most significant effect on the conversion yield. Reaction with low incubation time and low amount of enzyme showed the lowest conversion yield. Reaction with low enzyme amount and high incubation time resulted in an 88.3% yield, indicating that lower enzyme amounts can be compensated for by a longer reaction time. Inverse proportionality between reaction time and enzyme amount has been reported for many industrial enzyme-catalyzed processes [4].

Due to the low solubility of adipic acid in oleyl alcohol, it is difficult to obtain a homogeneous mixture of substrates. Therefore, mixing and mass transfer in the reaction system are factors to investigate, considering the high viscosity of the mixture in the absence of solvent. The results showed that employing 100 rpm agitation speed caused a significant 1.8-fold increase in the yield. However, the reaction yield was found to increase gradually at higher impeller speeds above 100 rpm (from 90.3% at 100 rpm to 96.4% at 500 rpm). It seems that mass transfer is not a highly limiting factor in the small reactor that was

Table 3 The analysis of variance (ANOVA)

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P* value
Model	3,419.52	10	341.95	819.54	<0.0001
A, Temperature	63.86	1	63.86	153.06	<0.0001
B, Time	1,309.36	1	1,309.36	3,138.08	<0.0001
C, Amount of enzyme	805.62	1	805.62	1,930.79	<0.0001
D, Agitation speed	61.28	1	61.28	146.87	<0.0001
AB	18.68	1	18.68	44.78	<0.0001
AC	25.28	1	25.28	60.58	<0.0001
BC	436.50	1	436.50	1,046.13	<0.0001
A^2	38.30	1	38.30	91.79	<0.0001
B^2	505.35	1	505.35	1,211.14	<0.0001
C^2	271.83	1	271.83	651.48	<0.0001
Residual	7.93	19	0.42		
Lack of fit	6.66	14	0.48	1.88	0.2521
Pure error	1.27	5	0.25		
Cor total	3,427.45	29			

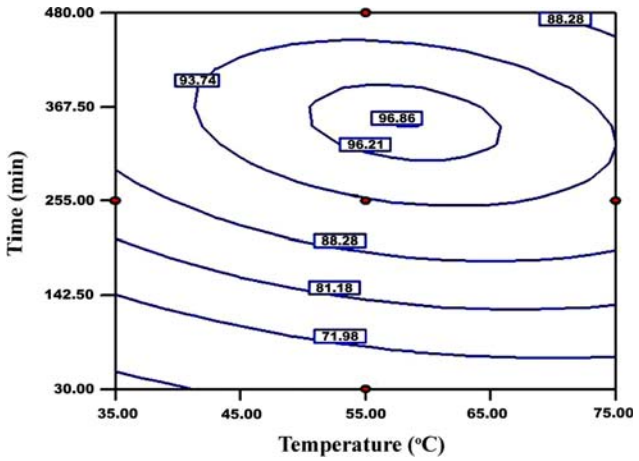

Fig. 1 Contour plots between two parameters, time and temperature, for synthesis of dioleyl adipate. Amount of enzyme and agitation speed are constant. The *numbers inside the contour plots* indicate conversion yield (%) at given reaction conditions

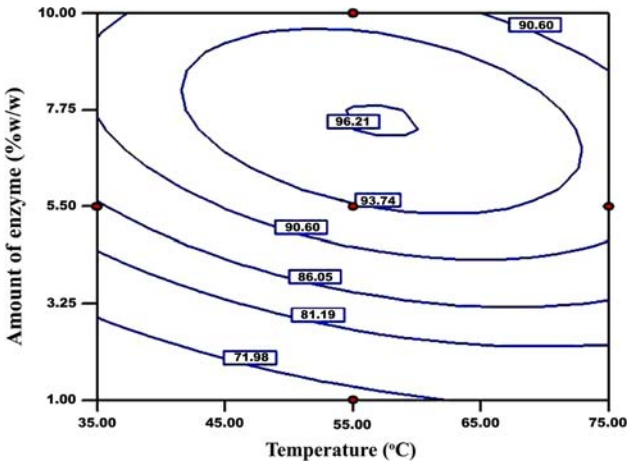

Fig. 2 Contour plots between two parameters, amount of enzyme and temperature, for synthesis of dioleyl adipate. Reaction time and agitation speed are constant. The *numbers inside the contour plots* indicate conversion yield (%) at given reaction conditions

used for the synthesis of adipate ester in this study [2]. According to the *F* value (146.87), impeller speed is less significant than other reaction parameters in the model (Table 3).

