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Optimization of Alkaline Pretreatment and Enzymatic Hydrolysis of Cassava Bagasse for the Production of Fermentable Sugar

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Abstract

The availability and reliability of energy sources and the consequences of their use on ecosystems are critical problems in Nigeria and across the globe. The quest for cleaner fuels for various consumer, industrial, and commercial applications has progressed in recent years. This study aims to assess the efficacy of the simultaneous saccharification and fermentation (SSF) process for extracting fermentable sugar from cassava bagasse. Response Surface Methodology (RSM) was used to determine the best alkaline pretreatment conditions. The highest overall fermentable sugar output was 582.209 mg/ml and was achieved using a central composite design. The optimized levels of the factors were obtained at a temperature of 87 °C, 45 minutes, and a 0.8M alkaline concentration. The pretreated condition was then used in enzymatic hydrolysis and fermentation. Biomass characterization showed cassava bagasse as a suitable feedstock for fermentable sugar biosynthesis because of its high hemicellulose and cellulose content.

1. Introduction

In recent years, the main driver behind the growth of bioenergy and biofuels has been the need for global energy security. Amid rising concerns about climate change and global warming, increasing crude oil prices, and restricting government policies on non-renewable energy sources, research has accelerated on finding an environmentally friendly substitute for fossil fuels. The process involved in converting this bagasse into bio-lignocellulosic biomass and then into biofuels is not straightforward as it requires additional chemical and physical pretreatment processes. Using agricultural waste as lignocellulose biomass provides a cost-friendly solution for producing biofuels, as over 40 million tons of waste plant material are discarded annually [1]. The most prevalent source of lignocellulosic materials utilized in the production of biofuels is waste products from agricultural activities, such as corn stalks [2], rice straw [3], wheat straw [4], [5], and sugarcane bagasse [6]. Hence, ways of facilitating the total bioconversion of such lignocellulosic waste into fermentable sugar for bioethanol generation have become an area of interest for many researchers.
Cassava (*Manihot spp.)* is one of the continent's most valuable cash crops and a staple food in western Africa because of the root crop's high carbohydrate content and comparatively low protein and vitamin content [7]. The yearly production of over 35 million tons of cassava from Nigeria's

plantations makes it the world's largest cassava producer[8]. Bagasse is one of the ample remnants attained from the extraction of cassava [9]. Bagasse is often discarded in the region around processing facilities because it contains a high percentage of organic materials and is biodegradable. Also, it is inappropriate disposal contributes to the pollution of the environment [10]. Considering its low ash level and high cellulose concentration, cassava bagasse has been identified as a promising carbon source for the biotechnological synthesis of highly significant commodities such as bioethanol, xylanase enzyme, antibiotics, biopolymers, and organic acids [10]. Cassava bagasse hydrolysis results in a glucose-rich hydrolysate that, with the help of the right microbe, might be converted into biofuels e.g., bioethanol, biobutanol [11], [12]. Lignin (15% -25%), cellulose (38% - 50%), and hemicellulose (23% -32%) are the constituent of the plant cell wall that makes up the lignocellulosic biomass [13].

Lignin is an amorphous layer that sits on top of the crystalline cellulose and hemicellulose that make up most of Lignocellulosic biomass [14]**.** Without pretreatment, the cellulase enzymes have a difficult time reaching the cellulose due to the presence of lignin and hemicellulose, which lowers the efficacy of the enzymatic hydrolysis [15]. With pretreatment, the cellulase enzymes can reach the cellulose due to lignin and hemicellulose, which lowers the efficacy of enzymatic hydrolysis. Hence, the pretreatment process achieves the following objectives: (1) Eliminate the protective lignin coating from the cellulose and hemicelluloses. (2) Decrease the crystalline structure of the cellulose. (3) Increase the porosity. (4) Split the outer layer so the enzyme can reach the substrate (sugar). (5) Decrease the number of inhibitors in the hydrolysate. Therefore, the most challenging phase is transforming lignocellulosic materials into fermentable sugars [16]. Before enzymes break down lignocellulose, it must undergo pretreatment to eliminate lignin, modify its compositional structure, or decrease its particle size. There are a variety of pretreatment alternatives that may be used, including physical, chemical, and biological processes [17]. The conventional pretreatment methods include steam explosion pretreatment and alkaline or dilute acid pretreatment. Among these options, alkaline pretreatment stands out due to its numerous appealing qualities. The alkaline pretreatment technique was shown to be the most successful and beneficial while operating at lower temperatures than the others [18]. Most of the chemicals used in alkaline processes are safe for the environment. These include: ammonia fiber explosion/expansion (AFEX), low-liquid ammonia (LLA), ammonia recycle percolation (ARP), soaking in aqueous ammonia (SAA), low-moisture anhydrous ammonia (LMAA), and other alkaline technologies using $Ca(OH)_2$, and NaOH [19], [20]. Nowadays, enzymatic hydrolysis is widely used to break down lignocellulosic biomass into fermentable monomer sugars. This is because sugars may be extracted from lignocellulosic material by enzymatic hydrolysis [21]. In comparison to chemical processes, this innovation may have improved selectivity, lower energy requirement, a more comfortable working environment, and fewer inhibition characteristics [22].

