# Optimized Synthesis of Lipase-Catalyzed Hexyl Acetate in *n*-Hexane by Response Surface Methodology

Chwen-Jen Shieh* and Shu-Wei Chang

Department of Food Engineering, Dayeh University, 112 Shan-Jiau Road, Da-Tsuen, Changhua 51505, Taiwan

Hexyl acetate, a short-chain ester with fruity odor, is a significant green note flavor compound and widely used in the food industry. The ability for immobilized lipase from *Mucor miehei* (Lipozyme IM-77) to catalyze the transesterification of hexanol with triacetin was investigated in this study. Response surface methodology and five-level−five-factor central composite rotatable design were adopted to evaluate the effects of synthesis variables, such as reaction time (2−10 h), temperature (25−65 °C), enzyme amount (10−50%; 0.024−0.118 BAUN), substrate molar ratio of triacetin to hexanol (1:1 to 3:1), and added water content (0−20%) on percentage molar conversion of hexyl acetate. The results showed that reaction temperature and substrate molar ratio were the most important parameters and that added water content had less of an effect on percent molar conversion. On the basis of canonical analysis, optimum synthesis conditions were as follows: reaction time, 7.7 h; temperature, 52.6 °C; enzyme amount, 37.1% (0.089 BAUN); substrate molar ratio, 2.7:1; and added water, 12.5%. The predicted value was 88.9% molar conversion, and the actual experimental value was 86.6% molar conversion.

**Keywords:** *Biosynthesis; contour plots; hexyl acetate; lipase; optimization; response surface*

## INTRODUCTION

Esters of short-chain alcohols derived from short-chain carboxylic acids (acetates, propionates, butyrates, etc.) represent an important class of flavoring materials, responsible for the fruity odors of many foods and fragrances (*1*). For instance, hexyl acetate is an extremely aromatic compound with green note flavor and widely used in the food industry. The present worldwide market for natural "green notes" is estimated to be 5−10 metric tons each year at a current market price of 2500−6000 U.S.$/kg. Traditionally, it has been isolated from natural sources or produced by chemical synthesis. There is a growing demand for natural flavors containing green notes represented by C-6 alcohol (hexanol) derivatives (*2*); however, this steadily growing demand has left these compounds in increasingly short supply (*3*). Therefore, the biosynthesis of such esters by lipase-catalyzed chemical reactions under mild conditions has become of much current commercial interest. An optimized enzymatic synthesis of the hexyl ester improves the conversion yield and reduces the costs of production in most favorable conditions. This should benefit manufacturers and be more appealing to the consumers.

The importance of the enzymatic synthesis with lipases (triacylglycerol hydrolases EC 3.1.1.3) as catalysts to produce a number of commercially important flavor esters by esterification in nonaqueous organic solvents has been emphasized in several works (*4*). The lipase-catalyzed esterification reactions for esters are reviewed, in which the parameters affecting the activities of lipase on esterification reaction include reaction time, synthesis temperature, added water content, pH memory, acyl donors, etc. (*5*). Response surface methodology (RSM) and central composite rotatable design (CCRD) are useful statistical techniques for investigation of the complex processes and have been successfully applied for optimizing of ester production by lipase (*6*, *7*).

Carvalho et al. reported that hexyl acetate was synthesized by the cutinase-catalyzed transesterification reaction of butyl acetate with hexanol in a reversed micelles system, and the optimization of the transesterification was described using RSM (*8*). Bourg-Garros et al. synthesized (*Z*)-3-hexen-1-yl butyrate by direct esterification using lipases *Mucor miehei* (Lipozyme IM) and *Candida antarctica* (Novozym 435) in hexane with high yield (>90%). In the absence of solvent at 60 °C, Novozym 435-catalyzed esterification afforded a yield of 80% (*2*). Bourg-Garros et al. investigated the use of immobilized lipase from *C. antarctica* (Novozym 435) to synthesize (*Z*)-3-hexen-1-yl acetate by direct esterification in *n*-hexane and a solvent-free medium. The ester yield reached 94% in <27 h in *n*-hexane at 70 °C in the presence of 2% (w/w reactants) of the lipase and 1.5 mol $L^{-1}$ of substrate (*3*).

