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# Study on influence factors and experimental optimization for lipase-catalyzed enantioselective esterification of (±)-2-methyl-1-butanol

**Qisong Liu, Yiwen Zhang, Quanyi Wang, Hang Song, Shun Yao***

**School of Chemical Engineering, Sichuan University, Chengdu, 610065, (P.R.CHINA)**

**E-mail: cusack@scu.edu.cn**

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## ABSTRACT

(±)-2-Methyl-butanol is a kind of useful solvent and important fine chemical. In this paper, related influence factors were investigated and response surface methodology was successfully applied to optimize lipase-catalyzed enantioselective esterification of (±)-2-methyl-butanol. The effects of were investigated. Then a quadratic polynomial regression model was used to analyze the experimental data at a 95% confidence level ($p<0.05$). The results indicated a significantly good fit to this model, and the response evaluated from the quadratic model showed a good agreement with the observed ones. The F-test and p-value indicated that reaction time, substrate molar ratio were the significant factors affecting the conversion of 2-methyl-butanol. The optimum reaction condition was established and the verified experimental trial was performed for validating the optimum points. Under the optimal condition, the conversion of (±)-2-methyl-butanol and the enantiomeric ratio exceeded 51.1% and 85.5%, respectively.

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## KEYWORDS

(±)-2-methyl-butanol;
Transesterification;
Enantiomeric excess;
Response surface methodology (RSM).

## INTRODUCTION

2-methyl-butanol also known as *tert*-amyl alcohol or amylene hydrate, as one of the isomers of amyl alcohol, is a kind of useful solvent and important fine chemical. By right of its characters of non-HAP (Hazardous Air Pollutant) solvent, it has been used in fuel and lubricating oil additives, flotation aids, manufacture of corrosion inhibitors, pharmaceuticals, paint solvent, chemical intermediate and extraction agent, *etc*[1-3]. In general, it is produced by optimal resolution of (±)-2-methyl-butanol which is synthesized chemically in the laboratories and industry. In recent years, enzyme-catalyzed reactions are used in more and more resolution of isomers as a highly selective method. And among them, enzymatic esterification has been investigated in some researches about the resolution of (±)-2-methyl-butanol in organic solvent[4-6]. Among several lipases investigated, the lipase from porcine pancreas was found to be highly steroselective for the esterification of (–)-2-methyl-butanol[6]. But the further researches on the effects and interaction of relative influence factors are needed to explore and the reaction conditions are not so ideal, which always result in low conversion rate.

Statistical optimization methods can overcome the limitations of classic empirical methods and are proved

to be a powerful tool for the optimization of the target conditions in chemical synthesis[7,8] including lipase-catalyzed reactions[9-12]. Among them, response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for designing experiments, building models and analyzing the effects of the several independent variables (factors)[13]. The main advantage of RSM is the decreasing number of experimental trials needed to evaluate multiple factors and their interactions. The study of the individual and interactive effects of these factors will be helpful in efforts to find the target values. Hence, RSM provides an effective tool for investigating the aspects affecting desired response if there are many factors and interactions in the experiment. In order to determine a suitable polynomial equation for describing the response surface, RSM can be employed to optimize the process.

The present work focuses on the parameters that affect lipase from porcine pancreas to catalyze the enantioselective esterification of (–)-2-methyl-butanol for separating (±)- 2-methyl-butanol using vinyl acetate as the acyl donor in organic solution. The main purpose of the study was to further understand the relationships between the factors (reaction time, temperature, enzyme loading, substrate molar ratio and pH value) and the response (enantiomeric excess (e.e.%), and enantiomeric ratio (E)); also to determine the optimal condition for enantiomeric resolution of (±)- 2-methyl-butanol using central composite rotatable design (CCRD) and response surface methodology (RSM).

## EXPERIMENTAL

### Materials and reagents

(±)-2-Methyl-butanol, (–)-2-methyl-butanol, lipase from porcine pancreas (PPL) were purchased from Sigma (St. Louis, MO, USA), vinyl acetate, *tert*-amyl acetate and acetone from Fisher Scientific (Fair Lawn, NJ, USA). All other regents were of analytical grade and obtained from local sources. The organic solvents were anhydrated by molecular sieves of 3A (Hangjia Biological and Pharmaceutical Tech. Ltd, Chengdu, China) before use.

### Esterification of 2-methyl-butanol

All enzymatic reactions were carried out in a temperature-controlled incubator shaker. In a typical experiment, 8.0 g (±)-2-methyl-butanol and 10.16 g vinyl acetate were added in a 250 ml screw-capped vial. The reaction was started by adding 1.3 g PPL and run by shaking at 260 rpm at designated temperature. Then, 1 ml of the diluted solution was analyzed. Control experiments were performed in the absence of PPL. As a result, no chemical acyl transfer reaction was detected.