Therefore, this study aims to produce fermentable sugar from the alkaline hydrolysis of lignocellulosic biomass (cassava bagasse), using sodium hydroxide for biomass pretreatment, followed by enzymatic hydrolysis. This approach to producing fermentable sugar from alkaline pretreatment using cassava bagasse addresses the problem of environmental pollution associated with the improper disposal of cassava bagasse waste. Also, this study explores the possibility of utilizing appropriate alkaline pretreatment conditions and provides relevant information on the optimal conditions for the alkaline pretreatment of cassava bagasse**.** The experimental design was carried out using RSM. A three-factor Central Composite Design (CCD) was employed for the experimental design. The responses obtained from the CCD were optimized using RSM. The effects of three independent variables (alkaline concentration, temperature, and time) were examined, and the required response was the fermentable sugar yield.

2.0 Materials and Methods

2.1 Material Collection and Preparation

The lignocellulosic biomass (cassava bagasse) needed was collected from an agro-based farm at Ikpoba Okha Local Government Area in Edo State, Nigeria. The bagasse samples were washed with clean water to remove dirt before being air dried to remove/reduce the moisture content in the biomass sample. This was done by placing the collected cassava bagasse on a large table exposed to sunlight, after which they were sundried for 24 hours. The material was dried when the moisture content was less than 10% by weight, and the observed weight change was less than 1% in 24 hours. The air-dried biomass was milled via a milling machine to reduce the biomass sample size. Then an appropriate sieve size of 250 microns was used to screen the particle size of the lignocellulosic biomass.

2.2 Estimation of Hemicellulose Content

5 mg of cassava bagasse was measured using an analytical weighing scale and deposited in a round bottom flask. Then, 250 ml of a 0.5 M NaOH solution was poured into the bagasse. The solution in the round bottom flask was heated on the heating mantle for an hour and then cooled. The pH was neutralized after diluting with distilled water, decanting, and filtering. The filtrates were dried on crucible plates in the oven for 24 hours. The dried sample was weighed using an analytical weighing scale.

2.3 Estimation of Lignin Content

A 5 mg dried cassava bagasse sample was measured, then a 1 M H_2SO_4 solution was added to it, and the resulting solution was heated in a water bath for 30 minutes to carbonize it. The carbonized sample was then allowed to cool for 1 hour, and washing was carried out using distilled water accompanied by decantation and filtration until a neutralized pH was ascertained, as indicated by the pH meter. The neutralized sample was then placed in a crucible plate and kept in the oven for 24 hours for drying. After drying, the weight of the dried sample was taken using an analytical weighing balance.

2.4 Neutralization

Neutralization was done to adjust the PH of the pretreated samples to a near-neutral state suitable for the analysis of sugar using dinitrosalicylic acid (DNS) solution. After pretreatment with sulphuric acid, the samples were cooled and neutralized with varying quantities of NaOH solution.

2.5 Hydrolysis

Enzymatic hydrolysis was carried out with the use of cellulase enzyme. The sample was pretreated under optimal conditions. The sample was neutralized using NaOH, and the pH was adjusted to 5.0. Afterward, 0.5g of cellulase enzyme was added and maintained at 50 °C for 24–72 hours.

2.6 Design of Experiment

The effects of the independent variables which were temperature, time, and alkaline concentration on cassava bagasse were observed and the corresponding responses of total sugars were investigated using RSM. For this study, we used a central composite design (CCD) with three factors (one for each independent variable) and three levels (one for each level of the independent variables). The actual factor levels corresponded to the coded factor levels as follows: X_1 = Temperature, X_2 = Alkaline Concentration and X_3 = Time.

