



A Multi-response Nonlinear Programming Model with an Inscribed Design to Optimize Bioreduction Conditions of (S)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13

Akın Özdemir¹ · Engin Şahin²

Received: 25 February 2023 / Accepted: 23 January 2024 / Published online: 28 February 2024
© The Author(s) 2024

Abstract

Asymmetric bioreductions have the potential to synthesize chiral alcohols when catalyzed by biocatalysts. Nevertheless, the (S)-phenyl (pyridin-2-yl)methanol ((S)-2) analgesic synthesis poses significant challenges concerning unsatisfactory substrate amount and production method. Thus, this study proposes an inscribed design-focused multi-response nonlinear optimization model for the asymmetric reduction of the phenyl(pyridin-2-yl)methanone (1) with *Leuconostoc pseudomesenteroides* N13 biocatalyst. From the novel inscribed design-focused multi-response nonlinear optimization model, optimization conditions of the reaction, such as pH = 6, temperature = 29 °C, incubation time = 53 h, and agitation speed = 153 rpm, were found. Also, the reaction conversion was predicted to be 99%, and the product of the enantiomeric excess (ee) was 98.4% under the obtained optimization conditions. (S)-2 was obtained with 99% ee, 99% conversion, and 98% yield while performing a validation experiment using the determined optimized conditions. In addition, 1 with the amount of 11.9 g was converted entirely to (S)-2 (11.79 g, 98% isolated yield) on a high gram scale. Also, this study is noted as the first example of the gram-scale production of (S)-2 using an optimization strategy and biocatalyst. Further, the applicability of the inscribed design-focused optimization model in biocatalytic reactions has been demonstrated and provides an effective process for the analgesic synthesis of (S)-2, which is a green, cost-effective method of producing chiral aryl heteroaryl methanol.

Keywords Inscribed design · Optimization · Asymmetric bioreduction · Biocatalysts · (S)-phenyl (pyridin-2-yl)methanol

List of Symbols

CI	Confidence interval
df	Degrees of freedom
$d(\hat{\mu}_1)$	The desirability function for the ee
$d(\hat{\mu}_2)$	The desirability function for the conversion
ee	Enantiomeric excess
E	PH
F	Temperature
G	Incubation period
H	Agitation speed
lower _c	Lower value for the conversion
lower _{ee}	Lower value for the ee

REML	Restricted maximum likelihood
RR	Randomized run
SR	Standard run
target _c	The target value for the conversion
target _{ee}	The target value for the ee
y ₁	The ee response
y ₂	The conversion response
$\hat{\mu}_1$	The estimated fitted function of the ee response
$\hat{\mu}_2$	The estimated fitted function of the conversion response
$\hat{\beta}_i$	The <i>i</i> th coefficient of the estimated function of the ee
$\hat{\delta}_i$	The <i>i</i> th coefficient of the estimated function of the conversion
$\varepsilon_1, \varepsilon_2$	Observed errors for Eqs. 1 and 2, respectively

✉ Engin Şahin
esahin@bayburt.edu.tr

¹ Department of Industrial Engineering, Faculty of Engineering, Ondokuz Mayıs University, 55139 Samsun, Turkey

² Department of Nutrition and Dietetics, Faculty of Health Sciences, Bayburt University, 69000 Bayburt, Turkey



1 Introduction

The creation of the chiral diarylmethanol derivatives has drawn much attention to building blocks in synthesizing medicines, agrochemicals, and other chiral ligands [1, 2]. Moreover, diarylmethanols are significant ingredients and precursors of physiologically active chemicals because of the vital biological activity of their different derivatives. For instance, the diarylmethanol derivative (*S*)-phenyl(pyridin-2-yl) methanol possesses analgesic and anticonvulsant properties [3]. Neobenodine, orphenadrine, and carbinoxamine have potent antihistaminic effects, as shown in Fig. 1 [4–7].

In high optical purity, many techniques have been established for building the chiral diarylmethanol core moiety [8, 9]. One of the most effective ways to reduce pro-chiral diaryl ketones is chemical reduction, and different chemical catalysts have been used to achieve extremely enantioselective (> 95% ee) reduction [5, 10, 11]. However, the direct asymmetric hydrogenation of 2-benzoylpyridine is challenging owing to the coordination effect of pyridines [12]. On the other hand, green biocatalytic approaches have become an alternative to chemical techniques in order to produce enantiomerically pure pyridyl phenylmethanols. Also, a promising method is to use biocatalysts, considering the asymmetric reduction of pro-chiral ketones. Some diaryl ketones were reduced with remarkable enantioselectivity by many biological catalysts [13]. However, a limited number of studies in the literature involve the biocatalytic asymmetric reduction of 2-benzoylpyridine [14]. It is also noted that a number of biocatalysts have been applied to the asymmetric reduction of various heterocyclic ketones involving AS2.2241, *Candida maris*, *Candida viswanathii*, *Daucus carota*, *Geotrichum candidum*, *Pseudomonas putida* UV4, *Rhodococcus ruber* DSM 44541 and 4333, *Rhodotorula* sp., and *Rhizopus arrhizus* [15–22]. Using the *Lactobacillus paracasei* BD101 biocatalyst, (*S*)-**2** was reported for the first time in the literature to be obtained in gram scale (5.87 g) with 93% yield as an enantiopure form [23].

The recent studies of obtaining bioreduction conditions are summarized as follows. Particularly, Kavi et al. [24] developed a Box–Behnken design-focused model to find optimum conditions of enantiopure (*S*)-1-(4-Methoxyphenyl) ethanol with whole cells of *Lactobacillus senmaizuke*. Further, Aksuoğlu et al. [25] proposed an *I*-optimal design-embedded model to optimize asymmetric bioreduction optimum conditions of 2-methyl-1-phenylpropan-1-one by *Lactobacillus fermentum* BY35. Next, Danouche et al. [26] optimized sulfate leaching from Phosphogypsum to find efficient bioreduction. Then, Che et al. [27] used a structure-guided rational design for a ketoreductase from *Candida glabrata* to acquire the asymmetric reduction of the ketone precursor 2-chloro-1-(3, 4-difluorophenyl) ethanone. Finally, Arnodo et al. [28] reported

the enantioselective reduction of 2-substituted cyclic imines associated with amines by imine reductases in non-traditional solvents.