Optimum conditions and model validation

Response surface methodology can present the optimum combination of parameters that can be selected to obtain the highest percentage of yield. The optimum conditions for synthesis of dioleyl adipate are presented in Table 4 along with their predicted and actual values. From an economic point of view, it would be desirable to use the lowest amount of enzyme to achieve maximum conversion of the substrates. The maximum yield (99.6%) was predicted under reaction conditions of 60.0°C, 323.7 min, amount of enzyme 6% w/w, and 500 rpm agitation speed. The actual experimental value obtained was 96.2% with 3.4% deviation. The minimum amount of enzyme that resulted in maximum yield of 95.5% was 2.5% w/w at 60.0°C, 437.9 min, and 500 rpm agitation speed. The results demonstrate that RSM can be effectively applied to optimize the solvent-free lipase-catalyzed synthesis of adipate ester.

From the results obtained, it seems that there is an equilibrium conversion of around 96% for solvent-free

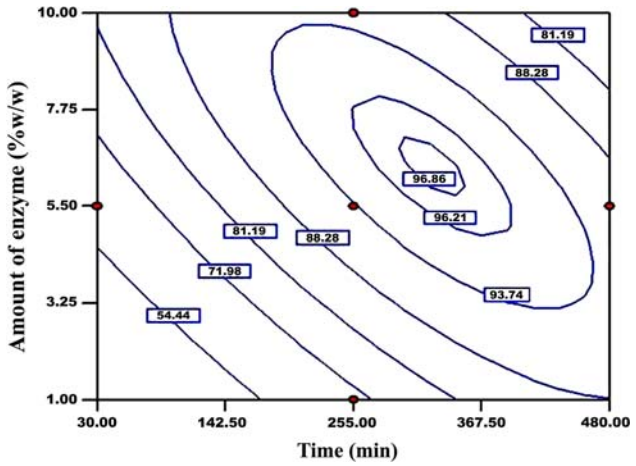

Fig. 3 Contour plots between two parameters, time and amount of enzyme, for synthesis of dioleyl adipate. Temperature and agitation speed are constant. The *numbers inside the contour plots* indicate conversion yield (%) at given reaction conditions

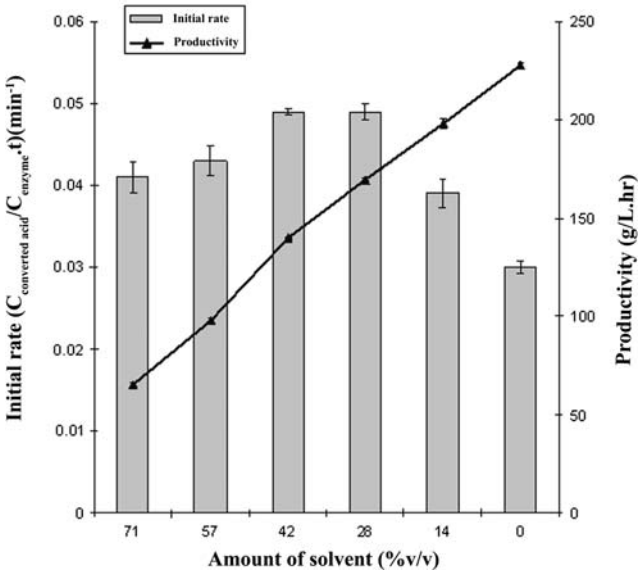

Fig. 4 Effect of solvent amount on the productivity and initial rate of adipate ester synthesis in 500-mL stirred-tank reactor. Reaction conditions: *T* 55°C, enzyme amount: 10% w/w, impeller speed: 500 rpm

lipase-catalyzed synthesis of dioleyl adipate ester. Removing water may shift the reaction to completeness. However, no efforts have been made for removing water because of the relatively high yield achieved in this study.

