JOURNAL OF SCIENCE TECHNOLOGY AND EDUCATION 14(1), MARCH, 2026
E-ISSN: 3093-0898, PRINT ISSN: 2277-0011; Journal homepage: www.atbufstejoste.com

Kinetic Study and Optimization of Lactic Acid Production from Corncob

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ABSTRACT

In this study, corncob, an agricultural waste, was utilized in the production of lactic acid, the process was optimized while the kinetics of the process was also investigated. The corncob was hydrolyzed using diluted Sulphuric acid, followed by the fermentation of the hydrolysate by lactobacillus delbrueckii to produce lactic acid. Experimental design based on central composite design (CCD) was used for the fermentation steps, while Response surface methodology (RSM) was used for the optimization of the process. For the fermentation step, glucose concentration, reaction time, temperature and pH were the process variables. The results obtained from the lactic acid fermentation process were then fitted into kinetic models to establish the kinetics of the process. The statistical analysis also showed that the lactic acid yield of 72.1% was obtained at optimized variables of temperature of $42^{o}C$, pH of 5.6, reaction time of 120h and glucose concentration of 10g/l. An excellent correlation exists between the predicted yields and the experimental yields, the coefficient of determination (R^2) given as 0.9654 indicates a well-fitted and reliable model. From the kinetic study of the lactic acid production process, there was an excellent fit to the first order kinetic model, this reveals a good agreement with the observed value of lactic acid yield (72.1%). Hence, useful kinetic parameters were determined based on the first order kinetic model. The values of the rate constant at $20^{o}C$, $30^{o}C$, $40^{o}C$, $50^{o}C$ and $60^{o}C$ are, $0.0026min^{-1}$; $0.0027min^{-1}$;$0.0033min^{-1}$;$0.042min^{-1}$and $0.004min^{-1}$ respectively The activation energy (Ea), needed for the conversion of corncob hydrolysate into lactic acid was found to be 12.79Kj/mol, while the pre-exponential factor was $2.145min^{-1}$ It is evident that corncob has the potentials, to produce high yield of lactic acid.

ARTICLE INFO
Article History
Received: August, 2025
Received in revised form: December, 2025
Accepted: February, 2026

KEYWORDS
Lactic acid, Corncob, PH, RSM and CCD

INTRODUCTION

Lactic acid is one of the most important organic acids which is extensively used around the globe in a range of industrial and biotechnological applications. Lactic acid is widely used in the food, cosmetic, pharmaceutical, and chemical industries and has received wide interest for use as a monomer for the biodegradable poly (lactic acid) production. Presently, the main growing application of lactic acid is in the production of biodegradable and renewable raw material-based poly lactic acid (PLA) polymers (Abdel-Rahman *et al*. 2013).

Lactic acid is industrially produced either by chemical synthesis or by microbial fermentation (biotechnological), a biological method has the advantage that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always gives racemic mixture of lactic acid (Ryu *et al*., 2003).

The biotechnological production of lactic acid has received a significant amount of interest

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recently, since it offers an alternative to environmental pollution caused by the petrochemical industry and the limited supply of petrochemical resources. The chemical way of producing lactic acid is rather too expensive and produces racemic mixture of the two forms of lactic acid, fermentation approach has become more successful because of increasing market demand for naturally produced lactic acid (Narayanan et al., 2004), with an estimated worldwide demand of 13,000-15,000T/year (Wee *et al*., 2006). Presently more than 95 % of industrial production of lactic acid is based on fermentation (Wee *et al*., 2006).

Corncob, a waste product of corn, contains large number of sugars that can be further utilized to produce various compounds (Yah *et al*., 2010). The bioconversion of lignocellulose material to biofuel from cheap non-edible materials such as corncob for renewal energy is imperative. Corncobs contain sufficient amount of cellulosic material, which is the best source of fermentable sugars. Corncob consists of polymers of mainly two types of sugars: glucose and xylose (Hsu *et al.,* 2011).

Lactic acid bacteria are widely used in the production of fermented foods where they are highly employed as starter cultures (Hansen, 2002). These organisms are able to fermentatively utilize carbohydrates and thus forming lactic acid as their major end product (Adams and Nicolaides, 1997). In this study, lactobacillus delbrueckii was used for the production of lactic acid.

Response surface methodology (RSM) is one of the most useful statistical optimization tools for biological and chemical processes. RSM has been increasingly used for various phases of an optimization process in fermentation. Factorial design of experiment, when combined with RSM, becomes very useful for designing experiments, developing models and evaluating the effects of variables in which a response of interest is influenced by several variables and the objective is to optimize this response.

In this work, the central composite design (CCD) of experiment was coupled with response surface methodology to investigate and optimize lactic acid production from corncob. The main objective of this study is to optimise and investigate the kinetics of lactic acid production from corncob.

There are numerous research articles on lactic acid production from agricultural raw waste, but the use of corncob for lactic acid production is not widely reported, among are, Oh *et al*., (2005) studied lactic acid production by using three different substrates which are wheat, corn and barley. They reported that the highest lactic acid production (0.94 g/g) was obtained when barley and corn were used as substrate. Nancib *et al*., (2009) investigated the production of lactic acid from date juice by the single and mixed culture system of Lactobacillus casei and Lactococcus lactis. Using the same parameters for both single and mixed culture with only 19 hours fermentation time, 150 rpm agitation and operating at 30^{o}C, they reported differences in lactic acid production. Farooq et al., (2012) investigated lactic acid production using culture of Lactobacillus delbrueckii. They reported that the highest lactic acid production (77.6g/l) took place after 7 days of fermentation at a temperature of 42^{o}C and no agitation used.

