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Comparative Optimization of Acid and Alkaline Pretreatment of Cassava Bagasse for Optimum Yield of Fermentable Sugar for the Production of Biobutanol

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Abstract

The constantly growing population of the world and its accompanying energy utilization as well as the search for cleaner and sustainable energy sources for various activities of mankind has pioneered various research work in the field of biofuels over the years. This study compared the effects of both acid and alkaline pretreatment methods on lignocellulosic biomass (cassava bagasse) by comparing their yields of fermentable sugar for the production of biobutanol. The Response Surface Methodology (RSM) tool was used to ascertain the optimum conditions for both pretreatment methods by optimizing the input variables which are acid/alkaline concentration, temperature, and time of pretreatment. Using a central composite design (CCD), the maximum fermentable sugar yield obtained from the acid pretreatment was 798.52 mg/L at 117.35 °C, 30.98 minutes, and 2.1 % (w/w) H_2SO_4, while that of the alkaline pretreatment was a sugar yield of 1382.36 mg/L, at 115.41 °C, 32.84 minutes and an alkaline concentration of 2.32% (w/w) NaOH. Overall, the results show that both pretreatment methods are effective for cassava bagasse but with alkaline having a higher yield.

1. Introduction

The ever-increasing population of the world, accompanied by its energy demands which are mainly met by the use of fossil fuel resources has put humanity on the brink of an extreme energy and environmental crisis [1]. Governments, as well as the scientific community, have been prompted to look for an alternate source of energy due to the rapidly depleting fossil fuels and their detrimental effects on the environment [2]. Apart from the exhaustion of fossil fuels, other worldwide environmental concerns such as global warming, the greenhouse effect, and climate change need to be addressed [3]. As a result, the world needs sustainable, alternative energy to replace fossil fuels. Under this circumstance, the current demand for petroleum fuels may be offset by the profitable production of biofuels. Biofuels are an eco-friendly alternative to non-renewable fossil fuels due to their lower carbon emissions [4-5].

The term “biofuel” simply means any fuel that comes from biomass, which includes animal waste and plant or algal matter. Biobutanol represents a promising biofuel that outperforms bioethanol in terms of energy content, octane number, low water solubility, and low corrosivity. Biobutanol can be made from a range of raw materials or renewable agricultural residues and crops using the acetone-butanol-ethanol (ABE) fermentation process by anaerobic bacteria [6].

Lignocellulosic biomass represents one of the best sources that can be effectively harnessed to serve as an alternative source of energy for mankind [7]. Lignocellulosic biomass is a plant composite which consists of lignin, cellulose and hemicellulose as parts of its cell wall structure. The cellulose and hemicellulose can easily be broken down into sugar monomers [8], which can further be utilized as a substrate for the fermentation process for biofuel production, while lignin is generally regarded as the glueing material that binds all the constituents of the lignocellulosic biomass together [9]. Lignocellulosic biomass such as wheat straw, cassava bagasse, corn cob, rice straw are readily available as they are usually generated from forestry and agricultural activities and are also generally considered as waste productions which are dumped indiscriminately in the environment [10]. In terms of landmass cultivated, Cassava (*Manihot spp*) is ranked seventh (7th) in the world’s most significant food crop, serving as the primary source of food for about 800 million people, especially in the poorest tropical regions like Sub-Sahara Africa [11]. However, the industrial processing of cassava crops generates a substantial amount of solid waste known as bagasse, which is typically discarded without proper treatment, posing significant environmental concerns.

Being a carbohydrate-rich product, cassava bagasse has been discovered as a promising potential carbon source for the biological synthesis of value-added products like bioethanol and biobutanol [12] . There are three major processes involved in converting these lignocellulosic biomasses into these value-added products: pretreatment, enzymatic hydrolysis, and fermentation [13]. Enhancing the yield and efficiency of the lignocellulosic biomass bioconversion requires pretreatment. The complex coexistence of cellulose, hemicellulose, and lignin in lignocellulosic biomass poses a challenge to its natural conversion into valuable products. In this sense, pretreatment is essential to breaking up the intricate nature of lignocellulosic biomass in order to produce value-added products in a sustainable manner [14].

The main goals of pretreatment processes are to increase the surface area of the biomass, dissolve hemicellulose and/or lignin, decrease biomass particle sizes, and free carbohydrates from their lignin bonding. The behaviour and physicochemical characteristics of every lignocellulosic feedstock vary. Therefore, it is vital to apply appropriate pretreatments that are based on the inherent properties of each raw material [15]. Various physical, chemical, and biological processes have been used for the pretreatment of lignocellulosic biomass. Some of the most widely used pretreatment methods are alkaline, acid, steam explosion, ozonolysis, liquid hot water, ammonia fibre explosion, CO_2 explosion, and wet oxidation. To maximize the yield of fermentable sugar during hydrolysis, it's critical to optimize the factors that affect its yield.

