ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Optimization of Lactic Acid Production from Cane Sugar Molasses

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ABSTRACT

The response surface methodology was employed to make mathematical modeling and optimization of the temperature and shaking time. The polynomial regression model equation $Y_1 = 0.68 - 0.021\ x_1 + 0.12\ x_2 + 0.024\ x_1 x_2 - 0.30\ x_1^2 - 0.13\ x_2^2$ obtained with temperature (X_1) and shaking time (X_2). The optimal temperature and shaking time were found to be 37.29°C and 9.57 hr respectively. The maximum % of lactic acid recovery was found to be 0.65. The simulation parameter revealed that the modeled values were in good agreement with simulated results.
Keywords: Response surface methodology, Optimization, Analysis of variance.

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ISSN: 0975-8585

INTRODUCTION

Lactic acid is widely used in the food, cosmetic, pharmaceutical, and chemical industries and has received increased attention for use as a monomer for the production of biodegradable poly (lactic acid). It can be produced by either biotechnological fermentation or chemical synthesis, but the former route has received considerable interest recently, due to environmental concerns and the limited nature of petrochemical feed stocks [1]. In the early 1960s, a method to synthesize lactic acid chemically was developed due to the need for heat stable lactic acid in the baking industry [2]. Recently, lactic acid consumption has increased considerably because of its role as a monomer in the production of biodegradable PLA, which is well-known as a sustainable bioplastic material [3,4].

Lactic acid is the common name given to 2-hydroxypropanoic acid. There are two optical isomers of lactic acid: L(+)-lactic acid and D(–)-lactic acid. Lactic acid is classified as GRAS (generally recognized as safe) for use as a food additive by the US FDA (Food and Drug Administration), but D(–)-lactic acid is at times harmful to human metabolism and can result in acidosis and decalcification [3]. Lactic acid can be obtained either through chemical synthesis or fermentative processes [5].

A number of byproducts and raw materials from the food and/or agriculture industries have been employed for microorganism growth due to their considerable availability and low cost. Examples include cheese whey, corn steep liquor, corn syrup, distillery yeast and molasses [6]. Biotechnological processes for the production of lactic acid usually include lactic acid fermentation and product recovery and/or purification. There have been numerous investigations on the development of biotechnological processes for lactic acid production, with the ultimate objectives to enable the process to be more efficient and economical [1]. Microorganisms that can produce lactic acid can be divided into two groups: bacteria and fungi [7]. However among the genus *Lactobacillus*, *Lactobacillu. Cassei* has appeared commonly in many investigations on the production of lactic acid [8].

Molasses, which a by-product of the sugar manufacturing process, is 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 [7]. However, among the genus *Lactobacillus*, *Lactobacillus casei* has appeared commonly in many investigations on the production of lactic acid [8].

The aim of the present research is to get better understanding of the relation between the variables (temperature, shaking time) and to determine the optimum conditions for the % recovery of lactic acid.

MATERIALS AND METHODS

The project work was conducted in a laboratory level to produce lactic sugar cane molasses as substrates. The production of lactic acid was done by using *lactobacillus casei.*

Sample collection

The sample of sugar cane molasses were collected from Rani sugar (M K Hubli). These samples were stored under refrigeration for the experiments.

Experimental design and data analysis: Central Composite Design (CCD)

RSM consists of a group of empirical techniques devoted to the evaluation of relations existing between a cluster of controlled experimental factors and the measured responses, according to one or more selected criteria. Prior knowledge and understanding of the process variables under investigation is necessary for achieving a realistic model. The significant variables obtained by using statistical experimental design technique called the response surface methodology and central composite design was used. According to the Central composite design, the total number of treatment combinations was $2^k + 2k + n_{o.}$ where 'k' is the number of independent variables and n_o is the number of repetition of experiments at the center point. A 2^2 factorial design with four axial points and six replicates at the center point with a total number of 13 experiments was employed for optimization of the medium components [9]. The central value (zero level) chosen for experimental design were the range of temperature (X_1) and Shaking time (X_2) studied in this work was between 25 to 50 and 3 to 12 respectively.