EXPERIMENTAL PROCEDURE

This section present step by step method used in implementing the study.

Corncob Preparation

The corn cobs (red and white) were collected from Ofana Aduma's farm in cross river state as a substrate for the production of lactic acid. The corncobs were washed with water to remove dirt and other impurities, then the washed shells were drained and oven dried at 60^{o}C to constant weight, the Cobs were further treated by breaking to small pieces with the aid of a hammer mill in such a way that it is suitable to be dried and grinded. Then, the corncobs were grinded using milling machine to the size of 2mm, and sieve analysis was performed on the samples. The corncobs were further reduced to powdered form to increase the surface area of the sample, to enhance the contact between hemicellulose and cellulose with dilute acid.

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Figure 2.1 shows the corncobs and it ground sample.

Fig. 2.1: Corncob and Grinded Corncob

Pretreatment

The purpose of the pre-treatment is to reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

Acid Pretreatment

In this study, dilute sulfuric acid pretreatment method with 1.5% concentration was used. Corn cob powder was pretreated inside an autoclave and heated at a temperature of 120°C, for 30 minutes. After that, it cooled and filtered. The filtrate was preserved in another conical flask prepared for the purpose of fermentation. The residue was washed twice by distilled water to remove sulfuric acid. It was then kept for hydrolysis. Corncobs powder was fed in batches, and every batch contained 50 g of screened corn cobs powder with a ratio of 10:1(v/w) water to the sample. (Zhang *et al*., 2011).

Dilute Acid Hydrolysis

The hydrolysis experiments were based on factorial design of experiment, employing the central composite design (CCD) method. The experimental runs were determined by using Design expert 11 software by Start Ease Corp. Thirty (30) experimental runs were carried out under different combination of process variables, as shown in Table 3.1. The hydrolysis reactions were performed in a 250ml conical flask which served as the batch reactor, six flasks (250ml) were taken containing the non-soluble (residue) corncob sample, different concentrations of Sulphuric acid were prepared, (according to the experimental design).

After hydrolysis process the samples were cooled at room temperature, the solid particles were separated from the liquid in the hydrolysate by vacuum filtration (to remove the non-fermentable lignin portion). After separation, the solid part was washed with distilled water two times. Finally, the soluble component was mixed with the previous filtered solution from the pre-treatment step for the next procedure. Wang (2003).

pH Adjustment

Before addition of any micro-organism to the above prepared samples (hydrolysed samples), pH of these samples were adjusted. As samples are acid hydrolyzed, a highly basic solution was added to bring the pH of the solution to 5.0, for this purpose, a highly concentrated NaOH solution was prepared by mixing water with sodium (Na) pellets. The NaOH solution was added drop wise to the samples with constant stirring until a pH of 5.0 was obtained (Wondale, 2012).

Figure 2.2 represent samples after Pretreatment and the sterilization equipment

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Figure 2.2: (a) Reduced sugar samples; (b) An Autoclave

Standardization of Glucose in the Corncob Hydrolysate

After the hydrolysis has been completed, the glucose contents were standardized by concentrating the solution through evaporation process using a rotary evaporator. The hydrolysate was evaporated at 100^{o}c for 45min. Different glucose concentrations were prepared and was used in the fermentation

Fermentation Process

Batch fermentations were done in 500mL Erlenmeyer flasks fitted with rubber stoppers, containing different concentration of the reduced sugar obtained from the hydrolysis step. The pH of the various concentrations of the reduced sugar were adjusted according to the required experimental conditions, by adding NaOH solution drop wise to the samples with constant stirring and were autoclaved at 120^{o}c for 15minutes for sterilization.

To 200ml of the hydrolysate, the following nutrients were added; 10 g peptone, 10 g Lab-Lemo meat extract, 5 g yeast extract, 1 g Tween 80, 2 g dipotassium hydrogen phos-phate, 5 g sodium acetate, 2 g triammonium citrate, 0.2 g $MgSO_4.7H_2O$ and 0.5 g $MnSO_4.4H_2O$. The pH was adjusted to 5, autoclaved at 121℃, and maintained for 15 minutes. Then, 1gram Lactobacillus delbrueckii (lactic acid bacterial) was added to the above 200 ml media in a 250 ml conical flask, next, the conical flask was properly covered with a rubber stopper. The various concentrations of the reduced sugar prepared, and media were mixed in a 500ml flasks with the ratio of 10 % (1% media with 10% sample). The prepared samples were inoculated in an incubator, set at different temperature according to the required experimental conditions.

The Fermentation of the corn cob hydrolysate was carried out according to the experimental design. Various experimental runs were carried out for the optimization process under different combination of process variables. At the end of each batch of the fermentation process, the reaction was stopped by raising the fermentation liquor's temperature to 80^{o}C and increasing the pH to 10. This procedure kills the organisms, solubilizes the calcium lactate and degrade some of the residual sugars.

Design of Experiment and Optimization of the fermentation parameters using RSM

RSM based on central composite experimental design was used to optimize the fermentation process parameters for lactic acid production. A five-level, four-factor design was employed in this study (Table 2.1). The fermentation process parameters investigated were Temperature (A), fermentation time (B), substrate concentration (C), and pH (D). The CCD generated 30 experimental runs that were

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randomized to minimize the unpredictable variations in the observed responses due to uncontrolled extraneous factors.

The experimental runs include 16 factorial points, 8 axial points and 6 centre points that provide information on the interior of the experimental regions to evaluate the curvature effect. Various experimental runs were carried out for the optimization.The effects of the operating conditions on the lactic acid yield were investigated and the optimal values were determined in this study. Table 2.1 shows the coded and actual factor levels for the experiment.

