Hilal Celik Kazici, Emine Bayraktar and Ülkü Mehmetoglu*

# Optimization of the asymmetric synthesis of chiral aromatic alcohol using freeze-dried carrots as whole-cell biocatalysts

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**Abstract:** Asymmetric reduction of ketones is an important transformation in organic synthesis, because chiral carbinols are useful bioactive compounds. In this study, bioreduction of acetophenone (ACP) for production of enantiomerically pure (S)-1-phenyl-ethanol was investigated and freeze-dried carrots were used as a source of alcohol dehydrogenases (ADHs). However, production of product was investigated systematically using response surface methodology (RSM). Before RSM, the effects of the initial substrate concentration, reaction time, temperature and pH on the bioreduction were studied. The best results for enantiomeric excesses (ee) and conversion (c) were obtained with >99% and 58%, respectively, for the reaction time 48 h, initial substrate concentration 1 mM, reaction temperature 33°C and pH 7. In the RSM, initial substrate concentration, concentration of plant cell, reaction time and stirring rate were chosen as independent variables. The predicted optimum conditions for a higher ee (>99%) and conversion (57.8%) were as follows: initial substrate concentration, 1 mM; concentration of plant cell, 25 g/l; reaction time, 52 h and stirring rate, 200 rpm. As a result of repeated experiments, the product was obtained as 0.6 mM at this optimum point and the values obtained demonstrated conformity with 0.578 mM value calculated by the model equation.

**Keywords:** acetophenone; asymmetric reduction; biocatalyst; optimization; (S)-1-phenyl-ethanol.

***Corresponding author: Ülkü Mehmetoglu,** Faculty of Engineering, Department of Chemical Engineering, Ankara University, Ankara, Turkey, e-mail: mehmet@eng.ankara.edu.tr
**Hilal Celik Kazici:** Faculty of Engineering, Department of Chemical Engineering, Yuzuncu Yil University, Van 65100, Turkey
**Emine Bayraktar:** Faculty of Engineering, Department of Chemical Engineering, Ankara University, Ankara, Turkey

## 1 Introduction

Asymmetric reduction of prochiral ketones by biocatalysts is an advantageous method to obtain chiral alcohols [1]. Chiral compounds with one chiral center can exist in two enantiomeric forms, both of which have the same chemical and physical properties in an achiral environment, but frequently have different biological activities [2]. Chemical synthesis of some of these compounds is extremely complicated and costly. Hence, biotransformations using plant cells and isolated enzymes have wide potential for production of pharmaceuticals, despite their disadvantages. Plant enzyme biocatalysts may be applied to the production of totally new drugs and also may be used to modify existing drugs by improving their bioactivity spectrum [3].

We studied asymmetric reduction of a group of aldehydes (or) ketones using the freeze-dried carrot as a whole-cell biocatalyst. Whole cells continue to be very popular sources of dehydrogenases for asymmetric synthesis [4]. Dehydrogenases have been widely used for the reduction of carbonyl groups of aldehydes or ketones and of carbon-carbon double bonds. The importance of the use of these enzymes is that a chiral product can potentially be obtained from a prochiral substrate [5].

In another study, fresh carrot was used as the plant cells and baker's yeast was used as the microbial cells. Prochiral ketones (indanone, tetralone and hydroxyl trimonoterpene ketone) were reduced with asymmetric synthesis. Using carrot biocatalyst higher conversion (c) and enantiomeric excess (ee) values were obtained than baker's yeast. For indanone: 95% ee and 99% c at the end of 2 h, for tetralone: 85% c and 45% ee at the end of 36 h, and for hydroxyl trimonoterpene ketone: 99% ee and 70% c at the end of 8 h were obtained [6].

In a further study, green peas *Pisum sativa* were used as biocatalysts in the enantioselective reduction of prochiral ketones. Various substituted acetophenones (ACPs) were converted into chiral secondary alcohols; the S-alcohols were obtained in all cases with ee ranging from 91% to 98% and yield ranging from 55% to 72%. The reduction

was achieved using sprouted green peas in aqueous buffer pH 7.0 at room temperature [7].