The present work focuses on the reaction parameters that affect lipase from *M. miehei* (Lipozyme IM-77)-catalyzed the transesterification of hexyl acetate using triacetin as acyl donor in *n*-hexane. Our purposes were to better understand relationships between the factors (reaction time, temperature, enzyme amount, substrate molar ratio, and added water content) and the response (percent molar conversion) and to determine the optimal conditions for hexyl acetate synthesis using CCRD and RSM analyses.

## MATERIALS AND METHODS

**Materials.** Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Lipozyme IM-77, 7.7 BAUN/g, water = 5.4% w/w)

* Author to whom correspondence should be addressed (telephone 886-4-853-0421; fax 886-4-853-4845; e-mail cjshieh@mail.dyu.edu.tw).

10.1021/jf001050q CCC: $20.00 
Published on Web 03/01/2001

**Table 1. Central Composite Rotatable Second-Order Design, Experimental Data, and Predicted Values for Five-Level−Five-Factor Response Surface Analysis**

| treatment[a] | time (h) $x_1$ | temp (°C) $x_2$ | enzyme (%[c] by wt of hexanol) $x_3$ | substrate molar ratio (triacetin/hexanol) $x_4$ | added $H_2O$ (% by wt of hexanol) $x_5$ | obsd yield (% molar conversion) $Y$ | predicted value[d] (% molar conversion) |
|---|---|---|---|---|---|---|---|
| 1 | −1 (4)[b] | −1 (35) | −1 (20) | −1 (1.5:1) | 1 (15) | 25.714 | 26.646 |
| 2 | 1 (8) | −1 (35) | −1 (20) | −1 (1.5:1) | −1 (5) | 38.820 | 41.026 |
| 3 | −1 (4) | 1 (55) | −1 (20) | −1 (1.5:1) | −1 (5) | 26.329 | 28.830 |
| 4 | 1 (8) | 1 (55) | −1 (20) | −1 (1.5:1) | 1 (15) | 31.083 | 36.256 |
| 5 | −1 (4) | −1 (35) | 1 (40) | −1 (1.5:1) | −1 (5) | 33.568 | 34.514 |
| 6 | 1 (8) | −1 (35) | 1 (40) | −1 (1.5:1) | 1 (15) | 44.509 | 48.127 |
| 7 | −1 (4) | 1 (55) | 1 (40) | −1 (1.5:1) | 1 (15) | 32.913 | 36.826 |
| 8 | 1 (8) | 1 (55) | 1 (40) | −1 (1.5:1) | −1 (5) | 42.200 | 47.387 |
| 9 | −1 (4) | −1 (35) | −1 (20) | 1 (2.5:1) | −1 (5) | 35.206 | 35.838 |
| 10 | 1 (8) | −1 (35) | −1 (20) | 1 (2.5:1) | 1 (15) | 54.355 | 57.660 |
| 11 | −1 (4) | 1 (55) | −1 (20) | 1 (2.5:1) | 1 (15) | 55.099 | 58.699 |
| 12 | 1 (8) | 1 (55) | −1 (20) | 1 (2.5:1) | −1 (5) | 63.278 | 68.151 |
| 13 | −1 (4) | −1 (35) | 1 (40) | 1 (2.5:1) | 1 (15) | 49.527 | 51.572 |
| 14 | 1 (8) | −1 (35) | 1 (40) | 1 (2.5:1) | −1 (5) | 60.940 | 64.258 |
| 15 | −1 (4) | 1 (55) | 1 (40) | 1 (2.5:1) | −1 (5) | 60.564 | 64.177 |
| 16 | 1 (8) | 1 (55) | 1 (40) | 1 (2.5:1) | 1 (15) | 78.836 | 85.121 |
| 17 | −2 (2) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 43.898 | 41.326 |
| 18 | 2 (10) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 79.511 | 69.047 |
| 19 | 0 (6) | −2 (25) | 0 (30) | 0 (2:1) | 0 (10) | 42.206 | 40.223 |
| 20 | 0 (6) | 2 (65) | 0 (30) | 0 (2:1) | 0 (10) | 67.729 | 56.675 |
| 21 | 0 (6) | 0 (45) | −2 (10) | 0 (2:1) | 0 (10) | 50.714 | 45.621 |
| 22 | 0 (6) | 0 (45) | 2 (50) | 0 (2:1) | 0 (10) | 73.284 | 65.340 |
| 23 | 0 (6) | 0 (45) | 0 (30) | −2 (1:1) | 0 (10) | 32.813 | 27.094 |
| 24 | 0 (6) | 0 (45) | 0 (30) | 2 (3:1) | 0 (10) | 80.877 | 73.560 |
| 25 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | −2 (0) | 63.716 | 58.596 |
| 26 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 2 (20) | 70.695 | 62.778 |
| 27 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 70.965 | 73.858 |
| 28 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 70.339 | 73.858 |
| 29 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 72.598 | 73.858 |
| 30 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 72.207 | 73.858 |
| 31 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 71.941 | 73.858 |
| 32 | 0 (6) | 0 (45) | 0 (30) | 0 (2:1) | 0 (10) | 72.061 | 73.858 |