In the literature, the phenyl(pyridin-2-yl)methanone (**1**) asymmetric reduction was investigated while conducting the classical techniques. As observed from the previous studies, the reaction conditions were obtained where one of them was constant, and other reaction conditions were accordingly found. This technique is not desirable for a couple of reasons. First, this technique did not observe the interaction, quadratic, and higher-order effects of the reaction conditions. Second, obtaining the reaction conditions takes lots of computation time. Third, this method is expensive because many experimental runs may be necessary to acquire the optimum reaction conditions. Based on these awarenesses, a mathematical optimization technique eliminates the disadvantages of the classical approach. The superiority of the mathematical optimization technique is to optimize the reaction conditions simultaneously. In addition, the interaction, quadratic, and higher-order effects are analyzed. Next, the number of experimental runs is less, so the reaction conditions are obtained cheaper and more quickly. Notice that a large amount of substrate is converted into a product based on the usability of the method and biocatalyst, for example, in pharmaceutical industries. The classical method used in the literature may improve the biocatalytic asymmetric reduction of **1** based on yield, substrate amount, and an efficient technique. It is also investigated that a mathematical optimization method has not been conducted to acquire optimum reaction conditions for the biocatalytic reduction of substrate **1**.

As observed from the literature, no multi-response nonlinear optimization model is presented to obtain the phenyl(pyridin-2-yl)methanone (**1**) asymmetric reduction conditions. This paper bridges the research gap between an inscribed design and a nonlinear optimization model for obtaining bioreduction conditions. This paper has three main contributions: First, we selected a biocatalyst, *Leuconostoc pseudomesenteroides* N13, from Turkish sourdough while catalyzing the enantioselective reduction of **1**. Second, the full second-order approximation function was presented to obtain unbiased estimates with restricted maximum likelihood (REML). The estimated responses of ee and conversion were found using the REML analysis. Third, we proposed a novel inscribed design-focused multi-response nonlinear optimization model to obtain optimum bioreduction conditions of (*S*)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13. In addition, a gradient-based solution technique was applied to solve the proposed nonlinear optimization model. We also tested the usability of the novel inscribed design-focused multi-response nonlinear optimization model, which has not been used in any biocatalytic reactions in the literature for the optimum reaction conditions, as shown in Fig. 2.

Fig. 1 Some examples of important aryl heteroaryl chiral compounds

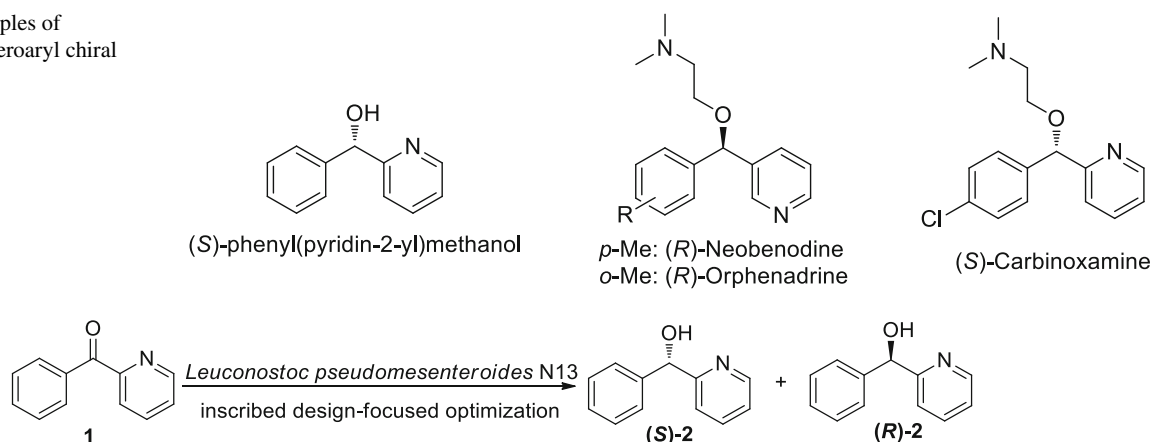


Fig. 2 Phenyl(pyridin-2-yl)methanone asymmetric reduction using the novel inscribed design-focused multi-response nonlinear optimization model in the presence of *Leuconostoc pseudomesenteroides* N13 biocatalyst

The novel inscribed design-focused multi-response nonlinear optimization model has been successfully used in a biocatalytic asymmetric reduction, and the reaction conditions have been optimized. Under optimized analgesic reaction conditions (**S**)-**2**, the conversion, yield, and high gram scale were obtained as 99%, 98%, and 11.79 g, respectively. Along the same lines, this research presents a practical and novel procedure in order to produce (**S**)-**2** with a biocatalyst. In addition, a novel inscribed design-focused multi-response nonlinear optimization model was proposed for the first time in the biocatalytic reduction of substrate **1**. (**S**)-**2** was also obtained using the selected biocatalyst for the first time at the most significant gram scale.

The organization of the remainder of this paper is presented as follows: The material and proposed methodology are explained in Sect. 2, including general information, the selected biocatalyst, general and gram-scale procedures, and the proposed optimization methodology. Results and discussions are elaborated on in Sect. 3. To sum up, Sect. 4 is related to the concluding remarks.

2 Material and Proposed Methodology

This section describes the material and proposed methodology and consists of five subsections: (1) General information and material, (2) Biocatalyst (*Leuconostoc pseudomesenteroides* N13), (3) A general procedure for the production of (**S**)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13, (4) Gram-scale procedure production of (**S**)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13, and (5) A novel inscribed design-focused multi-response nonlinear optimization model for the asymmetric reduction.

2.1 General Information and Material

The materials, such as substrate, solvents, and culture medium (MRS), were purchased by commercial providers. TLC (thin layer chromatography) was employed to track the progress using an ethyl acetate: hexane (05:95) solvent mixture. When using this mixture, (**S**)-**2** was purified using column chromatography. An Agilent 1260 HPLC system was applied to conduct chiral HPLC analysis involving a chiral detector and column. Then, polarimetry (Bellingham + Stanley ADP 220) was used to determine the specific optical rotation of the product. The produced racemic alcohol sample **2** was obtained while reducing **1** with NaBH₄ in MeOH. A Bruker (400 MHz) was used in order to record the spectra of the ¹³C-NMR and ¹H-NMR with the solvent CDCl₃. Next, (**S**)-phenyl (pyridin-2-yl)methanol [29]: M. p.: 72–73 °C, White solid, Yield: 98%, ¹H NMR with 400 MHz and CDCl₃, δ = 8.56–8.54 (m, 1H), 7.63–7.59 (m, 1H), 7.40–7.27 (m, 5H), 7.20–7.15 (m, 1H), 5.76 (s, 1H), 5.38 (bs, 1H (OH)); ¹³C NMR with 100 MHz and CDCl₃, δ = 160.9, 147.8, 143.2, 136.8, 128.6, 127.8, 127.1, 122.4, 121.3, 75.0; [α]_D²⁵ = 129.3 for *c* 1.684 and CHCl₃ > 99% ee; Chiralcel AD column, HPLC, hexane/*i*-PrOH, 90:10, 215 nm, flow rate = 0.8 mL/min, *t*_R (**S**) = 15.8 min. As provided in the supporting information, the HPLC condition of **1** is denoted as follows: Chiralcel AD column, 90:10, the flow rate of 0.8 mL/min, *n*-hexane/*i*-PrOH, 215 nm, and 9.3 min.