Biotransformation by cell cultures and hairy root cultures serves as an important tool in the structural modification of compounds possessing useful therapeutic activity. However, a major drawback of suspension cultures is the phenomenon of somaclonal variation, which may lead to unstable biochemical behavior [8]. Since variation and instability are the main problems associated with cell cultures, continuous screening is required to maintain highly productive lines [9].

The plant cell is easily available and cheap, and using plant cells for biocatalysis and biotransformation has been tried for many years. Unlike microbial biocatalysts like baker's yeast, information in association with enzymes from plants is scarce. However, activity of plant cells varies according to the source and seasons. This is a disadvantage [10]. Therefore, we are interested in the high enantiomerical bioreduction of aromatic ketone by freeze-dried biocatalysts. Also, we used whole cells in the study. The most important advantage of using whole cells is that the whole reaction system for cofactor regeneration is present within the cells themselves. Above all, whole cells are easier to obtain and cheaper than isolated enzymes [11].

This paper presents biotechnological processes for the production of chiral alcohols by reducing prochiral ketones with whole plant cell and investigates the operating conditions to obtain a high ee. The response surface methodology (RSM) method is used in bioreduction reactions with plant (freeze-dried carrot) biocatalysts.

# 2 Materials and methods

## 2.1 Chemicals

ACP, (R)-1-phenyl-ethanol, (S)-1-phenyl-ethanol and other chemical reagents were purchased from Sigma Aldrich. Carrots (*Daucus carota*) were obtained from a local market in Turkey. The external layer of the vegetable was removed and the rest was cut with a sterile slicer into small thin pieces (approximately 1 cm long slices).

## 2.2 Asymmetric reduction of ACP with freeze-dried carrot

Freshly cut *D. carota* root carrots weighing approximately 100 g were freeze-dried under conditions of 0.024 mbar and -50°C for 24 h and freeze-dried carrots were obtained as a result with a Labconco mark and (7752030) model lyophilizer. Finally, 15 g of carrots were obtained as freeze-dried weight and added into a 250 ml conical flask including 50 ml of 0.1 mM sodium phosphate buffer pH 7 with ACP (1 mM) (Sigma Aldrich). The reaction was incubated in an orbital shaker (150 rpm) at 33°C temperature. At the end of the bioreduction reaction (Figure 1), the filtrate was extracted with organic solvent [methyl tertiary butyl ether (MTBE), 3.5 ml (Sigma Aldrich)]. The organic phase was dried with anhydrous $Na_2SO_4$. The chemical yield and enantioselectivity were determined. All experiments were carried out twice and the averaged values are presented in this study. Alcohol dehydrogenases (ADHs) require cofactors and cofactors are quite expensive materials. Cofactors will run out the reaction proceeds. So, instead of pure enzymes, the plant is more economical to use as a source of ADH. Cofactors can be regenerated using the glucose/glucose dehydrogenase (GDH) in the plant cells [12].

## 2.3 RSM

**2.3.1 Application of central composite design:** In RSM, central composite design is the most convenient option for fitting a second order model. The total number of experiments for four variables were 30 ($=2^k+2k+6$), where $k$ is the number of independent variables. The results of thirty experiments were used to estimate the experimental error [13].

In the optimization process, the response can be involved to selected variables by linear or quadratic models. A quadratic model is given in Eq. (1):

$$\hat{y}_n = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \quad (1)$$

where $\hat{y}_n$ is the response, $\beta_0$ is the constant coefficient, $x_i$ ($i$=1–3) are noncoded variables and $\beta_i$ is the linear, $\beta_{ii}$ the quadratic and $\beta_{ij}$ ($i$ and $j$=1–3) the second-order interaction coefficients. The residuals, $\varepsilon_n$, for each experiment were computed as the difference between $y_n$ and $\hat{y}_n$, which are the residual of the $n$th experiment, the observed response and the predicted response, respectively.

The analysis of variance (ANOVA) data were computed by Design-Expert 8.0.7.1 for the purpose of obtaining the interaction between the processed variables and the response. The characteristic of the fit of the polynomial model was represented by the coefficient of determination ($R^2$) and the statistical significance was checked by the F-test using the same program [14].