A three-variable Central Composite Design (CCD) for Response Surface Methodology (RSM) was used in determine a varied set of conditions for pretreatment in order to arrive at the optimal condition for pretreatment. The coded and the actual levels denoted as X_1, X_2, and X_3, are shown below in Table 1. The total sugar yield was chosen as the intended response for optimization. The experimental design was developed using Design Expert 13.0 statistical software. The variables selected for the statistical model are as follows:

Table 1: Actual values for the process parameters

Variables	units	Symbols	Coded and actual levels		
			-1	0	1
Temperature	◦C	X_1	52.1619	70	87.8381
Alkaline concentration	g/ml	X_2	0.28428	0.55	0.817572
Time	Min	X_3	20.1349	35	49.8651

The relevance of the model was evaluated by computing its F-value and p-value. The effectiveness of the regression equation was measured by calculating a number of different coefficients of determination (R^2), including the actual R^2, the adjusted R^2, and the predicted R^2. Analysis of variance (ANOVA) was used for the statistical assessment of the model.

3.0 Results and Discussion

3.1 Compositional Analysis

After pre-treating cassava bagasse with different alkaline concentrations, the composition of the carbohydrate's cellulose, hemicellulose, and lignin were characterized. Alkaline pretreatment was mostly used to delignify cassava bagasse. Table 2 displays the findings of an analysis conducted on the cellulose, hemicellulose, and lignin content of cassava bagasse. This was done to figure out the optimum alkaline concentration needed to produce the highest composition of convertible compounds when fermentable sugar was being made using cassava bagasse as the feedstock.

Table 2: Composition of Cassava Bagasse after Characterization

	Compositions (wt%)		
Alkaline Concentration (%)	**Cellulose Composition**	**Hemicellulose Composition**	**Lignin Composition**
0.5% NaOH	65%	16%	3.3%
0.1% NaOH	35%	32.7%	6.3%
0.1% KOH	53.5%	16.7%	3%

Table 2 shows cassava bagasse's cellulose, hemicellulose and lignin content when treated with different percentage concentrations of alkaline solutions. Since cellulose and hemicellulose are part family of the polysaccharides, a higher concentration of these is more desirable in the production of fermentable sugars. Therefore, this pretreatment method that gives the least percentage composition

of lignin is most suitable. From Table 2, the lignin content was 3.3%, 6.3% and 3% when the NaOH concentration was 0.5%, 0.1%, and 0.1% KOH respectively. Thus, alkaline treatment using 0.1% KOH will have the highest yield of fermentable sugar.

3.2 Modelling and Optimization of Total Sugar Yield

Table 3: Input variables with actual and predicted yield of sugar obtained

		Factor 1	Factor 2	Factor 3	Yield	Yield
Std	Run	A: X_1	B: X_2	C:X_3		
		Temperature (◦C)	Alkaline Conc (g/ml)	Time (mins)	Actual Values (mg/ml)	Predicted Values (mg/ml)
3	1	52.1619	0.817572	20.1349	44.48	36.30
12	2	70	1	35	228.77	298.74
5	3	52.1619	0.282428	49.8651	322.48	266.78
7	4	52.1619	0.817572	49.8651	69.34	19.51
19	5	70	0.55	35	791.31	736.00
6	6	87.8381	0.282428	49.8651	379.34	444.67
1	7	52.1619	0.282428	20.1349	117.62	142.03
2	8	87.8381	0.282428	20.1349	342.33	376.73
8	9	87.8381	0.817572	49.8651	502.21	462.36
14	10	70	0.55	60	97.77	137.92
13	11	70	0.55	10	52.19	33.87
16	12	70	0.55	35	655.94	736.00
4	13	87.8381	0.817572	20.1349	423.10	463.37
15	14	70	0.55	35	791.31	736.00
9	15	40	0.55	35	56.71	145.53
20	16	70	0.55	35	791.31	736.00
10	17	100	0.55	35	782.28	715.29
17	18	70	0.55	35	711.71	736.00
11	19	70	0.1	35	481.96	433.82
18	20	70	0.55	35	678.18	736.00

The coded and actual values of the factors, X_1 (Temperature), X_2 (Alkaline Concentration) and X_3 (Time) as designed by design expert and their corresponding responses (actual and predicted values) are shown in Table 3.

From Table 3, it can be observed that a positive strong correlation exists between the actual and predicted values of the percentage sugar yield. This shows that the actual values of Sugar yield reasonably agree with the predicted values. Therefore, response surface methodology adequately modeled the process. Table 4 further emphasizes this profound strong correlation by the relative closeness of the adjusted and predicted R^2 values.

