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Statistical Optimization of Dilute Acid Hydrolysis of Corncob Biomass for Fermentable Sugar Production in Lactic Acid Bioprocessing

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ABSTRACT

This study evaluated dilute sulfuric acid hydrolysis for the conversion of corncob waste into glucose. The process was optimized using Response Surface Methodology (RSM) based on Central Composite Design (CCD). The effects of four process variables sulfuric acid concentration, temperature, reaction time, and biomass loading were investigated on reducing sugar yield. The optimum conditions for hydrolysis were determined as 0.8 M sulfuric acid concentration, 110°C temperature, 105 min reaction time, and 40 g biomass loading, which gave a maximum reducing sugar yield of 36.921 g/L. Statistical analysis showed that acid concentration, hydrolysis time, biomass loading, and temperature had significant effects on yield with p-values of 0.0270, 0.0050, 0.0001, and 0.0028, respectively. It was observed that high acid concentration and prolonged hydrolysis time led to a decline in sugar yield due to sugar degradation. The model showed excellent correlation between predicted and experimental yields, with a coefficient of determination (R^2) of 0.9654, indicating a well-fitted and reliable model. The results demonstrate that dilute acid hydrolysis of corncob is effective for glucose production and can be optimized using RSM for maximum yield.

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INTRODUCTION

The increasing demand for renewable energy and value added chemicals has driven research toward the utilization of lignocellulosic biomass as a sustainable feedstock. Corncob, a major agricultural residue generated during maize processing, is abundantly available and often underutilized. It contains approximately 35–45% cellulose, 30–35% hemicellulose, and 15–20% lignin, making it a promising substrate for the production of fermentable sugars such as glucose and xylose. These sugars can serve as precursors for biofuels, organic acids, and other biochemicals. (Ali et al., 2014). Hydrolysis is a critical step in converting lignocellulosic biomass into fermentable sugars. Among the various methods, dilute acid hydrolysis is widely adopted due to its simplicity, short reaction time, and effectiveness in breaking down hemicellulose into monomeric sugars.

Sulfuric acid is commonly used because it is inexpensive and provides high hydrolysis efficiency. However, the process is sensitive to operating conditions such as acid concentration, temperature, reaction time, and biomass loading. (Yah et al., 2010). Improper control of these parameters often results in low sugar yield and the formation of degradation products like furfural and 5-hydroxymethylfurfural (HMF), which inhibit downstream fermentation. (Amenaghawon et al., 2014). To maximize sugar yield while minimizing inhibitor formation, process optimization is essential. Response Surface Methodology (RSM) is a statistical technique that evaluates the effects of multiple variables and their interactions, allowing for efficient optimization with fewer experimental runs.

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Central Composite Design (CCD) under RSM has been successfully applied in optimizing biomass hydrolysis for sugar production. Several studies have reported the optimization of dilute acid hydrolysis for different feedstocks, but limited work has focused on corncob under a wide range of conditions using CCD-RSM to achieve maximum reducing sugar yield. (Adoga et al., 2026). Despite the potential of corncob as a feedstock, there remains a need to establish optimal hydrolysis conditions that balance high sugar yield with minimal degradation.

Understanding the influence of process variables and their interactions is crucial for developing an efficient and scalable process for glucose production. In this work, dilute sulfuric acid hydrolysis of corncob was investigated and optimized using RSM based on Central Composite Design. The effects of sulfuric acid concentration, temperature, reaction time, and biomass loading on reducing sugar yield were evaluated. Statistical analysis was performed to determine significant factors and develop a predictive model. The study addresses the gap in identifying optimal conditions for corncob hydrolysis to maximize glucose yield for subsequent bioconversion processes.