Table 2.1: Experimental range and levels of independent process variables for Lactic acid production

		Coded factor Levels				
Independent process Variables	**Symbols**	**-2**	**-1**	**0**	**+1**	**+2**
Temperature (degree Celsius)	A	20	30	40	50	60
Fermentation Time (hours)	B	12	48	84	120	156
Concentration (grams per litre)	C	1	4	7	10	13
pH	D	1.5	3	4.5	6	7.5

Determination of Lactic Acid Concentration

The concentration of lactic acid was determined following a modified method of Borshchevskaya et al., (2016). The method is based on the spectrophotometric determination of the coloured product of the reaction of lactate ions with iron (III) chloride. A calibration curve in the range of 0.3 to 10g/L of lactic acid was constructed by measuring standard samples using the spectrophotometer. 1% solution of iron (III) chloride was used to set blank on the spectrophotometer. 1 ml of lactic acid extract was added to 2ml of 1% iron (III) chloride solution in a quartz cuvette. The mixture was shaken to enhance interaction of the solvents before placing in a spectrophotometer at 390nm. The concentration of lactic acid in solution was read in PPM (part per million) by reference to the calibration curve.

For determination of percentage yield of the lactic acid produced, equation 2.1 was used

$$\text{Lactic acid yield } (\%) = \frac{\mathit{Concentration\ of\ lactic\ acid\ produced}}{\mathit{concentration\ of\ glucose\ used}} \times 10$$

Equation 2.1

RESULTS AND DISCUSSION

This section documents and discusses all the results obtained in this study, the production of lactic acid, the development of mathematical model and optimization of the lactic acid yield from the developed model, the kinetics of the lactic acid production, and finally.

Modelling and Optimization of Lactic Acid Production Process

Equation (3.1) and equation (3.2) are the quadratic model for lactic acid production in terms of coded parameters and actual parameters respectively. Table 3.1 shows the results obtained from the fermentation of corncob hydrolysate for the production of lactic acid, the results obtained were based on central composite design and were statistically analyzed, the process was also optimized using response surface methodology. Design expert software version 11 generated a quadratic mathematical model; Equation 3.1 shows the full quadratic regression model for the lactic acid production process in coded process variables while equation 3.2 shows the complete design equation in terms of actual parameters. Both equations 3.1 and 3.2 can be used to make predictions of the response for the given values of each factor.

An excellent correlation exists between the predicted yields and the experimental yields as shown in Figure 2.3. The coefficient of determination (R^2) given as 0.9654 indicates a well-fitted and reliable model.

Lactic acid Yield (%)(Coded Parameter) = $70.42 + 1.13A - 0.6042B + 0.4958C - 0.5542D - 0.7937AB + 0.3563AC + 0.5813AD + 0.8938BC$

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$- 2.11BD + 0.1438CD + 1.55A^2 + 0.8385B^2 + 1.76C^2 - 0.0615D^2$
Equation 3.1

Lactic Acid Yield (%) (Actual Parameter) = $100.26586 - 1.20021(\text{Temperature}) + 0.080305(\text{Time}) - 3.89190(\text{Concentration}) + 1.37917(\text{pH}) - 0.002205(\text{Temperature})(\text{Time}) + 0.011875(\text{Temperature})(\text{Concentration}) + 0.038750(\text{Temperature})(\text{pH}) + 0.008275(\text{Time})(\text{Concentration}) - 0.039005(\text{Time})(\text{pH}) + 0.031944(\text{Concentration})(\text{pH}) + 0.015510(\text{Temperature})^2 + 0.000647(\text{Time})^2 + 0.195949(\text{Concentration})^2 - 0.027315(\text{pH})^2$
Equation 3.2

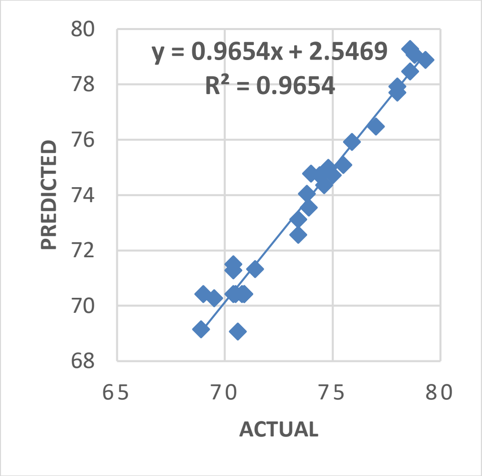

Figure 2.3: Correlation of predicted lactic acid yield vs actual lactic acid yield

Analysis of Variance (ANOVA) for the Lactic Acid Production Process

Table 3.2 indicates the statistical evaluation of the results carried out by an analysis of variance. Based on the ANOVA analysis obtained, temperature (A), time (B), glucose concentration (C) and pH were found to have a very significant effect ($p<0.05$) on lactic acid yield. Coefficient of determination (R^2) of the model was 0.9654, this value means that 96% of the variability was explained by the model and indicated that the model adequately represented the real relationship between the variables under consideration.

The adjusted determination coefficient ($R^2adj = 93.32\%$) was also able to confirm the significance of the model. The predicted R^2 is also in reasonable agreement with the adjusted R^2. This means that the data were well fitted by the model, which gives good estimates of system response within the experimental range.