Reactor productivity

One of the advantages of using a solvent-free system instead of an organic solvent is the higher volumetric productivity, defined as the mass of product formed per unit of reactor volume per hour, and thus saving in reactor design in large-scale production [19]. Figure 4 represents the volumetric productivity as well as the initial rates of the reaction using different percent volume of the solvent, *n*-hexane, per working volume of the reactor for the synthesis of dioleyl adipate. The selection of hexane (log $P = 3.5$) as solvent was based on prior studies in which several solvents including hexane, heptane, ethyl acetate, butanol, and acetonitrile were screened for activity via *C. antarctica* lipase-catalyzed esterification of adipic acid and different alcohols [1]. The use of solvent can reduce the viscosity of the reaction mixture (from 28.2 cp in SFS to 9.9 cp by using 71% v/v hexane) and thus increase the reaction rate by increasing the mass transfer of the substrates. Lower reaction rates in the highest amount of solvent could be due to inactivation or inhibition of enzyme by direct interaction of solvent with the water layer in the vicinity of enzyme or an increase in the inhibitory effects of substrates in the presence of organic solvent [6]. Although the initial rate in the solvent-free system is lower than in solvent-based ones, the volumetric productivity is greater in SFS than in hexane (Fig. 4). In addition to the extra cost for the reactor, performing the reaction in hexane involves the cost of hexane itself and also costly downstream processing including evaporation and recycling of solvent.

Table 4 Optimum conditions for solvent free lipase-catalyzed synthesis of dioleyl adipate

Experiment	Temperature (°C)	Time (min)	Enzyme amount (% w/w)	Agitation speed (rpm)	Predicted yield (%)	Actual yield (%)
1	61.4	477.0	1.0	500	90.8	87.5
2	60.0	437.9	2.5	500	96.0	95.5
3	57.1	324.0	6.3	300	97.0	96.0
4	60.0	323.7	6.0	500	99.6	96.2
5	57.3	328.2	6.2	100	93.8	93.5
6	61.9	255.0	5.5	250	93.3	93.6

Conclusion

Optimization of reaction parameters time, temperature, agitation speed, and amount of enzyme in solvent-free lipase-catalyzed esterification reaction of adipic acid and oleyl alcohol was successfully developed by central composite rotatable design and response surface methodology. The R^2 (0.9977) and ANOVA implied that the model satisfactorily represented the real relationship of reaction parameters and the response. The ability to achieve a high percentage of yield (about 96.0%) and higher volumetric productivity compared to a solvent-based system indicates that the solvent-free system has a great potential for enzymatic synthesis of adipate ester. The results will further facilitate upscaling of the process for large-scale production of dioleyl adipate ester.

