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Optimization of Fermentable Sugar Production from Lignocellulosic Biomass (Elephant Grass) Using Alkaline Pretreatment

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Abstract

Energy sources and supply in Nigeria and the world at large is a significant issue of concern, ranging from its shortage or low availability to its environmental effects. Advances have been made in obtaining a more environmentally friendly source of fuel which would be helpful for various domestic, industrial and commercial purposes. This research takes a shift from the regular usage of food crops and regular bioethanol fuel, holding to the fact that continuous usage of food crops tends to bring about a shortage of food crops and a high purchase cost. The research tends to evaluate the production of fermentable sugar obtained from simultaneous saccharification and fermentation (SSF) of Elephant grass with glucose supplementation. The optimum alkaline pretreatment condition was analyzed using the response surface methodology (RSM). It was found to be at a temperature of 65.832°C, a timing of 40 minutes and an alkaline concentration of 0.302M NaOH, which gave a sugar concentration yield of 564.282 mg/l. Enzymatic hydrolysis was performed, and cellulase was the enzyme of choice. Within the first 24 hours after hydrolysis, there was an overall increase of 15% in the total sugars. Because of the findings of this research, it is possible to conclude that elephant grass is an excellent and environmentally friendly feedstock option for the production of fermentable sugar.

1.0. Introduction

The growth of the economy today is largely dependent on the energy and transportation industries. The transportation industry is becoming more important, and by 2030 it is projected that over 1.3 billion automobiles will be in use throughout the world [1]. However, researchers have been looking into viable alternative energy sources like biofuels due to anxiety about fossil fuel depletion, greenhouse gas emissions, and the accelerated pace of energy consumption [2]. Notably, bioethanol accounts for the largest percentage (65%) of all biofuels [1]. Many nations are now pushing for ecologically sustainable bioethanol manufacturing practices. Bioethanol is made by a process called consolidated bioprocessing (CBP), which employs microorganisms capable of breaking down biomass into fermentable sugars and then fermenting those carbohydrates into ethanol [3] .
As a result of the worldwide shortfall of fossil fuels, lignocellulosic biomass has emerged as a key component in the production of next-generation biofuels. High potential exists for ethanol production from lignocellulosic materials because they are readily available, do not compete with food production, could use degraded agricultural lands for growing feedstock, and have the potential to use large quantities of agro-industrial wastes whose disposal is problematic for the environment

[4]. Hence, the potential for developing efficient and inexpensive lignocellulose biomass conversion into fuel ethanol, is now a topic of heightened attention among scientists throughout the world.
Lignocellulosic biomass includes lignin, hemicellulose, and cellulose, as its major components. The cellulose and hemicellulose fractions could be used for fermentable sugars or other sugar-to-product conversion processes because they contain sugar polymers. Unlike cellulose, the hemicellulose fraction is readily hydrolyzed in neutral or alkaline environments. Hence, the cellulose fractions must undergo more intense pretreatment for optimal enzymatic hydrolysis [5]. The complicated structure and inhibitors present are the main hindrances to using lignocellulose biomass as a feedstock. Therefore, the use of aggressive pretreatment techniques is required to liberate fermentable sugars from lignocellulose biomass [6].