## 2.4 Analysis

The concentrations of ACP, (R)-1-phenyl-ethanol and (S)-1-phenyl-ethanol were determined by high-pressure liquid chromatography (Thermo Finnigan Spectra System) with a 4.6 mm×50 mm Chiralcel-OB column (Daicel Chemical Ind. Ltd., France) using eluent n-hexane-iso-propanol, 95:5, flow rate of 0.8 ml/min and detection

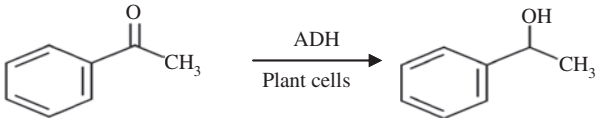


**Figure 1:** Asymmetric reduction of acetophenone (ACP) catalyzed by freeze-drying carrots.

monitored by UV (Eppendorf, Germany) absorption at 254 nm with a diode array detector.

The conversion percent (c%) was determined from the ratio of reacted substrate concentration ($C_0$-$C$) to its initial substrate concentration ($C_0$). In Eq. (2), $C$ is the substrate concentration in a certain reaction time:

$$c\% = \frac{C_0 - C}{C_0} \times 100 \quad (2)$$

The ee of (S)-1-phenyl-ethanol was calculated as follows:

$$ee\% = \frac{[C_{(S)\text{-}1\text{-}PE}] - [C_{(R)\text{-}1\text{-}PE}]}{[C_{(S)\text{-}1\text{-}PE}] + [C_{(R)\text{-}1\text{-}PE}]} \times 100 \quad (3)$$

Here, in Eq. (3), $C_{(R)\text{-}1\text{-}PE}$ and $C_{(S)\text{-}1\text{-}PE}$ are the concentrations of (R)-1-phenyl-ethanol and (S)-1-phenyl-ethanol, respectively [7].

## 3 Results and discussion

ACP was reduced to the corresponding enantiopure (S)-1-phenyl-ethanol with high enantioselectivity and chemical yield. The conversion and enantioselectivity of asymmetric synthesis of alcohol is significantly influenced by the initial substrate concentration, reaction time, temperature and pH, also, the effects of these parameters on the asymmetric synthesis of (S)-1-phenyl-ethanol were investigated. In this study, the effect of initial substrate concentration on the asymmetric reduction reaction was investigated in the range from 0.5 mM to 4 mM (Table 1).

From Table 1, initial substrate concentration had no effect on the ee, but conversion decreased with the increase of the initial ACP concentration. At the end of the reaction, >99% ee and 58% conversion was obtained for 1 mM initial substrate concentration; >99% ee and 22% conversion was obtained for 4 mM initial substrate concentration. This finding indicates the presence of substrate inhibition. When the initial ACP concentration is more than 1 mmol·l$^{-1}$ the conversion decreases sharply. In our previous study [15], fresh carrots had been used as biocatalysts and achieved >99% ee and 94% conversion for 1 mM and >99% ee and 62% conversion for 4 mM. According to this research, the conversion value is not high because carrots were frozen slowly at -50°C. Slow freezing allows ice crystals to grow outside cells [16] and causes damage by cell collapse. The damaged cells may have lost their activity and therefore can reduce conversion in terms of lyophilized carrots [17].

It was observed that (S)-1-phenyl-ethanol concentration increased when the concentration of the substrate increased (Table 1). (S)-1-Phenyl-ethanol 0.39 mM was obtained for 0.5 mM substrate concentration and 0.88 mM (S)-1-phenyl-ethanol was obtained for 4 mM substrate concentration. However, due to the remaining substrates in the batch, the system is preferable to work at 1 mM.

The effect of the reaction time on the asymmetric reduction reaction catalyzed by freeze-dried carrot (*D. carota*) was investigated in the range of 6–72 h (Table 1). Reaction conversion did not change much after 48 h. At the end of reaction, >99% ee and 12% c for 6 h and >99% ee and%58 c for 48 h were obtained.

The effect of reaction temperature on the asymmetric reduction reaction was investigated in the range from 25°C to 40°C (Table 1). High ee values were obtained at all temperatures. Ee values were unaffected by temperature. However, c% was affected from the change of temperature. A maximal value of 58 c% was obtained at 33°C. Enzyme catalysts are dependent on temperature because of the protein structure. Therefore, reduced conversion was observed due to the inactivation of ADH enzyme at high temperature.