Experimental procedure

The sterilized conical flask was added with 50 ml of molasses collect from Rani sugar industry and is diluted with 50ml of double distilled water. 5ml of 20% H_2SO_4 is added the conical flask. The medium of the conical flask was adjusted by using 4 M KOH. The dry yeast and 4 g of $CaCO_3$ is added. Than a pure culture of *Lactobacillus Cassei* is inoculate to the flask. The sample was kept in laminar air flow for a period of 72 hours for fermentation to take place. A detailed methodology for the optimization process is given for run number 1 (Table 1). The samples were kept in continuous shaking incubator according to run number 1 shown in Table 1. Fermentation medium was filtered through Whitman filter paper and the filtrate was used for the estimation of sugars and lactic acid. Total sugars were estimated using Lane and Eynon method by titration with Fehling's solution [10]. Lactic acid was estimated by high performance liquid chromatography [11].

Table 1 Experimental data obtained for % of lactic acid recovered

Run	Temperature $x_1(\equiv X_1)$	Shaking Time $x_2(\equiv X_2)$	% of lactic acid recovered
1	-1 (25.0)	-1 (3.00)	0.346
2	+1 (50.00)	+1 (3.00)	0.1
3	-1 (20.00)	-1 (12.00)	0.3
4	+1 (50.00)	+1 (12.00)	0.15
5	-1.414 (19.82)	0 (7.50)	0.016

6	+1.414 (55.18)	0 (7.50)	0.18
7	0 (37.50)	-1.414 (1.14)	0.1
8	0 (37.50)	+1.414 (13.86)	0.758
9	0 (37.50)	0 (7.50)	0.675
10	0 (37.50)	0 (7.50)	0.675
11	0 (37.50)	0 (7.50)	0.675
12	0 (37.50)	0 (7.50)	0.675
13	0 (37.50)	0 (7.50)	0.675

RESULT AND DISCUSSION

The application of response surface methodology offers, on the basis of parameter estimate, an empirical relationship between the response variable and the test variables under consideration. Multiple regression analysis of the experimental data (using Design- Expert software) gave the following second order polynomial equation in terms of % of lactic acid recovered result shown in Table 1.

The expression obtained in terms of coded factors is given by the equation, $Y_1 = 0.68 - 0.021\, x_1 + 0.12\, x_2 + 0.024\, x_1 x_2 - 0.30\, x_1^2 - 0.13\, x_2^2$. Goodness of fit was examined using second order model equation with independent and dependent variables. The coefficient of determination (R^2) is 0.8330 which indicates that model response to total variability by 83.30% to various responses given. Analysis of variance (ANOVA) was used for statistical testing of the model which is part of test for adequacy and significance of the model. Models reliability was tested using Fisher's statistical test (F). The results of statistical testing using ANOVA are given in Table 2.

Table 2 Analysis of Variance (ANOVA) Table for the effect of temperature and pressure on out feed

Source	Sum of squares	Degrees of freedom	Mean square	F value	Probability (P) > F
Model	0.80	5	0.16	6.98	0.0120 significant
Error	0.16	7	0.023		

Probability with a value of less than 0.05 signifies that the model terms are significant. Table 2 indicates that the model was highly significant. The F value ($F_{0.025\,(5,\,7)} = 5.29$) obtained from the standard distribution table, so the null hypothesis is rejected at 2.5% α level of significance. From Figure 1 it can be observed that a stationary point exists inside the range based on the shape of the contour plot. The response surface plot shown in Figure 2 for the chosen model Y_1 illustrates the three dimensional relationship. CCD demonstrated that optimum of % of lactic acid recovered. This result indicates that two variables had mutually dependent influence on the % of lactic acid recovered can determined. The optimal temperature and shaking time were found to be 37.29°C and 9.57 hr respectively. The maximum % of lactic acid recovered was found to be 0.65.