Response Surface Methodology (RSM) is widely used to identify correlations between input and response variables in multi-factor regression analysis of experimental results. It has been discovered that the response surface approach, which is based on statistically designed trials, is highly helpful in multivariable process optimization [16].

The aim of this study therefore is to compare the effects of both acid and alkaline pretreatment methods on cassava bagasse by comparing their optimum yields of fermentable sugar for the production of biobutanol, using H_2SO_4 and NaOH for acid and alkaline pretreatment respectively. RSM was used to carry out the experimental design making use of a three-factorial Central Composite Design (CCD) approach to carry out optimization studies to determine the optimum pretreatment conditions (acid/alkaline concentration, temperature, and time) of both the acid and alkaline methods.

2. Materials And Methods

2.1 Feedstock collection and preparation

Drying, grinding, and sieving were all part of the preparation stage. Cassava bagasse was collected from an agro-based farm at Isihor, Edo State, Nigeria. The sample was sun-dried for a total of 30 days to decrease moisture and enhance size reduction. The biomass was certified dried when the observable weight change in 24 hours was less than 1%. The dried biomass sample was ground and sieved after drying to achieve a uniform surface area of 1.5 mm to aid contact between the biomass and the reagents for pretreatment. The sample was then stored in airtight bags and kept in a clean and dry environment.

2.2 Estimation of hemicellulose content

5 mg was measured from the sieved cassava bagasse biomass sample with an analytical weighing scale and placed in a round bottom flask. 250 ml of 0.5 M NaOH solution was added to the sample inside the round bottom flask. The solution was then heated for 60 minutes and then allowed to cool. The pH of the cooled solution was then neutralized after several rounds of diluting with distilled water. The neutral solution was decanted and filtered. The filtrate was then placed in a crucible and allowed to dry in an oven for 24 hours. The dried mass was ascertained with an analytical weighing balance.

2.3 Estimation of lignin content

5 mg was measured from the sieved cassava bagasse biomass sample with an analytical weighing scale and placed in a round bottom flask. Added to it was a 1 M H_2SO_4 solution. The mixture was then heated for 30 minutes in a water bath. The resulting carbonized mixture was then allowed to cool for 1 hour. The pH of the cooled solution was then neutralized after several rounds of diluting with distilled water. The neutral solution was decanted and filtered. The filtrate was then placed in a crucible and allowed to dry in an oven for 24 hours. The dried mass was obtained using an analytical weighing balance.

2.4 Pretreatment of cassava bagasse feedstock

Experiments on feedstock pretreatment were conducted with a constant solid-to-liquid ratio of 5% wt/vol, while the acid/alkaline concentration, temperature, and pretreatment time changed between 1% and 3% vol/vol, 100 ^oC and 130 ^oC, and 15 and 45 minutes, respectively. The solutions were heated in an autoclave (with screw-capped pyrex bottles) at different time ranges as stipulated by the design of the experiment. The samples were then removed and allowed to cool. After cooling, the samples were placed in a beaker and neutralized and the solutions were then separated using a Buchner funnel [16]. 3 ml of the filtrate was taken with a syringe and placed in a test tube, 1 ml of Dinitro Salicylic acid (DNS) solution was added and the resulting solution in the test tube was boiled for 5 minutes until a colour change was observed. A UV spectrophotometer set at a wavelength of 540 nm was then used to measure the absorbance of the solution which was in turn used to determine its sugar content making use of the standard glucose curve.

2.5 Design of experiment

This work was done using a Central Composite Design (CCD) with three factors as its input for optimization. Three independent variables: acid concentration/alkaline concentration (A), time (B), and temperature (C) were used at varied conditions for the pretreatment of cassava bagasse to get the optimum levels for pretreatment. Using Response Surface Methodology (RSM) for optimization, total sugar yield was selected as the response parameter for optimization. Table 1 below shows the coded and actual levels represented as A, B, and C. Different iterations of the experimental design were carried out. The Design Expert 13.0 software was used to implement the experimental design. The F-value and p-value were used to ascertain the importance of the model. The different R^2 values were used to study the efficiency of the regression model while the statistical significance of the model was tested using the Analysis of variance (ANOVA).