Table 4: Model Summary Statistics

Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	
Linear	264.09	0.2767	0.1411	-0.0292	1.588E+06	
2FI	287.80	0.3021	-0.0201	-0.6859	2.601E+06	
Quadratic	**79.01**	**0.9595**	**0.9231**	**0.7643**	**3.637E+05**	**Suggested**
Cubic	58.75	0.9866	0.9575	0.8113	2.912E+05	Aliased

The predicted and adjusted R^2 value of the quadratic model is the highest among the models shown in Table 4. Therefore, we may infer that the quadratic model provides the most accurate description of the connection between the response and the independent variables. The correlation between the dependent and independent variables is shown in further detail in Table 5.

Table 5: Analysis of Variance (ANOVA) for Response Surface Quadratic Model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1.480E+06	9	1.645E+05	26.35	< 0.0001	significant
A-A	3.919E+05	1	3.919E+05	62.77	< 0.0001	
B-B	22025.21	1	22025.21	3.53	0.0898	
C-C	13069.17	1	13069.17	2.09	0.1785	
AB	35103.05	1	35103.05	5.62	0.0392	
AC	1613.60	1	1613.60	0.2585	0.6222	
BC	2376.76	1	2376.76	0.3807	0.5510	
A^2	1.682E+05	1	1.682E+05	26.95	0.0004	
B^2	2.462E+05	1	2.462E+05	39.44	< 0.0001	
C^2	7.613E+05	1	7.613E+05	121.96	< 0.0001	
Residual	62426.97	10	6242.70			
Lack of Fit	42909.05	5	8581.81	2.20	0.2038	not significant
Pure Error	19517.91	5	3903.58			
Cor Total	1.543E+06	19				

The results for test of significance for every regression coefficient and ANOVA as shown in Table 5 implied that the model was significant because of the low p-value (< 0.05) and lack of fit was not significant. From the analysis of variance in Table 5, it can be concluded that the quadratic model was adequately sufficient to model the interaction between the independent and response variables.

3.3 Regression model

The empirical model describes the correlation between the process variables actually examined and the sugar yield in percentage terms. For a given level of each coded component, the reaction may be predicted using the corresponding equation.

Yield = -3,089.61 + 51.2739 X_1 + 1,038.46B + 81.0255C + 13.8784AB + -0.0535595AC - 4.33351BC + -0.339546A^2 + -1,825.78B^2 + -1.04017C^2

The model developed represent cassava bagasse sugar yield as (Y) as a function of Temperature (X_1), alkaline concentration (X_2) and Time (X_3).

3.4 Analysis of Response Surface Plots

A. Interaction between Time and Alkaline concentration

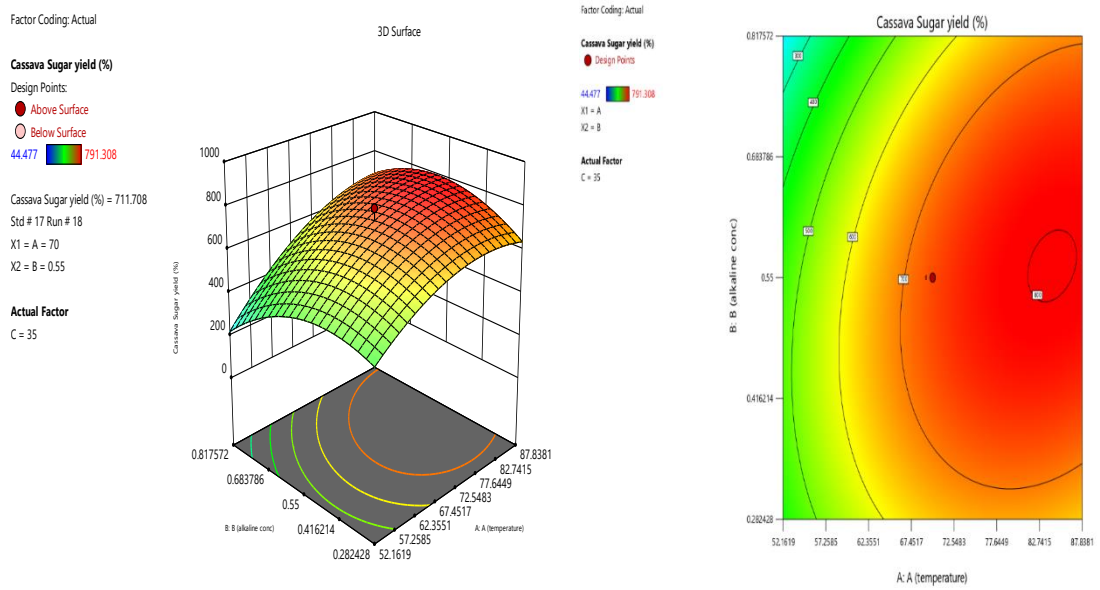

Figure 1: Contour plot and surface plot modelling the effect of alkaline concentration and time on total fermentable sugar yield

