Optimum of lipase-catalyzed hexyl laurate using substrate as solvent in a continuous packed-bed reactor

Running head: Optimization of continuous production on hexyl laurate by lipase

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Abstract

BACKGROUND: Hexyl laurate was applied in many cosmetic industries and synthesized by chemical methods with some difficulties in cost, environmental pollution, and by-products. In this study, Lipozyme® IM77 (from *Rhizomucor miehei*) to catalyze the direct-esterification of hexanol and lauric acid in a solvent-free system by utilizing a continuous packed-bed reactor, in which could overcome difficulties of the above. Response surface methodology (RSM) and 3-level-3-factor Box-Behnken design were employed to evaluate the effects of synthesis parameters, such as reaction temperature (45–65 °C), mixture flow rate (0.25–0.75 mL/min) and concentration of lauric acid (100–300 mM) on production rate (μmol/min) of hexyl laurate by direct-esterification.

RESULTS: The production rate was significantly affected to the mixture flow rate and lauric acid concentrate. Based on the analysis of ridge-max, the optimum synthesis conditions for hexyl laurate were as follows: 81.58 ± 1.76 μmol/min at 55 °C, 0.5 mL/min flow rate and 0.3 M lauric acid.

CONCLUSION: The optimization of lipase-catalyzed synthesis of hexyl laurate by Lipozyme® IM-77 in a continuous packed-bed reactor was successfully developed by Box-Behnken design and RSM.

Kewords: hexyl laurate; lipase-catalyzed; optimization; packed-bed reactor; solvent-free

Introduction

Hexyl esters with a "green note" flavor, derived from medium-chain carboxylic acids such as hexyl laurate, are used as important emollient materials in many cosmetic industrial applications.[1] In general, hexyl laurate was synthesized by chemical methods in industry with some difficulties in cost, environmental pollution, and by-products. The lipase-catalyzed esterification had some advantages to overcome difficulties above, such as reacted in mild conditions, reduced by-products formulation, specificity of substrates, biodegradable, and decreased cost.[2]

The lipase-catalyzed hexyl ester was successfully performed in *n*-hexane.[1, 3] The organic solvents with toxicity and flammability were major cause of disadvantageous and higher costs to disburse for safety requirements. The enzymatic processes in solvent-free systems are interested for the ester synthesis with the considerable simplification and reduced environmental hazard.[4]

Additional advantages of reactions in a solvent-free system are savings in reactor design in large-scale process and reduction of separation costs.[5, 6]

Enzymatic synthesis with a continuous bioreactor could either satisfy consumers' need for "natural quality" or lower production cost on industrial applications. In present, four types of bioreactor used in microorganism, zooblast, and plant cells, such as stirred-tank reactor, packed-bed reactor, membrane reactor, and fluidized-bed reactor.[7–10] Packed-bed enzyme reactors are the most frequently

* Superscripts should consistently follow punctuation marks. See section 5i of the stylesheet ("Instructions to Authors").

used reactors for immobilized lipases. They are best used continuously on a commercial scale so as to minimize labor and overhead costs.[11] Some works reported the lipase-catalyzed ester synthesis in continuous packed-bed bioreactor, such as butyl oleate[12], ethyl oleate[13], structured lipids[7] and erythrityl laurate.[14] Yang et al.[15] reported the optimization of lipase-catalyzed glycerolysis in a bioreactor by response surface methodology (RSM).

The present work focuses on the reaction parameters that affect lipase from *Rhizomucor miehei* (Lipozyme® IM77) catalyzed synthesis of hexyl laurate in a packed-bed reactor with a solvent-free system. The main objectives of this work were to better understand relationships between the reaction variables (reaction temperature, flow rate and concentration of lauric acid) and the response (production rate); and to obtain the optimum conditions for hexyl laurate synthesis using Box-Behnken design and RSM.

Materials and methods

Materials

Immobilized lipase from *Rhizomucor miehei*, Lipozyme® 77 (7.7 Batch Acidolysis Unit of Novo, 7.7 BAUN/g; water content, 5.1%) was supplied from NOVO Nordisk Bioindustrials (Bagsvaerd, Denmark); Lauric acid (99% pure), hexanol (98% pure) and tributyrin (99% pure) were purchased from

* The phrase within [ ] needs clarification via consultation, for the grammar does not sound correct. This revision sounds better but might not convey the precise meaning intended: in a 4 Å molecular sieve.