[a] The treatments were run in a random order. [b] Numbers in parentheses represent actual experimental amounts. [c] The activity of 10% Lipozyme IM-77 is 0.024 BAUN. [d] The predicted values are derived from eq 2.

from *M. miehei* (presently named *Rhizomucor miehei*) supported on macroporous weak anionic resin beads was purchased from Novo Nordisk Bioindustrials, Inc. (Bagsvaerd, Denmark). Hexanol (98% pure), triacetin (99% pure), and tributyrin (99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). A 4 Å molecular sieve was purchased from Davison Chemical (Baltimore, MD), and *n*-hexane was obtained from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were of analytical reagent grade.

**Experimental Design.** A five-level−five-factor CCRD was employed in this study, requiring 32 experiments (*7*, *9*). The fractional factorial design consisted of 16 factorial points, 10 axial points (2 axial points on the axis of each design variable at a distance of 2 from the design center), and 6 center points. The variables and their levels selected for the study of hexyl acetate synthesis were as follows: time, 2−10 h; temperature, 25−65 °C; enzyme amount, 10−50% by weight of hexanol (0.024−0.118 BAUN); substrate molar ratio, 3:1 to 1:1, triacetin/hexanol; and amount of added water, 0−20% by weight of hexanol. Table 1 shows the independent factors ($x_i$), levels, and experimental design in terms of coded and uncoded.

**Esterification Method.** All materials were dehydrated by 4 Å molecular sieve for 24 h. Hexyl acetate synthesis was carried out in screw-capped test tubes (16 × 125 mm). Hexanol (100 mM, 30.66 mg) and different molar ratios of triacetin were added to 3 mL of *n*-hexane, followed by different amounts of added water and enzyme. The mixtures of hexanol, triacetin, and Lipozyme IM-77 were stirred in an orbital shaking water bath (200 rpm) at different reaction temperatures and reaction times (Table 1).

**Extraction and Analysis.** The enzyme and any residual water were removed by passing reaction media through an anhydrous sodium sulfate column. Before sample analysis, the reactant was taken to mix with an equal volume of an internal standard solution (50 mM tributyrin). Then analysis was done by injecting a 1 μL aliquot in a splitless mode into a Hewlett-Packard 4890 gas chromatograph (Avondale, PA) equipped with a flame ionization detector. A DB-5 fused-silica capillary column (30 m × 0.32 mm i.d.; film thickness = 1 μm; J&W Scientific, Folsom, CA) was used. Injector and detector temperatures were set at 280 and 300 °C, respectively. Oven temperature was maintained at 50 °C for 2 min, elevated to 200 °C at a rate of 50 °C/min, held for 4 min, and then increased up to 300 °C at a rate of 70 °C/min. Nitrogen was used as carrier gas. The percentage yield (percent molar conversion) was defined as (millimoles of hexyl acetate/millimoles of initial hexanol) × 100% and was estimated using peak area integrated by on-line software Hewlett-Packard 3365 Series II ChemStation (Avondale, PA).

**Statistical Analysis.** The experimental data (Table 1) were analyzed by the response surface regression (RSREG) procedure to fit the following second-order polynomial equation (*10*)

$$Y = \beta_{k0} + \sum_{i=1}^{5}\beta_{ki}x_i + \sum_{i=1}^{5}\beta_{kii}x_i^2 + \sum_{i=1}^{4}\sum_{j=i+1}^{5}\beta_{kij}x_ix_j \qquad (1)$$

where $Y$ is response (percent molar conversion); $\beta_{k0}$, $\beta_{ki}$, $\beta_{kii}$, and $\beta_{kij}$ are constant coefficients; and $x_i$ are the uncoded independent variables. Canonical analysis was one part of the RSREG SAS output and the RIDGE MAX option was used to compute the estimated ridge of maximum response for increasing radii from the center of the original design. Holding one of three variables constant created individual contour plots.