### Analysis method

The HPLC analysis was carried out on a Waters 2487 series liquid chromatography system (Waters, USA), equipped with CBL Model 515 HPLC pump, Waters 2487 Dual λ absorbance detector (Waters, USA), a model 100 column heater (Photolectron Technology, USA) and JASCO MODEL OR-2090 optical rotation detector (JASCO, Japan). Chromatographic parameters such as peak areas, retention times, theoretical plates, etc. were calculated using the Allchrom Plus Client/Service workstation (Multilink Services Co., Ltd, USA). The GC analysis was performed with an SQ-206 GC equipped with a splitless/split injector, a flame-ionization detector, and a PEG-20M column (0.25 lm film thickness, 30 m length, 0.25 mm I.D.). The injector and detector were set at 190 and 250°C, respectively, and the flow rate of the carrier gas $N_2$ was 25ml $min^{-1}$. Chromatographic data were acquired and analyzed by the N2000 workstation (Zhida Information Engineering Co., Ltd, Hangzhou, China).

### Calculation of enantioselectivity

The enantiomers of the (±)-2-methyl-butanol and of the product (±)-*tert*-amyl acetate were baseline separated in the HPLC analysis. The conversion in percentage was calculated fromthe following equation:

$$c = \frac{P_- - P_+}{P_- + P_+ + S} \times 100\% \quad (1)$$

The enantioselectivity for each reaction was expressed by enantiomeric excess (e.e.*P*%) and enantiomeric ratio (E-value), where *S*, $P_-$ and $P_+$ stand for 2-methyl-butanol and the products of (–) and (+)-*tert*-amyl acetate, respectively.

$$e.e._p\% = \frac{P_- - P_+}{P_- + P_+} \times 100\% \quad (2)$$

$$E_P = \frac{\ln[1 - c(1 + e.e._p)]}{\ln[1 - c(1 - e.e._p)]} \quad (3)$$

$$E_S = \frac{\ln[(1-c)(1-e.e._s)]}{\ln[(1-c)(1+e.e._s)]} \quad (4)$$

where $S$, $P_-$ and $P_+$ stand for 2-methyl-butanol and the products of (–) and (+)-*tert*-amyl acetate, respectively.

## Determination ranges of variables

Before arranging an experimental design with a central composite rotatable design (CCRD), the effects of various reaction conditions, including reaction time, temperature, enzyme loading, substrate molar ratio and pH were tested by varying one factor successively while keeping the others unchanged and the results were shown in Figure 1 and Figure 2. As shown in Figure 1a, the time course for the enantioselective esterification of (±)- 2-methyl-butanol by PPL at 30°C. The conversion of 2-methyl-butanol increased to 53% after 13 h; therefore, the range of reaction time from 7 to 19 h was chosen in this study.

The selection of reaction time range must be extremely precise in the study of CCRD, otherwise, the optimal condition of synthesis could not be found within the experimental region through the analyses of statistics and contour plots. Also, as shown in Figure 1a, the $e.e._p$ exceeded 94.4% at initial stage of the reaction, and then followed a slight decline. The reason was supposed to be that (–)-2-methyl-butanol was esterified preferentially initially, and with the proceeding of the enantioselective esterification of (±)- 2-methyl-butanol, the reaction probability of (+)-2-methyl-butanol increased with the increasing consumption of (–)-2-methyl-butanol. As shown in Figure 1b, an increase in temperature increased the conversion of (±)-2-methyl-bu-