**Table 1:** The effect of independent model parameter on the c% and ee% values.

| $C_{so}$ (mM)[a] | c% | ee% | $C_{(S)\text{-}1\text{-}PE}$ (mM) | Time (h)[b] | c% | ee% | Temperature (°C)[c] | c% | ee% | pH[d] | c% | ee% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.5 | 78 | >99 | 0.39 | 6 | 12 | >99 | 25 | 10 | >99 | 6.0 | 43 | >99 |
| 1 | 58 | >99 | 0.58 | 12 | 15 | >99 | 30 | 49 | >99 | 6.5 | 71 | >99 |
| 2 | 36 | >99 | 0.72 | 24 | 33 | >99 | 33 | 58 | >99 | 7.0 | 58 | >99 |
| 4 | 22 | >99 | 0.88 | 48 | 58 | >99 | 40 | 60 | >99 | 7.5 | 61 | >99 |
| | | | | 72 | 80 | >99 | | | | 8.0 | 35 | >99 |

[a]Effect of substrate concentration was carried out with the cell concentration 30 g/l, phosphate buffer 0.1 M (pH=7), time 48 h, temperature 33°C and stirring rate 150 rpm.

[b]Effect of reaction time was carried out with the substrate concentration 1 mM, cell concentration 30 g/l, phosphate buffer 0.1 M (pH=7), temperature 33°C and stirring rate 150 rpm.

[c]Effect of reaction temperature was carried out with the substrate concentration 1 mM, cell concentration 30 g/l, phosphate buffer 0.1 M (pH=7) and stirring rate 150 rpm.

[d]Effect of reaction pH was carried out with the substrate concentration 1 mM, cell concentration 30 g/l, phosphate buffer 0.1 M, time 48 h, temperature 33°C and stirring rate 150 rpm.

Each enzyme has a pH range where it works best. Enzymes are affected by extreme acidic and basic environments. They usually work in a neutral medium. The effect of pH on the asymmetric reduction reaction was investigated in the range from 6 to 8 (Table 1). Generally, the enzymes demonstrate high activity at a specific pH. In this study, optimal pH was found to be 7 for lyophilized carrots. Values of ee were not affected by pH. After identifying the concentration of the substrate, reaction time, reaction temperature and pH of media, other reaction conditions were evaluated using the RSM. Independent variables are determined as substrate concentration, the concentration of the lyophilized biocatalyst carrots, stirring speed, reaction time and response variable is determined as (S)-1-phenyl-ethanol concentration obtained as the product (Table 2).

Central composite design experiments for optimization of these parameters were performed to place the maximum $C_{(S)\text{-}1\text{-}Phenyl\text{-}Ethanol}$ by Design Expert 8.0.7.1. Combinations of different factors levels were created and responses and arguments were based on their relationship with each other.

The RSM experiments performed and the results obtained under the operational conditions are listed in (Table 3) and quadratic model equations (second order polynomial equation) is given below for the actual value (Eq. 4):

$$C_{(S)\text{-}1\text{-}Phenyl\text{-}Ethanol} = 0.20710 + 6.55477.10^{-3} C_h - 5.85893.10^{-3} rpm + 7.9105.10^{-3} t + 0.80883 C_{SO} + 7.50000.10^{-8} C_h rpm - 7.44048.10^{-8} C_h t + 3.94737.10^{-6} C_h C_{SO} + 2.97619.10^{-8} rpmt - 1.05263.10^{-6} rpm C_{SO} + 4.27632.10^{-4} t C_{SO} - 1.04993.10^{-4} C_h^2 + 1.95647.10^{-5} rpm^2 - 6.83161.10^{-5} t^2 - 0.39314 C_{SO}^2 \quad (4)$$

The statistical significance of the quadratic model was evaluated by the analysis of variance (ANOVA) (Table 4). The effect of each variable was determined by the F-test, and the smaller the p-value, the more significant was the effect of the variables; the R-squared value provided a measure of the variability in the actual response values that could be explained by the experimental factors and their interactions [18].