## RESULTS AND DISCUSSION

RSM is a useful statistical technique for investigating complex synthesis processes, especially for the optimum

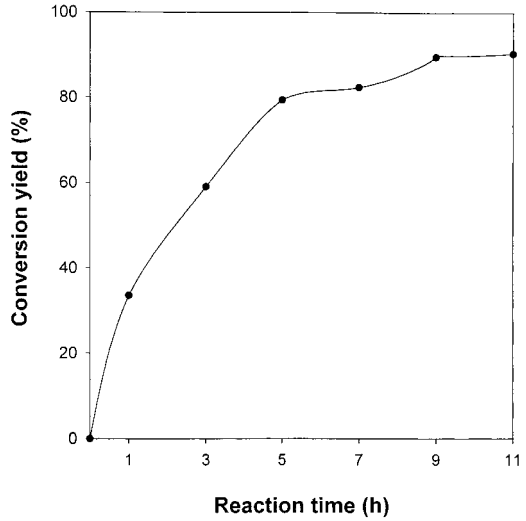


**Figure 1.** Time course of transesterification of hexanol with triacetin by Lipozyme IM-77. The reaction was carried out at 45 °C in 3 mL of hexane containing 100 mM hexanol, substrate molar ratio of 2:1 (triacetin/hexanol), 30% (weight of hexanol) Lipozyme IM-77, and without added water. The activity of 10% Lipozyme IM-77 is 0.024 BAUN.

synthesis of lipase-catalyzed reaction. Compared with one-factor-at-a-time design, which has been adopted most often in the literature, the five-level−five-factor CCRD employed in this study was more efficient in reducing the experimental runs and time for investigating the optimal condition of short-chain ester synthesis.

The time course for the transesterification of hexanol with triacetin by Lipozyme IM-77 is shown in Figure 1. The percent molar conversion of hexyl acetate increased up to 80% at 9 h; therefore, the range of reaction time from 2 to 10 h was chosen in this experiment. The selection of reaction time range needs to be extremely precise in the CCRD; otherwise, the optimal condition of synthesis cannot be found within the experimental region through the analyses of statistics and contour plots. Also, as shown by Figure 1 the conversion was >80% after 9 h, so the commercially immobilized Lipozyme IM-77 was added in the reaction system directly and the pH was not controlled to increase the yield during the reaction. Therefore, the ionization of the amino acid residues from the enzyme protein hydration of pH control did not affect the esterification reaction in this study.

The RSREG procedure for SAS was employed to fit the second-order polynomial eq 1 to the experimental data—percent molar conversions (Table 1). Among the various treatments, the greatest molar conversion (80.9%) was treatment 24 (6 h, 45 °C, 30% enzyme, molar ratio 3:1, and 10% added water), and the smallest conversion (only 25.7%) was treatment 1 (4 h, 35 °C, 20% enzyme, substrate molar ratio 1.5:1, and 15% added water). From the SAS output of RSREG, the second-order polynomial eq 1 is

$$Y = -242.247 + 16.898x_1 + 4.617x_2 + 2.700x_3 + 61.965x_4 + 1.548x_5 - 1.167{x_1}^2 - 0.044x_2x_1 - 0.064{x_2}^2 + 0.015x_3x_1 + 0.003x_3x_2 - 0.046{x_3}^2 + 1.183x_4x_1 + 0.848x_4x_2 + 0.134x_4x_3 - 23.531{x_4}^2 - 0.025x_5x_1 - 0.000014x_5x_2 + 0.007x_5x_3 + 0.613x_5x_4 - 0.132{x_5}^2 \quad (2)$$

**Table 2. Analysis of Variance for Joint Test**

| factor | degrees of freedom | sum of squares | prob > $F^a$ |
|---|---|---|---|
| time ($x_1$) | 6 | 1829.115 | 0.0155 |
| temp ($x_2$) | 6 | 1890.785 | 0.0138 |
| enzyme amount ($x_3$) | 6 | 1214.255 | 0.0561 |
| substrate molar ratio ($x_4$) | 6 | 4608.530 | 0.0004 |
| added water content ($x_5$) | 6 | 385.045 | 0.5034[b] |

[a] Prob > $F$ = level of significance. [b] Not significant at $p = 0.1$.