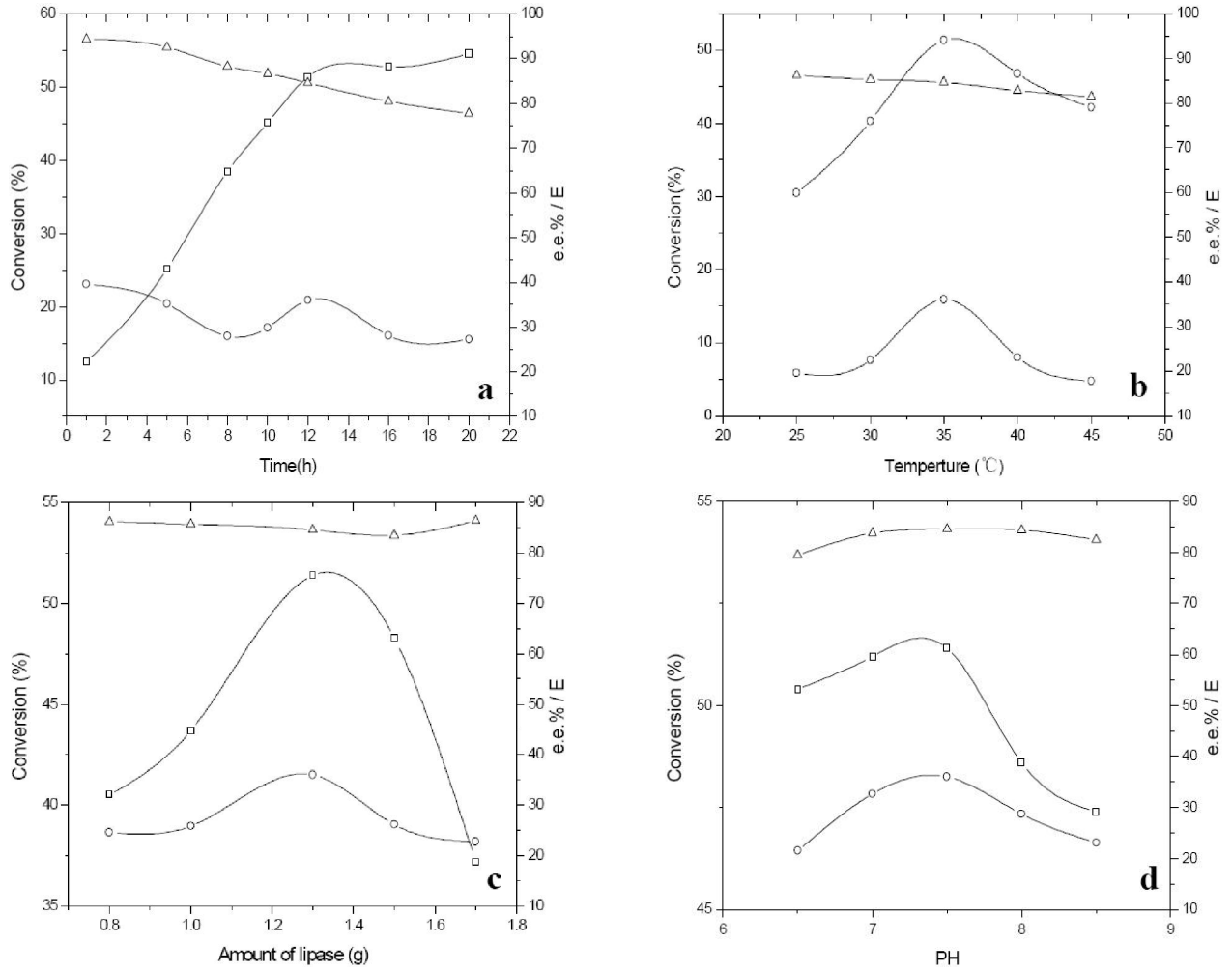


**Figure 1 : Effects of reaction time (a), reaction temperature (b), the amount of lipase (c) and pH value (d); □: substrate conversion percent; △: production enantiomericexcess percent; ○: production enantiomeric ratio. (Conditions of 1a: Enzyme loading: 1.25 g, MR=1.3:1, T=35°C, pH=7.5; Conditions of 1b: Enzyme loading: 1.25 g, MR=1.3:1, t=13h, pH=7.5; Conditions of 1c: MR=1.3:1, t=13h, T=35°C, pH=7.5; Conditions of 1d: Enzyme loading: 1.25 g, MR=1.3:1, t=13h, T=35°C)**

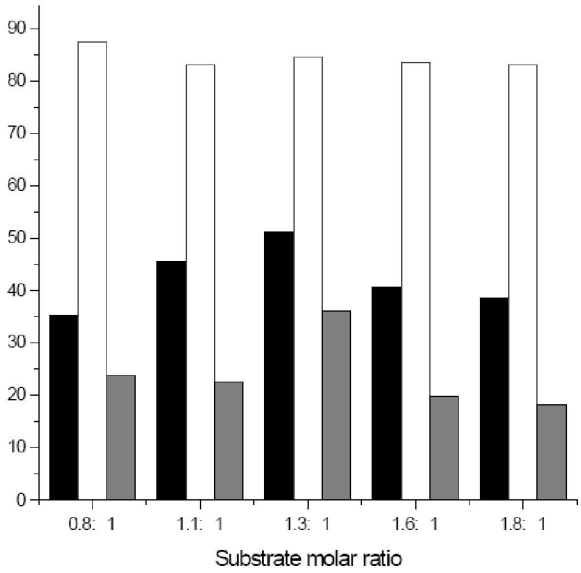


**Figure 2 : Effect of substrate molar ratio (■: substrate conversion percent; □: production enantiomeric excess percent; ▣: production enantiomeric ratio) (Conditions: Enzyme loading:1.25 g, t=13h, T=35°C, pH=7.5).**

tanol up to 35°C, and then the conversion decreased with a higher temperature. Therefore, 35°C was chosen as the center point temperature (T). Similarly, the conversion of (±)-2-methyl-butanol was subtle changed with an increase in enzyme loading, however, an increase in enzyme loading caused an increment in initial reaction rate. When enzyme loading was up to 1.25g, the reaction rate began to decrease (Figure 1c). Thus, a 1.25g enzyme loading was chosen as the center point. Also can be shown in Figure 1c, the conversion was increased with the substrate molar ratio (vinyl acetate:(±)-2-methyl-butanol) increased up to 1.3:1. So, the substrate molar ratio 1.3:1 was chosen as the center point. Finally, an increase in pH caused a growth in the conversion with the pH up to 7.5 (Figure 1d), and then the conversion decreased with a higher pH. Hence, the pH range of 6.5-8.5 was chosen finally.