The lack of fit p value of 0.3307 indicates that the model did not show lack of fit. The responses reveal that linear model term (B), (C), (D), interactive model term (AB), (AD), (BC), and quadratic model term (D^2) are significant ($p<0.05$), but the model terms, (A), (BD), (A^2), (B^2) and (C^2) were more significant than the other terms (P-values are less than 0.0001). The P-value greater than 0.1 means that the model terms are insignificant. Also, the F value of 29.93 implies that the model is significant.

Table 3.3 indicates the Fit Statistics for the lactic acid yield. Adequate precision measures the signal to noise ratio due to random error, a ratio greater than 4 is desirable, and the ratio of 17.067 for the production of lactic acid indicates an adequate signal. Therefore, the model can be used to navigate the design space. The coefficient of variation (CV) indicates the degree of precision with which the treatments are compared. Usually, the higher the CV, the less reliable is the experiment. In these experiments, the low CV (1.15%) indicates highly reliable experimental results.

Table 3.2: ANOVA for Quadratic Model of Lactic Acid Production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	299.79	14	21.41	29.93	**< 0.0001**	**significant**
A-Temperature	30.60	1	30.60	42.77	< 0.0001	
B-Time	8.76	1	8.76	12.24	0.0032	

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Source	Sum of Squares	df	Mean Square	F-value	p-value	
C-Concentration	5.90	1	5.90	8.25	0.0116	
D-pH	7.37	1	7.37	10.30	0.0058	
AB	10.08	1	10.08	14.09	0.0019	
AC	2.03	1	2.03	2.84	0.1127	
AD	5.41	1	5.41	7.56	0.0149	
BC	12.78	1	12.78	17.86	0.0007	
BD	70.98	1	70.98	99.20	< 0.0001	
CD	0.3306	1	0.3306	0.4621	0.5070	
A^2	65.99	1	65.99	92.22	< 0.0001	
B^2	19.29	1	19.29	26.96	0.0001	
C^2	85.31	1	85.31	119.22	< 0.0001	
D^2	0.1036	1	0.1036	0.1448	0.7089	
Residual	10.73	15	0.7155			
Lack of Fit	8.10	10	0.8104	1.54	0.3307	not significant
Pure Error	2.63	5	0.5257			
Cor Total	310.53	29				

Fit Statistics for the Lactic Acid Production Process

Table 3.3 : Fit Statistics for Quadratic Model of Lactic Acid Production

Statistical Parameter	Value
Standard Deviation	0.8459
Mean response	73.69
Coefficient of variance (%)	1.15
R^2	0.9654
Adjusted R^2	0.9332
Predicted R^2	0.8375
Adequate precision	17.0673

Parametric Effects of Interactive Factors on Yield of Lactic acid

Results obtained from the fermentation of lactic acid from corncob hydrolysate, different glucose (reducing sugar) concentration were subjected to fermentation at different temperature, time and pH in a specifically design medium. The result indicated that, the lactic acid production was affected significantly by the selected fermentation time, temperature, substrate concentration and pH. Similarly, the first order interactions of all the variables were highly significant ($P<0.05$) except the interaction between AC and CD (Temperature and glucose concentration, glucose concentration and pH). From the results in Table 3.2, two-dimensional contour and 3D response plots were obtained (Figures 3.1 to 3.6).

The 3D-surface plot depicted in Figure 3.1 shows the interaction between temperature and fermentation time, with the glucose concentration and pH constant at the center point. The effect of temperature and fermentation time were studied at various points. Effect of temperature was studied at 20^{o}C, 30^{o}C, 40^{o}C, 50^{o}C and 60^{o}C while effect of fermentation time was studied at 12, 48, 84, 120 and 156 hours.

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Increase in temperature leads to an accompanying increase in the lactic acid yield, this is because, higher temperature stimulated the rapid growth of lactic acid bacteria resulting in a rapid decline in pH, and consequently leads to a higher yield of lactic acid. However, the lactic acid production decreased above a temperature of 50 ^{o}C, this is because the lactic acid bacteria responsible for the conversion of sugar to lactic acid has optimum growth between 20^{o}C and 45^{o}C. In this study, the optimum temperature for the lactic acid production process was found to be 42^{o}C. Also, as the fermentation time increases, the lactic acid yield increases too, this is because utilization of total sugar increases as the fermentation time increases, thus reducing the available sugar content in the media (Farooq *et al.*, 2012). The optimum fermentation time found in this study was 120hours.

It is evident from observing Figure 3.2, that as temperature and glucose concentration start rising, lactic acid yield increases slightly.

The 3D-surface plot depicted in Figure 3.3 shows the interaction between temperature and pH, there is an observed increase in the lactic acid yield as the pH increases.

In this study, an optimum lactic acid yield of 72.117% was observed at a pH of 5.6 and glucose concentration of 10grams per liter. However, beyond the optimum pH, a decrease in the yield of lactic acid is observed, this is because the microorganism (lactic acid bacteria), did not alter its metabolic pathway in the pH range of 5.0-7.0. Furthermore, increase in initial pH beyond 6.5 did not improve the lactic acid production. Possibly, the higher initial pH must have brought too much stress on the microorganism metabolic abilities (Vijayakumar *et al.*, 2008). Similar observations were noticed for the plots of Figure 3.4 and 3.6. Reduction in yield is noticeable when the process variables were increased beyond their optimum points.

Table 3.4 shows the optimum values of each process variable for the lactic acid production process. The optimum temperature is 42^{o}C; the optimum fermentation time is 120 hours; the optimum pH is 5.6 and the optimum glucose concentration is 10 grams per liter, giving an optimum lactic acid yield of 72.117%.

This result is in conformity with the optimal values of lactic acid production process from other lignocellulose substrate.