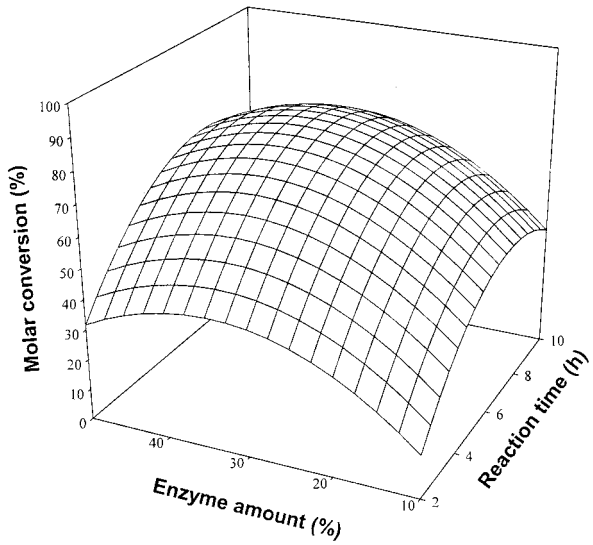


**Figure 2.** Response surface plot showing the effect of enzyme amount, reaction time, and their mutual interaction on hexyl acetate synthesis. Other synthesis parameters (reaction temperature, substrate molar ratio, and added water content) are constant at zero level.

With very small $P$ value (0.0015) from the analysis of variance (ANOVA) and a satisfactory coefficient of determination ($R^2 = 0.921$), the second-order polynomial model (eq 2) was highly significant and adequate to represent the actual relationship between the response (percent molar conversion) and the significant variables. Furthermore, the overall effect of the five synthesis variables on the percent molar conversion of hexyl acetate was further analyzed by a joint test (Table 2). The results revealed that the time ($x_1$), temperature ($x_2$), enzyme amount ($x_3$), and substrate molar ratio ($x_4$) were the important factors, exerting a statistically significant overall effect ($p < 0.1$) on the response molar conversion of hexyl acetate; and water content ($x_5$) had a less significant effect ($p > 0.1$) on this reaction. The importance of control of the water content in lipase-catalyzed hexanol esterification has been frequently emphasized in the literature (*11*). However, water content in the experimental scale (0−20%, w/w) of this study was the least important factor compared to the other reaction parameters from the statistical analysis. Besides, the reaction was done by transesterification in which triacetin, not acetic acid by direct esterification, was employed as acyl donor. No additional esterification water was produced along the conversion. Therefore, added water content was constant at zero level (10%) in the following discussion.

Enzyme amount and reaction time were investigated in the range of 10−50% and 2−10 h, respectively. Figure 2 shows the effect of enzyme amount, reaction time, and their mutual interaction on hexyl acetate synthesis at 45 °C, substrate molar ratio 2:1, and added water

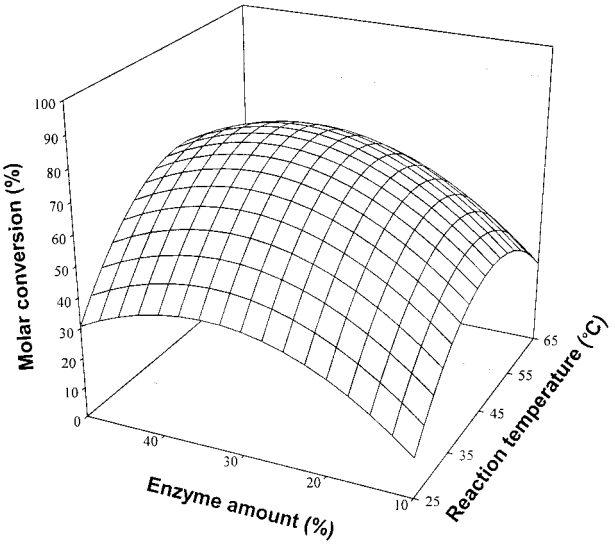


**Figure 3.** Response surface plot showing the effect of reaction temperature, enzyme amount, and their mutual interaction on hexyl acetate synthesis. Other synthesis parameters (reaction time, substrate molar ratio, and added water content) are constant at zero level.