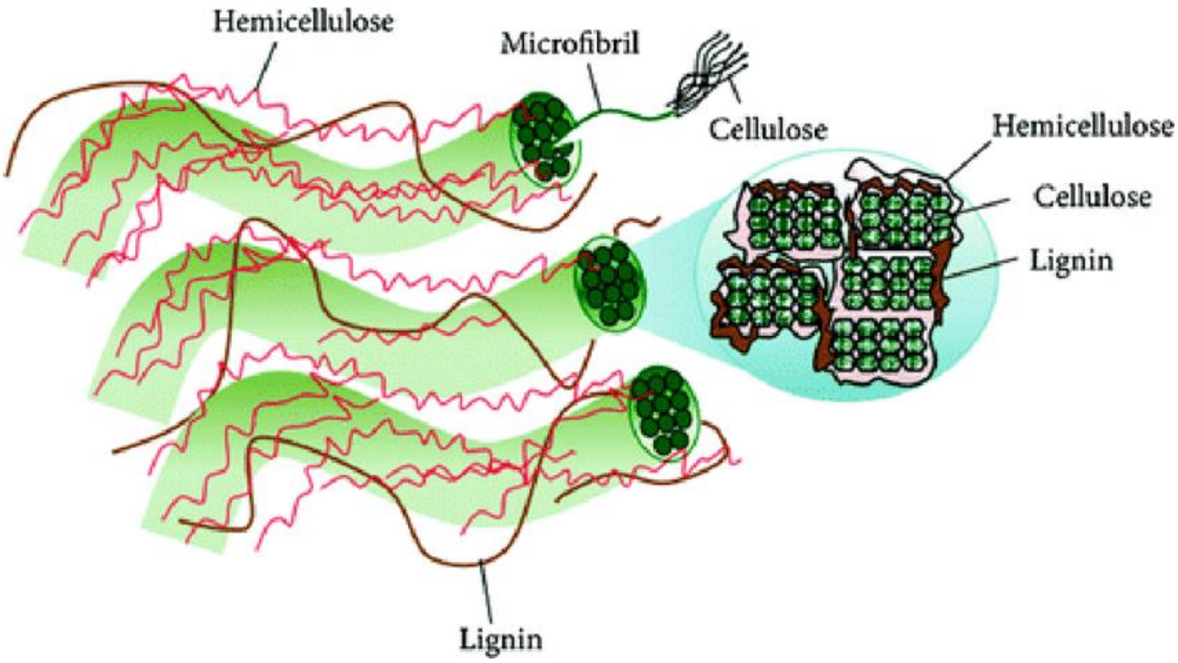

Figure 1: Structure of Lignocellulosic Biomass

Pretreatment is a vital part of the cellulose conversion process because it modifies the microstructure of cellulosic biomass so that enzymes may more easily break down the polymers and carbohydrates to produce fermentable sugars. The lignocellulosic biomass will undergo treatment in order to reduce crystallinity, promote porosity, and remove lignin and hemicellulose[7].
For cellulose and hemicellulose to be hydrolyzed (enzymatically) more quickly and advantageously, lignocellulosic biomass must first undergo pretreatment [8]. Pretreatment, which accounts for at least 20% of the total manufacturing expenses, is the most expensive phase in these various processes [8]. The conventional method of pretreatment methods includes physical methods of pretreatment, physicochemical pretreatment (Ammonia fiber expansion (AFEX), steam explosion pretreatment, etc.), and chemical pretreatment (alkaline pretreatment or dilute acid pretreatment). Alkali treatment requires less pressure, temperature, and environmental conditions than other pretreatment techniques. Compared to acid pretreatment, alkali pretreatment minimizes the quantity of sugar that is damaged and makes it possible and simple to extract and recover caustic salt. The alkaline pretreatment uses reagents that are not corrosive and are relatively affordable, such as calcium hydroxide (lime), sodium carbonate, and sodium hydroxide to remove the lignin component from lignocellulosic biomass.
There are several types of lignocellulose biomass that have been documented in the literature. They include: rice straw [9], elephant grass [10], palm wood [11], switch grass [12], agricultural waste [13], and cotton spinning wastes from textile mills [14] have been used as starting material for bioethanol production. In contrast to other lignocellulosic biomass, elephant grass is an excellent

feedstock because of its high biomass output, year-round availability, and little nutritional needs[15].

Elephant grass (*Pennisetum purpureum*) is a lignocellulosic material that is gaining popularity because to its excellent carbon dioxide absorption, high biomass yield, potential for rapid development and sustainability. It is native to Africa and has been utilized as animal feed by several European countries. It is frequently characterized as "invasive" or "opportunistic" due to its tenacity, drought resistance, feed quality, adequate seed size, and disease tolerance. At the same time, elephant grass delivers rusticity, aggression, perennity, and palatability [16]. It is a crop that can grow throughout the year and in any season. It is also beneficial as a feedstock for the creation of biofuels since it has a short production cycle (1 month), is affordable (low procurement cost), is not a food crop, is incredibly hardy and thrives in a range of environments, including wet and dry, grows all over the country [17].