2.2 Biocatalyst (*Leuconostoc pseudomesenteroides* N13)

Leuconostoc pseudomesenteroides N13 was previously identified and preserved in the lab from the Turkish sourdough. As previously described in the literature, the biocatalyst was isolated, dried, and stored [30].



2.3 A General Procedure for the Production of (S)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13

Dry *Leuconostoc pseudomesenteroides* N13 (25 mg) was added to Erlen Mayer (250 mL) involving MRS broth (100 mL), which was freshly prepared, and the mixture was agitated at 153 rpm for two hours at 29 °C. The reaction medium's pH was 1 M HCl to 6.0. And then, the mixture was stirred for an extra two hours. After, the mixture was added to 1 mmol of **1** and went for 53 h at 29 °C and 153 rpm. Then, the mixture was centrifuged, and the liquid part was separated. The liquid phase was extracted in order to determine the conversion rate, and then, the organic layer was dried and dissolved with the isopropanol. After a small amount of crude product was filtered through a small silica column, the sample was used to detect the conversion rate and the ee values based on the chiral HPLC analysis. Next, the crude product was purified in column chromatography with ethyl acetate: hexane solvent mixture (05:95). The NMR analysis was performed to characterize the product. Comparing the optical rotation values to the literature value allowed us to ascertain their absolute configuration. The reaction transformation was examined by comparing the ketone peak with the alcohol peaks. The product could not be obtained in the trials without the use of a biocatalyst. Notice that the relevant spectra can be seen in the supporting information.

2.4 Gram-Scale Procedure Production of (S)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13

Dry *Leuconostoc pseudomesenteroides* N13 (250 mg) was added in 5-L Erlen Mayer containing 1000 mL MRS broth, which was freshly prepared, and the mixture was agitated (153 rpm) for two hours at 29 °C. Then, the reaction medium's pH was 1 M HCl to 6.0, and the mixture was stirred for an additional two hours. After that, the mixture was added to 65 mmol of **1** and stirred for 53 h at 29 °C and 153 rpm. As a result of the reaction, conversion, ee, and purification of the product were carried out as stated above. Characterization of the product, conversion of the substrate, and ee of the product were carried out as described above.

2.5 A Novel Inscribed Design-Focused Multi-response Nonlinear Optimization Model for the Asymmetric Reduction

The details of each step are explained as follows. The first step is planning, and the experimenters decide the design-related issues, including which variables are hard or easy to change. The levels of one or more design variables may be hard to change for an experiment. Thus, the design should be split

into a whole plot and subplot. Further, the response surface design is appropriate for building a second-order approximation response [31–33]. The second-order response function has three main advantages as follows [31]: (1) flexibility, (2) easy estimations, and (3) practicality for actual experiments. Therefore, a novel inscribed design is performed when combining the split plot and response surface approaches. In addition, the presented design is rotatable in this paper to provide prediction variance without changing the experimental design space. Also, the inscribed design provided the process stability as another property. Thus, these properties are important in choosing the inscribed design in this paper over the other experimental methods.

The design variables and two responses are denoted in Table 1 for the experiment. The design variables and their coded and uncoded levels were specified based on the experimenters' knowledge, experience, and expectations. Also, the specified coded and uncoded levels are consistent with the previous studies. Note that adjusting pH may be complicated for this particular experiment. Therefore, the pH is defined as the hard-to-change design variable. An inscribed design consists of factorial, center, and axial points. Due to the nature of the inscribed design, eight factorial points were used in each group to observe the main and interaction effects. Next, three center points were enough to run for each center point group when providing process stability. In addition, the total number of axial points was specified as ten in Table 2, and they were sufficient to allow curvature estimation. Therefore, as shown in Table 2, thirty-five experimental runs were conducted, including three groups of center points, two groups of factorial points, two groups of the hard-to-change design variable of axial points, and one group of the three easy-to-change design variables of axial points. Each experimental run was randomly performed in order to eliminate the experimental prejudices.

The finding of the estimated response functions is another important step in analyzing significant model parameters. For this particular purpose, the REML analysis was conducted for each response. Also, unbiased estimates are provided by using the REML. In this paper, the full second-order approximation function is used to estimate the model terms for the response. Therefore, the estimated fitted response functions, $\hat{\mu}_1$ and $\hat{\mu}_2$, are denoted for the ee and conversion, respectively, as follows:

$$\begin{aligned}\hat{\mu}_1 = & \hat{\beta}_0 + \hat{\beta}_1 E + \hat{\beta}_2 F + \hat{\beta}_3 G + \hat{\beta}_4 H \\ & + \hat{\beta}_{12} EF + \hat{\beta}_{13} EG + \hat{\beta}_{14} EH + \hat{\beta}_{23} FG \\ & + \hat{\beta}_{24} FH + \hat{\beta}_{34} GH + \hat{\beta}_{11} E^2 \\ & + \hat{\beta}_{22} F^2 + \hat{\beta}_{33} G^2 + \hat{\beta}_{44} H^2 + \varepsilon_1 \\ \hat{\mu}_2 = & \hat{\delta}_0 + \hat{\delta}_1 E + \hat{\delta}_2 F + \hat{\delta}_3 G + \hat{\delta}_4 H + \hat{\delta}_{12} EF \\ & + \hat{\delta}_{13} EG + \hat{\delta}_{14} EH + \hat{\delta}_{23} FG\end{aligned}\quad (1)$$



Table 1 The four design variables and two responses

Notation	Name of the design variable	Units	Type	Coded/uncoded low for factorial points	Coded/uncoded high for factorial points
<i>E</i>	pH		Numeric	− 1/4.5	+ 1/5.50
<i>F</i>	Temperature	°C	Numeric	− 1/25.00	+ 1/35.00
<i>G</i>	Incubation period	hour	Numeric	− 1/36.00	+ 1/60.00
<i>H</i>	Agitation speed	rpm	Numeric	− 1/125.00	+ 1/175.00