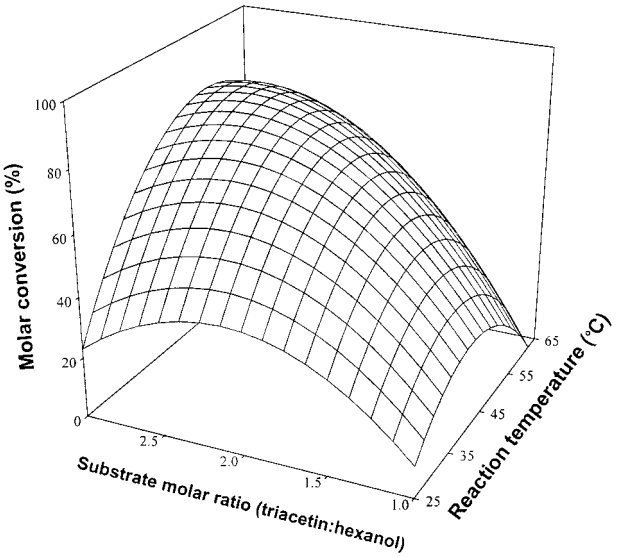


**Figure 4.** Response surface plot showing the effect of substrate molar ratio, reaction temperature, and their mutual interaction on hexyl acetate synthesis. Other synthesis parameters (reaction time, enzyme amount, and added water content) are constant at zero level.

amount of 10%. At the lowest reaction time (2 h) with the lowest enzyme amount (10%), molar conversion was only 10%. A reaction with an enzyme amount of 30% and a reaction time of 8 h led to the maximum molar conversion (>70%). The effect of varying enzyme amount and reaction temperature on esterification at constant reaction time (6 h), substrate molar ratio (2:1), and added water content (10%) is show in Figure 3. At any given temperature from 25 to 65 °C, an increase of enzyme amount led to higher yields. A reaction with a moderate reaction temperature (55 °C) and the highest enzyme amount favored maximal yield, and an increase of temperature up to 65 °C resulted in less esterification at any given enzyme amount because of the inhibition of the enzyme by temperatures >55 °C, indicating that the optimal temperature for lipase IM-77 was ∼55 °C. Figure 4 represents the effect of varying substrate molar ratio and reaction temperature on esterification at 6 h, 45 °C, and 10% added water amount. At low substrate molar ratio (1.0) and any reaction temperature, the yield was low (≤20%). A reaction with high substrate molar ratio (2.5−3.0) and high reaction temperature (55 °C) produced maximal molar conversion (∼80%). An increase in substrate molar ratio up to 3.0 resulted in less molar conversion at any given temperature. The reason high substrate molar ratio decreased the yield was that lipase was inhibited by the accumulation of acetic acid from the release of triacetin.

The relationships between reaction factors and response can be better understood by examining the planned series of contour plots (Figure 5) generated from the predicted model (eq 2) by holding constant the enzyme amount (10, 20, and 30%) and substrate molar ratio (2:1, 2.5:1, and 3:1). Figure 5A−C represent the same substrate molar ratio (2:1), and Figure 5A,D,G represent the same enzyme amount (10%). Such an application could be adopted to study the synthesis variables simultaneously in a five-dimensional space. Reaction time ($x_1$) and temperature ($x_2$) were the most important variables for hexyl acetate synthesis with small $p$ values (see Table 2) and considered to be indicators of effectiveness and economical performance. The amount of added water content was kept (10%) with

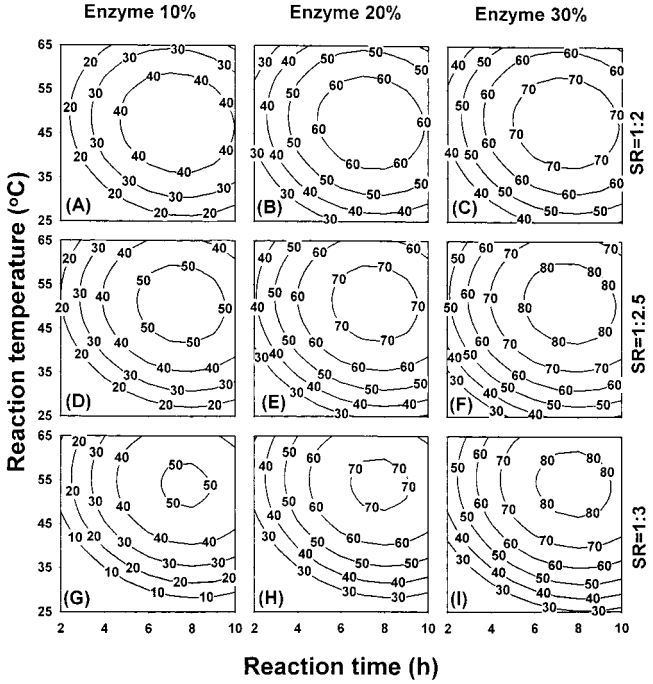


**Figure 5.** Contour plots of percent molar conversion of hexyl acetate at an added water content of 10%. Enzyme amount was by weight of hexanol. The numbers inside the contour plots indicate molar conversions at given reaction conditions.