Hence, the primary goal of this project is to biosynthesize fermentable sugar from lignocellulosic biomass (elephant grass), with the use of diluted sodium hydroxide for pretreatment and response surface methodology for optimization. This approach to the production of fermentable sugar from elephant grass involves the determination of the lignin, hemicellulose, and cellulose content of elephant grass, the alkaline pretreatment of elephant grass, and provide relevant information on the optimal conditions for the pretreatment process. It also investigates the potential of elephant grass to be converted into a source of biofuels e.g. bioethanol, and biobutanol. Experimental design was done using response surface methodology (RSM). A three-factor Central Composite Design (CCD) was employed for the experimental design. The responses obtained from the CCD were optimized using RSM. Three independent variables (alkaline concentration, temperature and time) were examined, and the dependent variable was the fermentable sugar yield.

2.0. Materials and Methods

2.1. Feedstock sourcing and preparation

Elephant grass**,** as shown in Figure 2, was readily found in a nearby uncultivated land at BDPA region of Ugbowo, Edo State, Nigeria.

Figure 2: Elephant grass

Prior to the pre-treatment phase, elephant grass was mowed, rinsed with clean water, and air dried at room temperature. As part of the preparation process, the raw materials were hammer milled to a size of 2 mm. Then the ground elephant grass was further dried in an electric oven at 40°C until the moisture content was less than 10% by weight before being placed in sealed plastic bags. To further examine the chemical makeup, a portion of the elephant grass sample was further ground down to 0.5 mm using a centrifugal mill.

2.2 Feedstock Analysis

2.2.1 Estimation of Hemicellulose Content

As described by [7], 5g of the sieved elephant grass sample was weighed using an analytical weighing balance. A 0.5M NaOH solution was added to a 250ml portion of the weighed-out sample. After that, the mixture was cooked to a controlled temperature for 60 minutes in a round bottom flask. The resulting solution was removed from the heating mantle and allowed to cool.
The cellulose and lignin residue were then put to a sizable beaker after the solution had been filtered and the sample had cooled. The residue was then given a large amount of distilled water and washed using the decantation technique. The washing cycle was repeated as needed until the pH was balanced. Once there was no longer any water present, the residue was transferred to a crucible plate and dried in a 105°C oven. The sample was removed from the oven after it had finished drying completely and weighed using an analytical weighing scale [18].

2.2.2 Estimation of lignin Content

The amount of lignin in the dried residue might be determined by method as described as [19]. For this procedure, lignin content is estimated by boiling 100ml of 0.5M H2SO4 for two hours and allowing the residue rest in the solution for 24 hours at room temperature. Until neutral pH of 7 was obtained, the undissolved residue was repeatedly washed with a large amount of water. The residue was weighed using an analytical weighing scale after four hours of drying in a 105^{o}C oven, and cooling in a desiccator.

2.3 Pre-treatment of Elephant grass

5g of the grinded elephant grass was weighed on an analytical weighing balance before being transferred to a conical flask to begin the pre-treatment process for elephant grass. The sample was then combined with 100ml of a sodium hydroxide solution that was created by combining different concentrations of sodium hydroxide pellets with 100ml of water. the solution was heated in an autoclave for different time interval, the sample was removed from the autoclave and allowed to cool.
After cooling, the pre-treated sample was placed in a beaker, neutralized with a solution of 2M sulphuric acid, and the resulting solution was filtered through filter paper.

2.4 Determination of Sugars using Dinitro Salicylic acid (DNS) method

3 ml of the filtrate was metered out with a syringe and put to a boiling tube following filtration. Additionally, 1ml of DNS solution was measured and added to the boiling tube's measured filtrate. The boiling tube's solution was heated on a hot plate and brought to a boil for five minutes. Finally, a UV spectrophotometer was used to measure the solution's absorbance.