References

1. Abdul Rahman MB, Zaidan UH, Basri M, Hussein MZ, Rahman RNZA, Salleh AB (2008) Enzymatic synthesis of methyl adipate ester using lipase from *Candida rugosa* immobilized on Mg, Zn and Ni of layered double hydroxides (LDHs). J Mol Catal B Enzym 50:33–39. doi:10.1016/j.molcatb.2007.09.020
2. Betts JI, Baganz F (2006) Miniature bioreactors: current practices and future opportunities. Microb Cell Fact 5:1–14. doi:10.1186/1475-2859-5-21
3. Bezbradica D, Mijin D, Siler-Marinkovic S, Knezevic Z (2007) The effect of substrate polarity on the lipase-catalyzed synthesis of aroma esters in solvent-free systems. J Mol Catal B Enzym 45:97–101. doi:10.1016/j.molcatb.2006.12.003
4. Chang SW, Shaw JF, Shieh CJ (2003) Optimization of enzymatically prepared hexyl butyrate by lipozyme IM-77. Food Technol Biotechnol 41:237–242
5. Foresti ML, Ferreira ML (2005) Solvent-free ethyl oleate synthesis mediated by lipase from *Candida antarctica* B adsorbed on polypropylene powder. Catal Today 107:23–30. doi:10.1016/j.cattod.2005.07.053
6. Graber M, Irague R, Rosenfeld E, Lamare S, Franson L, Hult K (2007) Solvent as a competitive inhibitor for *Candida antarctica* lipase B. Biochim Biophys Acta 1774:1052–1057
7. Gryglewicz S (2001) Enzyme catalyzed synthesis of some adipic esters. J Mol Catal B Enzym 15:9–13. doi:10.1016/S1381-1177(00)00246-0
8. Gunawan ER, Basri M, Abdul Rahman MB, Salleh AB, Rahman RNZA (2005) Study on response surface methodology of lipase-catalyzed synthesis of palm-based wax ester. Enzyme Microb Technol 37:739–744. doi:10.1016/j.enzmictec.2005.04.010
9. Jeong GT, Park DH (2006) Response surface methodological approach for optimization of enzymatic synthesis of sorbitan methacrylate. Enzyme Microb Technol 39:381–386. doi:10.1016/j.enzmictec.2005.11.046
10. Jimenez AE, Bermudez MD (2008) Imidazolium ionic liquids as additives of the synthetic ester propylene glycol dioleate in aluminium–steel lubrication. Wear 265:787–798. doi:10.1016/j.wear.2008.01.009
11. Keng PS, Basri M, Abdul Rahman MB, Salleh AB, Rahman RNZA, Ariff A (2005) Optimization of palm based wax esters production using statistical experimental designs. J Oleo Sci 54:519–528
12. Kumar A, Gross RA (2000) *Candida antarctica* lipase B catalyzed polycaprolactone synthesis: effects of organic media and temperature. Biomacromolecules 1:133–138. doi:10.1021/bm990510p
13. Macedo GA, Pastore GM, Rodrigues MI, Davrieux F, Boulanger R, Caro Y, Figueroa-Epinoza MC, Graille J (2004) Optimising the synthesis of isoamyl butyrate using *Rhizopus* sp. lipase with a central composite rotatable design. Process Biochem 39:687–692. doi:10.1016/S0032-9592(03)00153-5
14. Petersson AEV, Gustafsson LM, Nordblad M, Borjesson P, Mattiasson B, Adlercreutz P (2005) Wax esters produced by solvent-free energy-efficient enzymatic synthesis and their applicability as wood coatings. Green Chem 7:837–843. doi:10.1039/b510815b
15. Radzi SM, Basri M, Salleh AB, Ariff A, Mohammad R, Abdul Rahman MB, Rahman RNZRA (2005) High performance enzymatic synthesis of oleyl oleate using immobilised lipase from *Candida antarctica*. Electron J Biotechnol 8:291–298. doi:10.2225/vol8-issue3-fulltext-4
16. Rodrigues RC, Volpato G, Ayub MAZ, Wada K (2008) Lipase-catalyzed ethanolysis of soybean oil in a solvent-free system using central composite design and response surface methodology. J Chem Technol Biotechnol 83:849–854. doi:10.1002/jctb.1879
17. Rosu R, Yasui M, Iwasaki Y, Yamane T (1999) Enzymatic synthesis of symmetrical 1,3-diacylglycerols by direct esterification of glycerol in solvent-free system. J Am Oil Chem Soc 76:839–843. doi:10.1007/s11746-999-0074-7
18. Sandoval G, Condoret JS, Monsan P, Marty A (2002) Esterification by immobilized lipase in solvent-free media: kinetic and thermodynamic arguments. Biotechnol Bioeng 78:313–320. doi:10.1002/bit.10224
19. Santos JC, Bueno T, Molgero da Ros PC, de Castro HF (2007) Lipase-catalyzed synthesis of butyl esters by direct esterification in solvent-free system. J Chem Technol Biotechnol 82:956–961. doi:10.1002/jctb.1780
20. Trubiano G, Borio D, Errazu A (2007) Influence of the operating conditions and the external mass transfer limitations on the synthesis of fatty acid esters using a *Candida antarctica* lipase. Enzyme Microb Technol 40:716–722. doi:10.1016/j.enzmictec.2006.06.003
21. Villeneuve P, Barea B, Sarrazin P, Davrieux F, Boulanger R, Caro Y, Figueroa-Epinoza MC, Graille J (2003) Synthesis of pyroglutamic acid fatty esters though lipase-catalysed esterification with medium chains alcohols. Enzyme Microb Technol 33:79–84. doi:10.1016/S0141-0229(03)00081-4
22. Vosmann K, Wiege B, Weitkamp P, Weber N (2008) Preparation of lipophilic alkyl (hydroxy) benzoates by solvent-free lipase-catalyzed esterification and transesterification. Appl Microbiol Biotechnol 80:29–36. doi:10.1007/s00253-008-1534-y
23. Xing C, Cheng Z, Qu J (2005) Synthesis of dioctyl adipate catalyzed by magnetic nanometer-sized solid superacids. Chem React Eng Technol 21:239–243