MATERIALS AND METHODS

Materials and Substrate Preparation

Red and white corn cobs were collected from Ofana Aduma's farm in Cross River State, Nigeria, and used as the lignocellulosic substrate. The cobs were washed with tap water to remove dirt and impurities, drained, and oven-dried at 60°C to constant weight. The dried cobs were mechanically reduced to small pieces using a hammer mill, then ground in a milling machine to obtain particles of approximately 2 mm size. Sieve analysis was performed, and the powdered sample was stored in airtight containers to increase surface area and enhance contact between the biomass and dilute acid during hydrolysis. The lactic acid-producing strain Lactobacillus delbrueckii IFST-1 was obtained from the Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria. All chemicals used were of analytical grade. Figure 1 shows the raw corn cob and the ground sample. (Adoga et al., 2026)

Pretreatment

Pretreatment was carried out to reduce cellulose crystallinity, increase porosity, and improve sugar yield while minimizing carbohydrate degradation and inhibitor formation. Dilute sulfuric acid pretreatment was employed. Corn cob powder was mixed with 1.5% H_2SO_4 at a liquid-to-solid ratio of 10:1 (v/w). The mixture was placed in an autoclave and heated at 120°C for 30 min. After cooling, the slurry was filtered. The filtrate was preserved for fermentation, while the solid residue was washed twice with distilled water to remove residual acid and retained for hydrolysis. (Hu et al., 2010)

Dilute Acid Hydrolysis

Hydrolysis experiments were designed using Response Surface Methodology based on Central Composite Design (CCD) in Design Expert 11 software. Thirty experimental runs were conducted to study the effects of biomass loading, acid concentration, temperature, and reaction time. The hydrolysis reactions were carried out in 250 mL conical flasks serving as batch reactors. Weighed amounts of pretreated corn cob were placed in flasks, and prepared H_2SO_4 solutions of varying concentrations were added according to the experimental design. The flasks were sealed with aluminium foil and placed in an autoclave at the specified temperature and time. After hydrolysis, the samples were cooled to room temperature and vacuum filtered to separate the liquid hydrolysate from the solid residue containing non-fermentable lignin. The solid residue was washed twice with distilled water, and the filtrate was combined with the pretreatment liquor for analysis. (Miller, 1959)

pH Adjustment and Hydrolysate Preparation

Before fermentation, the pH of the hydrolysate was adjusted to 5.0 using a concentrated NaOH solution added dropwise with constant stirring. The hydrolysate was then sterilized in an autoclave at 121°C for 15 min.

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Glucose concentration in the hydrolysate was concentrated by evaporation using a rotary evaporator at 100°C for 45 min to obtain the desired sugar levels for fermentation.

Analytical Methods

Glucose concentration was determined using the 3,5-dinitrosalicylic acid (DNS) method. Reducing sugars reduce DNS to 3-amino-5-nitrosalicylic acid under alkaline conditions, producing a colour change measurable at 540 nm. The DNS reagent was prepared by dissolving 10 g NaOH, 2 g phenol, 0.5 g sodium sulphite, 182 g Rochelle salt, and 10 g 3,5-dinitrosalicylic acid in 800 mL distilled water, then making up to 1 L. The reagent was stored at 4°C in a dark bottle. A glucose standard curve was prepared using stock solutions of 25–100 µg/mL. Absorbance was measured at 540 nm, and the reducing sugar concentration in the hydrolysate was calculated from the calibration curve. Sugar yield was calculated as:

$$\text{Sugar Yield (\%)} = \frac{gram\ of\ Sugar}{raw\ material\ used} \times 100 \qquad (1)$$

Experimental Design and Optimization

Thirty experiments were designed using CCD with four independent variables: biomass loading, acid concentration, temperature, and time. The coded and actual levels of the variables are presented in Table 1. The experimental design matrix is shown in Table 2, while fig 2 shows Samples after pretreatment and sterilization equipment. Analysis of Variance (ANOVA) was performed to determine the significance of the model terms and their interactions. The response surface model was used to predict the optimum conditions for maximum reducing sugar yield. (Adoga et al., 2026).