**Table 2:** Experimental range and of the independent variables.

| Variables | Units | -1 Level | +1 Level |
|---|---|---|---|
| Cell concentration (Ch) | g/l | 10 | 50 |
| Stirring rate | rpm | 100 | 200 |
| Reaction time (t) | H | 12 | 96 |
| Substrate concentration ($C_{So}$) | mM | 0.1 | 2 |

**Table 3:** Full factorial central composite design matrix of four variables and natural units along with observed responses ($C_{S\text{-}1\,PE}$).

| Run | A:Ch (g/l) | B:stirring rate (rpm) | C:t (h) | D:$C_{So}$ (mM) | $C_{S\text{-}1\,PE}$ (mM) |
|---|---|---|---|---|---|
| 1 | 10.00 | 100.00 | 96.00 | 2.00 | 0.109 |
| 2 | 30.00 | 150.00 | 100.20 | 1.05 | 0.570 |
| 3 | 30.00 | 205.00 | 54.00 | 1.05 | 0.570 |
| 4 | 10.00 | 200.00 | 96.00 | 2.00 | 0.109 |
| 5 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 6 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 7 | 10.00 | 100.00 | 96.00 | 0.10 | 0.080 |
| 8 | 50.00 | 100.00 | 96.00 | 2.00 | 0.108 |
| 9 | 50.00 | 200.00 | 12.00 | 0.10 | 0.077 |
| 10 | 50.00 | 100.00 | 12.00 | 2.00 | 0.038 |
| 11 | 10.00 | 200.00 | 96.00 | 0.10 | 0.080 |
| 12 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 13 | 10.00 | 200.00 | 12.00 | 2.00 | 0.037 |
| 14 | 50.00 | 100.00 | 96.00 | 0.10 | 0.080 |
| 15 | 52.00 | 150.00 | 54.00 | 1.05 | 0.500 |
| 16 | 50.00 | 200.00 | 12.00 | 2.00 | 0.0371 |
| 17 | 10.00 | 200.00 | 12.00 | 0.10 | 0.077 |
| 18 | 50.00 | 100.00 | 12.00 | 0.10 | 0.076 |
| 19 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 20 | 8.00 | 150.00 | 54.00 | 1.05 | 0.410 |
| 21 | 50.00 | 200.00 | 96.00 | 2.00 | 0.109 |
| 22 | 10.00 | 100.00 | 12.00 | 0.10 | 0.077 |
| 23 | 30.00 | 150.00 | 54.00 | 2.09 | 0.146 |
| 24 | 50.00 | 200.00 | 96.00 | 0.10 | 0.080 |
| 25 | 30.00 | 150.00 | 7.80 | 1.05 | 0.150 |
| 26 | 30.00 | 150.00 | 54.00 | 0.01 | 0.007 |
| 27 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 28 | 10.00 | 100.00 | 12.00 | 2.00 | 0.037 |
| 29 | 30.00 | 150.00 | 54.00 | 1.05 | 0.570 |
| 30 | 30.00 | 95.00 | 54.00 | 1.05 | 0.560 |

In Table 4, the probability value for response $C_{S\text{-}1PE}$ ($p=0.0001<0.05$) demonstrated a high significance for the regression model, and the insignificant probability for the lack of fit ($p=0.083>0.05$) indicated that the regression analysis is effective. This proves that the model equation as expressed in Eq. (4) provides a suitable model to describe the response of the value of the product concentration.

As shown from Table 4 the model F-value of 19.05 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Furthermore, a value of the coefficient of determination ($R^2=0.95$) was calculated. The high value $R^2$ (0.95) demonstrates that the model adequately represented the real relationship among the independent variables.