Notation	Name of the response	Units	Number of runs	Minimum	Maximum
<i>y</i> ₁	Enantiomeric excess (ee)	%	35	60	98
<i>y</i> ₂	Conversion	%	35	55	98

$$\begin{aligned}
& + \hat{\delta}_{24}FH + \hat{\delta}_{34}GH + \hat{\delta}_{11}E^2 \\
& + \hat{\delta}_{22}F^2 + \hat{\delta}_{33}G^2 + \hat{\delta}_{44}H^2 + \varepsilon_2
\end{aligned} \quad (2)$$

As indicated in (1) and (2), two response functions are obtained from the inscribed design. The objective of the novel inscribed multi-response nonlinear optimization model is to maximize the two response functions; therefore, objective functions are defined in terms of the desirability of each response. In addition, the design space limitations of the four design variables should be incorporated in order to prevent meaningless results. Further, each desirability value should be defined between zero and one. The highest value of desirability is wanted for each response. The novel inscribed design-focused multi-response nonlinear optimization model is denoted in order to obtain optimum bioreduction conditions of (*S*)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13 as follows:

$$\begin{aligned}
& \text{maximize } \{d(\hat{\mu}_1), d(\hat{\mu}_2)\} \\
& \text{subject to } -1 \leq E, F, G, H \leq +1 \\
& \text{where} \\
& d(\hat{\mu}_1) = \begin{cases} 0, & \hat{\mu}_1 < \text{lower}_{ee} \\ \left(\frac{\hat{\mu}_1 - \text{lower}_{ee}}{\text{target}_{ee} - \text{lower}_{ee}} \right), & \text{lower}_{ee} \leq \hat{\mu}_1 \leq \text{target}_{ee} \\ 1, & \hat{\mu}_1 > \text{target}_{ee} \end{cases} \\
& d(\hat{\mu}_2) = \begin{cases} 0, & \hat{\mu}_2 < \text{lower}_c \\ \left(\frac{\hat{\mu}_2 - \text{lower}_c}{\text{target}_c - \text{lower}_c} \right), & \text{lower}_c \leq \hat{\mu}_2 \leq \text{target}_c \\ 1, & \hat{\mu}_2 > \text{target}_c \end{cases} \\
& \text{Solution technique: A gradient - based procedure} \\
& \text{Find: Optimum bioreduction conditions}
\end{aligned} \quad (3)$$

Design Expert 12.0.3.0 (Minneapolis, MN 55143) was applied to statistical analysis and plotting the graphs. Next, a gradient-based solution procedure was used to solve the novel inscribed design-focused multi-response nonlinear optimization model in (3). For this particular purpose, MATLAB R2014a (Natick, Massachusetts 01760) with an optimization

toolbox was run in order to solve the proposed optimization model.

3 Results and Discussion

This section consists of three subsections as follows: (1) the results and discussion of the statistical analysis, (2) the results and discussion of the novel inscribed design-focused multi-response nonlinear optimization model, and (3) Gram-scale production of (*S*)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13.

3.1 The Results and Discussion of the Statistical Analysis

The statistical analysis was conducted for the ee. R^2 (the maximum coefficient of determination) and R^2_{adj} (the adjusted coefficient of determination) values are 0.9837 and 0.9693, respectively. Notice that R^2 and R^2_{adj} values are 0.9385 and 0.9254, respectively, when dealing with the linear regression model. Based on these values, the quadratic (second-order) regression model is better than the linear regression model. Also, a good model fit is provided associated with the R^2 and R^2_{adj} values of the quadratic regression model for the ee response. The REML (restricted maximum likelihood) analysis and variance components are depicted in Table 3. Based on the whole plot and subplot, the model is significant. In this model, *E* (pH), *F* (temperature), *H* (agitation speed), and F^2 (temperature) are significant model terms based on the Kenward–Roger *p*-values, where *p*-values are less than 0.05. Next, the group variance component accounts for 90.35% of the total variability, as shown in Table 3.

The full second-order approximation response function of the ee is obtained as follows:

$$\begin{aligned}
\hat{\mu}_1 = & 84.00 + 7.73E - 1.46F - 0.13G + 0.71H \\
& - 0.31EF + 0.81EG - 0.06EH
\end{aligned}$$



Table 2 The thirty-five runs inscribed design for the experiment

SR	Group	RR	Design variables				Responses	
			<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>y</i> ₁	<i>y</i> ₂
30	1	1	5.00	30	48	150	84	87
32	1	2	5.00	30	48	150	84	87
31	1	3	5.00	30	48	150	84	87
34	2	4	5.00	30	48	150	84	87
35	2	5	5.00	30	48	150	84	87
33	2	6	5.00	30	48	150	84	87
24	3	7	5.00	20	48	150	81	77
27	3	8	5.00	30	72	150	85	94
28	3	9	5.00	30	48	100	82	71
25	3	10	5.00	40	48	150	70	59
26	3	11	5.00	30	24	150	83	71
29	3	12	5.00	30	48	200	85	92
13	4	13	5.50	25	36	175	83	88
15	4	14	5.50	25	60	175	84	90
14	4	15	5.50	35	36	175	81	80
12	4	16	5.50	35	60	125	80	73
16	4	17	5.50	35	60	175	81	84
9	4	18	5.50	25	36	125	81	73
11	4	19	5.50	25	60	125	83	79
10	4	20	5.50	35	36	125	80	70
18	5	21	5.00	30	48	150	84	87
17	5	22	5.00	30	48	150	84	87
19	5	23	5.00	30	48	150	84	87
21	6	24	4.00	30	48	150	69	61
20	6	25	4.00	30	48	150	69	61
1	7	26	4.50	25	36	125	65	57
6	7	27	4.50	35	36	175	64	61
3	7	28	4.50	25	60	125	61	64
7	7	29	4.50	25	60	175	60	65
8	7	30	4.50	35	60	175	63	70
4	7	31	4.50	35	60	125	61	65
5	7	32	4.50	25	36	175	66	64
2	7	33	4.50	35	36	125	60	55
23	8	34	6.00	30	48	150	98	98
22	8	35	6.00	30	48	150	98	98

$$+ 0.44FG + 0.31FH - 0.31GH \\ - 0.41E^2 - 3.77F^2 - 1.64G^2 - 1.77H^2 \quad (4)$$