Table 1: Experimental Range and Levels of Independent Process Variables for Acid Hydrolysis of Corncob

		Coded Factor Levels				
Independent Process Variables	**Symbols**	**-2**	**-1**	**0**	**+1**	**+2**
Biomass Loading (grams)	A	10	20	30	40	50
Acid Concentration (Molar)	B	0.2	0.4	0.6	0.8	1.0
Temperature (degree Celsius)	C	80	90	100	110	120
Time (Minutes)	D	60	75	90	105	120

Table 2: Central Composite Design Matrix for Acid Hydrolysis of Corncob

Run	**Biomass Loading (grams)**	**Acid Concentration (Molar)**	**Temperature (degree Celsius)**	**Time (Minutes)**
1	20	0.4	90	75
2	40	0.4	90	75
3	20	0.8	90	75
4	40	0.8	90	75
5	20	0.4	110	75
6	40	0.4	110	75
7	20	0.8	110	75
8	40	0.8	110	75
9	20	0.4	90	105
10	40	0.4	90	105
11	20	0.8	90	105
12	40	0.8	90	105
13	20	0.4	110	105
14	40	0.4	110	105
15	20	0.8	110	105

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Run	Biomass Loading (grams)	Acid Concentration (Molar)	Temperature (degree Celsius)	Time (Minutes)
16	40	0.8	110	105
17	10	0.6	100	90
18	50	0.6	100	90
19	30	0.2	100	90
20	30	1	100	90
21	30	0.6	80	90
22	30	0.6	120	90
23	30	0.6	100	60
24	30	0.6	100	120
25	30	0.6	100	90
26	30	0.6	100	90
27	30	0.6	100	90
28	30	0.6	100	90
29	30	0.6	100	90
30	30	0.6	100	100

Fig. 1: Corn cob samples: (a) Raw corn cob (b) Ground corn cob powder

Fig 2: Samples after pretreatment and sterilization equipment: (a) Reducing sugar samples. (b) Autoclave

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RESULTS AND DISCUSSION

Standard Curve for Reducing Sugar Determination

The calibration curve for glucose determination was established using the 3,5-dinitrosalicylic acid (DNS) method at concentrations of 25, 50, 75, and 100 µg/mL. Absorbance was measured at 540 nm. As shown in Table 1, absorbance increased linearly with glucose concentration. Linear regression gave the equation $y = 0.0109x - 0.0925$ with $R^2=0.9993$, confirming excellent linearity and suitability for quantifying reducing sugars in the hydrolysate. The standard curve is shown in Fig. 3.