2.5 Design of Experiment

A three factor Central Composite Design (CCD) was employed for the design of the experiment. The responses obtained from the CCD were optimized using RSM in order to arrive at the optimal condition for pretreatment. In order to optimize total sugar yield (response), a central composite design (factors, k = 3) with three input variables – temperature, time and alkaline concentration coded as A, B, and C respectively were factors to be optimized. The actual and the coded levels of the independent variables are shown in Table 1. The experimental design was developed using Design Expert 13.0 statistical software. The variables selected for the statistical model are as follows:

Table 1: Coded and uncoded values for each variable of CCD

	Compositions (wt %)		
Alkaline Concentration (%)	**Cellulose Composition**	**Hemicellulose Composition**	**Lignin Composition**
0.5% NaOH	33.33%	17.67%	34%
1% NaOH	44.67%	12%	22%
3% NaOH	34%	10%	32.33%

Table 2: Characterization of elephant grass

Variables	Units	Symbols	Coded and actual levels		
			-1	0	+1
Temperature	℃	A	40	70	100
Time	Min	B	20	40	60
Alkaline concentration	%	C	0.1	0.3	0.5

3. Results and Discussion

3.1 Compositional Analysis

Table 2 shows the composition of elephant grass's cellulose, hemicellulose and lignin content when treated with different percentage alkaline concentration. Cellulose and hemicellulose being part of the family of the polysaccharides, a higher concentration of these is more desirable in producing fermentable sugars. Hence, the treatment method with the most negligible percentage of lignin composition will be the most suitable.

From Table 2, the lignin content was 34%, 22% and 32.33% when the NaOH concentration was 0.5%, 1% and 3%, respectively. Thus, alkaline treatment using 1% NaOH will yield the highest fermentable sugar yield. The sum of the composition of cellulose and hemicellulose (56.67%) is similar to the result (53.1%) obtained by Eliana et al. (2014) when they pre-treated elephant grass with 1% NaOH.

3.2 Modelling and Optimization of Total Sugar Yield

The coded and actual values of the factors, A (Temperature), B (Time), and C (Alkaline Concentration) as designed by design expert version 13.0, and their corresponding responses (actual and predicted values) are shown in Table 3.

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

Run	**Factor 1 A:A temperature**	**Factor 2 B:B alkaline conc.**	**Factor 3 C:C time**	**Response 3 Elephant grass Sugar yield %**	
				Actual	**Predicted**
1	52.1619	0.817572	20.1349	388.81	375.94
2	70	1	35	438.45	469.46
3	52.1619	0.282428	49.8651	473.64	449.30
4	52.1619	0.817572	49.8651	473.19	445.85
5	70	0.55	35	694.29	692.58
6	87.8381	0.282428	49.8651	275.23	245.82
7	52.1619	0.282428	20.1349	355.42	341.43
8	87.8381	0.282428	20.1349	406.86	391.92
9	87.8381	0.817572	49.8651	386.56	358.27
10	70	0.55	60	348.92	393.57
11	70	0.55	10	442.51	457.65
12	70	0.55	35	694.29	692.58
13	87.8381	0.817572	20.1349	560.28	542.34
14	70	0.55	35	694.29	692.58
15	40	0.55	35	388.81	415.14
16	70	0.55	35	694.29	692.58
17	100	0.55	35	350.48	383.95
18	70	0.55	35	694.29	692.58
19	70	0.1	35	317.11	345.88
20	70	0.55	35	694.29	692.58

From Table 3, it can be observed that a positive strong correlation exists between the predicted and actual 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. From Table 4; the models under investigation include the linear, cubic, quadratic, and two-factor interaction (2FI) models.

Table 4. Model summary statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	159.65	0.0568	-0.1200	-0.3078	5.655E+05	
2FI	168.28	0.1486	-0.2443	-0.7464	7.551E+05	
Quadratic	**31.23**	**0.9774**	**0.9571**	**0.8296**	**73680.22**	**Suggested**
Cubic	38.64	0.9793	0.9344	-3.5661	1.974E+06	Aliased

The quadratic model was recommended as the most appropriate model based on the findings of the model summary statistics as it had the best values for R-squared and adjusted R-squared. The quadratic model, therefore, captures the connection between the input variables and the response the best. Given that the lack of fit is insignificant, Table 4 suggests the quadratic model as a suitable fit to reflect the effects of the independent variables since it's p-value < 0.05.