Table 1: Coded and actual values of factors

Variables	Units	Symbols	Coded and actual levels		
			-1	0	1
Acid concentration/alkaline concentration	%	A	1	2	3
Time	Min	B	15	30	45
Temperature	◦C	C	100	115	130

3.0 Results and Discussion

3.1 Compositional analysis of cassava bagasse

Table 2 presents the results of the characterization analysis conducted on cassava bagasse, demonstrating the varying percentages of cellulose, hemicellulose, and lignin content in response to different concentrations of alkaline solutions. Cellulose, constituting the primary structural element in plant cell walls, is highly crystalline, providing substantial resistance to enzyme hydrolysis. Hemicellulose acts as a pivotal link between cellulose and lignin, enhancing the rigidity of the cellulose-hemicellulose-lignin network.

The relative ease of hydrolyzing cellulose and hemicellulose compared to lignin underscores the preference for higher concentrations of cellulose and hemicellulose in the quest for optimal fermentable sugar production. Thus, pretreatment methods resulting in lower lignin composition are considered more favourable. According to the data in Table 2, the lignin content was measured as 3%, 6.3%, and 3.3% for alkaline concentrations of 0.5% NaOH, 0.1% NaOH, and 0.1% KOH, respectively.

Table 2: Composition of cassava bagasse from the characterization analysis

Alkaline Concentration	Cellulose (%)	Hemicellulose (%)	Lignin (%)
0.5% NaOH	70	16.7	3
0.1% NaOH	35	32.7	6.3
0.1% KOH	53.7	16	3.3

3.2 Modelling and optimization of total sugar yield for acid pretreatment

3.2.1 Appropriate model determination and model fit statistics

Different models were used to interpret the relationship between the independent variables and the response parameter. The quadratic model offered the most accurate and statistically viable correlation between the input levels and the response. Table 3 shows the model summary statistics and from the adjusted and predicted R^2 values of the quadratic model which was the highest of the different models examined, it can adequately be concluded that the quadratic model provides a sufficient description of the relationship between the response variable and the input variables.

Table 3: Model summary statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	207.08	0.0345	-0.1465	-0.3221	9.395E+05	
2FI	229.22	0.0389	-0.4047	-1.3746	1.688E+06	
Quadratic	**57.23**	**0.9539**	**0.9124**	**0.8849**	**81778.03**	**Suggested**
Cubic	67.31	0.9617	0.8789	0.9335	47271.57	Aliased

Table 4: Model fit statistics

Std. Dev.	57.23	**R^2**	0.9539
Mean	531.42	**Adjusted R^2**	0.9124
C.V. %	10.77	**Predicted R^2**	0.8849
PRESS	81778.03	**Adeq Precision**	12.8517

Table 4 presents a predicted R^2 value of 0.8849, which aligns reasonably well with the adjusted R^2 value of 0.9124, with a difference of less than 0.2. Additionally, Adequate Precision evaluates the signal-to-noise ratio, where a ratio above 4 is considered favourable. The model's Adequate Precision ratio is 12.8517, indicating a sufficient signal.

3.2.2 Analysis of variance (ANOVA) for response surface quadratic model

Table 5: Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.779E+05	9	75321.23	22.99	< 0.0001	significant
A-Acid conc.	4858.48	1	4858.48	1.48	0.2512	
B-Time	1419.33	1	1419.33	0.4333	0.5252	
C-Temp	18272.76	1	18272.76	5.58	0.0398	
AB	366.50	1	366.50	0.1119	0.7449	
AC	2140.43	1	2140.43	0.6534	0.4377	
BC	572.65	1	572.65	0.1748	0.6847	
A^2	4.297E+05	1	4.297E+05	131.17	< 0.0001	
B^2	1.195E+05	1	1.195E+05	36.49	0.0001	
C^2	2.140E+05	1	2.140E+05	65.33	< 0.0001	
Residual	32756.62	10	3275.66			
Lack of Fit	5608.82	5	1121.76	0.2066	0.9458	not significant
Pure Error	27147.80	5	5429.56			
Cor Total	7.106E+05	19				

The model is significant, according to the model's F-value of 22.99. Model terms are significant if the P-value is less than 0.0500. Significant model terms in this instance are C, A^2, B^2, and C^2. The lack of Fit F-value of 0.21 indicates that the difference between the pure error and the lack of fit is not significant. Table 5's ANOVA and test of significance results for the model indicate that the model was significant due to its low p-value (<0.05).