Table 3. Glucose Concentration and Corresponding Absorbance

Glucose Concentration	Absorbance
25	0.1820
50	0.4411
75	0.7350
100	0.9901

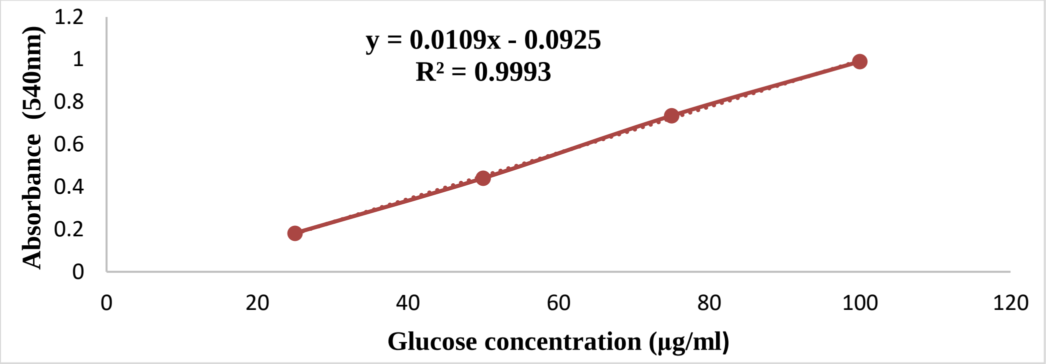


Fig. 3. Standard curve for glucose determination using DNS Method

Model Development for Glucose Yield

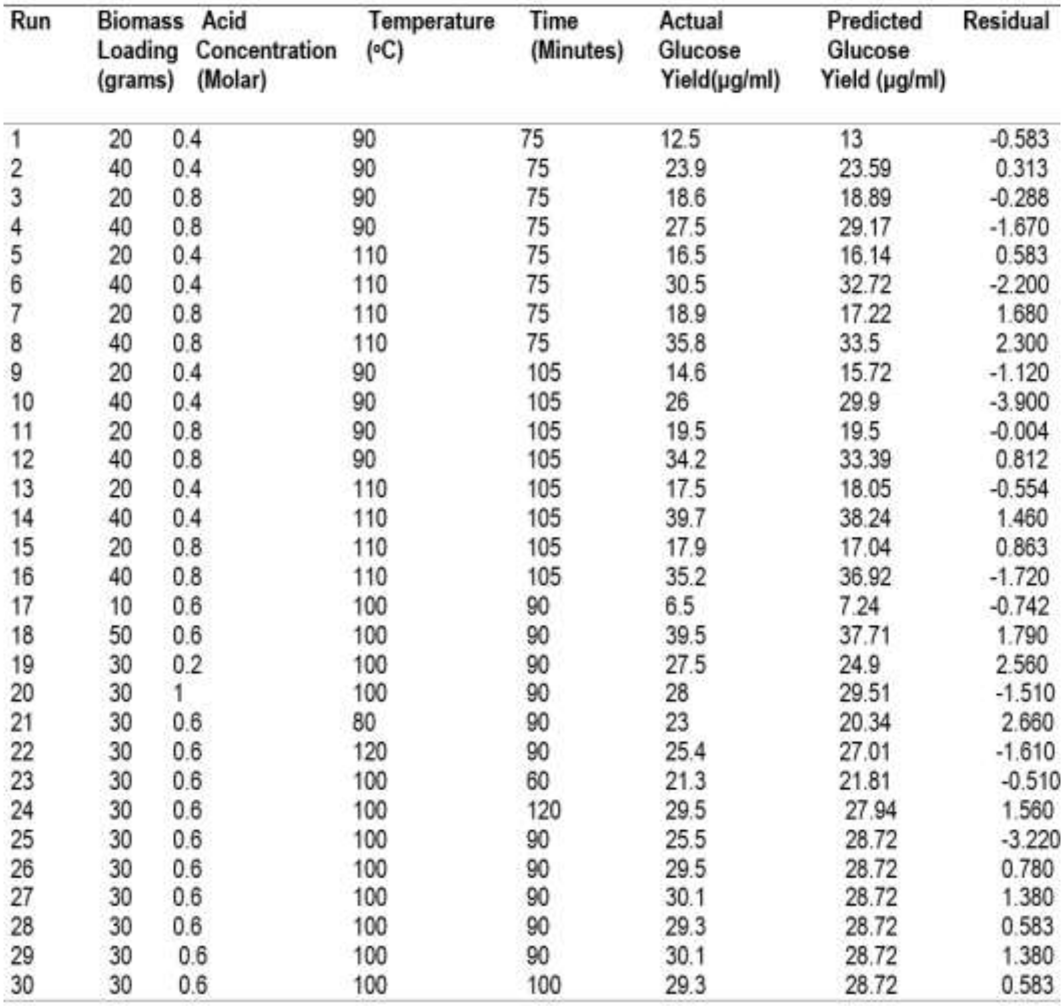
A Central Composite Design was used to study the effects of biomass loading, acid concentration, temperature, and reaction time on reducing sugar yield. Thirty experimental runs were conducted. The experimental and predicted yields are presented in Table 4. The quadratic regression model in coded terms is:

$$\text{Reduced sugar yield } (\mu g/ml) = +28.72 + 7.62A + 1.14B + 1.67C + 1.53D - 0.0750AB + 1.50AC + 0.9000AD - 1.20BC - 0.5250BD - 0.2000CD - 1.56A^2 - 0.3729B^2 - 1.26C^2 - 0.9604D^2 \qquad (2)$$