## Experimental design

Response surface methodology (RSM) was employed to analyze the operating conditions of 2-methyl-butanol acylation to obtain a high percent conversion and high enantiomeric excess. The experimental design was carried out by five chosen independent process variables at five levels, and related experimental range and the central points were shown in TABLE 1.

The software of Design-Expert 6.0 was used for designing and analyzing the experimental data. The coded values of these factors were obtained according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (5)$$

where $x_i$ is the coded value of the factor, $X_i$ is the real value of the factor, $X_0$ is the real value of the factor at the center point, and $\Delta X_i$ is the step change value of the factor. The independent variables (factors) and their levels, real values as well as coded values were presented in TABLE 1. The enantiomeric excess (e.e.$_p$%) and the percent conversion of 2-methyl-butanol (c%) were the responses of the experimental design. The model equation was used to predict the optimum value and subsequently to elucidate the interaction between the factors. The quadratic equation model for predicting the optimal point was expressed according to Eq.:

$$Y = \beta_0 + \sum_{i=1}^{5}\beta_i x_i + \sum_{i=1}^{5}\beta_{ii} x_i^2 + \sum_{i=1}^{4}\sum_{j=i+1}^{5}\beta_{ij} x_i x_j \quad (6)$$

where $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are regression coefficients ($\beta_0$ is constant term, $\beta_i$ is linear effect term, $\beta_{ii}$ is squared effect term, and $\beta_{ij}$ is interaction effect term), and Y is the predicted response value.

**TABLE 1 : Coded levels for independent factors used in the experimental design**

| Factors | Symbol | Coded levels | | | | |
|---|---|---|---|---|---|---|
| | | -2 | -1 | 0 | 1 | 2 |
| Reaction time (h) | $x_1$ | 7 | 10 | 13 | 16 | 19 |
| Reaction temperature (°C) | $x_2$ | 25 | 30 | 35 | 40 | 45 |
| Substrate molar ratio | $x_3$ | 0.7:1 | 1.0:1 | 1.3:1 | 1.6:1 | 1.9:1 |
| Enzyme loading (g) | $x_4$ | 0.75 | 1.0 | 1.25 | 1.5 | 1.75 |
| PH | $x_5$ | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 |

## RESULTS AND DISCUSSION

### RSM experiments and fitting the models

A central composite rotatable design (CCRD) was employed to design the experiments. According to statistical theory, five factors consists of 30 experiments, including 15 factorial points (cubic point) and 11 axial points (star point) as well as four replicates at the center point. Four replications at the centre of the design were used to estimate the pure error. The results at each point based on experimental design were shown

in TABLE 2, which also given the experimental data of the response value, e.e.$_p$% and c%. The coded values of each factor in brackets correspond to the real value of the factor levels. For each factor, a conventional level was set at zero as a coded level. The runs were randomized for statistical reasons.