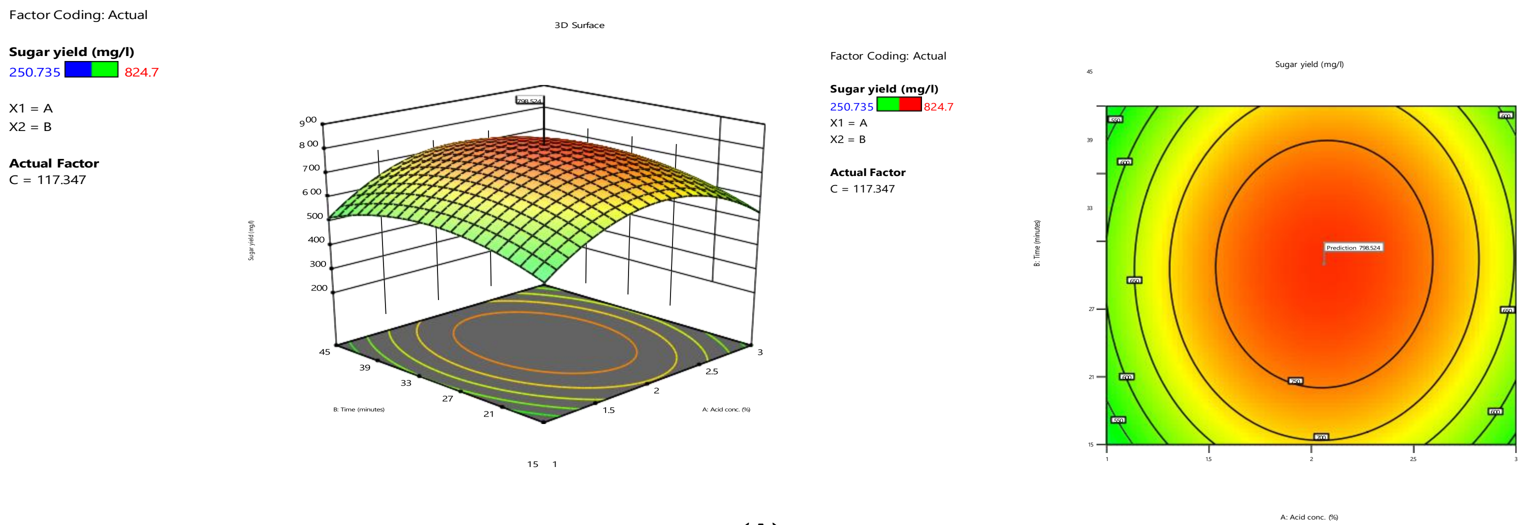
3.2.3 Regression analysis

To fit the response to the model, regression analysis was used, and the yield of fermentable sugar as Y, A – Acid concentration, B – reaction time, and C- reaction temperature. Equation 1 articulates the relationship between the response (the fermentable sugar yield) and the independent factors in terms of actual factors.

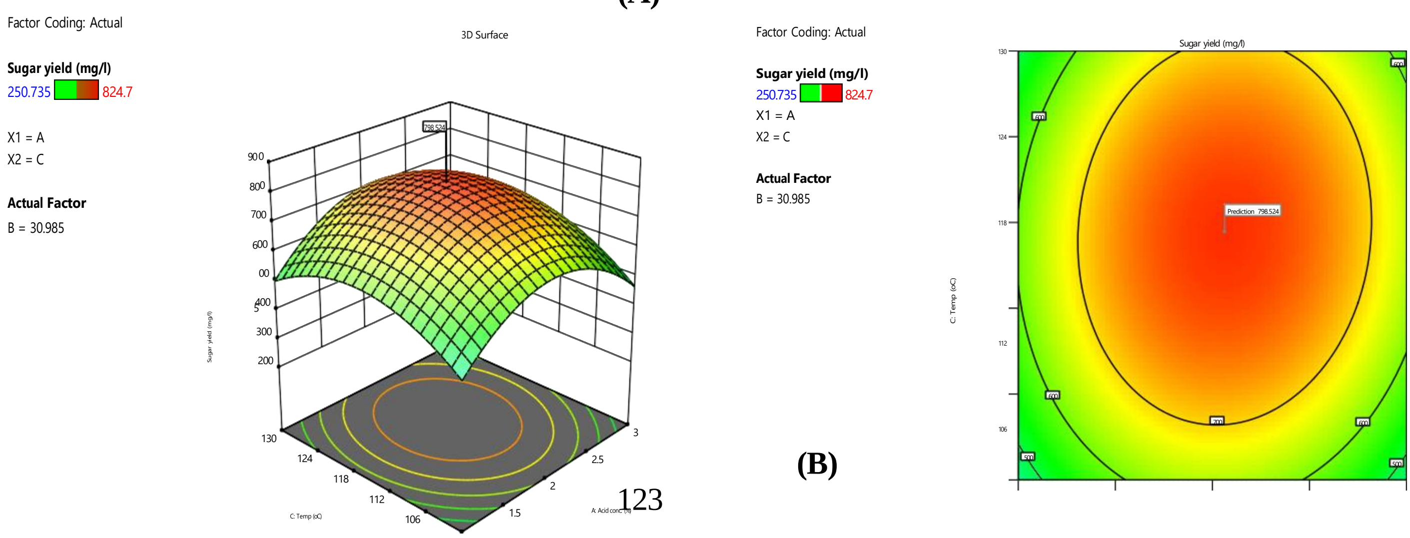
$$Y = -7353.83547 + 570.60642A + 19.73821B + 123.69756C + 0.451230AB + 1.09047AC + 0.037603BC - 172.67156A^2 - 0.404755B^2 - 0.541600C^2 \qquad (1)$$