Sigma-Aldrich Co. (St Louis, MO, USA); Molecular sieve 4 Å, ~~was purchased~~ from Davison Chemical (Baltimore, MD, USA); Sodium hydroxide, ~~was purchased~~ from Katayama Chemical Co. (Japan).

Avoid repeating the same verb in parallel grammatical structures in closely related clauses.

Preparation of hexyl laurate

Lipozyme® IM-77 was employed as ~~a~~ the biocatalyst to perform ~~the~~ a direct esterification of hexanol by lauric acid. All materials were dehydrated ~~in~~ overnight [~~by molecular sieve 4 Å~~ in a 4 Å molecular sieve (?)]. Before the reaction, lauric acid and hexanol were ~~well-~~thoroughly mixed in a feeding flask. The esterification reaction was ~~carried out~~ implemented in a packed-bed type reactor consisting of a stainless steel tube 25 cm in length and ~~with a 0.25 cm~~ an of 0.25 cm inner diameter. The mixture was pumped through a continuous reactor (packed-bed column with 1.5 g Lipozyme® IM-77) ~~at~~ under the designed conditions. The ~~whole~~ entire system was placed in ~~the~~ a temperature-controlled chamber to prevent any possible ~~temperature~~ gradient.

Analytical methods

Analysis of hexyl laurate

The formation of hexyl laurate was ~~determined~~ achieved by injecting a 1 μL aliquot in a splitless mode into gas chromatograph (Hewlett Packard 6890, Avondale, PA, USA) equipped with a flame-ionization detector (FID) and ~~a~~ an MXT-65TG fused

silica capillary column (30 m × 0.25 mm i.d.; film thickness 1 μm; Restek, Bellefonte, PA, USA). Injector and FID temperatures were set at 250 and 280 °C, respectively. The oven temperature was maintained at 130 °C, for 3 min, elevated to 180 °C at a rate of 10 °C/min, held for 7 min. Nitrogen was used as carrier gas. The percentage (molar conversion) was defined as (mmole of hexyl laurate ÷ mmole of initial hexanol) x 100% and was estimated using peak area integrated by on-line software Hewlett Packard 6890 Series II ChemStation. The production rate (μmol/min) was calculated with the equation below: (percentage molar conversion × mixture flow rate × mmole of initial hexanol).

Experimental design and statistical analysis

A 3-level-3-factor Box-Behnken design with three replicates at the center was employed in this study, requiring 15 experiments with a continuous packed-bed reactor in solvent-free system [16]. The variables were reaction temperature (45–65 °C), mixture flow rate (0.25–0.75 mL/min) and concentration of lauric acid (100–300 mM). The experimental data (Table 1) were analyzed by the response surface regression (RSREG) procedure to fit the following second-order polynomial equation [16]:

$$Y = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} X_i X_j \quad (1)$$

1* Is this an acronym? Will your readers know its meaning?
2* The sentence within [ ] is definitely ungrammatical, as originally written; however, your precise intended meaning should be clarified in consultation with your editor.
3* Processes/procedures are "carried out" [better: "implemented"]; chemical compounds are formed.
Alternative: The production of hexyl laurate was implemented as . . . .

Where Y is response (production rate); β_{k0}, β_{ki}, β_{kii} and β_{kij} are constant coefficients; and X_i the uncoded independent variables. The option of RIDGE-MAX was employed to compute the estimated ridge of maximum response for increasing radii from the centre of the original design.

Results and discussion

The application of lipase for esterification reaction in organic media or solvent-free system has increased significantly in the last decade. For the design of suitable reactors, control systems and process optimization, kinetic information on the rate of product formation and the effects of changes in operating conditions are necessary.[17] Generally, packed-bed reactors are the most in common used system for large-scale lipase synthesis product applications,[18] They are the best continuous way which can minimize labor, overhead costs and further the process control to conform all commercial demands from industry enterprise.[11]

Lipase-catalyzed hexyl laurate of one-factor-at-a-time design

2* 3* The hexyl laurate was carried out at constant enzyme-loaded 1.5 g in a continuously-packed-bed reactor with solvent-free (Figure 1–3). Figure 1 showed the lauric acid concentrate affected the molar conversion (%) and production rate

* Again, you wrote an ungrammatical sentence [ ]. The revision might not convey your precise intended meaning; hence, consultation with the editor is needed.