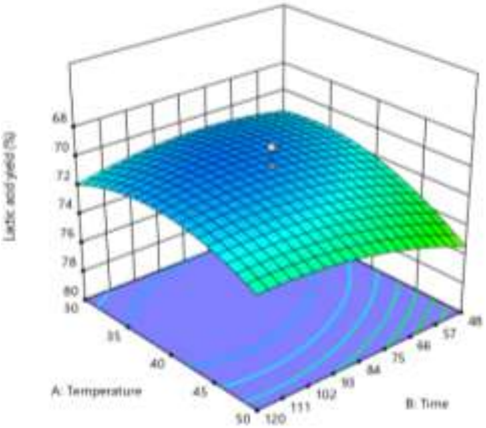

Figure 3.1: 3D plot for the interactive effect between temperature and fermentation time on Lactic acid yield

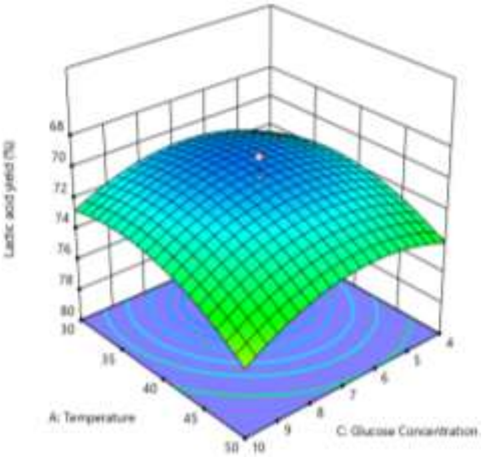

Figure 3.2: 3D plot for the interactive effect between temperature and glucose concentration on Lactic acid yield.

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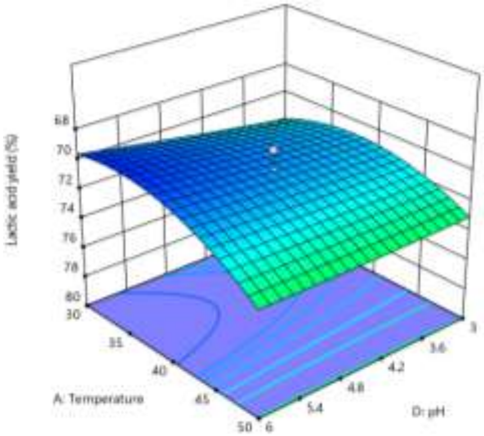

Figure 3.3: 3D plot for the interactive effect between temperature and pH on Lactic acid yield

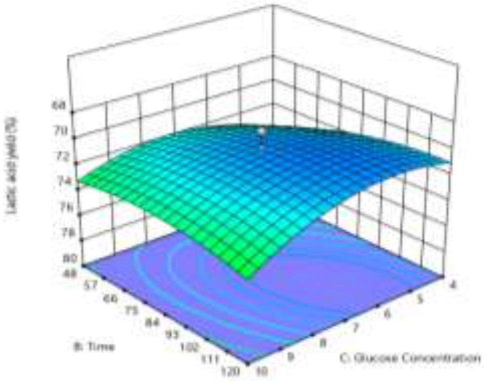

Figure 3.4: 3D plot for the interactive effect between time and glucose concentration on Lactic acid yield

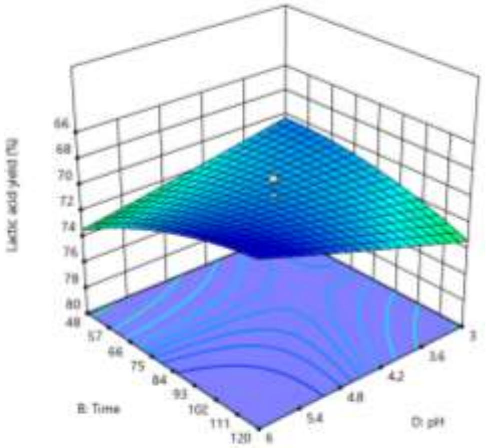

Figure 3.5: 3D plot for the interactive effect between time and pH on Lactic acid yield

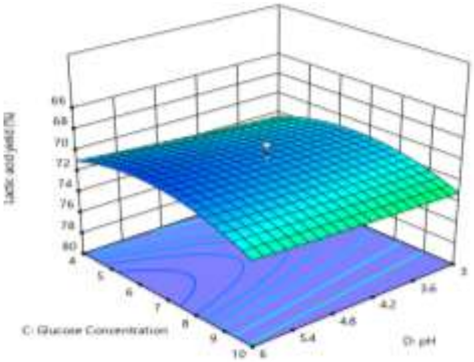

Figure 3.6: 3D plot for the interactive effect between glucose concentration and pH on Lactic acid yield

Optimization solution

Table 3.4: Optimized Values of Process Variables for Lactic Acid Production

Parameters	Value
Temperature(°C)	42
Time(minutes)	120
Glucose concentration(grams/litre)	10
pH	5.6
Lactic acid Yield (%)	72

Kinetics of Lactic Acid Production

In this section, the fermentation of corncob hydrolysate as substrate by lactobacillus delbrueckii (lactic acid bacteria), was critically investigated to obtain certain useful kinetic parameters and to determine the effect of temperature, pH, substrate concentration and fermentation time on the rate of fermentation.

The model (design equation in terms of actual parameters), generated for the lactic acid

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production process was used in carrying out the kinetic study of the process. The optimized values of the process variables obtained from the optimization step were put into the model, temperature and reaction time were varied, while the pH and glucose concentration were held constant at the optimum levels to calculate lactic acid yield.