The regression model formulated shows cassava bagasse fermentable sugar yield as (Y) as a function of Acid concentration (A), Time (B), and Temperature (C).

3.2.4 Response surface plots



(A)



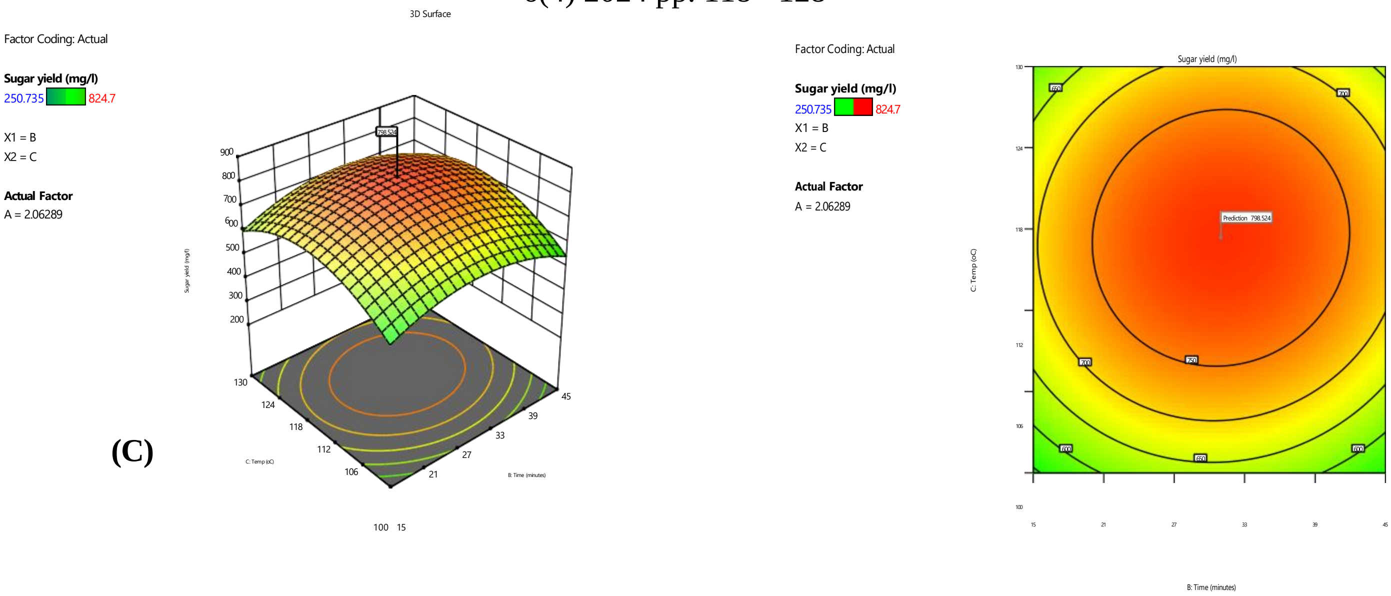
(B)

100

1 1.5 2 2.5 3

A: Acid conc. (%)

100 1



(C)

Figure 1: 3D plots showing the interaction between (A) Acid concentration and reaction time (B) Temperature and acid concentration (C) Temperature and reaction time

3.2.5 Numerical optimization and model validation

Utilizing the created model, the design space was explored and factor choices aligned to maximize the yield of fermentable sugar were identified. The ideal settings, which included a pretreatment temperature of 117.35 ^{o}C, a time of 30.98 minutes, and an acid concentration of 2.1 % (w/w) H_2SO_4, were selected from among the many solutions—more than 100 in total—based on the greatest desirability score. The result of this combination was a fermentable sugar yield of 798.52 mg/L. To determine the success of this study, the optimum values of the input variables were taken to the lab and used to conduct a three-time repeating experiment. the average yield of fermentable sugar was 800.05 mg/L. The response model's validity was confirmed by the close correlation seen between the experimental yield (800.05 mg/L) and the predicted yield (798.52 mg/L).