less significant effect on response in the optimization studies. In general, all nine contour plots in Figure 5 exhibited similar behaviors in that predicted molar conversion increased with the reaction time; however, it decreased over 8 h. Likewise, a reaction temperature of ∼55 °C gave a higher percent molar conversion than 45 and 65 °C. The reaction of 30% enzyme and a substrate molar ratio of 2.5:1 (Figure 5F) possessed more predicted molar conversion than the others.

The optimum synthesis of hexyl acetate was determined by the ridge maximum analysis and the canonical analysis (*10*, *12*). The method of ridge analysis computes the estimated ridge of maximum response for increasing

**Table 3. Estimated Ridge of Maximum Response for Variable Percent Molar Conversion**

| coded radius | estimated responses (% conversion) | SE | $x_1$ (h) | $x_2$ (°C) | $x_3$ (%) | $x_4$ (triacetin/ hexanol) | $x_5$ (%) |
|---|---|---|---|---|---|---|---|
| 0 | 73.858 | 3.292 | 6.000 | 45.000 | 30.000 | 2.000 | 10.000 |
| 0.2 | 79.236 | 3.257 | 6.369 | 46.240 | 31.344 | 2.151 | 10.208 |
| 0.4 | 83.393 | 3.260 | 6.728 | 47.673 | 32.729 | 2.296 | 10.553 |
| 0.6 | 86.368 | 3.612 | 7.070 | 49.213 | 34.134 | 2.437 | 11.037 |
| 0.8 | 88.191 | 4.629 | 7.391 | 50.810 | 35.542 | 2.575 | 11.659 |
| 1.0 | 88.888 | 6.384 | 7.690 | 52.433 | 36.944 | 2.710 | 12.414 |

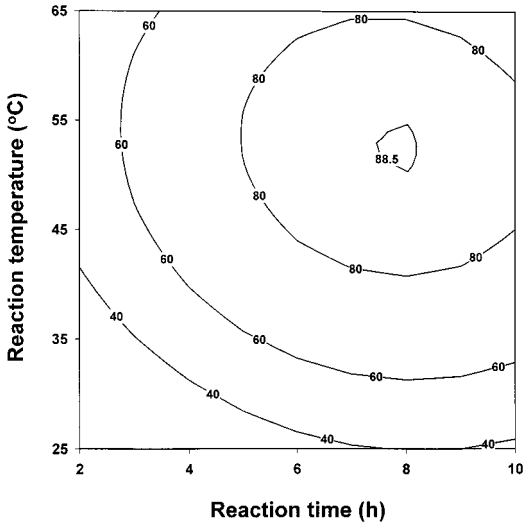


**Figure 6.** Contour plots showing response behavior of reaction time and temperature of the optimum synthesis condition at the stationary point (enzyme amount, 37.1%; molar ratio, 2.7:1; added water content, 12.5%) suggested by canonical analysis.

radii from the center of the original design. The ridge maximum analysis (Table 3) indicated that maximum molar conversion was 88.9 ± 6.4% at 7.7 h, 52.4 °C, 36.9% enzyme amount, 2.7:1 substrate molar ratio, and 12.4% added water content at the distance of the coded radius of 1.0. The optimum point was also determined by canonical analysis. The stationary point (reaction time = 7.7 h, reaction temperature = 52.6 °C, 37.1% or 0.089 BAUN enzyme amount, substrate molar ratio = 2.7:1, and added water content = 12.5%), values of variables at which the first derivative of the response was zero, was located exactly in the experimental region with the predicted value of 88.9%. The canonical analysis based on the stationary point resulted in the equation

$$Y = 88.897 - 11.466W_1^2 - 16.451W_2^2 - 18.312W_3^2 - 19.139W_4^2 - 33.791W_5^2 \quad (3)$$

where $W_1$, $W_2$, $W_3$, $W_4$, and $W_5$ are eigenvalues based on coded data and $Y$ is the molar conversion of hexyl acetate (percent). All eigenvalues were negative, indicating that the predicted response surface of the stationary point is shaped like a maximum. The response behavior of reaction time and synthesis temperature (Figure 6) was followed while the other reaction parameters were held constant at the suggested optimum point. The maximum value (88.5%) was predicted to be near a combination of 7.7 h and 53 °C. Because results from both maximum ridge analysis and canonical analysis indicated similar conclusions, the reaction condition (reaction time = 7.7 h, synthesis temperature = 53 °C, 37.1% or 0.089 BAUN enzyme amount, 2.7:1 substrate molar ratio, and 12.5% added water content) was recommended as the optimization for hexyl acetate synthesis with 88.9% molar conversion in this study.