Values of "Prob>F" <0.0500 indicate model terms are significant. In this case C, $C^2$, $D^2$ are significant model terms. Values >0.1000 indicate the model terms are not

**Table 4:** Analysis of variance (ANOVA) for quadratic model.

| Sum of source | Squares | df | Mean square | F-value | p-Value Prob>F |
|---|---|---|---|---|---|
| Model | 1.48 | 14 | 0.11 | 19.05 | <0.0001 |
| A-Ch | $5.235\times10^{-4}$ | 1 | $5.235\times10^{-4}$ | 0.094 | 0.7628 |
| B-rpm | $8.080\times10^{-6}$ | 1 | $8.080\times10^{-6}$ | $1.458\times10^{-3}$ | 0.9700 |
| C-t | 0.031 | 1 | 0.031 | 5.67 | 0.0309 |
| D-$C_{So}$ | $2.372\times10^{-4}$ | 1 | $2.372\times10^{-4}$ | 0.043 | 0.8389 |
| AB | $9.000\times10^{-8}$ | 1 | $9.000\times10^{-8}$ | $1.624\times10^{-5}$ | 0.9968 |
| AC | $6.250\times10^{-8}$ | 1 | $6.250\times10^{-8}$ | $1.128\times10^{-5}$ | 0.9974 |
| AD | $9.000\times10^{-8}$ | 1 | $9.000\times10^{-8}$ | $1.624\times10^{-5}$ | 0.9968 |
| BC | $6.250\times10^{-8}$ | 1 | $6.250\times10^{-8}$ | $1.128\times10^{-5}$ | 0.9974 |
| BD | $4.000\times10^{-8}$ | 1 | $4.000\times10^{-8}$ | $7.216\times10^{-6}$ | 0.9979 |
| CD | $4.658\times10^{-3}$ | 1 | $4.658\times10^{-3}$ | 0.84 | 0.3738 |
| $A^2$ | $8.149\times10^{-3}$ | 1 | $8.149\times10^{-3}$ | 1.47 | 0.2441 |
| $B^2$ | $7.301\times10^{-3}$ | 1 | $7.301\times10^{-3}$ | 1.32 | 0.2691 |
| $C^2$ | 0.059 | 1 | 0.059 | 10.57 | 0.0054 |
| $D^2$ | 0.44 | 1 | 0.44 | 79.29 | <0.0001 |
| Residual | 0.083 | 15 | $5.543\times10^{-3}$ | | |
| Lack of fit | 0.083 | 10 | $8.314\times10^{-3}$ | | |
| Pure error | 0.000 | 5 | 0.000 | | |
| Cor total | 1.56 | 29 | | | |

$R^2$=0.95; Adeq Precision=10.709.

significant. The high F value of $(C)^2$ and $(D)^2$ implied that there was not a simple linear correlation between the variables and response $C_{S\text{-}1PE}$. The low F value between the factors A and B, A and C, A and D, B and C, B and D, C and D meant that there was weak mutual interaction between them.

The response surfaces obtained according to the RSM analysis for each $C_{S\text{-}1PE}$ value are shown in Figures 2 and 3. Response surface plots provide a method to predict the $C_{S\text{-}1PE}$ value for different values of the test variables.

The 3D-plots were drawn to define the basic and interactive effects of the independent variables on the $C_{S\text{-}1PE}$. The response surfaces based on these factors are shown in Figure 2A. 3D-plot demonstrates the effect of time and cell concentration on the product concentration. The other variables are held at zero level. From the analysis of the response surface plots, the effect of time displayed a more significant influence on the response surface in comparison to concentration of the cell. Product concentration increased with the increase in the concentration of biocatalysis, relatively, but increasing the concentration of cells caused mass transfer restrictions and the product concentration was unchanged in this case.

Figure 2B demonstrates the 3D-plot of the effect of time and concentration of substrate on the product concentration. The concentration of product increased with time and then the product was not affected, but the concentration of the product which increased with increasing