Tables 3.6 to 3.10 show lactic acid yield at various temperature and time for first order kinetics, while Tables 3.5 to 3.9 show lactic acid yield at various temperature and time for second order kinetics. Also, Figure 3.7 to 3.12 show the plot of –ln (1-X_A) versus time for the first order kinetic model, while Figure 3.13 to 3.16 show the plot of $\frac{1}{1-X_A}$ versus time for the second order kinetic model.

First Order Kinetics

Table 3.5: Lactic acid yield at a temperature of 20°C for a range of 30 – 150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	-ln(1-X_A)
20	30	0.510	0.71335
	60	0.540	0.77653
	90	0.580	0.86750
	120	0.610	0.94161
	150	0.640	1.02165

Table 3.6: Lactic acid yield at a temperature of 30°C for a range of 30 – 150 minute

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	-ln(1-X_A)
30	30	0.650	1.04982
	60	0.680	1.13943
	90	0.710	1.23787
	120	0.725	1.29098
	150	0.748	1.37833

Table 3.7: Lactic acid yield at a temperature of 40°C for a range of 30-150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	-ln(1-X_A)
40	30	0.685	1.15518
	60	0.728	1.30195
	90	0.734	1.32426
	120	0.782	1.52326
	150	0.786	1.54178

Table 3.8: Lactic acid yield at a temperature of 50°C for a range of 30-150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	-ln(1-X_A)
50	30	0.650	1.04982
	60	0.685	1.15518
	90	0.709	1.23443
	120	0.766	1.45243
	150	0.785	1.53712

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Table 3.9: Lactic acid yield at a temperature of 60°C for a range of 30-150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	$-\ln(1-X_A)$
60	30	0.528	0.75078
	60	0.530	0.75502
	90	0.538	0.77219
	120	0.545	0.78745
	150	0.545	0.78745