3.3 ANOVA (Analysis of variance) for Quadratic model

Table 5 displays the outcome for the significance tests for the analysis of variance for the quadratic model. The result suggests that the model is statistically significant due to its minute p-value (< 0.05) and lack of fit being not significant.

Table 5: ANOVA for Quadratic Model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.226E+05	9	46957.20	48.13	< 0.0001	significant
A	1173.60	1	1173.60	1.20	0.2984	
B	18434.78	1	18434.78	18.90	0.0015	
C	4955.66	1	4955.66	5.08	0.0479	
AB	6716.89	1	6716.89	6.88	0.0254	
AC	32252.37	1	32252.37	33.06	0.0002	
BC	720.68	1	720.68	0.7387	0.4102	
A^2	1.547E+05	1	1.547E+05	158.56	< 0.0001	
B^2	1.462E+05	1	1.462E+05	149.88	< 0.0001	
C^2	1.284E+05	1	1.284E+05	131.61	< 0.0001	
Residual	9756.13	10	975.61			
Lack of Fit	9756.13	5	1951.23			
Pure Error	0.0000	5	0.0000			
Cor Total	4.324E+05	19				

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.

3.4 Regression model

Regression analysis was employed to fit the response to the model. With the yield of fermentable sugar as Y, **A – reaction temperature, B – alkaline concentration and C- reaction time**; The interaction between the dependent (response) and independent (predictors) components is expressed in terms of coded variables in Equation 1, while real variables are used to show the relationship in Equation 2.

$$Y = +692.58 - 9.27A + 36.74B - 19.05C + 28.98AB - 63.49AC - 9.49BC - 103.61A^2 - 100.73B^2 - 94.39C^2 \quad (1)$$

The equation 1 illustrates the relationship in terms of coded factors can be used to estimate the response for given concentrations of each element. The high levels of the components are by convention expressed as +1 and the low levels as -1. The coded equation may be used to establish the relative relevance of the components by contrasting the factor values.

$$Y = -2244.88916 + 50.10614A + 1343.53160B + 46.69381C + 6.07086AB - 0.239453AC - 2.38627BC - 0.325600A^2 - 11406.96550B^2 - 0.427159C^2 \quad (2)$$

The Equation 2 was stated in terms of the real components, and this enables one to predict the reaction for certain concentrations of each element.