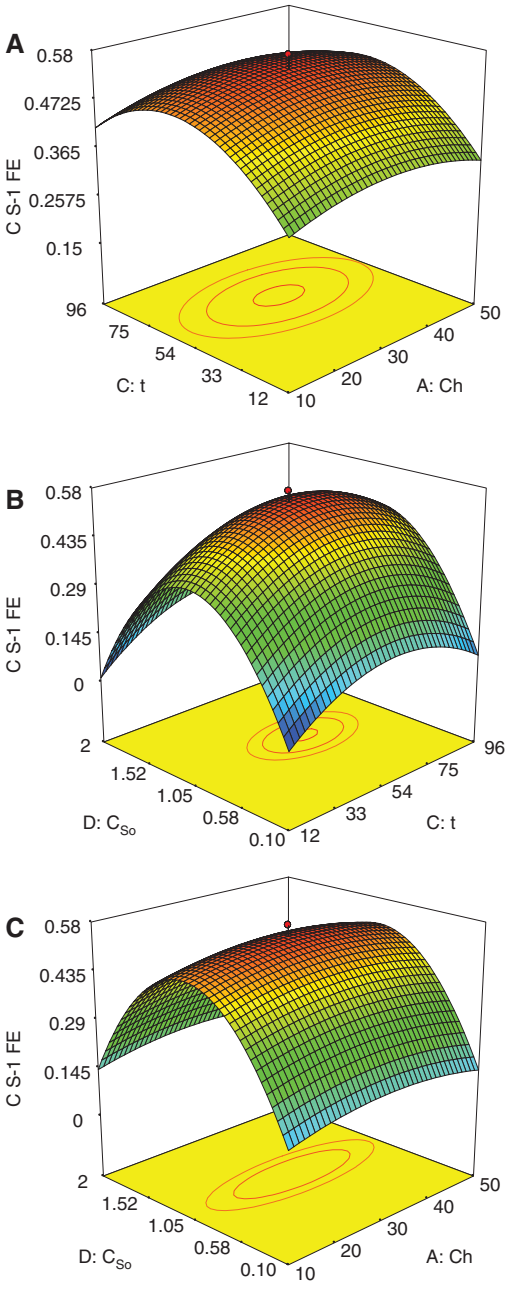


**Figure 2:** (A) 3D-plot between any two parameters for the concentration of product. Effect of time and concentration of the cell; (B) effect of time and concentration of the substrate; (C) effect of concentration of the cell and concentration of the substrate.

concentration of the substrate is then reduced, which was probably caused by substrate inhibition.

At high concentrations, some substrates also inhibit the enzyme activity. Substrate inhibition occurs with about 20% of all known enzymes. It happens when two molecules of substrate can bind to the enzyme, and thus block activity [8]. The effect of concentration of substrate and concentration of cells on the reaction is shown in Figure 2C.

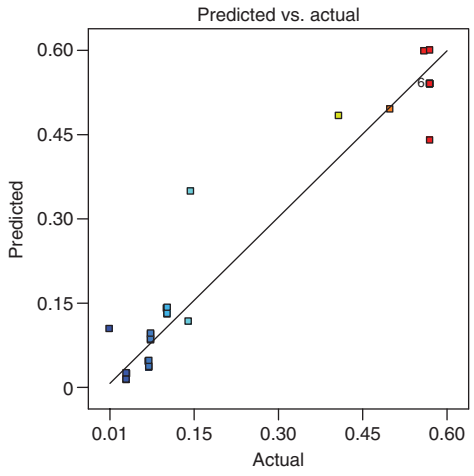


**Figure 3:** Comparison between the predicted and the observed values.

It was observed that concentration of the product did not change much with increasing concentrations of biocatalyst. Because additional mass transfer limitation can occur with increasing catalyst loading in a liquid-solid (catalyst) reaction, or the amount of enzyme can be more than the substrate concentration, no free substrate was available to bind the excess enzyme. By contrast, with the increase of initial substrate concentration to a certain value, S-1-phe concentration increased and then above this substrate concentration decreased because of substrate inhibition.

As a result of experiments using the optimum conditions, the product concentration was found to be 0.578 mM. Optimum conditions are determined to be the highest concentration of the product making the solution in the second degree of the model equations. Optimum initial substrate concentration, stirring rate, reaction time and biocatalyst concentration were found to be 1 mM, 200 rpm, 52 h and 25 g/l, respectively. Design Expert software package was used for the solution of the equation. The results of the model equations solved using these optimum conditions gives a value of 0.578 mM, the highest concentration of the product.

To appreciate the optimization technique, the observed and predicted values of the $C_{S\text{-}1PE}$ are compared in Figure 3. As shown, the predicted values of the response from the model accorded well with the observed values.

