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Optimization Lactic Acid Production from Molasses Renewable Raw Material through Response Surface Methodology with *Lactobacillus Casei* M-15

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Abstract

Lactic acid is one of the functional and valuable compounds utilized in food, pharmaceutical and chemical industries while Poly lactic acid (PLA) is a biodegradable polymer that has a variety of applications. In recent years, microbial conversion of renewable raw materials has become an important objective in industrial biotechnology. Sugarcane molasses can be considered as potential renewable raw materials in PLA production. The objective of this study is to optimized fermentation medium and conditions to obtain maximum lactic acid production and Colony Forming Unit (log CFU/mL) through response surface methodology (RSM). The maximum lactic acid production (38.33%) and log CFU/mL (8.30) by *Lactobacillus casei* M-15 was under 3.82% of molasses and 8.02% of inoculum level within 24 hr at 37 °C respectively. This process will be advantageous for increasing yields of lactic acid and enhancing productivity by optimization technique. Moreover, it can reduce waste disposal and pollution and can selectively produce by sustainable agriculture such as agriculture material. In addition, the high-performance of lactic acid-producing microorganisms, qualified renewable raw materials and effective fermentation processes will be benefit for bioplastic technologies.

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Keywords: Lactic acid, *Lactobacillus casei*, molasses, poly lactic acid, renewable raw material

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1. Introduction

Lactic acid has numerous applications in the food, chemical, textile, pharmaceutical, and other industries. Recently, there has been a great demand for lactic acid, as it can be used as a monomer for the production of the biodegradable polymer Poly Lactic Acid (PLA), which is an alternative to synthetic polymers derived from petroleum resources. While only racemic DL-lactic acid is produced through chemical synthesis, a desired stereoisomer (i.e. an optically pure L- or D-lactic acid) could be produced through a fermentative production of renewable resources if the proper microorganisms are chosen for lactic acid fermentation [1]. Molasses is a by-product of the sugar manufacturing process. It is also used as an animal feed as well as for ethanol and yeast production. The most abundant sugar is sucrose, the high concentration of which raises the viscosity of the liquid [2]. However, among the genus *Lactobacillus, Lactobacillus casei* has appeared commonly in many investigations on the production of lactic acid [3] and [4]. In the present study, it was described optimized fermentation medium and conditions to obtain maximum lactic acid production and colony forming unit (log CFU/mL) with *Lactobacillus casei* M-15 grown in molasses using response surface methodology (RSM).

2. Materials and Methods

2.1. Microorganisms, Medium and Culture Conditions

A lactic acid producing strain of *Lactobacillus casei* M-15 used in this study were identified by 16S rDNA technique and the cultures were maintained at -80 °C in 20% glycerol stocks and grow in Man Rossa de Sharpe (MRS) broth. The inoculum was prepared by transferring glycerol stock culture (1 mL) to an Erlenmeyer flask containing 20 mL of MRS medium and incubated at (37±1) °C for 12 h. Initial pH of the medium was adjusted to 6.5±02. Then, the mediums were autoclaved at 15 psi and 121 °C for 15 min. The production medium were inoculated with differences percentage of inoculum level (V/V) (5, 7.5, 10) and percentage of molasses concentration (Guang-yi Company, Taiwan) (W/V) = 1, 5.5, 10 in the modified MRS medium at (37±1) °C for 24 h. The composition of modified MRS medium was (in g/L): peptone 10, yeast extract 5, meat extract 10, sodium acetate 5, K_2HPO_4 2, Tween 80 1, $(NH_4)_2HC_6H_5O_7$ 2, $MgSO_4 \cdot 7H2O$ 0.1 and $MnSO_4 \cdot 4H_2O$ 0.05. The total values of modified MRS medium were 50 mL in 250 mL Erlenmeyer flask.

2.2. Analytical Procedure

At the end of fermentation, the fermented materials were centrifuged at 10,000xg within10 min, 4 °C, micro filtered (0.22 μm) and frozen at -4°C and supernatants were analyzed. Lactic acid concentrations were determined using a High Performance Liquid Chromatograph (HPLC) (Hitachi, L-7100 PUMP 220V 60HZ, Japan), equipped with a tunable UV detector set at 210 nm. RP-18 column (244 mm x 4 mm, Lichrocart) was eluted with 60% MeOH as a mobile phase at a flow rate of 0.7 mL/min. The injection volume was 20 μL for analysis, which was maintained at room temperature. The cell concentration was carried by Colony Forming Unit (CFU/mL).

2.3. Experimental Design and Statistical Analysis

RSM is a collection of experimental strategies, mathematical methods, and statistical inference which enable an experimenter to make efficient empirical exploration of the system of interest. According to this design, 13 experiments were conducted containing six replications at the center point. The independent

variables selected for the study of production of lactic acid were: percentage of molasses concentration and percentage of inoculum level. Actual variables and their corresponding coded levels are presented in Table 1. The response variable was fitted by a second order model in order to correlate the response variable (Lactic acid concentration) to the independent variables. The model equation is represented as:

$$Y=\beta_0 + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum \beta_{ij} \chi_i \chi_j \quad (1)$$

Where, *Y* is the predicted response; *βo* is the intercept; *βi* is the linear coefficient; *βii* is the quadratic coefficient and *βij* is the interaction coefficient. The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). This analysis included the Fisher's *F-test,* correlation coefficient *R*, determination coefficient *R2* which measures the goodness of fit regression model. It also includes the student's *t-value* for the estimated coefficients and the associated probabilities *p(t)* [5] and [6]. Analysis of variance (ANOVA) was performed and threedimensional response surface curves were plotted by Design Expert (version 7.0, Stat-Ease, Inc., Minneapolis, USA) statistical package to study the interaction among components.