First Order Kinetic Plots

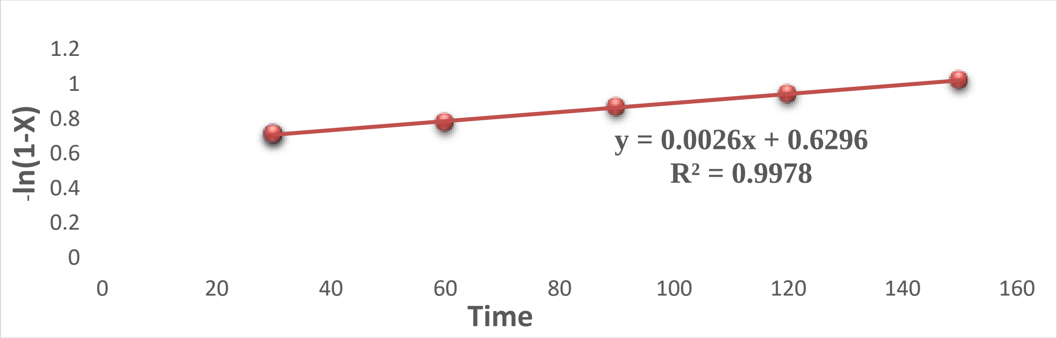

Figure 3.7: Plot of -ln (1-XA) versus time for lactic acid Production at 20°C

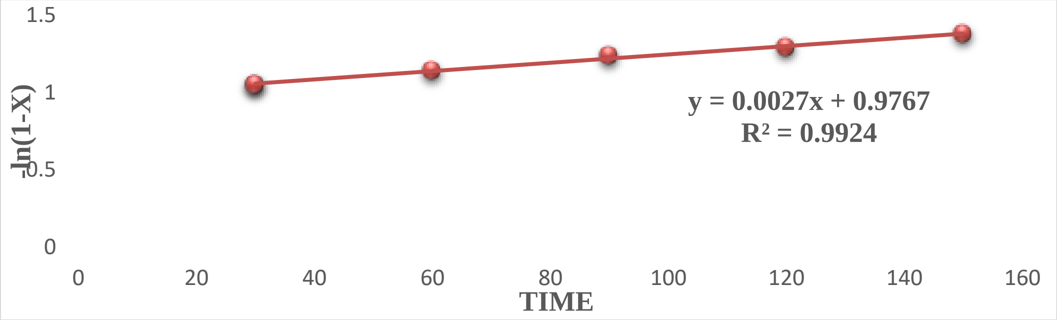

Figure 3.8: Plot of -ln (1-XA) versus time for lactic acid Production at 30°C

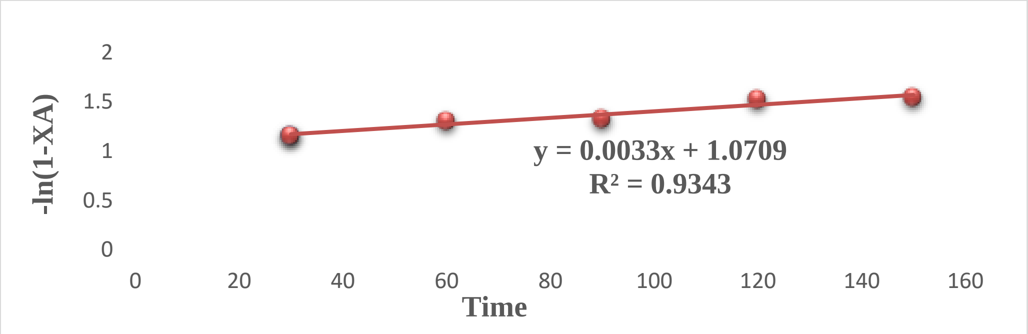

Figure 3.9: Plot of -ln (1-XA) versus Time for Lactic Acid Production at 40°C

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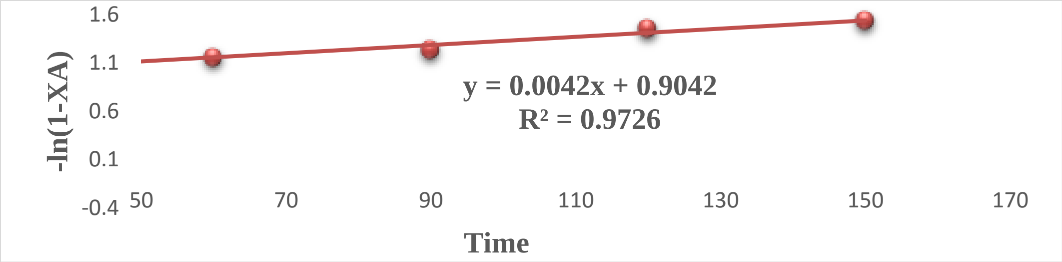

Figure 3.10: Plot of -In (1-XA) versus time for Lactic acid Production at 50°C

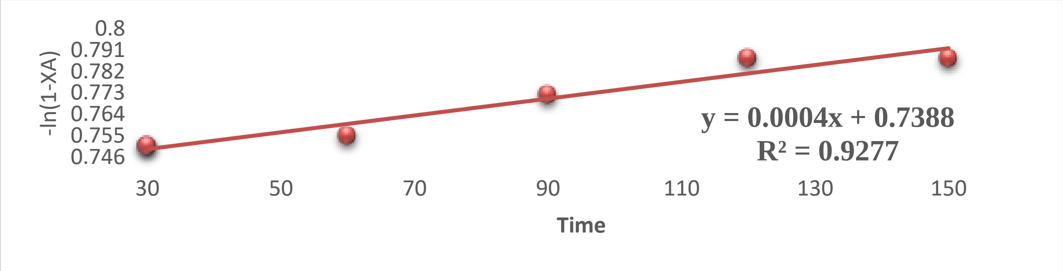

Figure 3.11: Plot of -In (1-XA) versus time for Lactic acid Production at 60°C

Second Order Kinetics

Table 3.12: Lactic acid yield at a temperature of 20°C for a range of 30 – 150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	1/(1-X)
20	30	0.510	2.04082
	60	0.540	2.17391
	90	0.580	2.38095
	120	0.610	2.56410
	150	0.640	2.77777

Table 3.13: Lactic acid yield at a temperature of 30°C for a range of 30 – 150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	1/(1-X)
30	30	0.650	2.85714
	60	0.680	3.12500
	90	0.710	3.44828
	120	0.725	3.63636
	150	0.748	3.96825

Table 3.14: Lactic acid yield at a temperature of 40°C for a range of 30-150 minutes

Temperature (°C)	Time (minutes)	Lactic acid yield, X_A	1/(1-X)
40	30	0.685	3.174603
	60	0.728	3.676471
	90	0.734	3.759398
	120	0.782	4.587156
	150	0.786	4.672897

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Table 3.15: Lactic acid yield at a temperature of 50^{o}C for a range of 30-150 minutes

Temperature (^{o}C)	Time (minutes)	Lactic acid yield, X_A	1/(1-X)
50	30	0.650	2.85714
	60	0.685	3.17460
	90	0.709	3.43643
	120	0.766	4.27350
	150	0.785	4.65116

Table 3.16: Lactic acid yield at a temperature of 60^{o}C for a range of 30-150 minutes

Temperature (^{o}C)	Time (minutes)	Lactic acid yield, X_A	1/(1-X)
60	30	0.528	2.11864
	60	0.530	2.12766
	90	0.538	2.16450
	120	0.545	2.19780
	150	0.545	2.19780

Rate Constant, Pre-exponential Factor, Activation Energy and Rate law

According to the regression coefficient (R^2), there is an excellent fit to the first order kinetic model. Hence, the rate constant, activation energy and pre-exponential factor were calculated based on the first order kinetic model. The activation energy (Ea) and the pre-exponential factor A, were obtained from the plot of lnK versus 1/T as shown in Figure 4.39

Determination of Rate Constant, Activation Energy, Pre-exponential Factor and Rate Law

This study assumes an irreversible reaction mechanism of first and second order for the Lactic acid fermentation process. Figures 3.7 to 3.11 show the plot of -ln (1 – XA) versus time for the first order kinetic model while Figures 4.34 to 4.38 show the plot of $\frac{1}{1-XA}$ versus time for the second order kinetic model. Values of the regression coefficient (R^2), indicated a better fit to the first order kinetic model. Hence, the rate constant, activation energy and pre-exponential factor were calculated based on the first order kinetic model.

First order reaction model was selected and reaction rate constants were found as a function of temperature and glucose concentration, the values of the rate constant at various temperatures is shown in Table 3.17 From the table, at a temperature of 20^{o}C, the rate constant is 0.0026 min^{-1}; at 30^{o}C, it is 0.0027 min^{-1}; at 40^{o}C, it is 0.0033 min^{-1}; at 50^{o}C, it is 0.042 min^{-1} and at 60^{o}C, it is 0.004 min^{-1}.

There was an observed increase in the rate constant between 20^{o}C and 50^{o}C. However, at temperatures above 50^{o}C, the rate constant steadily decreased, this is because, at temperatures above 50^{o}C the lactic acid bacteria (*lactobacillus delbrueckii*) responsible for the conversion of simple sugar to lactic acid begins to die, hence the rate of conversion declined as the temperature exceeds the optimum.