Figure 3 shows actual (S)-1-phenyl-ethanol concentration versus those obtained from the model equation. Additionally, the same figure proves that the predicted data of the response from the empirical model is well in agreement with the observed ones ($R^2$=0.95). The product was obtained as a result of repeated experiments; 0.6 mM is the optimum point, and is consistent with the calculated value of 0.578 mM, the value obtained by the model equation. The result showed a significantly good fit to this model, and the response evaluated from the quadratic model showed a good agreement with the observed ones.

## 4 Conclusion

In this work, reaction characteristics were studied in detail using freeze-dried carrots as the biocatalyst. The effects of various factors on the reaction were investigated, such as initial substrate concentration, reaction time, temperature and pH. The best results were obtained as >99% and 58% for ee and conversion, respectively, under the following conditions: reaction time 48 h, initial substrate concentration 1 mmol·$l^{-1}$, reaction temperature 33°C and pH 7.

According to this research, the conversion value is not high because, carrots were frozen slowly and at -50°C. Slow freezing allows ice crystals to grow outside cells, causing damage by cell collapse and rupture. Fast freezing determines ice crystals to grow inside cells with very little cell separation and much less damage [17].

RSM was successfully applied to determine the optimum operation conditions. The results showed a significantly good fit to this model and the response evaluated from the quadratic model showed a good agreement with the observed ones. Under the optimal reaction conditions (reaction time 52 h, initial concentration of substrate 1 mmol·$l^{-1}$, concentration of cell 25 g/l and mixing speed 200 rpm), concentration of ($C_{(S)\text{-}1\text{-}Phenyl\text{-}Ethanol}$) was obtained as 0.578 mM. As a result of repeated experiments, the product wa obtained as 0.6 mM in this optimum point and the value obtained demonstrated conformity with 0.578 mM calculated by the model equation.

The results indicate that freeze dried carrots can be used as a biocatalyst for the asymmetric reduction reaction of prochiral aromatic ketones when the freeze-drying conditions will be improved. There is no study in the literature like this. Therefore, it is important to work toward filling this gap.

## Nomenclature

| | |
|---|---|
| ACP | acetophenone |
| ADH | alcohol dehydrogenase |
| $C_0$ | initial substrate concentration |
| $C$ | the substrate concentration in a certain reaction time |
| c% | conversion percent |

$C_{(R)\text{-}1\text{-}PE}$ concentration of (R)-1- phenyl-ethanol
$C_{(S)\text{-}1\text{-}PE}$ concentration of (S)-1-phenyl-ethanol
ee enantiomeric excess

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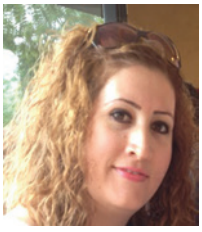
## Bionotes



**Hilal Celik Kazici**

Hilal Celik Kazici received her PhD from the University of Ankara during the period of 2007–2014. Currently, she is working at Yuzuncu Yil University. Her research has included biotechnology, enzymes, biotransformation, and bioprocess. She is serving as reviewer for journals such as *Journal of Ecobiotechnology* and *Biological and Chemical Research*. She is a member of the Biotechnology Association and the Turkish Chamber of Chemical Engineers.

**Emine Bayraktar**

Emine Bayraktar received her PhD from the Department of Chemical Engineering, University of Ankara (1991–1998). Currently, she is working at the University of Ankara. Her research has included organic acid production and separation, bioreactor modelling, reverse micelles, bioremediation, response-surface methods implementation, production of polylactic acid. She is serving as an editor for journals like the *Turkish Journal of Biochemistry*.

**Ülkü Mehmetoglu**

Ülkü Mehmetoglu received her PhD from the Department of Chemical Engineering, University of Hacettepe, during the period of 1977–1984. Currently, she is working at the University of Ankara. Her research has included fermentation process for organic acids, amino acids, and antibiotic production, extractive fermentation, mathematical modelling of bioreactors, enzymatic and microbial biotransformation, enantioselective production of drug precursor, biochemical production using plant cell culture, and microbial waste water treatment. She is serving as a member of editorial board for journals such as *Process Biochemistry*. She is a member of the Turkish Chamber of Chemical Engineers.