3.3 Modelling and optimization of total sugar yield for alkaline pretreatment

3.3.1 Appropriate model determination and model fit statistics

A comparative analysis was performed between the quadratic, cubic, linear, and two-factor interaction (2FI) models to determine which model best characterized the connection between the input and the output. The quadratic model turned out to be the most appropriate. The quadratic model has the largest anticipated and adjusted R^2 value, according to the model summary statistics findings displayed in Table 6. The response's relationship with the independent variables is best described by the quadratic model.

Table 6: Model summary statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	387.79	0.1319	-0.0309	-0.2369	3.428E+06	
2FI	392.66	0.2768	-0.0569	-0.7732	4.915E+06	
Quadratic	**71.98**	**0.9813**	**0.9645**	**0.9253**	**2.071E+05**	**Suggested**
Cubic	73.48	0.9883	0.9630	0.8678	3.664E+05	Aliased

Table 7: Model fit statistics

Std. Dev.	71.98	**R^2**	0.9813
Mean	873.03	**Adjusted R^2**	0.9645
C.V. %	8.24	**Predicted R^2**	0.9253
PRESS	207100	**Adeq Precision**	21.1740

Table 7 shows there is less than 0.2 difference between the adjusted R^2 and the predicted R^2, indicating a satisfactory agreement. The Adeq Precision which calculates the signal-to-noise ratio should be ideally higher than 4. In this case, a value of 21.174 shows sufficiency.

3.3.2 Analysis of variance (ANOVA) for quadratic model

Using the Central composite design of experiment and the 3D response surface graphs, the impact of the various parameters on the yield of fermentable sugar was investigated. The model response was fitted using regression analysis. A is for alkaline concentration, B is for reaction duration, C is for reaction temperature, and Y is the yield of fermentable sugar.

Table 8: Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.720E+06	9	3.022E+05	58.33	< 0.0001	significant
A-Alkaline conc.	3.283E+05	1	3.283E+05	63.37	<0.0001	
B-Time	29762.28	1	29762.28	5.74	0.0375	
C-Temp	7408.21	1	7408.21	1.43	0.2593	
AB	1.477E+05	1	1.477E+05	28.51	0.0003	
AC	2398.74	1	2398.74	0.4630	0.5117	
BC	2.517E+05	1	2.517E+05	48.59	<0.0001	
A^2	8.978E+05	1	8.978E+05	173.31	<0.0001	
B^2	7.011E+05	1	7.011E+05	135.33	<0.0001	
C^2	7.391E+05	1	7.391E+05	142.67	<0.0001	
Residual	51805.60	10	5180.56			
Lack of Fit	20869.15	5	4173.83	0.6746	0.6619	not significant
Pure Error	30936.45	5	6187.29			
Cor Total	2.772E+06	19				