**TABLE 2 : Experimental design and results of the 1/2 CCRD design**

| Trial | Variable level | | | | | Response value | | E |
|---|---|---|---|---|---|---|---|---|
| | Reaction time (h) | Reaction temperature (°C) | Substrate molar ratio | Enzyme loading (g) | pH | e.e.% | c% | |
| | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $Y_1$ | $Y_2$ | |
| 1 | 7 | 35 | 1.3 | 1.25 | 7.5 | 76.3 | 31.5 | 10.45 |
| 2 | 10 | 40 | 1.6 | 1.0 | 8 | 81.9 | 40.9 | 17.71 |
| 3 | 13 | 25 | 1.3 | 1.25 | 7.5 | 79.2 | 45 | 16.71 |
| 4 | 13 | 35 | 1.3 | 1.25 | 7.5 | 85.2 | 49 | 31.62 |
| 5 | 13 | 35 | 1.3 | 0.75 | 7.5 | 83.3 | 43.5 | 21.17 |
| 6 | 10 | 40 | 1 | 1.5 | 8 | 76.5 | 39.7 | 12.32 |
| 7 | 13 | 35 | 1.3 | 1.75 | 7.5 | 82.4 | 43.2 | 19.61 |
| 8 | 13 | 35 | 1.3 | 1.25 | 7.5 | 86.5 | 50.2 | 39.22 |
| 9 | 13 | 35 | 1.3 | 1.25 | 8.5 | 82.5 | 47.4 | 23.13 |
| 10 | 10 | 30 | 1.0 | 1.5 | 7.0 | 75.5 | 37.3 | 11.09 |
| 11 | 16 | 40 | 1.0 | 1.5 | 7.0 | 80.4 | 49.2 | 21.55 |
| 12 | 10 | 30 | 1.6 | 1.5 | 8.0 | 80.5 | 41.3 | 16.30 |
| 13 | 13 | 35 | 0.7 | 1.25 | 7.5 | 82.4 | 46.2 | 21.81 |
| 14 | 13 | 35 | 1.9 | 1.25 | 7.5 | 83.2 | 48.5 | 25.81 |
| 15 | 10 | 30 | 1.0 | 1.0 | 8.0 | 80.1 | 37.3 | 14.44 |
| 16 | 10 | 40 | 1.6 | 1.5 | 7.0 | 81.5 | 39.7 | 16.71 |
| 17 | 13 | 35 | 1.3 | 1.25 | 7.5 | 86.4 | 49.2 | 35.95 |
| 18 | 13 | 45 | 1.3 | 1.25 | 7.5 | 83.4 | 43.2 | 21.12 |
| 19 | 13 | 35 | 1.3 | 1.25 | 7.5 | 87.8 | 49.8 | 43.67 |
| 20 | 13 | 35 | 1.3 | 1.25 | 6.5 | 83.5 | 48.4 | 26.32 |
| 21 | 16 | 30 | 1.0 | 1.5 | 8.0 | 81.1 | 48.3 | 21.70 |
| 22 | 16 | 40 | 1.0 | 1.0 | 8.0 | 80.4 | 47.2 | 19.65 |
| 23 | 19 | 35 | 1.3 | 1.25 | 7.5 | 73.8 | 49.6 | 19.27 |
| 24 | 13 | 35 | 1.3 | 1.25 | 7.5 | 86.8 | 50.1 | 40.15 |
| 25 | 16 | 30 | 1.0 | 1.0 | 7.0 | 73.1 | 47.3 | 12.55 |
| 26 | 10 | 30 | 1.6 | 1.0 | 7.0 | 78.5 | 38.3 | 13.39 |
| 27 | 16 | 30 | 1.6 | 1.5 | 7.0 | 80.1 | 51.3 | 23.92 |
| 28 | 13 | 35 | 1.3 | 1.25 | 7.5 | 87.6 | 49.4 | 41.35 |
| 29 | 16 | 35 | 1.0 | 1.0 | 6.5 | 75.3 | 50.2 | 16.02 |
| 30 | 16 | 40 | 1.6 | 1.5 | 8.0 | 77.1 | 52.3 | 20.43 |
| 31 | 16 | 30 | 1.6 | 1.0 | 8.0 | 74.1 | 48.3 | 13.75 |
| 32 | 10 | 40 | 1.0 | 1.0 | 7.0 | 86.5 | 37.3 | 23.02 |

## Conversion of 2-methyl-butanol (c%)

The effects of factors as well as their interactions on the c% could be discussed from the Pareto chart illustrated by Figure 3. The length of each bar was proportional to the absolute value of its associated regression coefficient or estimated effect. The order in the bars was displayed corresponded to the order of the size of the effect. The chart included a vertical line that corresponded to the 95% limit indicating statistical significance. A factor was, therefore, significant if its corresponding bar crossed this vertical line. As indicated in Figure 3, several different conclusions could be obtained: (1) the conversion of 2-methyl-butanol was greatly affected by reaction time ($x_1$), substrate molar ratio ($x_3$), and a quadratic terms of $x_1^2$, $x_2^2$, $x_3^2$, $x_4^2$; (2) the second-order effects of reaction time ($x_1$) and substrate molar ratio ($x_3$) were less significant than their respective first-order effects; (3) the regression coefficient of reaction temperature and enzyme loading were negative, which suggested that too high temperature and too much enzyme loading would not benefit the conversion of 2-methyl-butanol. Similarly, the effects of the terms would be positively correlated if the coefficients were positive. According to the statistical method, the data were fitted to a response surface model to effectively evaluate the true relationship between the c% and the factors. A quadratic regression model was obtained

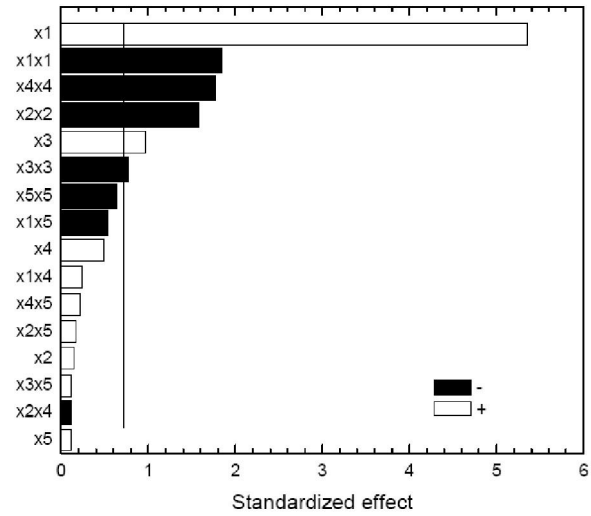


**Figure 3 : Pareto chart of standardized effects for the model of percent conversion. Positive effects are in pink and negative effects are in red. The line indicates the confidence level of 95%, and factors with standardized effect values to the right of this line are statistically significant.**

by using coded values from the estimation of data:

$$Y_1 = 50.62+5.35x_1+0.97x-1.84x_1^2 -1.58x_2^2-0.77x_3^2-1.77x_4^2 \quad (7)$$

where $x_i$ was the coded value of each factor.

