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Research Article

Optimization of Concentrated Sulphuric Acid Hydrolysis of Gadam Sorghum Stalks Found in Kenya for Fermentable Sugar Production

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Gadam sorghum stalks are agricultural residues which can be hydrolyzed into fermentable sugars that can be used to produce bioethanol which is a renewable source of energy. In order to produce bioethanol from lignocellulosic biomass such as Gadam sorghum stalks, several processes including hydrolysis are involved. However, the use of lignocellulosic biomass for bioethanol production is hindered by the low yield of fermentable sugars obtained during hydrolysis. The lack of sufficient information on optimal conditions governing hydrolysis of lignocellulosic biomass leads to inefficient process which hinders the economic viability of large-scale bioethanol production. The objective of this study was to optimize reaction conditions involved in concentrated sulphuric acid hydrolysis of Gadam sorghum stalks. During hydrolysis, the conditions that were varied included temperature (40°C–80°C), time (30-90 minutes), and concentration of acid (30%-70%, *w*/*w*). Central composite rotatable design was used to optimize and establish optimum level of hydrolysis conditions. Response surface methodology and analysis of variance were used to interprete the results. The results of hydrolysis revealed that the highest yield of glucose was 87.54% (*w*/*w*) which was realized at 60°C hydrolysis temperature, 60 minutes hydrolysis period, and 50% (*w*/*w*) concentration of sulphuric acid. In addition, the lowest glucose yield was 45.59% (*w*/*w*) which was realized at 60°C hydrolysis temperature, 60 minutes hydrolysis period, and 16.36% (*w*/*w*) sulphuric acid concentration. Concentrated sulphuric acid hydrolysis of Gadam sorghum stalks results in high yield of fermentable sugars. These results reveal that Gadam sorghum stalks are viable substrates for the production of fermentable sugars.

1. Introduction

Biofuels are increasingly being used as sources of energy as the world economy tends to substitute fossil fuels due to global warming and declining supplies [1]. In Kenya, the consumption of fossil fuels has been on the rise mainly due to an increase in transportation of people and goods, increased industrial activities, and population growth. Biofuels such as bioethanol, biodiesel, and biogas are candidate substitute to fossil fuels [1, 2]. Bioethanol is biodegradable, highly oxygenated, and less polluting when compared to fossil fuels [3]. It is used in the transportation sector through blending with gasoline to form a combustible mixture such as E10 which is a blend consisting of 90% gasoline and 10% bioethanol, cooking and lighting in rural and urban homesteads, and as industrial solvent [1, 3, 4].

Bioethanol can be produced from starch (corn, wheat)- and sucrose (sugar)-based substrates or lignocellulosic biomass (LGB). Bioethanol produced from starch- and sucrose-based substrates is referred to as first-generation bioethanol (1GBE). Materials containing cellulose, hemicellulose, and lignin are referred to as LGB. Bioethanol produced from LGB is referred to as second-generation bioethanol (2GBE). Examples of LGB include materials such as agricultural residues (wheat straws, maize cobs, and sorghum stalks), perennial grasses, woody biomass, energy crops, aquatic plants, and municipal solid waste [5, 6]. LGB are nonfood substrates that can be used to produce biofuels without affecting food supply. These materials have been identified as potential substrates for the production of 2GBE [7].

The main process steps involved in the production of 2GBE include pretreatment, saccharification/hydrolysis, fermentation, product recovery, distillation, and dehydration [4, 8, 9]. Pretreatment is meant to prepare LGB for effective hydrolysis [4, 9]. The breakdown of cellulose and hemicellulose found in LGB to glucose and xylose, respectively, is referred to as hydrolysis [4, 10]. The structure and composition of LGB determines to a greater extent the success of the hydrolysis process [9]. LGB can be hydrolyzed using enzymes and or acids (dilute or concentrated acid). The concentration of acid during dilute acid hydrolysis of LGB is normally below 10% (w/w) while during concentrated acid hydrolysis, it is above 10% (w/w) [11]. The hydrolysis process (whether acid or enzymatic) is also governed by reaction parameters such as temperature, time, pH, particle size of biomass and solid to liquid ratio (SLR) [4, 12].