In actual terms

$$\text{Reduced sugar yield } (\mu g/ml) = -198.367 - 0.319583(\text{Corncob concentration}) + 93.7708(\text{Acid concentration}) + 2.7175(\text{Temperature}) + 0.928889(\text{Time}) - 0.035(\text{Corncob concentration})(\text{Acid concentration}) + 0.015(\text{Corncob concentration})(\text{Temperature}) + 0.006(\text{Corncob concentration})(\text{Time}) - 0.6(\text{Acid concentration})(\text{Temperature}) - 0.175(\text{Acid concentration})(\text{Time}) - 0.00133333(\text{Temperature})(\text{Time}) - 0.0156042(\text{Corncobconcentration})^2 - 9.32292(\text{Acidconcentration})^2 - 0.0126042(\text{Temperature})^2 - 0.00426852(\text{Time})^2 \qquad (3)$$

Table 4: Central Composite Design for



Run	Biomass Loading (grams)	Acid Concentration (Molar)	Temperature (°C)	Time (Minutes)	Actual Glucose Yield(µg/ml)	Predicted Glucose Yield (µg/ml)	Residual
1	20	0.4	90	75	12.5	13	-0.583
2	40	0.4	90	75	23.9	23.59	0.313
3	20	0.8	90	75	18.6	18.89	-0.288
4	40	0.8	90	75	27.5	29.17	-1.670
5	20	0.4	110	75	16.5	16.14	0.583
6	40	0.4	110	75	30.5	32.72	-2.200
7	20	0.8	110	75	18.9	17.22	1.680
8	40	0.8	110	75	35.8	33.5	2.300
9	20	0.4	90	105	14.6	15.72	-1.120
10	40	0.4	90	105	26	29.9	-3.900
11	20	0.8	90	105	19.5	19.5	-0.004
12	40	0.8	90	105	34.2	33.39	0.812
13	20	0.4	110	105	17.5	18.05	-0.554
14	40	0.4	110	105	39.7	38.24	1.460
15	20	0.8	110	105	17.9	17.04	0.863
16	40	0.8	110	105	35.2	36.92	-1.720
17	10	0.6	100	90	6.5	7.24	-0.742
18	50	0.6	100	90	39.5	37.71	1.790
19	30	0.2	100	90	27.5	24.9	2.560
20	30	1	100	90	28	29.51	-1.510
21	30	0.6	80	90	23	20.34	2.660
22	30	0.6	120	90	25.4	27.01	-1.610
23	30	0.6	100	60	21.3	21.81	-0.510
24	30	0.6	100	120	29.5	27.94	1.560
25	30	0.6	100	90	25.5	28.72	-3.220
26	30	0.6	100	90	29.5	28.72	0.780
27	30	0.6	100	90	30.1	28.72	1.380
28	30	0.6	100	90	29.3	28.72	0.583
29	30	0.6	100	90	30.1	28.72	1.380
30	30	0.6	100	100	29.3	28.72	0.583

Optimization of Acid Hydrolysis of Corncob

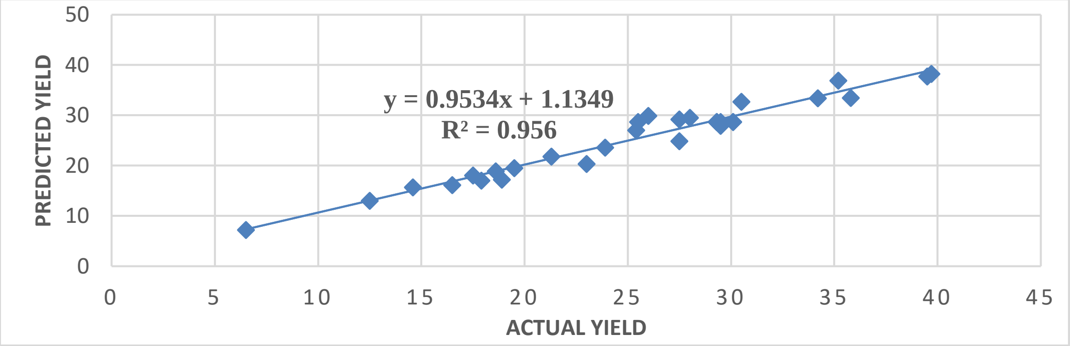


Fig 4: Predicted vs actual glucose yield

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Table 5. ANOVA for quadratic model of acid hydrolysis