From Figure 1, an increase in temperature from 52 ºC to about 87 ºC is followed by an increase in sugar concentration yield. This total fermentable sugar yield was directly increased by an increase in alkaline concentration up to a point (0.6 g/L) after which a steady decline was observed.
Figure 2 shows a steady increase in the sugar concentration yield as the temperature increases from 52 º C to 87 º C. Also, with increasing time, sugar yield increased from 20 minutes to 35 minutes until a steady decline was observed in the 40th minute.

B. Interaction between Temperature and Time

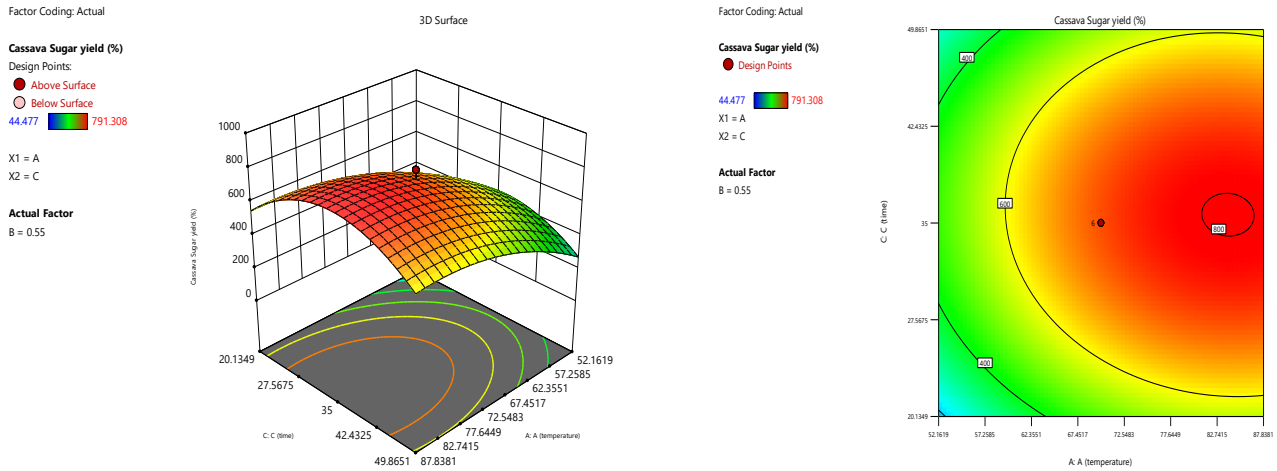

Figure 2: Contour plot and surface plot modelling the effect of temperature and time on total fermentable sugar yield

C. Interaction between Temperature and Alkali concentration

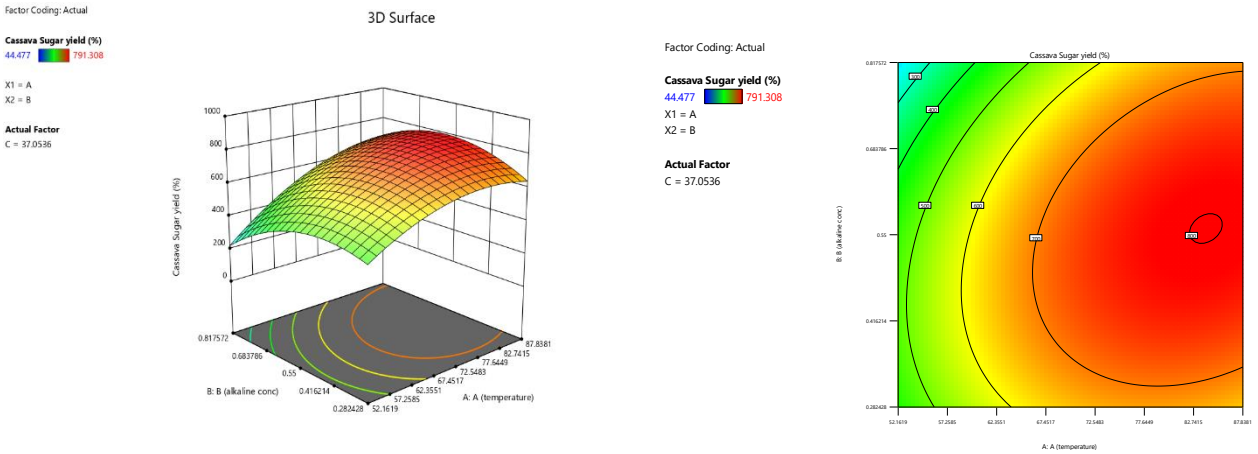

Figure 3: Contour plot and surface plot modelling the effect of Temperature and Alkaline concentration on total fermentable sugar yield