3.5 Analysis of Response Surface Plots

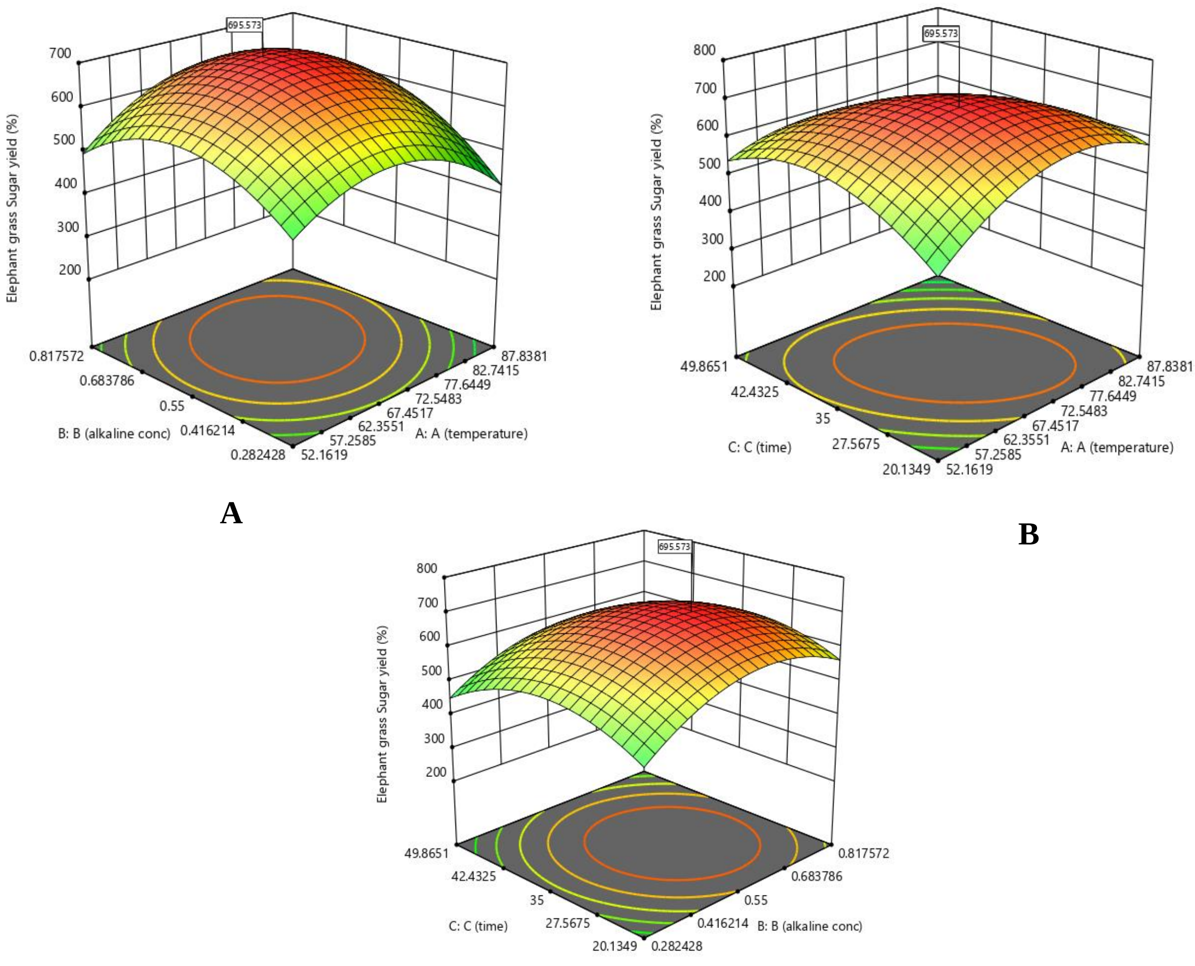

Figure 2: 3D response surface plot on (A) effect of alkaline concentration and temperature on total sugar yield; (B) effect of temperature and time on total sugar yield; (C) effect of time and alkaline concentration on total sugar yield

From Figure 2A, an initial increase in elephant grass sugar yield with an increase in reaction temperature and alkaline concentration is observed until a decline then appears as temperature and alkaline concentration are increased beyond 70^0C and 0.55% respectively. Similarly, Figure 2B shows an initial increase in elephant grass sugar yield with an increase in reaction temperature and time. From the plot, a steep increase in sugar yield was observed for the first 20 minutes of the reaction and declines after 35 minutes while for temperature its increase favored an increase in sugar yield until it becomes greater than 70^0C. Also, From Figure 2C an initial increase in the total sugar yield with an increase in reaction time and alkaline concentration is observed. This continues to a point before a drop then appears at a point where time and alkaline concentration are increased beyond 35 mins and 0.55% respectively.