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value significant**
Model	1731.34	14	123.67	23.73	< 0.0001
A-Biomass loading	1392.33	1	1392.33	267.14	< 0.0001
B-Acid con	31.28	1	31.28	6	0.027
C-Temperature	66.67	1	66.67	12.79	0.0028
D-Time	56.43	1	56.43	10.83	0.005
AB	0.09	1	0.09	0.0173	0.8972
AC	36	1	36	6.91	0.019
AD	12.96	1	12.96	2.49	0.1357
BC	23.04	1	23.04	4.42	0.0528
BD	4.41	1	4.41	0.8461	0.3722
CD	0.64	1	0.64	0.1228	0.7309
A^2	66.79	1	66.79	12.81	0.0027
B^2	3.81	1	3.81	0.7319	0.4057
C^2	43.57	1	43.57	8.36	0.0112
D^2	25.3	1	25.3	4.85	0.0436
Residual	78.18	15	5.21		
Lack of Fit	64.61	10	6.46	2.38	0.1753
Pure Error	13.57	5	2.71		
Cor Total	1809.52	29			

Statistical Analysis and Model Adequacy

ANOVA results are given in Table 5. The model is significant with F-value of 23.73 and p-value <0.0001. Biomass loading, acid concentration, temperature, and time significantly affected yield with p-values of <0.0001, 0.0270, 0.0028, and 0.0050 respectively. The interaction AC and quadratic terms A^2, C^2, D^2 were also significant. Lack-of-fit was not significant (p = 0.1753), confirming model fit. Fit statistics in Table 5 show R^2=0.9568, adjusted R^2=0.9165, and predicted R^2=0.7835. Adequate precision of 19.20 indicates the model is suitable for optimization.

Table 5. Fit statistics for the model

Statistical parameter	**Value**
Standard deviation	2.28
Mean of response	25.39
Coefficient of variance (%)	8.99
R^2	0.9568
Adjusted R^2	0.9165
Predicted R^2	0.7835
Adequate precision	19.2008

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Effect of Process Variables on Glucose Yield

The response surface plots in Fig. 5-10 illustrates the interactions. Glucose yield increased with biomass loading and acid concentration up to 40 g and 0.8 M. Further increase led to sugar degradation. Yield also increased with temperature and time up to 110°C and 105 min, beyond which degradation products formed.

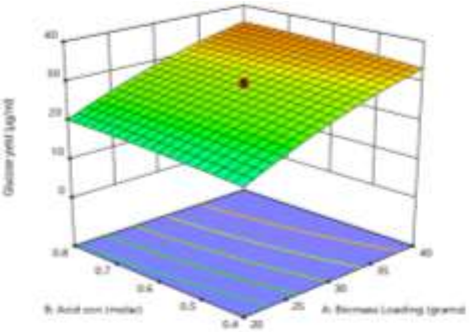

Fig. 5. 3D plot: biomass loading vs acid concentration.

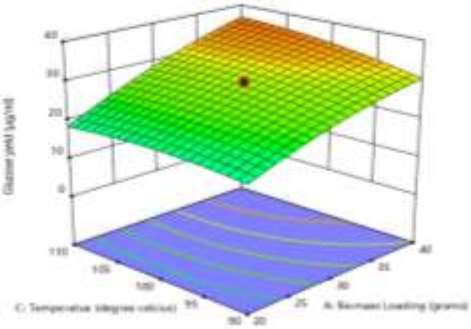

Fig. 6. 3D plot: biomass loading vs temperature.