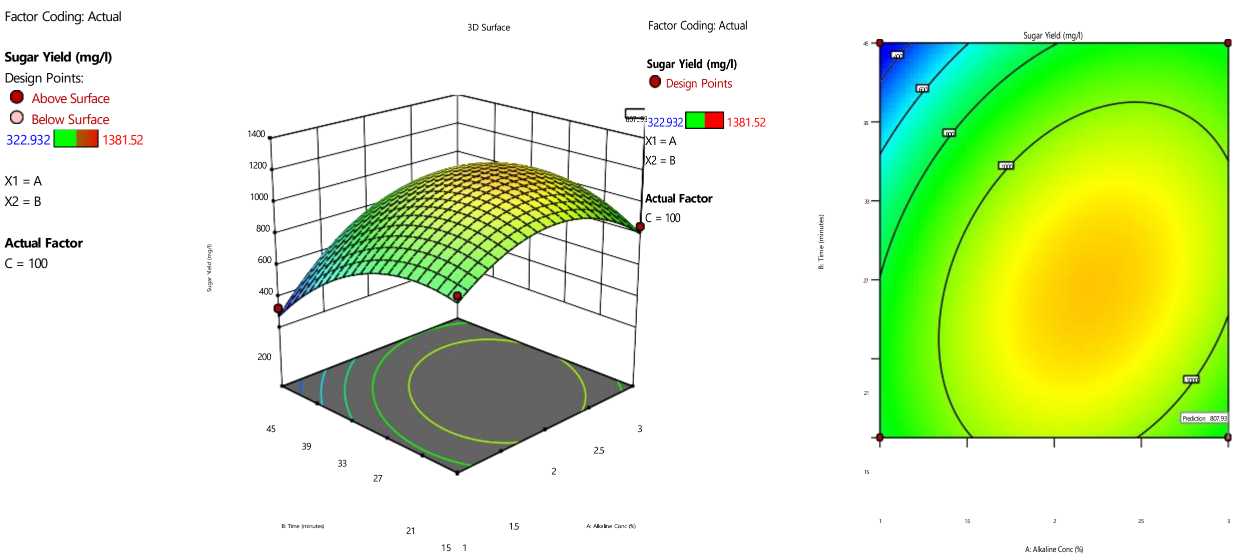
The model is considered significant with an F-value of 58.33, indicating that there is only a 0.01% chance that such a large F-value is due to random noise. Model terms are deemed significant when their P-values are below 0.0500. In this case, the significant terms are A, B, AB, BC, A^2, B^2, and C^2. If a term's P-value exceeds 0.1000, it is not considered important. Reducing the model by removing unimportant terms (while maintaining hierarchy) may improve its performance. Based on pure error, the Lack of Fit F-value of 0.67 implies that the Lack of Fit is not statistically significant, with a 66.19% probability that this value is due to noise. A minor Lack of Fit is desirable, as it indicates the model is suitable.

3.3.3 Regression analysis

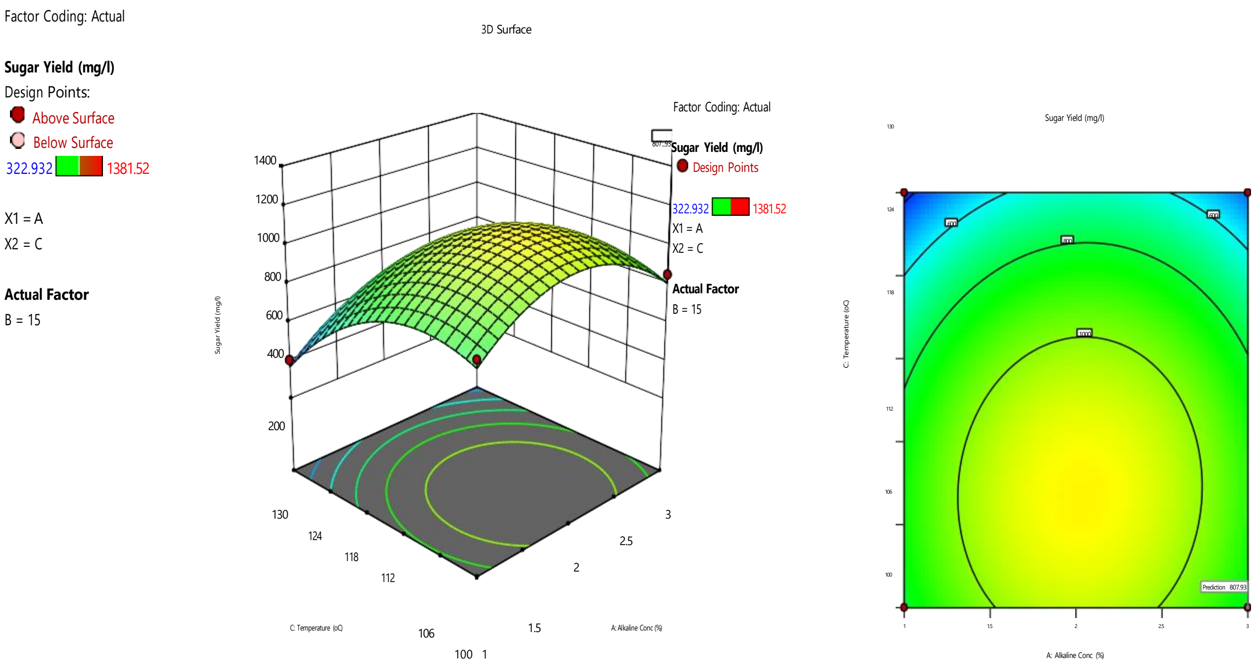
Equation 2 describes the relationship between the response (the fermentable sugar yield) and the variables that are independent in terms of actual factors. Regression analysis was used to fit the response to the model, with the yield of fermentable sugar as Y, A - Alkaline concentration, B - Reaction time, and C - Reaction temperature.