Cellulose, hemicellulose, and lignin form a complex structure which hinders effective hydrolysis of LGB which in turn leads to a reduction in the yield of fermentable sugars thus contributing to high production costs [4, 13–15]. Cellulose found in LGB consists of amorphous and crystalline fractions [15, 16]. The crystalline fraction requires concentrated acid for effective decrystallization [16]. Dilute acids hydrolyze the hemicellulosic fraction of LGB and partly the amorphous fraction of cellulose [11, 15–17]. On the other hand, temperature has an impact during hydrolysis such that the high temperature used in dilute acid hydrolysis of LGB leads to degradation of glucose to hydroxymethylfurfural (HMF), which is a fermentation inhibitor [16, 18]. Acid hydrolysis of LGB is preferred over enzymatic hydrolysis due to high sugar yields, faster reactions, less costly process, and less sensitive to changes in process conditions, and pretreatment of LGB is not necessary [15, 19].

The main challenges encountered during enzymatic hydrolysis of LGB include slow reaction arising from limited access of enzymes to cellulose, adsorption of enzymes by lignin which prevents the enzymes from reaching the cellulose fraction of LGB, the presence of crystalline fraction in cellulose that is difficult to degrade using enzymes, the high cost of enzymes, inhibition of enzymes brought about by cellobiose which is an intermediary product that is produced during enzymatic hydrolysis of cellulose, and the deactivation of enzymes due to prolonged usage [3, 4, 20–23]. Other challenges associated with enzymatic hydrolysis of LGB lay in finding the most suitable pretreatment process and less costly enzyme cocktails [24]. Several advances in enzyme research have been realized that have enabled a reduction in enzyme loading which has been found to reduce the cost of enzymes used in hydrolysis of LGB [3, 4]. The highly complex and recalcitrance nature of LGB tends to resist hydrolysis using enzymes; thus, the use of chemicals such as acids is preferred [24]. Acid hydrolysis of LGB is influenced by factors such as hydrolysis temperature, reaction time, particle size of LGB, concentration of acid, and SLR. This is due to the fact that different LGB differ in terms of structure and composition [12, 25].

The main advantages of concentrated acid hydrolysis over dilute acid hydrolysis of LGB include higher reaction rates, mild operating conditions (temperature and pressure), higher sugar recovery (over 90% yield), and minimum degradation of fermentable sugars, and the process is flexible towards different substrates [17, 21, 24]. The main challenges that exist in concentrated acid hydrolysis of LGB include the need for corrosion resistant reactors and acid recovery process so as to make the entire process economically feasible [17, 24]. Despite these challenges, concentrated acid hydrolysis of LGB has been claimed to be a low-cost process of hydrolyzing LGB [24].

The economic viability of biomass to bioethanol conversion process depends on the yield of fermentable sugars during the hydrolysis process. The yield is normally impacted negatively by the disintegration of these sugars into fermentation inhibitors. Therefore, the hydrolysis method chosen must ensure minimum degradation of fermentable sugars [26].

There has been renewed interest in concentrated acid hydrolysis of LGB due to improved sugar-acid separation processes [17]. According to Janga et al. [13], LGB is mixed with concentrated acid in order to decrystallize cellulose in a reaction where the concentrated acid disrupts inter- and intramolecular hydrogen bonding responsible for the crystallinity found in cellulose. The acid tends to decrystallize the crystalline portion of cellulose converting it into amorphous cellulose which is easily hydrolyzed into glucose under mild conditions with minimum formation of fermentation inhibitors [13, 16, 27]. The economic viability of concentrated acid hydrolysis of LGB can be improved through selection of optimum reaction conditions involved in the process. The selection of hydrolysis conditions that results in high yield of fermentable sugars can be achieved through optimization [28]. During optimization, the yield of fermentable sugars is improved due to low formation of fermentation inhibitors [14].

Gadam/Sila sorghum is a new variety of sorghum that was introduced in Bungoma, Siaya, Kitui, Makueni, Tharaka Nithi, and Machakos Counties in Kenya [29]. The major features that distinguish it from the usual varieties of sorghum include drought resistance, fast growing, and high yielding variety [29]. The use of Gadam sorghum grain in beer production has been on the rise in Kenya [29, 30]. Gadam sorghum grain is a promising raw material for beer manufacture due to its high carbohydrate content [30]. Farmers are being encouraged to venture into Gadam sorghum farming. The production of Gadam sorghum is being promoted by various organizations spearheaded by the Ministry of Agriculture (MOA), Kenya Agricultural and Livestock Research Organization (KALRO), the Provincial Administration, Smart Logistics Ltd., Equity Bank, and East African Breweries Limited (EABL) [30].

The Kenyan Government has been promoting the use of indigenous sources of energy. To this end, the Government has developed a national policy on energy [31]. This policy advocates for the development and use of biofuels.