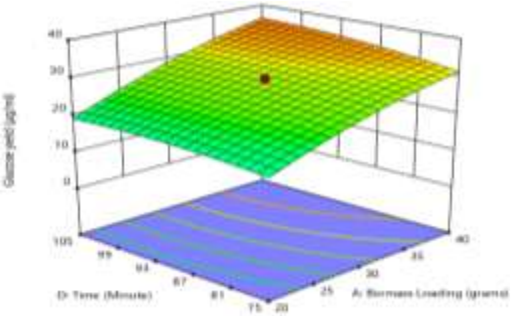

Fig. 7. 3D plot: biomass loading vs time

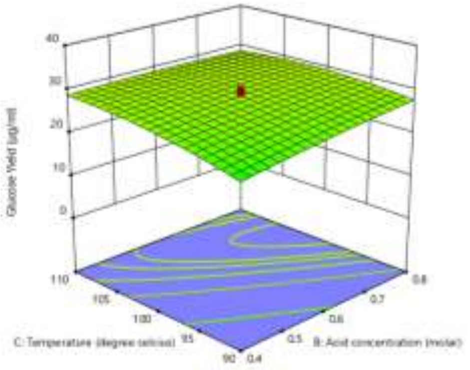

Fig. 8. 3D plot: Temperature vs Acid concentration

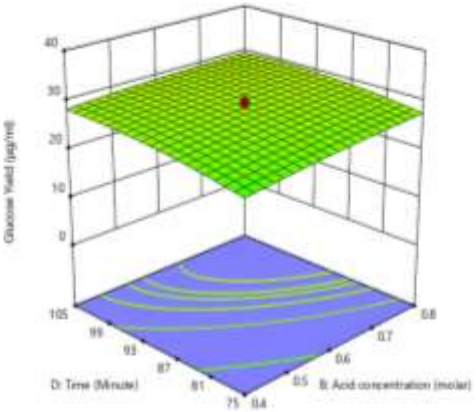

Fig. 9. 3D plot: Time vs Acid concentration

3.5 Optimization of Hydrolysis Conditions

Numerical optimization gave optimum conditions at 40 g biomass loading, 0.8 M acid concentration, 110°C, and 105 min. The predicted glucose yield was 36.9 g/L with desirability 0.975, as shown in Table 6. These conditions maximize sugar yield while minimizing degradation**.**

Table 6. Optimized process variables for acid hydrolysis of corncob

Parameters	Values	Desirability
Biomass loading (grams)	40	
Acid concentration (molar)	0.8	
Temperature (degree Celsius)	110	
Time (hours)	105	
Glucose Yield(g/l)	36.	0.975

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CONCLUSION

In this study, corncob, an agricultural waste, was successfully utilized for glucose production through dilute sulfuric acid hydrolysis. Response Surface Methodology based on Central Composite Design was employed to optimize the process and evaluate the effects of sulfuric acid concentration, temperature, reaction time, and biomass loading on reducing sugar yield. The optimum hydrolysis conditions were determined as 0.8 M sulfuric acid concentration, 110°C temperature, 105 min reaction time, and 40 g biomass loading. Under these conditions, the maximum reducing sugar yield obtained was 36.921 g/L. Statistical analysis indicated that acid concentration, hydrolysis time, biomass loading, and temperature had significant effects on yield, with p-values of 0.0270, 0.0050, 0.0001, and 0.0028, respectively.

It was observed that excessively high acid concentration and prolonged hydrolysis time led to a decline in sugar yield due to the degradation of monosaccharides into inhibitory by-products. The developed quadratic model showed excellent agreement between predicted and experimental values, with a coefficient of determination R^2=0.9654, confirming the reliability and adequacy of the model for predicting glucose yield. The results demonstrate that dilute acid hydrolysis of corncob is an effective method for producing fermentable sugars, and Response Surface Methodology provides a reliable approach for process optimization. The findings suggest that corncob can serve as a promising low-cost feedstock for bio-based chemical production, contributing to waste valorization and sustainable biorefinery development. Future work may focus on fermentation of the optimized hydrolysate for lactic acid production and scaling up the process for industrial application..

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