$$Y = -10539.02483 + 748.92700A - 46.85358B + 203.98563C + 9.05844AB + 1.15440AC + 0.788399BC - 249.59893A^2 - 0.980283B^2 - 1.00652C^2 \quad (2)$$

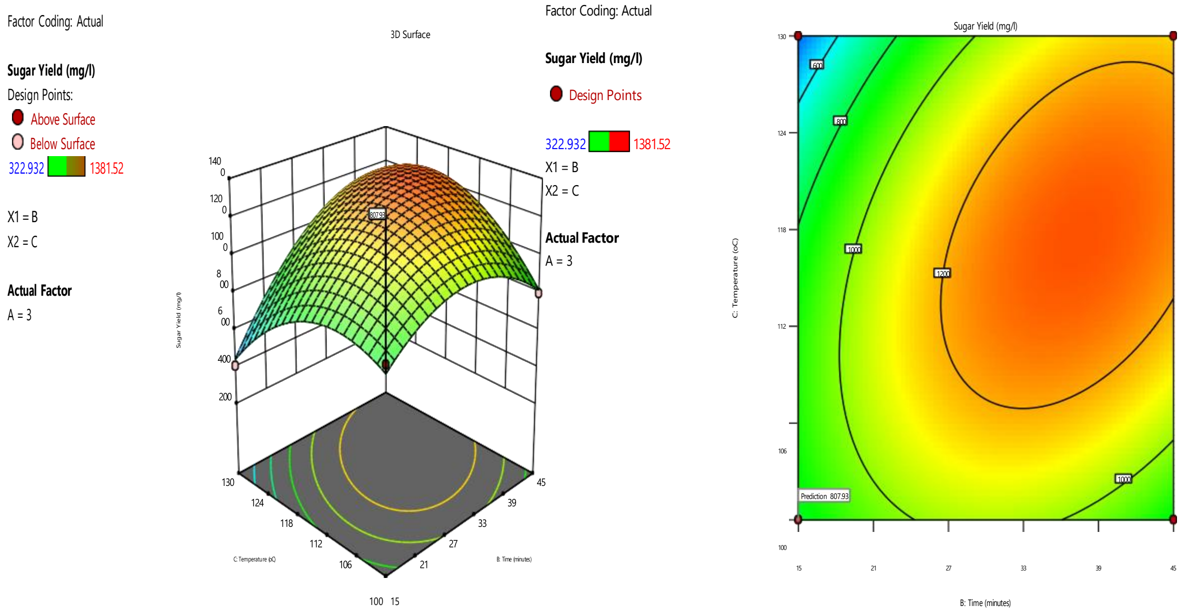
3.3.1 3.3.4 Response surface plots



(A)



(B)



(C)

Figure 2: 3D plots showing the interaction between (A) Alkaline concentration and reaction time (B) Temperature and alkaline concentration (C) Temperature and reaction time

3.3.5 Numerical optimization and model validation

Utilizing the created model, the design space was explored and factor choices aligned to maximize the yield of fermentable sugar were identified. The ideal settings, which included a pretreatment temperature of 115.41 ^oC, a time of 32.84 minutes, and an alkaline concentration of 2.32 % (w/w) NaOH, were selected from among the many solutions—more than 100 in total—based on the greatest desirability score. The result of this combination was a fermentable sugar yield of 1382.36 mg/L. To determine the success of this study, the optimum values of the input variables were taken to the lab and used to conduct a three-time repeating experiment. the average yield of fermentable sugar was 1383.02 mg/L. The response model's validity was confirmed by the close correlation seen between the experimental yield (1383.02 mg/L) and the predicted yield (1382.36 mg/L).

4. Conclusion

The primary focus of this research was the comparative optimization of acid and alkaline pretreatment methods on cassava bagasse by comparing their optimum yields of fermentable sugars and their optimum conditions for pretreatment. The acid pretreatment gave an optimum sugar yield of 798.52 mg/L at optimum dilute acid pretreatment conditions of a reaction time of 30.98 minutes at a temperature of 117.35 ◦C and with an acid concentration of 2.1 % (w/w) H_2SO_4. The alkaline pretreatment gave a sugar yield of 1382.36 mg/L at optimum levels of a reaction time of 32.84 minutes at a temperature of 115.41 ◦C and with an alkaline concentration of 2.32 % (w/w) NaOH. The alkaline pretreatment condition gave more yield of fermentable sugar when compared with the acid despite both being taken through the same range of input conditions. However, both types of pretreatments are efficient for cassava bagasse pretreatment.

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