(μmol/min); ~~In figure 1 indicated~~ the production rate (μmol/min) was more significant ~~increased than~~ molar conversion (%) with ~~increased~~ lauric acid concentrate at ~~temperature~~ 55 °C and ~~mixture~~ flow rate 0.5 mL/min ~~condition~~. When ~~lauric acid~~ concentrate increased from 0.1 to 0.3 M, production rate ~~was~~ ~~advanced~~ significantly from 26 to 89 μmol/min; ~~but~~ molar conversion ~~was not~~ ~~apparent variation~~. [Figure 2] showed ~~the~~ reaction molar conversion ~~was affected~~ more significantly ~~in~~ different mixture flow rate. When the mixture flow rate was increased (0.25 to 1.5 mL/min), molar conversion ~~was~~ decreased (83% to 15%) rapidly. [~~The result proved to get~~ minimum molar conversion ~~due to the enzyme~~ ~~and substrate mixture contact time decreased~~. Figure 3 indicated ~~hexyl~~ laurate ~~carried out~~ at 45 to 65 °C. The reaction production rate increased (19.85 to 37.48 μmol/min) at temperature ~~from~~ 45 to 55 °C; However, the production rate ~~decreased~~ was induced by the reaction at temperature 65 °C. ~~In~~ this result ~~expressed~~ enzyme inactivity due to protein denature in the higher temperature.

Model fitting

The major objective of this ~~paper~~ is the development and evaluation of a statistical approach to better understand the affinity between the variables of a lipase-catalyzed direct esterification reaction in ~~solvent-free~~. The RSM conjugated with ~~3-factor-3-level~~ Box-Behnken design was more efficient in reducing the

1* Is this abbreviation for "equation" prescribed by the stylesheet of the target journal?
2* A native speaker of English would more likely say/write "for representing."

experimental runs and time for investigating the optimized synthesis of hexyl laurate in this study. The RSREG procedure was employed to fit the second-order polynomial eqn. (1) fairly well (coefficient of determination, $R^2 = 0.993$) to the experimental data—μmol/min (Table 1). Among the various treatments, the highest production rate obtained in no.9 (87.44 μmol/min) at 55 °C, 0.75 mL/min and lauric acid concentrate 0.3 M; and the lowest production rate was in no.12 (20.75 μmol/min) was treatment (55 °C, 0.25 mL/min and lauric acid concentrate 0.2 M). From the SAS output of RSREG, the second-order polynomial eqn. (2) obtained below:

$$Y = -249.907 + 8.594X_1 + 69.345X_2 + 262.787X_3 - 0.076X_1X_1 - 0.225X_1X_2 - 73.037X_2X_2 - 1.472X_3X_1 + 167.95X_3X_2 - 15.808X_3X_3 \quad (2)$$

Analysis of variance (ANOVA) is presented in Table 2 indicated that the second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (μmol/min) and the significant variables with very small p-value (0.0001). Furthermore, the overall effect of the three synthesis variables on the production rate of hexyl laurate was further analyzed by a joint test (Table 3). Results revealed that the mixture flow rate were the most important parameters, and exerting a statistically significant overall effect ($p < 0.01$) on the production rate of hexyl laurate.

1* In headers and captions use headline grammar, which omits "the."
2* Why is "ridge max" in ALL-CAPS on p. 8 and in all-lower-case here and elsewhere? Your ALL-CAPS made me think that the phrase might be an acronym. Be consistent!

Interrelationship of variables

The relationships between the reaction factors and the response can be better understood by examining the planned series of contour plots generated from the predicted model (eqn. (2)) by holding constant either the mixture flow rate, the temperature and or the lauric acid concentrate (Figure 4). Figure 1–3 represents depict the same range of [diction & S-V agr] synthesis temperature s (45 to 65 °C) and lauric acid concentrate ions (0.1 to 0.3 M). [VT] Then, Thus the production rate was varied varying in the mixture flow rate s of 0.25, 0.5, and 0.75 mL/min, respectively.

1* *The optimum conditions obtained and model verification* O

2* The optimum point was determined by ridge-max [hyphen] analysis, [comma] which The method of ridge analysis computes the estimated ridge of in the maximum response for increasing the radii from the center of the original design. This The ridge max analysis [comma] (Table 4) listed in [delete parentheses] [comma] showed revealed a maximum production rate was of 81.58 ± 1.76 μmol/min at 54 °C, a flow rate was of 0.5 mL/min and a concentration of 0.3 M lauric acid.