3.6 Numerical optimization of alkaline hydrolysis of elephant grass sugar yield

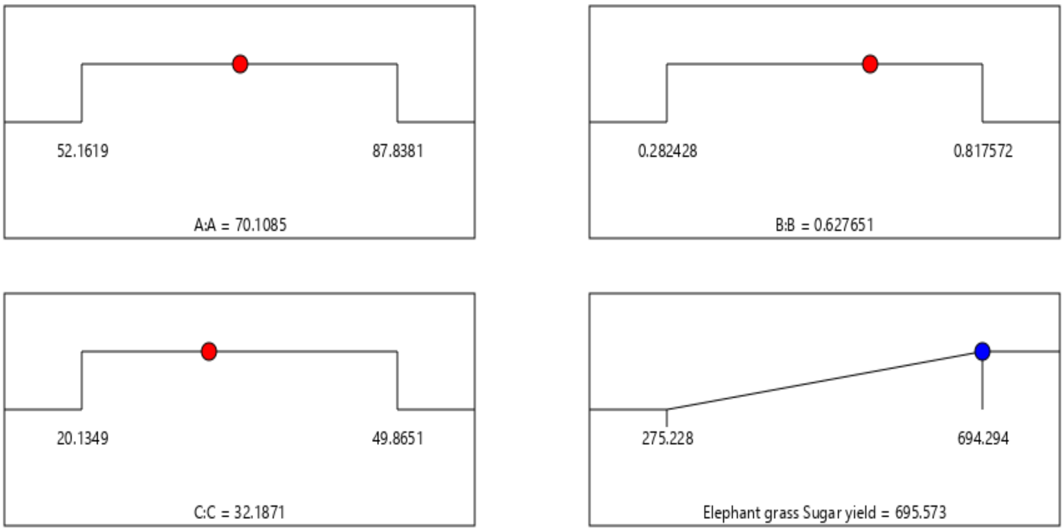

Figure 3: Optimum conditions for elephant sugar yield

Numerical Optimization was executed using RSM modelling to determine the conditions of the input variables (A: temperature, B: alkaline concentration, C: time), that gives the optimum total sugar yield. From the result obtained, the conditions favoring optimal sugar yield of 695.573 mg/L was observed to be at a temperature of 70^0C, alkaline concentration of 0.627g/l NaOH, and at a timing of 32 minutes.

3.7. Limitations of the Study

The study has three potential limitations which includes:

- Uncertainties associated with the use of Response Surface Methodology (RSM) to analyze the optimal alkaline pretreatment conditions. It is worth noting that like most statistical models, RSM are based on certain assumptions and considering these uncertainties associated with the use of RSM needs to be explored. Also, RSM fall short in optimization studies when compared to more recent machine learning models such as ANN and ANFIS. Thus, future research should examine the use of such model or a combination of two or more.
- Another limitation is the potential impact of variability in the feedstock characteristics. The study may have been conducted using one type of elephant grass, which could have different characteristics than other types depending on the specie. Variations in the feedstock characteristics may significantly affect the efficiency of the process and the yield of fermentable sugar. Hence, future studies could conduct further research to explore the impact of using different types of elephant grass on the yield of fermentable sugar. Identifying the

optimal variety of the elephant grass can improve the process's efficiency and be more accurate about the expected yield of fermentable sugar.

- One limitation of the study is the dearth of prior literature study on the production of fermentable sugar from elephant grass. Given the scarcity of literature on this topic, it is challenging to evaluate the comparability of this study to other research and the accuracy of the results obtained. Consequently, future research should attempt to conduct an extensive and systematic literature review to address research gaps, identify best practices, and obtain significant insights that are crucial to the study.

4 Conclusion

The study aimed to optimize the process parameters of the conversion of alkaline-treated elephant grass into fermentable sugar to be used as suitable feedstock for further industrial processing. Independent factors investigated using response surface methodology (RSM) were reaction time, alkaline concentration, and the reaction temperature which optimized the yield of total fermentable sugar. The following deductions can be made from this study:

1. The overall statistical model was significant
2. The reaction temperature, alkaline concentration as well as reaction time all affect the yield of elephant grass total sugar.
3. The fermentable sugar yield from elephant grass was significantly influenced by the time-temperature, time-alkaline concentration, and alkaline concentration-time relationships.
4. It was determined that the regression analytical method used to optimize process parameters for the yield of fermentable sugar was beneficial, since there was a high degree of agreement between actual and projected values.
5. At a temperature of 70^{0}C, a reaction time of 32 minutes and 0.627g/l NaOH concentration, an optimal yield of fermentable sugar (695.573 mg/L) was obtained.

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