Estimation of the pre-exponential factor and activation energy for lactic acid fermentation from corncob was based on the Arrhenius Equation (Equation 2.14). The slope of the linear regression between In k and 1/T (Figure 3.12) was expressed in terms of -Ea/R with InA as the intercept. The value of the universal gas constant, R was assumed to be 8.314 J.mol-1.K-1, the activation energy, Ea needed for the conversion of glucose into lactic acid was estimated to be 12.79 kJ/mol. The pre-exponential factor, A was calculated to be 2.145 min-1.

The rate laws for the lactic acid production process (fermentation process) at various temperatures are shown in Table 3.18.

At 20^{o}C, the rate law is -rA = 0.0026CA; at 30^{o}C, the rate law is -rA = 0.0027CA, at 40^{o}C, the rate law is -rA = 0.033CA, at 50^{o}C, the rate law is -rA = 0.0042CA and at 60^{o}C, the rate law is -rA = 0.0004CA.

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Table 3.17: Values of rate constant, K at various temperatures

Temperature, °C (Kelvin)	Rate constant K	lnK	1/T (K^{-1})
20 (293)	0.0026	-5.952	0.0034
30 (303)	0.0027	-5.915	0.0033
40 (313)	0.0033	-5.714	0.0032
50 (323)	0.0042	-5.473	0.0031
60 (333)	0.0004	-7.824	0.0030

Table 3.18: Rate law at various temperatures

Temperature, °C (Kelvin)	Rate law
20 (293)	$-r_A = 0.0026C_A$
30 (303)	$-r_A = 0.0027C_A$
40 (313)	$-r_A = 0.0033C_A$
50 (323)	$-r_A = 0.0042C_A$
60 (333)	$-r_A = 0.0004C_A$

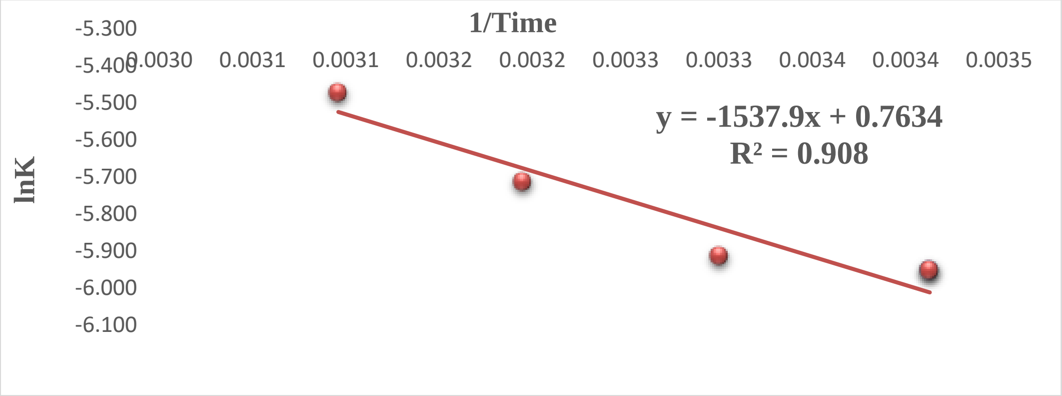

Figure 3.12: Arrhenius plot of ln k against 1/T for the lactic acid Production process

CONCLUSSION

Based on the results obtained from this study and the subsequent discussion of results, the following conclusions can be established from the study. Lactic acid was successfully produced from corncob through fermentation process using lactic acid bacteria. Response surface methodology (RSM) coupled with design expert was successfully used to model and optimize the process. The results indicated that the lactic acid produced was affected significantly by the selected fermentation time, temperature, glucose concentration and pH. From the numerical optimization carried-out using design expert 11 software, the optimum operating conditions for the maximum lactic acid production were temperature 42°C, pH 5.6, reaction time 120 hours and glucose concentration of 10gram per liter, while the optimum yield of lactic acid for this condition was found to be 72.12.6%.

The developed model was tested and validated for adequacy by substituting random experimental values as input parameters and the output parameters from the developed model were close to the experimental values. Based on this study, it is evident that the chosen method of optimization was efficient, and reliable. From this, it can be concluded that the selected model was adequate to fit the data of response variable.

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To date, no reports are available in the literature regarding optimization of acid hydrolysis of corncob. In the present work, sulfuric acid hydrolysis of corncob was successfully carried out using factorial experiment design method, by employing the central composite design (CCD), and using response surface method (RSM) to optimize the hydrolysis process.

Kinetics of the lactic acid production process was investigated; the kinetics of the process was best described by the first order kinetic model and useful kinetic parameters were determined. The values of the rate constant at various temperatures were determined to be, 0.0026 min^{-1} at $20^{o}C$; 0.0027 min^{1} at $30^{o}C$; 0.0033 min^{-1} at $40^{o}C$; 0.042 min^{-1} at $50^{o}C$ and 0.004 min^{-1} at $60^{o}C$. The value of the universal gas constant, R was assumed to be 8.314 J.mol-1.K-1, the activation energy, Ea needed for the conversion of glucose into lactic acid was estimated to be 12.79 kJ/mol. The pre-exponential factor A was calculated to be 2.145 min-1.

The rate laws for the lactic acid production process (fermentation process) at various temperatures are at $20^{o}C$, the rate law is -rA = 0.0026CA; at $30^{o}C$, the rate law is -rA = 0.0027CA, at $40^{o}C$, the rate law is -rA = 0.033CA, at $50^{o}C$, the rate law is -rA = 0.0042CA and at $60^{o}C$, the rate law is -rA = 0.0004CA.

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