Figure 3 shows that the sugar yield increased with an increase in alkaline concentration until a steep decline was observed at 0.6 g/l up until 0.8 g/l. The relationship between time and the sugar concentration yield is the same. There was a steady increase in sugar concentration yield until the 43rd minute, after which a steady decline was observed.

3.5 Optimization of Fermentable sugar yield

The optimum conditions of the input variables were determined by carrying out statistical optimization using the RSM model. The maximum total sugar yield obtained was 582.309 mg/ml.

The optimum fermentable sugar yield was obtained at a temperature of 87 °C, an alkaline concentration (KOH) of 0.8 g/l, and 45 minutes.

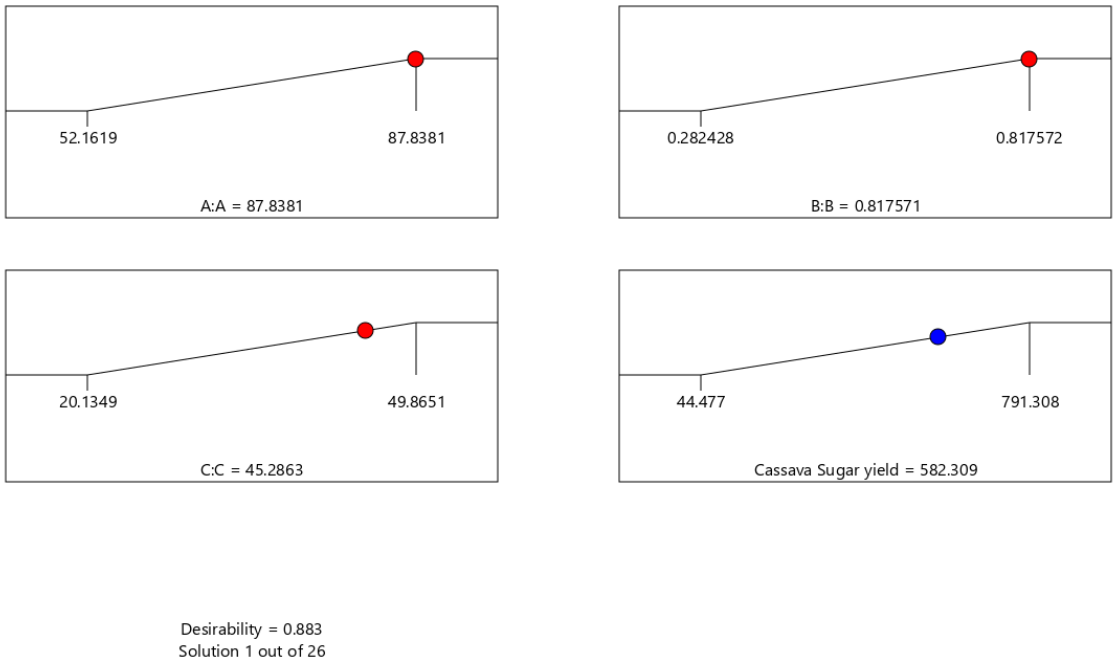

Figure 4: Optimum Total Yield Concentration

4.0. Conclusion

This study investigated the viability of employing RSM to optimize the pretreatment duration, alkali concentration, and temperature necessary for the biosynthesis of fermentable sugar from agricultural waste (cassava bagasse). The best conditions predicted by the model were obtained at a temperature of 87.8 °C for 45 minutes with a KOH concentration of 0.8 g/l, yielding a maximum concentration of fermentable sugar of 582,309 mg/l. This research argues that lignin and extractives were effectively removed by KOH pretreatment, which improved substrate accessibility for the enzymes and, presumably, enhanced the efficiency of enzymatic hydrolysis. The outcomes of this research indicate that cassava bagasse may be utilized sustainably to extract fermentable sugars for use in biofuel production and other value-added products.

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