The conversion response was statistically analyzed. Particularly, R^2 and R^2_{adj} values are 0.9655 and 0.9347, respectively. Note that R^2 and R^2_{adj} values are 0.8655 and 0.8366, respectively, when the regression model is linear. In terms of R^2 and R^2_{adj} values, the quadratic (second-order) regression

model is superior to the linear regression model. In addition, a good model fit is obtained based on the R^2 and R^2_{adj} values of the quadratic regression model for the conversion response. In Table 4, the REML analysis and variance components are denoted. The model is significant based on the REML analysis, including the whole part and subplot. In the model, *E* (pH), *F* (temperature), *G* (incubation period), *H* (agitation speed), *EH* (Ph* agitation speed), E^2 (Ph²), F^2 (temperature²), G^2 (incubation period²), and H^2 (agitation



Table 3 REML analysis and variance components for the ee response

REML analysis for the ee response					
Source	Term df	Error df	<i>F</i> -value	<i>p</i> value	
Whole plot	2	4.220	12.550	0.0167	*
<i>E</i>	1	4.220	24.970	0.0065	*
<i>E</i> ²	1	4.220	0.136	0.7300	**
Subplot	12	13.890	6.230	0.0009	*
<i>F</i>	1	16.080	20.970	0.0003	*
<i>G</i>	1	16.080	0.154	0.6998	**
<i>H</i>	1	16.080	4.950	0.0408	*
<i>EF</i>	1	16.080	0.642	0.4347	**
<i>EG</i>	1	16.080	4.340	0.0536	**
<i>EH</i>	1	16.080	0.026	0.8747	**
<i>FG</i>	1	16.080	1.260	0.2785	**
<i>FH</i>	1	16.080	0.642	0.4347	**
<i>GH</i>	1	16.080	0.642	0.4347	**
<i>F</i> ²	1	4.400	11.340	0.0242	*
<i>G</i> ²	1	4.400	2.150	0.2098	**
<i>H</i> ²	1	4.400	2.490	0.1829	**
Variance components for the ee response					
	Variance	Standard error	95% CI low	95% CI high	
Group	22.87	16.45	− 9.37	55.12	
Residual	2.43	0.8585	1.35	5.62	
Total	25.31				

*Denotes the significant source

**Denotes the nonsignificant source

speed²) are significant model terms associated with the Kenward–Roger *p*-values, where the *p*-values are specified as less than 0.05. Then, the group variance component explains 55.48% of the total variability, as depicted in Table 3.

The full second-order approximation response function of the conversion is obtained as follows:

$$\begin{aligned}\hat{\mu}_2 = & 87.00 + 9.07E - 2.42F + 3.67G + 4.50H - 1.50EF \\ & - 0.75EG + 1.75EH + 0.63FG - 0.13FH \\ & - 0.63GH - 2.11E^2 - 5.91F^2 - 2.29G^2 - 2.54H^2\end{aligned}\quad (5)$$

3.2 The Results and Discussion of the Novel Inscribed Design-Focused Multi-response Nonlinear Optimization Model

The novel inscribed multi-response nonlinear optimization model was effectively applied in finding the optimum bioreduction conditions of (*S*)-phenyl (pyridin-2-yl)methanol using *Leuconostoc pseudomesenteroides* N13. Based on the

optimization results, each desirability function was obtained one for the ee and conversion. It was reported that the most desirable responses were acquired for the experiment. The bioreduction conditions were acquired as follows: *E* (pH) = 6.0, *F* (temperature) = 29 °C, *G* (incubation period) = 53 h, and *H* (agitation speed) = 153 rpm. In addition, the estimated values of the ee and conversion were calculated as 98.4% and 99%, respectively.

The validation of the results is necessary to verify bioreduction conditions from the proposed multi-response nonlinear optimization model. Thus, an experiment called validation was performed using the optimum bioreduction conditions of the four design variables from the novel inscribed multi-response nonlinear optimization model. The results of the validation experiment showed that the ee and conversion were investigated as > 99% and > %99, respectively. It is examined that the validation experiment results are consistent with the novel inscribed multi-response nonlinear optimization model results when analyzing the ee, conversion, and yield. It is also concluded that the novel inscribed multi-response nonlinear optimization model is



Table 4 REML analysis and variance components for the conversion response

REML analysis for the conversion					
Source	Term df	Error df	F-value	p value	
Whole plot	2	5.44	33.64	0.0009	*
<i>E</i>	1	5.34	60.82	0.0004	*
<i>E</i> ²	1	5.53	6.46	0.0472	*
Subplot	12	14.3	15.19	< 0.0001	*
<i>F</i>	1	16.46	17.37	0.0007	*
<i>G</i>	1	16.46	39.97	< 0.0001	*
<i>H</i>	1	16.46	60.21	< 0.0001	*
<i>EF</i>	1	16.46	4.46	0.0503	**
<i>EG</i>	1	16.46	1.12	0.3062	**
<i>EH</i>	1	16.46	6.07	0.0251	*
<i>FG</i>	1	16.46	0.7743	0.3916	**
<i>FH</i>	1	16.46	0.031	0.8624	**
<i>GH</i>	1	16.46	0.7743	0.3916	**
<i>F</i> ²	1	6.87	44.96	0.0003	*
<i>G</i> ²	1	6.87	6.74	0.0362	*
<i>H</i> ²	1	6.87	8.29	0.0242	*
Variance components for the conversion					
	Variance	Standard error	95% CI low	95% CI high	
Group	10.07	8.05	– 5.71	25.86	
Residual	8.07	2.81	4.51	18.43	
Total	18.15				

*Denotes the significant source

**Denotes the nonsignificant source

valuable and practical for the asymmetric bioreduction of (*S*)-phenyl (pyridin-2-yl)methanol using *Leuconostoc pseudomesenteroides* N13.

Figures 3, 4, 5 show the contour plots of the desirability, ee, and conversion while dealing with the paired design variables. As depicted in Fig. 1, a high pH level is desirable in order to acquire the highest desirability. On the other hand, a low-temperature level is desirable for asymmetric reduction. Further, the incubation period is around 53 h in order to obtain the most desirable for the experiment. Also, the agitation speed is desirable around the medium level, as observed in Fig. 1. It is investigated that the maximized ee and conversion values are obtained at the high pH level, a low-temperature level, an upper middle-temperature level, and around a medium agitation speed level, as well in Figs. 2 and 3. Therefore, desirability is efficiently acquired in order to maximize the estimated values of the ee and conversion. Notice that observations from Figs. 3, 4, 5 are in agreement with the novel inscribed multi-response nonlinear optimization results.