## Enantiomeric excess

Similar to Figure 3, Figure 4 denoted the effects of factors as well as their interactions on enantiomeric excess. Compared with Figure 3, several conclusions could be drawn from Figure 4: (1) reaction time was the most significant factor affecting the enantiomeric excess; (2) reaction temperature produced a significant effect on the enantiomeric excess, although it was not important for the conversion of menthol; (3) also, significant interaction was found between time and enzyme loading. As aforementioned, the data were fitted to a response surface model to effectively evaluate the true relationship between enantiomeric excess and the factors. A quadratic regression model was obtained by using coded values from the estimation of data:

$$Y_1 = +88.14-1.02x_1+1.04x_2+1.80x_1x_4-1.25x_2x_4-1.03x_2x_5 +x_3x_4-3.32x_1^2-1.75x_2^2-1.38x_3^2-1.37x_4^2-1.33x_5^2 \quad (8)$$

where $x_i$ was the coded value of each factor.

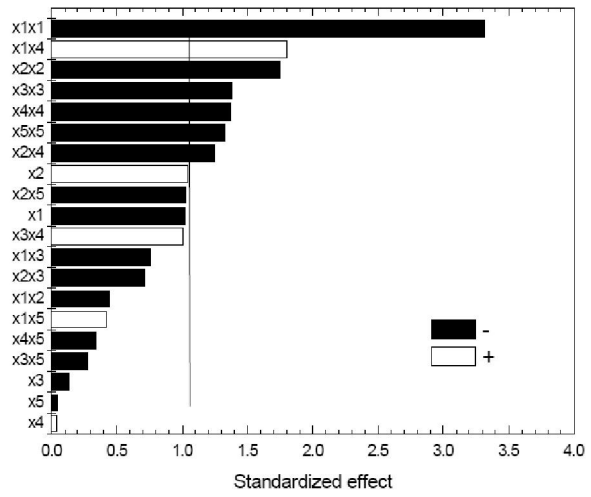


**Figure 4 : Pareto chart of standardized effects for the model of enantiomeric excess. Positive effects are in pink and negative effects are in red. The line indicates the confidence level of 95%, and factors with standardized effect values to the right of this line are statistically significant.**

## Analysis of variance (ANOVA) and adequacy test of the models

For the model fitted, software generated model coefficients, F and p-values (Prob>F, which indicates the insignificant probabilities) and hence one could justify the significance of each experimental variable. The corresponding variable would be more significant if the absolute F-value became larger and the p-value became smaller[14]. According to the analysis results (given in *Supplementary Information* of this article), the regression quadratic models were both highly significant ($p < 0.0001$) and the lack of fit was insignificant ($p > 0.05$), which indicated that the two models were adequate to explain most of the variability for the c% and the e.e.$_p$%, respectively.

To evaluate the optimization technique, the observed and predicted values of the c% were compared and the results were presented in Figure 5. As can be seen, the predicted values of the response from the model accorded well with the observed values. Consequently, this model could be used to navigate the design space.

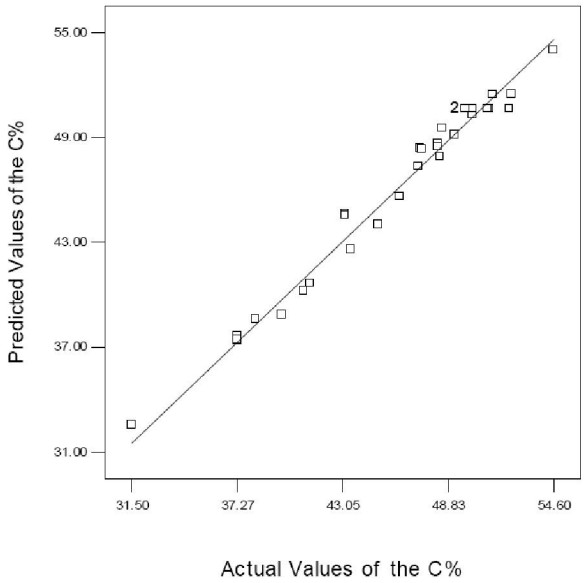


**Figure 5 : Comparison between the predicted and the observed conversion of 2-methyl-butanol.**

## Mutual effect of factors on the conversion of 2-methyl-butanol

The 3D-plots of response surfaces were used to illustrate the main and interactive effects of the independent variables on the conversion of 2-methyl-butanol. The response surfaces based on these factors were shown in Figure 6. Figure 6a represented the 3D-plot of the effect of reaction time and temperature on the reaction. From the analysis of the response surface plots, reaction time exhibited a more significant influence on the response surface in comparison to reaction temperature. At initial temperature, the conversion of