The validity of the predicted model was examined by experiments at the aforementioned [diction & syntax] suggested optimum conditions of the above. The predicted value was 81.58 μmol/min, [hyphen] obtained by ridge-max analysis, and the actual value was 87.44 μmol/min, 1* as carried out implemented in the experiments. Thus, the optimization of lipase-catalyzed synthesis of hexyl laurate by Lipozyme® IM-77 was successfully developed by Box-Behnken

design and RSM.

Conclusion

In our research, an
According to our study, the optimal production rate of 87.44 μmol/min was
by Lipozyme® IM-77
obtained in solvent-free with a continuous packed-bed reactor by Lipozyme® IM-77. in a solvent-free system

[syntax]

The production rate was significantly affected by the mixture flow rate and the concentration of lauric
was achieved at and a
acid concentrate. The optimal production rate in temperature 55 °C, mixture flow
of
rate 0.55 mL/min.

[delete comma]

Acknowledgment

The authors wish to express appreciation to Dr. Cheryl Rutledge, Department of English, Da Yeh University, for her editorial assistance.

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Table 1. 3-level-3-factor Box-Behnken design and experimental data for response surface analysis

Treatment # [a]	Factors			Production rate (μmol/min) Y	Predicted value
	Temperature (°C) X_1	Flow rate (ml/min) X_2	Concentration [c] (mM) X_3		
1	1(65)[b]	1(0.75)	0(200)	41.86	44.14
2	1(65)	-1(0.25)	0(200)	38.17	36.51
3	-1(45)	1(0.75)	0(200)	47.80	49.46
4	-1(45)	-1(0.25)	0(200)	41.85	39.58
5	1(65)	0(0.5)	1(300)	68.44	69.20
6	1(65)	0(0.5)	-1(100)	21.64	20.26
7	-1(45)	0(0.5)	1(300)	74.96	76.34
8	-1(45)	0(0.5)	-1(100)	22.27	21.51
9	0(55)	1(0.75)	1(300)	87.44	84.41
10	0(55)	-1(0.25)	-1(100)	25.02	24.13
11	0(55)	1(0.75)	1(300)	66.37	67.27
12	0(55)	-1(0.25)	-1(100)	20.75	23.78
13	0(55)	0(0.5)	0(200)	54.56	54.62
14	0(55)	0(0.5)	0(200)	54.76	54.62
15	0(55)	0(0.5)	0(200)	54.55	54.62

[a] The treatments were run in a random order.

[b] Numbers in parenthesis represent actual experimental amounts.

[c] Concentration of lauric acid.

delete verbosity

R

-s

If the Note does not constitute a complete sentence as in a (as revised) and c, do not punctuate with a period—unless prescribed by the editorial style of the target journal.

Table 2. Analysis of continuous synthesis variance of hexyl laurate for joint test

Factor	Degrees of freedom	Sum of squares	Prob > F[a]
Temperature (X_1)	4	260.283	0.0213
Flow rate (X_2)	4	301.878	0.0156
Concentrate (X_3)	4	5463.911	0.0001

[a] prob > F = level of significance

Table 3. Estimated ridge of maximum response for variable production rate

Coded radius	Estimated response	Standard error	Uncoded factor values		
			X_1	X_2	X_3
0.0	54.618	1.647	55.000	0.500	0.200
0.2	59.917	1.628	54.837	0.509	0.209
0.4	65.259	1.580	54.670	0.519	0.239
0.6	70.648	1.541	54.498	0.531	0.258
0.8	76.087	1.577	54.323	0.544	0.277
1.0	81.578	1.762	54.147	0.557	0.296

[verbose]

Table 4. ANOVA for synthesis variables pertaining to the response.

Source	Df	Sum of squares	Prob > F[a]
Model	9	5929.242	0.0001
Linear	3	5572.943	0.0001
Quadratic	3	275.835	0.0115
Crossproduct	3	80.464	0.1161
Lack of fit	3	40.700	0.001
Pure error	2	0.027	
Total error	5	40.728	
R^2	0.993		

[a]prob > F = level of significance.

1* Brevity is desirable in legends/captions. Yours are too verbose. Use headline grammar except where a complete sentence is necessary and unavoidable.