3.3 Gram-Scale Production of (*S*)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13

The industrial applicability of the method and biocatalyst used in biocatalytic asymmetric reduction studies is significant. Therefore, to demonstrate the industrial applicability of the technique and the biocatalyst, a large amount of the substrate must be converted into the product under optimized conditions. Note that substrate concentration affects selectivity, conversion, and ee [15]. Therefore, various experiments were carried out using various amounts of the substrate under optimized bioreduction conditions in order to convert the maximum amount of substrate into the product. When using 65 mmol (11.9 g) of the substrate under optimized conditions, (*S*)-2 was obtained as 11.79 g with > 99% conversion and 98% yield as enantiopure form, as depicted in Fig. 6.

When the amount of substrate was more than 65 mmol, both the selectivity and conversion decreased. For example, when the amount of substrate was 75 mmol, the reaction conversion was 81%, and the ee was 89% for the product.

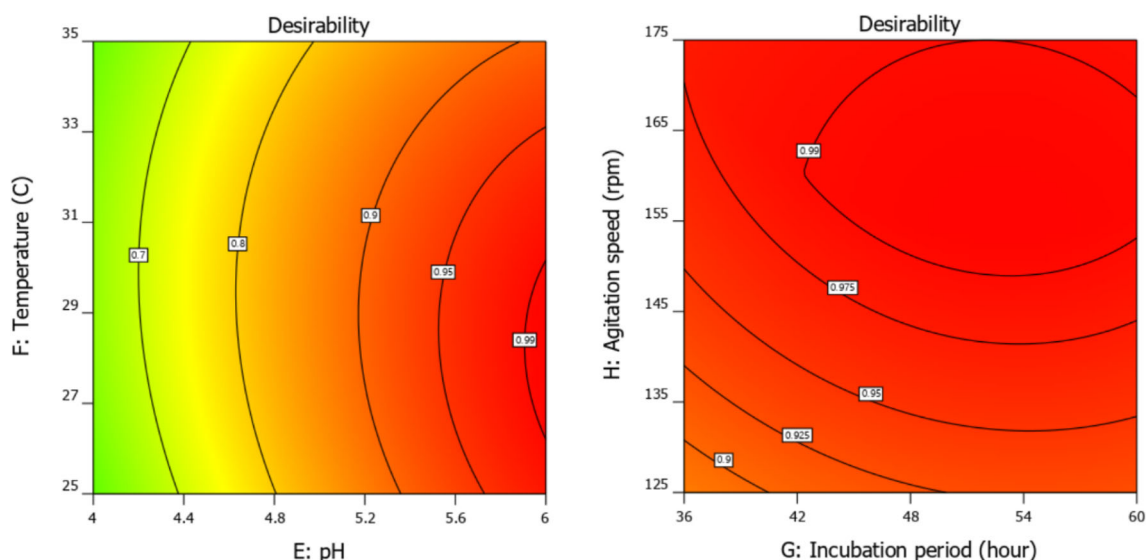


Fig. 3 Contour plots of the desirability

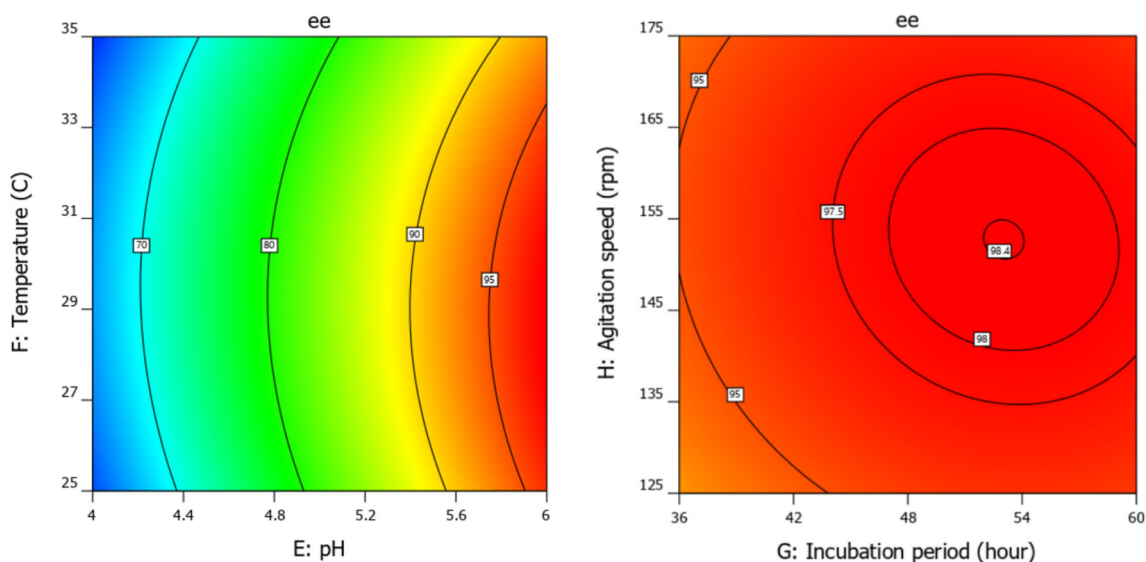


Fig. 4 Contour plots of the ee response

The reason is the substrate toxicity, and biocatalyst decreased activity, as presented in the literature [30].

4 Conclusion

In this study, the enantioselective reduction of aryl heteroaryl ketone **1** catalyzed by *Leuconostoc pseudomesenteroides* N13 was performed effectively with a novel inscribed design-focused multi-response nonlinear optimization model to give the corresponding enantiomerically pure alcohol (*S*)-**2**. Note that the proposed multi-response nonlinear optimization model has not been used in the literature to obtain optimum

reaction conditions when dealing with the REML analysis for estimating the ee and conversion responses. Also, it has been shown that the novel inscribed design-focused multi-response nonlinear optimization model can be successfully applied in biocatalytic reactions. After the optimization phase, the bioreduction conditions of (*S*)-phenyl (pyridin-2-yl)methanol by *Leuconostoc pseudomesenteroides* N13 were found as: pH = 6.0, temperature = 29 °C, incubation period = 53 h, and agitation speed = 153 rpm. After the validation study, the ee and conversion were obtained as > 99% and > %99, respectively. Further, (*S*)-**2** was obtained as 11.79 g with > 99% conversion and 98% yield as enantiopure form when 65 mmol (11.9 g) of the substrate was used under optimum bioreduction conditions. In addition, this research indicates