The adequacy of the predicted model here was examined by additional independent experiments at the suggested optimal synthesis conditions. The predicted value was 88.9% obtained by canonical analysis, and the actual value was 86.6 ± 2.9%. Chi-square test ($p$ value = 0.991, degrees of freedom = 7) indicated that observed values were significantly the same as the predicted values and the generated model adequately predicted the percent molar conversion (*13*). Thus, the optimization of lipase-catalyzed synthesis for hexyl acetate by Lipozyme IM-77 was successfully developed by CCRD and RSM.

## LITERATURE CITED

(1) Bauer, K.; Garbe, D.; Surburg, H. *Common Fragrance and Flavor Materials*; VCH Publishers: New York, 1990.

(2) Bourg-Garros, S.; Razafindramboa, N.; Pavia, A. A. Synthesis of (*Z*)-3-hexen-1-yl Butyrate in Hexane and Solvent-Free Medium Using *Mucor miehei* and *Candida antarctica* Lipases. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1471−1475.

(3) Bourg-Garros, S.; Razafindramboa, N.; Pavia, A. A. Optimization of Lipase-Catalyzed Synthesis of (*Z*)-3-hexen-1-yl Acetate by Direct Esterification in Hexane and Solvent-Free medium. *Enzyme Microb. Technol.* **1998**, *22*, 240−245.

(4) Chatterjee, T.; Bhattacharyya, D. K. Synthesis of Monoterpene Esters by Alcoholysis Reaction with *Mucor miehei* Lipase in a Solvent-Free System. *J. Am. Oil Chem. Soc.* **1998**, *75*, 651−655.

(5) Ahmad, R. W.; Anderson, W. A.; Moo-Young, M. Ester Synthesis in Lipase-Catalyzed Reactions. *Enzyme Microb. Technol.* **1998**, *23*, 438−450.

(6) Krishna Hari, S.; Manohar, B.; Divakar, S.; Karanth, N. G. Lipase-Catalyzed Synthesis of Isoamyl Butyrate: Optimization by Response Surface Methodology. *J. Am. Oil Chem. Soc.* **1999**, *76*, 1483−1488.

(7) Shieh, C.-J.; Lou, Y.-H. Five-Factor Response Surface Optimization of the Enzymatic Synthesis of Citronellyl Butyrate by Lipase IM77 from *Mucor miehei*. *J. Am. Oil Chem. Soc.* **2000**, *77*, 521−526.

(8) Carvalho, C. M. L.; Serralheiro, M. L. M.; Cabral, J. M. S.; Aires-Barros, M. R. Application of Factorial Design to the Study of Transesterification Reactions Using Cutinase in AOT-Reversed Micelles. *Enzyme Microb. Technol.* **1996**, *21*, 117−123.

(9) Cochran, W. G.; Cox, G. M. *Experimental Designs*; Wiley: New York, 1992.

(10) SAS. *SAS User's Guide*; SAS Institute Inc.: Cary, NC, 1990.

(11) Linko, Y.-Y.; Lamsa, M.; Huhtala, A.; Linko, P. Lipase-Catalyzed Transesterification of Rapeseed Oil and 2-Ethyl-1-hexanol. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1411−1414.

(12) Shieh, C.-J.; Lai, Y.-F. Application of Response Surface Methodology to the Study of Methyl Glucoside Polyester Synthesis Parameters in a Solvent-Free System. *J. Agric. Food Chem.* **2000**, *48*, 1124−1128.

(13) Ott, L. *An Introduction to Statistical Methods and Data Analysis*; PWS-Kent Publishing: Boston, MA, 1988.

Received for review August 24, 2000. Revised manuscript received January 2, 2001. Accepted January 4, 2001. This research was supported by the National Science Council (NSC-89-2313-B-212-005), Taiwan, Republic of China.

JF001050Q