2* See Note 2, p. 8. Similar remarks are applicable here, except that phrases rather than sentences are desirable.

4* Do you mean [a 1.5 g enzyme-loaded constant]?

1* **Figure legends**

2* **Figure 1**. The hexyl laurate was carried out at constant enzyme loaded 1.5 g in a continuously synthesis reaction; (■) Molar conversion (left label); (△) production rate (right label); The reaction carried out at temperature 55 °C and mixture flow rate 0.5 mL/min.

3* It is obvious that "55 °C" is a "temperature." Reduce verbosity by omitting the obvious

Alternative: Production of hexyl laurate implemented as . . .

Figure 2. The hexyl laurate was carried out at constant enzyme loaded 1.5 g in continuously synthesis reaction. (■) Molar conversion (left label); (△) production rate (right label). The reaction carried out at temperature 55 °C and lauric acid concentrate 0.1 M.

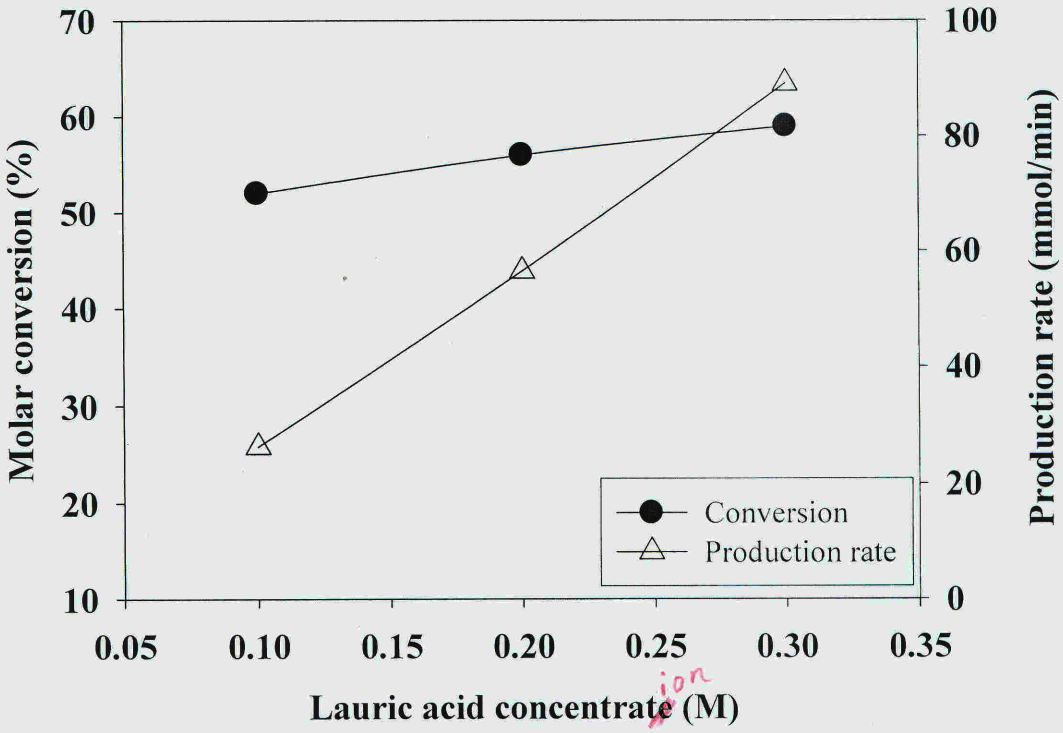
Revise in the same syntax and diction as Fig 1, with only the concentration differing.

Figure 3. The hexyl laurate was carried out at constant enzyme loaded 1.5 g in continuously synthesis reaction. (■) Molar conversion (left label); (△) production rate (right label); The reaction carried out at mixture flow rate 1.5 mL/mi and lauric acid concentrate 0.1 M.

Revise in same syntax & diction as Figs 1&2.