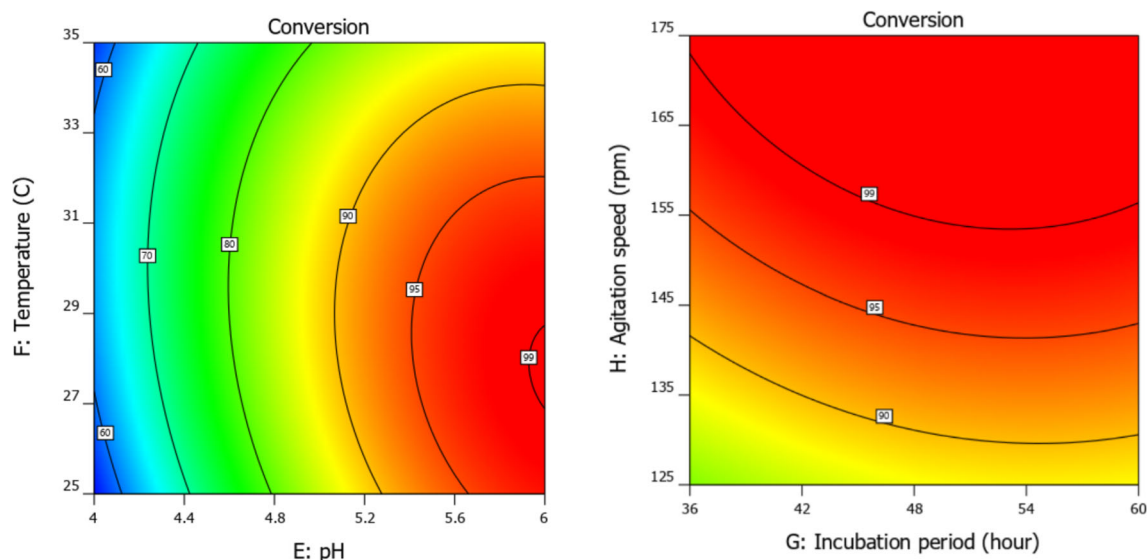
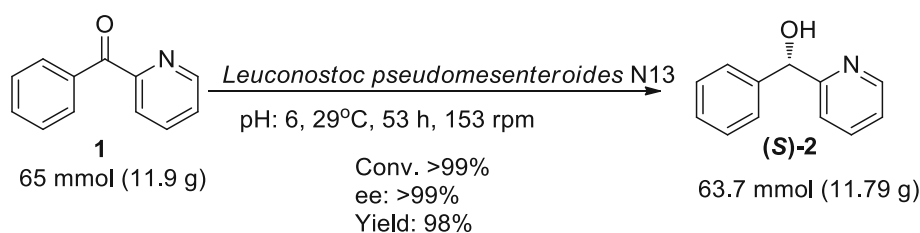


Fig. 5 Contour plots of the conversion

Fig. 6 Gram-scale production of (S)-2



that *Leuconostoc pseudomesenteroides* N13 is an effective aryl heteraryl ketone reduction biocatalyst. It is still a difficult task in organic synthesis to reduce diaryl ketones in a high ee, but this biocatalyst and the novel inscribed design-focused multi-response nonlinear optimization model can hold great promise in this regard. Next, a green and efficient biocatalytic strategy was presented in order to produce a pharmaceutically important analgesic, enantiopure (S)-2, which was firstly developed by the inscribed design-focused multi-response nonlinear optimization model with the excellent conversion, ee, yield, and high gram scale. Finally, this procedure has economic potential for the practical synthesis of pharmaceutically effective compounds since it efficiently produces (S)-2.

The limitation of this study is that only the controllable design variables are considered. Besides, the experimental procedure in this paper did not consider noise design variables. On the other hand, both controllable and noise design variables could be considered for asymmetric bioreductions. Another possible future work is developing a nonlinear optimization model when considering both controllable and noise design variables for asymmetric bioreductions. Further, the biocatalyst in this paper could be carried out with different biocatalysts for future research.

Supplementary Data

¹H-NMR and ¹³C-NMR spectrums and chiral and racemic HPLC chromatograms of the product (S)-2 could be found in the supplementary data.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13369-024-08773-5>.