3. Results and Discussion

In the present study, the relationship between lactic acid production (g/L), log CFU/mL and two independent variables (percentage of molasses concentration and percentage of inoculum level) were investigated. The optimum values of parameters for maximum lactic acid production and log CFU/mL were determined using statistical Central Composite Design (CCD) according to design matrix which is given in Table 1 and Table 2. The data was analysed by multiple regression analysis using the Design Expert Software. The following polynomial equation was derived to represent lactic acid production (g/L) (*Y1*), log CFU/mL (*Y2*) as a function of the independent variables tested. The experimental data (Table 2) were analysed using statistical methods appropriate to the experimental design used. Multiple regression analysis of the experimental data gave the following second order polynomial equation:

$$Y_1=26.54+1.34x_1+2.32x_2-0.11x_1x_2-0.06x_1^2-0.12x_2^1 \quad (2)$$

$$Y_2=6.88+0.12x_1+0.27x_2-0.009x_1x_2-0.005x_1^2-0.01x_2^1 \quad (3)$$

A summary of the analysis of variance (ANOVA) for the selected quadratic model was shown in Table 3 and 4. The correlation measures for testing the goodness of fit of the regression equation were the multiple correlation coefficients R and the determination coefficient R2. The value of R (0.8615, 0.8701) for Eq. (2 and 3) being close to 1, indicated a high degree of correlation between the observed and predicted values. The value of the determination coefficient R2 (0.7422, 0.7571) and adjust R2 (0.5581, 0.5837) suggested that model may explain 55.81 and 58.37% of the total variation in response respectively. The analysis of variance (ANOVA) for response surface quadratic model was summarized in Table 3 and Table 4. The model F-value were 4.03, 4.36 and the F-value for lack of fit were 10.71, 128.84 The P-value for the model (0.0483, 0.0401) and for lack of fit (0.0221, 0.0002) led to the same conclusion respectively. The coefficient estimate and the corresponding Prob > F-values suggested that all the independent variables studied had significant effects on lactic acid production (g/L), log CFU/mL. The analyses also showed that there were significant interactions between percentage of molasses concentration and percentage of inoculum level.

Table 1. Central Composite Design (CCD) experimental range and levels of the independent variables

	Factor	-α	-1	0	+1	+α
x_1	Molasses concentration (%)	0.86	1	5.5	10	11.86
x_2	Inoculum level (%)	3.96	5	7.5	10	11.03

Table 2. CCD experimental design used in RSM studies by using two independent variables showing observed lactic acid production and log CFU/mL

Run	x_1	x_2	Y_1 Lactic acid production (g/L)	Y_2 log CFU/mL
1	1	5	34.43	7.89
2	10	5	36.43	8.17
3	1	10	37.26	8.23
4	10	10	34.18	8.07
5	0.86	7.50	38.01	8.27
6	11.86	7.50	35.41	7.83
7	5.50	3.96	38.29	8.04
8	5.50	11.04	36.78	8.17
9	5.50	7.50	37.43	8.30
10	5.50	7.50	38.43	8.27
11	5.50	7.50	38.50	8.27
12	5.50	7.50	38.36	8.27
13	5.50	7.50	38.45	8.27

Table 3. ANOVA for response surface quadratic model of lactic acid production

Source	Sum of Squares	df	Mean Square	*F*-Value	*P*-value Prob > F	
Model	21.21	5	4.24	4.03	0.0483	significant
Lack of Fit	6.55	3	2.18	10.71	0.0221	
Pure Error	0.81	4	0.20			
Cor Total	28.58	12				

R= 0.8615, *R*-Squared = 0.7422, *Adj R*-Squared = 0.5581

To solve the regression equation and analyze the response surface plots, the optimal values of the test variables in coded unit were percentage of molasses concentration (x_1) and percentage of inoculum level (x_2) respectively. The optimization of lactic acid production (g/L), log CFU/mL by *Lactobacillus casei* M-15 was x_1= 3.82, x_2= 8.02, Y_1= 38.33 g/L, Y_2 = 8.30 and desirability= 0.980.

Table 4. ANOVA for response surface quadratic model of log CFU/mL

Source	Sum of Squares	df	Mean Square	*F*-Value	*P*-value Prob > F	
Model	0.22	5	0.044	4.36	0.0401	significant
Lack of Fit	0.070	3	0.023	128.84	0.0002	
Pure Error	7.200E-004	4	1.800E-004			
Cor Total	0.29	12				

R= 0.8701, *R*-Squared = 0.7571, *Adj R*-Squared = 0.5836

4. Conclusion

The optimization results from RSM and CCD illustrated the maximum lactic acid production (38.33%) and maximum log CFU/mL (8.30) by *Lactobacillus casei* M-15 was under 3.82% of molasses and 8.02% of inoculum level within 24 hr at 37 °C. It was significantly different from one another ($p < 0.05$) as determined by one-way ANOVA. Therefore, this optimum condition by RSM and CCD can be adapted to increase percentage of lactic acid production (g/L) and log CFU/mL with molasses by-product of sugar cane. However, a well-established study on microorganism producing lactic acid with renewable raw material using RSM technique still requires more research efforts to be generalized and applied to a wider scope of lactic acid industries. In addition, the high-performance of lactic acid-producing microorganisms, qualified renewable raw materials and effective fermentation processes will be benefit for bioplastic technologies.

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