Figure 4. Contour plots of production rate of continuously synthesis hexyl laurate (keep the temperature condition as constant); Enzyme loaded 1.5 g, numbers inside the contour plots indicate the production rate at given reaction conditions.

headline grammar


70
60
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Molar conversion (%)
100
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Production rate (mmol/min)
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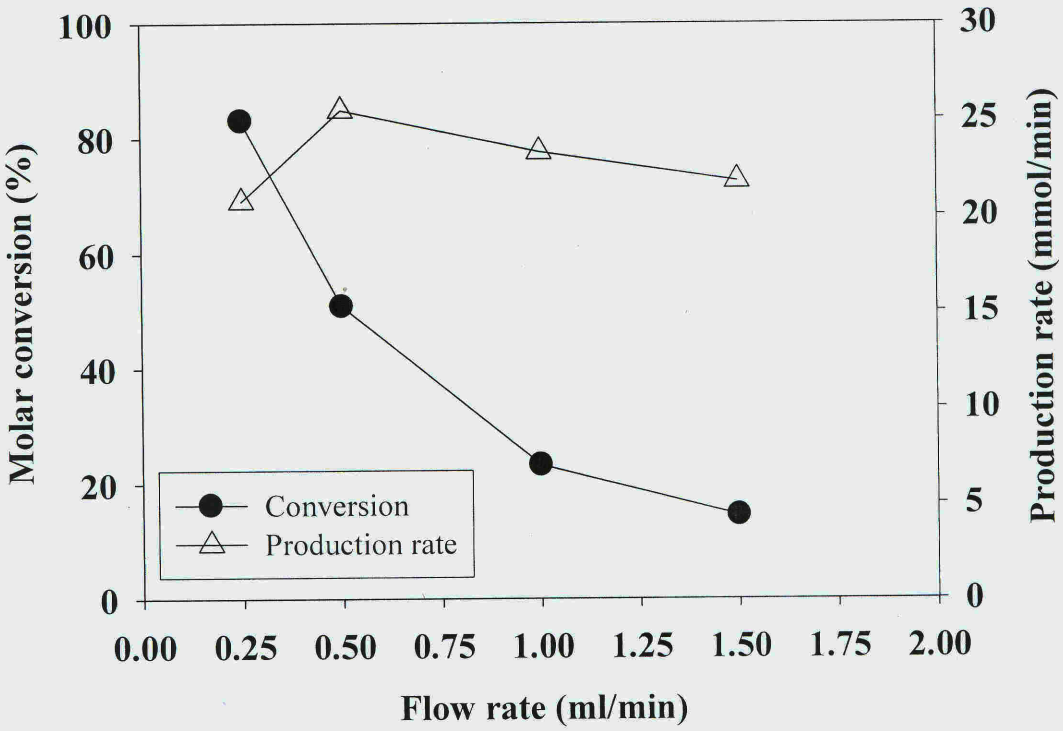
Figure 1.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Molar conversion (%)
Production rate (mmol/min)
Flow rate (ml/min)
Conversion
Production rate

Figure 2.