2-methyl-butanol increased as the time was increased, which reflected a general effect of temperature on the reaction rate. Subsequently, the conversion of 2-methyl-butanol emerged a peak with a maximum value around 13 h and then declined, possibly because of the depletion of (-)-2-methyl-butanol. Figure 6b depicted the enzyme loading and substrate molar ratio effect on the response. As can be seen, enhancing enzyme loading could bring about high conversion of 2-methyl-butanol, but excess amount of enzyme would influence the mass transfer of the reaction and led to the decline of the conversion of 2-methyl-butanol. On the other hand, both the increase of vinyl acetate amount, *i.e.* and the decrease of substrate molar ratio could increase the conversion of 2-methyl-butanol. Excess vinyl acetate would lead to conversion decrease, which was probably caused by the substrate inhibition.

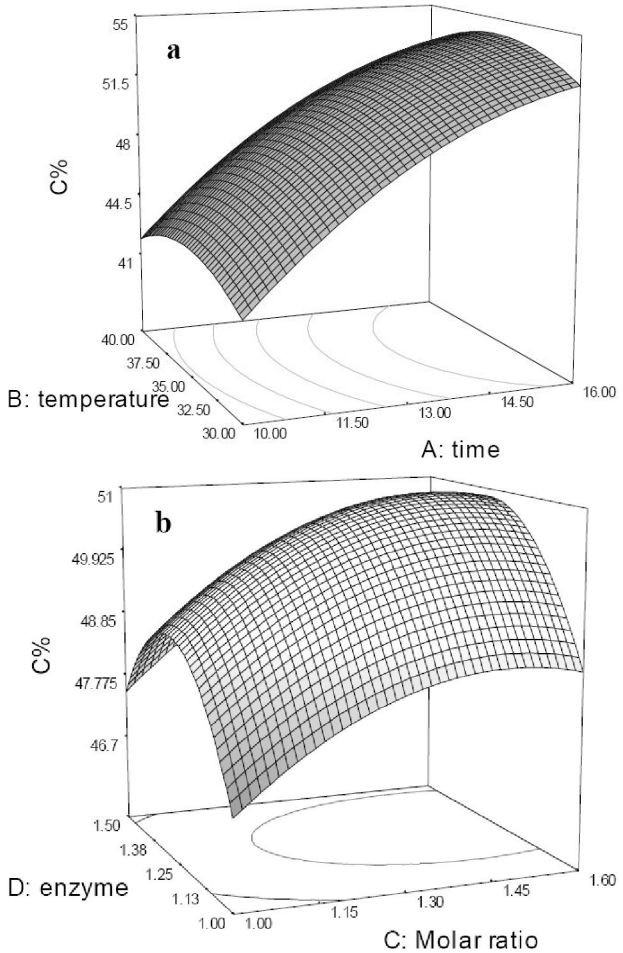


**Figure 6 : 3D-plot between any two parameters for the conversion of 2-methyl-butanol (Conditions of 6a: Enzyme loading: 1.25 g, MR=1.3:1, pH=7.5; Conditions of 6b: t=13h, T=35°C, pH=7.5).**

In addition, significant interactions were found between enzyme loading and substrate molar ratio. In Figure 6b, the response surface of 2-methyl-butanol conversion showed a net peak of 52.4% at 1.25g of enzyme loading and 1.3:1 of substrate molar ratio. Overall, reaction time, temperature and enzyme loading were the most important variables for the conversion of enantioselective esterification of (±)-2-methyl-butanol.

## Mutual effect of factors on the enantiomeric excess

The effects of these five factors as well as their interactive effects on the enantiomeric excess can be reflected in Figure 7. Figure 7a denoteed the two-dimensional contour plots of the effect of reaction time and temperature. As indicated, reaction temperature performed a very significant influence on the enantiomeric excess, and the response was expected to exhibit a monotonic increase with decrease of temperature. That was to say, low temperature was more favorable for improving stereospecificity. One possible explanation was that low temperature could increase the "rigidity" of the lipase, which enhanced the enantioselective recognition capability of the stereospecificity "pocket"; while high temperature increased the "flexibility" of the lipase and therefore brought down the recognition capability, which was similar to the previous report[15]. In Figure 7a, the enantiomeric excess showed a decreasing trend along with the reaction time course, which could be attributed to the fact that, with the proceeding of the reaction, the increasing consumption of (–)-2-methyl-butanol resulted in the incremental reaction probability of (+)-2-methyl-butanol.

The effect of enzyme loading on the enantiomeric excess was shown in Figure 7b. As depicted, keeping other experimental conditions constant, the enantiomeric excess would slightly increase with enzyme loading. A reaction with enzyme concentrations of 1.25-1.35 g and reaction time of 12-14 h led to over 86% enantiomeric excess. With the increase of substrate molar ratio, there was a slight decrease in the response value. As mentioned above, the effect of these factors on enantioselective esterification of 2-methyl-butanol could be studied by using response surface methodology.