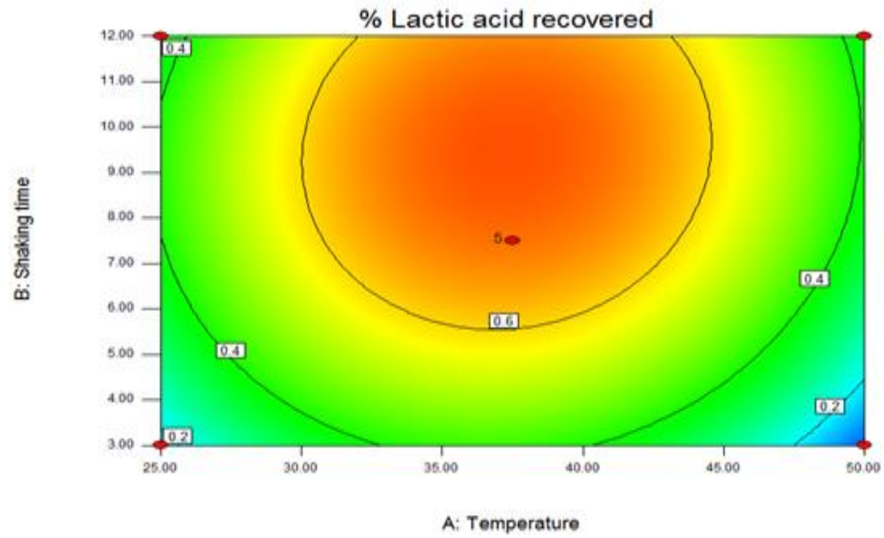

Figure 1. Isoresponse contour plots showing the effect of temperature (°C) and shaking time (hr) and their interactive effect on the % of lactic acid recovered

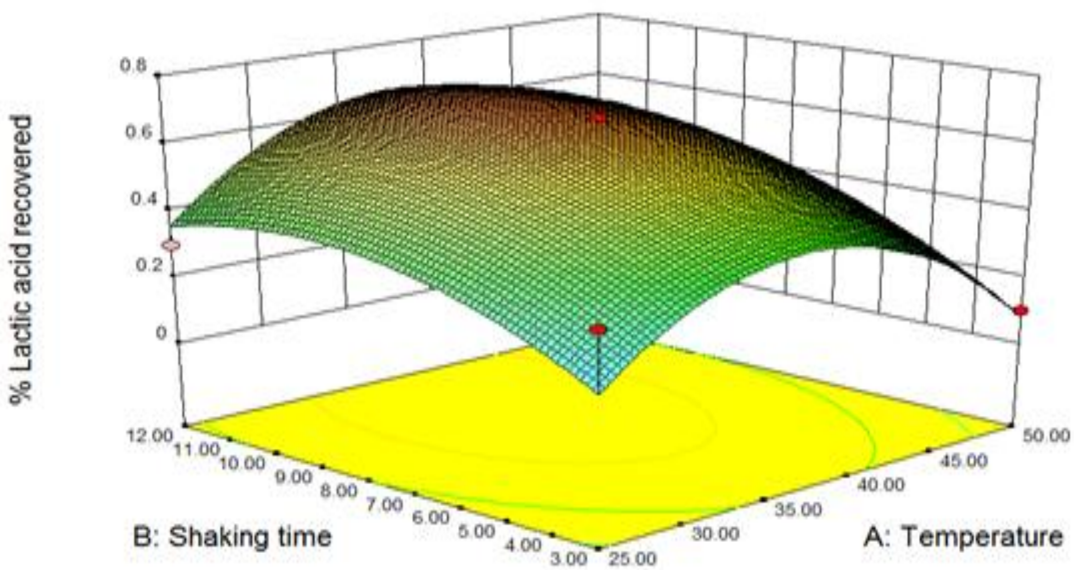

Figure 2. Response surface plot showing the effect of temperature (°C) and shaking time (hr) and their interactive effect on the % of lactic acid recovered

CONCLUSION

The study has demonstrated the use of a central composite design by determining the conditions leading to the optimum % of lactic acid recovered. The optimal temperature and shaking time were found to be 37.29°C and 9.57 hr respectively. The maximum % of lactic acid recovered was found to be 0.65. This methodology could therefore be successfully employed to any process, where an analysis of the effects and interactions of many experimental factors are referred. Response surface plots are very helpful in visualizing the main effects and interaction of its factors. Thus, smaller and less time consuming experimental designs could generally suffice the optimization of many fermentation processes.

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