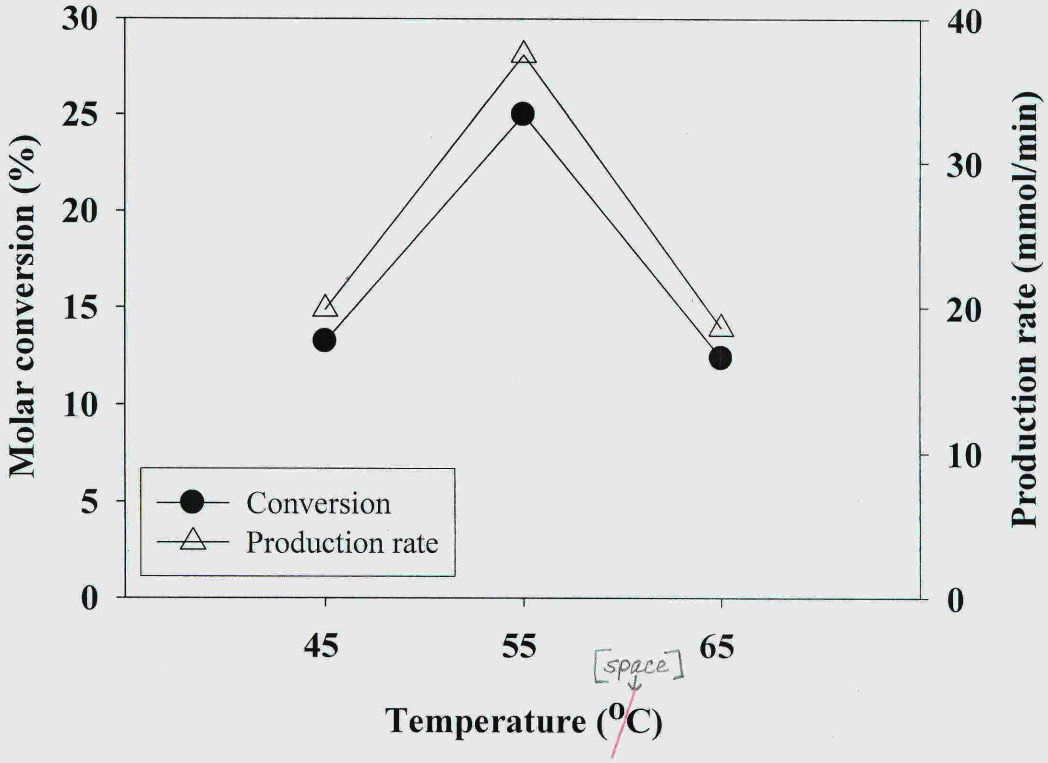

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Figure 3.

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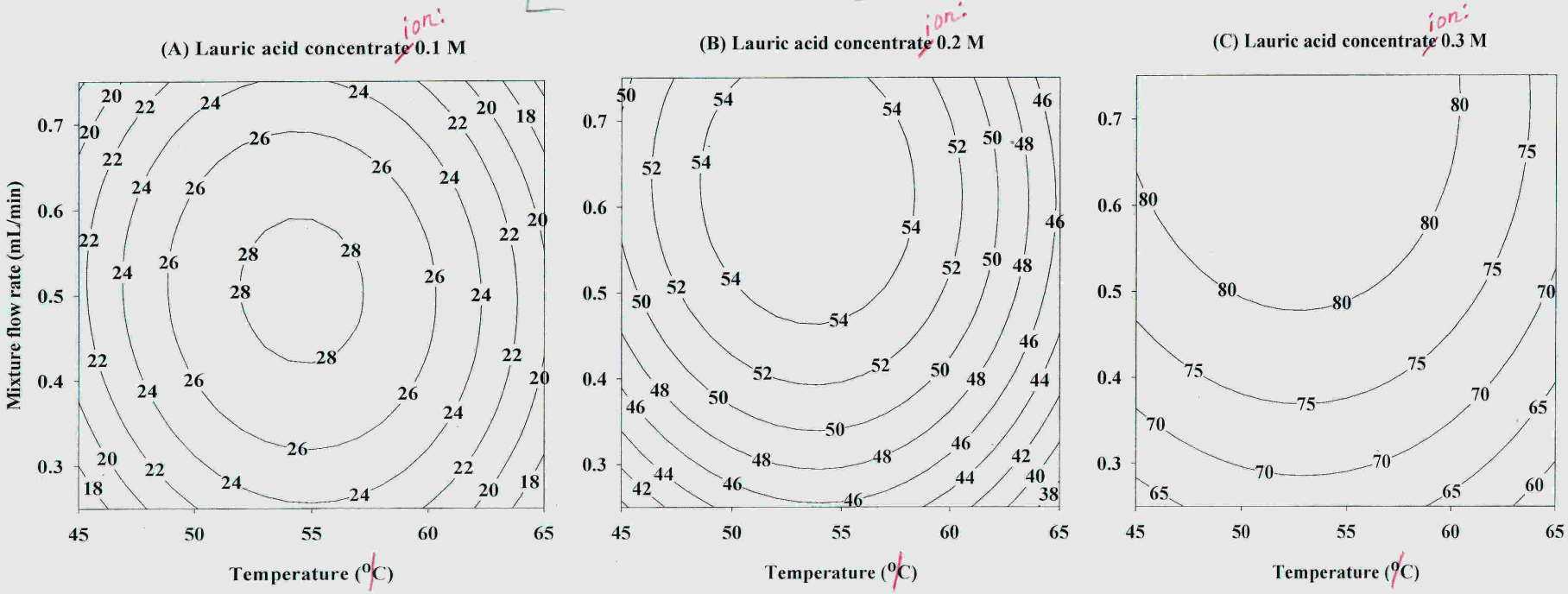

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Figure 4.

The author wishes to express appreciation to **Cheryl Rutledge** for her editorial assistance.

This is not the appropriate place for acknowledging your English editor. The proper position is one immediately preceding the References.