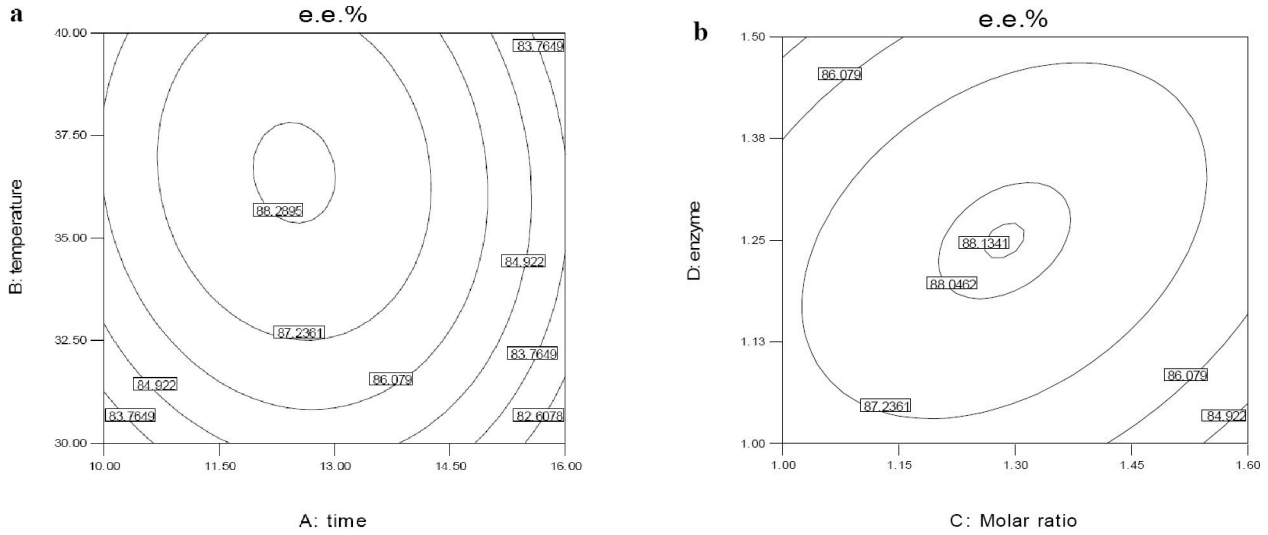


**Figure 7 : Contour polts between any two parameters for the production enantiomeric excess of esterification of 2-methyl-butanol (Conditions of 7a: Enzyme loading: 1.25 g, MR=1.3:1, pH=7.5; Conditions of 7b: t=13 h, T=35°C, pH=7.5).**

## Attaining optimum conditions and model verification

As known to all, it is of general interest for developing industrial processes for the enantioselective esterification of (±)-2-methyl-butanol useful for food additives and cosmetic formulations as well as medicine industry. Based on the above discussion, it was possible to obtain a high degree of conversion and high enantiomeric ratio through searching for the optimum point. Hence, one set of predicted reaction conditions were given by the model (TABLE 3). To validate the predicted results, experiments using the improved formula were performed, and the observed values were shown in TABLE 3. Based on the solution given by the design, experiments were established at the fixed conditions. The experimental values were found to be reasonably close to the predicted ones, which confirmed the validity and adequacy of the predicted models. In addition, under these conditions, the enantiomeric ratios (E) have also been calculated and the E-value was 31.5, which were much higher than the previous report[7].

**TABLE 3 : Optimum conditions found by the model and verification of the model**

| Predicted value | | | | | | | Experimental value | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T(h) | t(℃) | Molar ratio | Enzyme loading(g) | pH | e.e.% | c% | E | e.e.% | c% | E |
| $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $Y_1$ | $Y_2$ | | | | |
| 11.76 | 38.47 | 1.19 | 1.14 | 7.33 | 88.60 | 46.28 | 38.1 | 86.22 | 47.31 | 31.5 |

## CONCLUSIONS

The lipase from porcine pancreas was used as a biocatalyst to perform enantioselective esterification of (±)-2-methyl-butanol. Response surface methodology was successfully applied to determine the operation conditions for optimizing the conversion of 2-methyl-butanol and enantiomeric ratio. The results indicated a significantly good fit to this model, and the response evaluated from the quadratic model showed a good agreement with the observed ones. The F-test and p-value indicated that reaction time and substrate molar ratio were the significant factors affecting the conversion of 2-methyl-butanol.

Moreover, the optimum operation condition was established. Furthermore, the experimental values agreed well with the values predicted in optimized conditions. By the optimum model, the conversion of 2-methyl-butanol and the E-value could exceed 53% and 40, respectively. The experimental conditions allowed a fast, quantitative and maximum enantiomeric resolution of (±)-2-methyl-butanol.

## SUPPLEMENTARY INFORMATION

Supplementary Information of the analysis results

of variance and adequacy test of the models in tables is available and free of charge from Editorial Office on request.

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