Acknowledgements The authors of this paper would like to express their gratitude to the Central Research Laboratory of Bayburt University for the HPLC analysis and Prof. Enes Dertli from Yıldız Technical University for providing the biocatalyst.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Özdemir, A.; Şahin, E.: Modified distance optimization method for the asymmetric bioreduction conditions of phenyl (thiophen-2-yl) methanone by *Weissella paramesenteroides* N7. *Tetrahedron* **139**, 133431 (2023)
- Baydaş, Y.; Kalay, E.; Şahin, E.: Production of enantiomerically enriched chiral carbinols using whole-cell biocatalyst. *Biocatal. Biotransformation* **40**, 29–37 (2022)
- Takemoto, M.; Yamamoto, Y.; Achiwa, K.: Effect of inhibitor or immobilization on reduction of benzoylpyridines by baker's yeast. *Chem. Pharm. Bull.* **44**(4), 853–855 (1996)
- Vyas, V.K.; Clarkson, G.J.; Wills, M.: Enantioselective synthesis of bicyclopentane-containing alcohols via asymmetric transfer hydrogenation. *Org. Lett.* **23**(8), 3179–3183 (2021)
- Qian, L.; Tang, X.; Wang, Y.; Liu, G.; Huang, Z.: Asymmetric transfer hydrogenation of diaryl ketones with ethanol catalyzed by chiral ncp pincer iridium complexes. *Chin. J. Chem.* **40**(10), 1131–1136 (2022)
- Patel, R.; Singh, B.; Sharma, A.; Saraswat, J.; Dohare, N.; Ud din Parry, M.; Khan, A.A.: Interaction and esterase activity of albumin serums with orphenadrine: a spectroscopic and computational approach. *J. Mol. Struct.* **1239**, 130522 (2021)
- Soliman, R.M.; Rostom, Y.; Mahmoud, A.M.; Fayez, Y.M.; Mostafa, N.M.; Monir, H.H.: Novel fabricated potentiometric sensors for selective determination of carbinoxamine with different greenness evaluation perspectives. *Microchem. J.* **187**, 108381 (2023)
- Zhou, J.; Xu, G.; Ni, Y.: Stereochemistry in asymmetric reduction of bulky–bulky ketones by alcohol dehydrogenases. *ACS Catal.* **10**(19), 10954–10966 (2020)
- Wu, K.; Yan, J.; Wang, X.; Yin, X.; Shi, G.; Yang, L.; Shao, L.: Efficient synthesis of bepotastine and cloperastine intermediates using engineered alcohol dehydrogenase with a hydrophobic pocket. *Appl. Microbiol. Biotechnol.* **105**(14–15), 5873–5882 (2021)
- Asai, K.; Miura, M.; Hirano, K.: Palladium-catalyzed cross-coupling reaction of diarylmethanol derivatives with diboryl-methane. *J. Org. Chem.* **87**(11), 7436–7445 (2022)
- Liu, Z.Q.; Tao, J.; Zhuang, X.; Hong, C.M.; Luo, Z.; Wu, Y.F.; Liu, T.L.: Rhodium (III)-catalyzed aryl borrowing amination of diaryl methanols containing pyridine-directing groups. *Adv. Synth. Catal.* **363**(23), 5279–5283 (2021)
- Xu, J.; Zhou, S.; Zhao, Y.; Xia, J.; Liu, X.; Xu, J.; Zhang, J.: Asymmetric whole-cell bioreduction of sterically bulky 2-benzoylpyridine derivatives in aqueous hydrophilic ionic liquid media. *Chem. Eng. J.* **316**, 919–927 (2017)
- Truppo, M.D.; Pollard, D.; Devine, P.: Enzyme-catalyzed enantioselective diaryl ketone reductions. *Org. Lett.* **9**, 335–338 (2007)
- Takemoto, M.; Achiwa, K.: Synthesis of optically active aphenylpyridylmethanols with baker's yeast. *Chem. Pharm. Bull.* **42**, 802–805 (1994)
- Salvi, N.A.; Chattopadhyay, S.: Laboratory scale-up synthesis of chiral carbinols using *Rhizopus arrhizus*. *Tetrahedron Asymmetry* **27**, 188–192 (2016)
- Kawano, S.; Horikawa, M.; Yasohara, Y.; Hasegawa, J.: Microbial enantioselective reduction of acetylpyridine derivatives. *Biosci. Biotechnol. Biochem.* **67**, 809–814 (2003)
- Garrett, M.D.; Scott, R.; Sheldrake, G.N.: Enantiocomplementary preparation of (S)- and (R)-1-pyridylalkanols via ketone reduction and alkane hydroxylation using whole cells of *Pseudomonas putida* UV4. *Tetrahedron Asymmetry* **13**, 2201–2204 (2002)
- Yang, W.; Xu, J.H.; Xie, Y.; Xu, Y.; Zhao, G.; Lin, G.Q.: Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2. 2241 and synthesis of the b-blocker (R)-nifenalol. *Tetrahedron Asymmetry* **17**, 1769–1774 (2006)
- Nakamura, K.; Fujii, M.; Ida, Y.: Asymmetric reduction of ketones by *Geotrichum candidum* in the presence of amberlite™ XAD, a solid organic solvent. *J. Chem. Soc. Perkin. Trans.* **1**, 3205–3211 (2000)
- Soni, P.; Kaur, G.; Chakraborti, A.K.; Banerjee, U.C.: Candida viswanathii as a novel biocatalyst for stereoselective reduction of heteroaryl methyl ketones: a highly efficient enantioselective synthesis of (S)-a-(3-pyridyl)ethanol. *Tetrahedron Asymmetry* **16**, 2425–2428 (2005)
- Aldabalde, V.; Arcia, P.; Gonzalez, A.; Gonzalez, D.: Enzymatic synthesis of chiral heteroaryl alcohols using plant fragments as the only biocatalyst and reducing agent. *Green Chem. Lett. Rev.* **1**, 25–30 (2007)
- Stampfer, W.; Edegger, K.; Kosjek, B.; Faber, K.; Kroutil, W.: Simple biocatalytic access to enantiopure (S)-1-heteroarylethanol employing a microbial hydrogen transfer reaction. *Adv. Synth. Catal.* **346**, 57–62 (2004)
- Şahin, E.; Serencam, H.; Dertli, E.: Production of enantiomerically pure (S)-phenyl (pyridin-2-yl) methanol with *Lactobacillus paracasei* BD101. *Biocatal. Biotransform.* **37**(6), 448–454 (2019)
- Kavi, M.; Özdemir, A.; Dertli, E.; Şahin, E.: Optimization of biocatalytic production of enantiopure (S)-1-(4-methoxyphenyl) ethanol with *Lactobacillus senmaizuke* using the Box–Behnken design-based model. *Arab. J. Sci. Eng.* **47**(5), 5849–5858 (2022)
- Aksuoglu, S.; Özdemir, A.; Serencam, H.; Dertli, E.; Şahin, E.: Optimization of asymmetric bioreduction conditions of 2-methyl-1-phenylpropan-1-one by *Lactobacillus fermentum* BY35 Using *I*-optimal design-based model. *Arab. J. Sci. Eng.* **47**(5), 6505–6514 (2022)
- Danouche, M.; Bounaga, A.; Boulif, R.; Zeroual, Y.; Benhida, R.; Lyamlouli, K.: Optimization of sulfate leaching from phosphogypsum for efficient bioreduction in a batch bioreactor using a sulfate-reducing microbial consortium. *Chem. Eng. J.* **475**, 146072 (2023)
- Che, C.; Zhang, W.; Men, Y.; Li, H.; Qin, B.; Jia, X.; You, S.: Development of an enzymatic process for the synthesis of (1S)-2-chloro-1-(3, 4-difluorophenyl) ethanol, the key intermediate of ticagrelor. *Mol. Catal.* **537**, 112963 (2023)
- Arnodo, D.; De Nardi, F.; Parisotto, S.; De Nardo, E.; Canana, S.; Salvatico, F.; Prandi, C.: Asymmetric reduction of cyclic imines by imine reductase enzyme in non-conventional solvents. *ChemSusChem*. (2023). <https://doi.org/10.1002/cssc.202301243>
- Şahin, E.: Production of enantiopure chiral aryl heteroaryl carbinols using whole-cell *Lactobacillus paracasei* biotransformation. *Synth. Commun.* **50**(4), 549–557 (2020)
- Şahin, E.: Production of (R)-1-(1,3-benzodioxol-5-yl)ethanol in high enantiomeric purity by *Lactobacillus paracasei* BD101. *Chirality* **30**, 189–194 (2018)
- Myers, R.H.; Montgomery, D.C.; Anderson-Cook, C.M.: Response Surface Methodology: Process and Product Optimization Using Designed Experiments, p. 1–12. Wiley, Hoboken, NJ (2016)
- Özdemir, A.; Cho, B.R.: A nonlinear integer programming approach to solving the robust parameter design optimization problem. *Qual. Reliab. Eng. Int.* **32**, 2859–2870 (2016)
- Özdemir, A.: Development of a multiple response-based mixed-integer nonlinear optimization model with both controllable and uncontrollable design factors. *Comput. Ind. Eng.* **164**, 107901 (2022)