Bioethanol has been identified as a candidate biofuel whose production from biomass is envisaged. The policy has identified several challenges that impede the production of biofuels in Kenya. These challenges include lack of efficient technologies for the production and conversion of biomass into biofuels, lack of adequate data on the use and sustainable production of biofuels and lack of sufficient and sustainable substrates for the production of biofuels [31].

After the energy crisis of 1970s, Kenya began large-scale production of bioethanol from sugar cane molasses [1]. Currently, the leading company in bioethanol production from sugar cane molasses is the Agro Chemical and Food Corporation (ACFC) situated in Muhoroni, Kisumu County. ACFC was established in 1978 with a capacity of about 45000 liters per annum [1]. Mumias Sugar Company (MSC) situated in Kakamega County used to produce bioethanol from sugar cane molasses but has since shut down due to mismanagement and lack of support from the government.

Large-scale production of bioethanol has stagnated over the years especially in Sub-Saharan Africa (SSA) [1, 4]. The main challenge has been the lack of sufficient, low cost, and sustainable substrates [1]. In addition, inefficient bioethanol production processes especially hydrolysis contribute to the production of costly bioethanol [4]. In Kenya, the main substrates for bioethanol production are starch- and sugar-based in nature. However, the use of these classes of substrates is being discouraged because they are mainly used as food sources for human beings and animals. It is projected that the use of these substrates will continue causing food insecurity and the production of costly bioethanol [1, 3, 4, 14, 32]. On the other hand, cultivation of energy crops dedicated for biofuel production affects food supply due to competition for land, thereby leading to food insecurity [1]. The production of 2GBE on a large-scale especially in SSA is still undergoing improvements in order to reduce production costs. The use of LGB and optimization of the hydrolysis process in order to improve on the yield of fermentable sugars are some of the ways of overcoming these challenges.

Several studies involving the production of fermentable sugars from agricultural residues such as wheat straws, barley straws, rice straws, banana pseudostem, and corn stover have been reported in literature. Kim [9] did a study on fermentable sugars production from sodium hydroxide pretreated soybean straws. Legodi et al. [15] reported on fermentable sugars production from banana pseudostem.

Agricultural residues are preferred over starch- and sucrose-based substrates because they are available at low cost, they are sustainable and their production does not require any additional land [1, 4]. Despite the above advantages, no study has been reported in literature concerning the use of Gadam sorghum stalks as substrate for the production of fermentable sugars in Kenya. To address this gap, this research study is aimed at optimizing conditions involved in concentrated sulphuric acid hydrolysis of Gadam sorghum stalks for the production of fermentable sugars.

The average consumption of gasoline in Kenya was about 166 million liters per month between January and June 2019 [33]. Under the E10 policy, where 90% gasoline is blended with 10% bioethanol, about 200 million liters of bioethanol will be required every year for blending with gasoline. In addition, Nairobi, which is the capital city of Kenya requires about 120 million liters of bioethanol per year for cooking against a production capacity of 1.8 million liters per year [1, 33]. The grains of a sorghum plant account for about 7.0% of the total weight while the stalks account for 70-75% [34]. The annual production of sorghum grain in Kenya was 117,000 metric tonnes in 2016 [35]. This production rate has the potential of producing over 1.2 million tonnes of sorghum stalks in Kenya [34]. In order to meet the bioethanol demand in Kenya, about 0.815 million tonnes of Gadam sorghum stalks are required [33]. Therefore, the availability of sorghum stalks that can support large-scale bioethanol production in Kenya is quite promising. Large-scale production of bioethanol in Kenya will help address the existing challenges in energy provision through blending of gasoline with bioethanol thus reducing the quantity of gasoline that is imported into the country. In order to achieve effective decrystallization of LGB using concentrated acid, it is important to have proper control and management of reaction conditions involved in the process [13].

The main objective of this study was to optimize reaction conditions involved in concentrated sulphuric acid hydrolysis of Gadam sorghum stalks found in Kenya for the production of fermentable sugars. The use of concentrated acid in hydrolysis of LGB has continued to be of interest, and it is necessary to test the same on agricultural residues such as Gadam sorghum stalks. This will provide information on the applicability of this method of hydrolysis on a wide variety of LGB such as Gadam sorghum stalks that have not been studied in literature. Most studies reported in literature involve two-stage concentrated acid hydrolysis of LGB [11, 13, 24]. The current study reports on the findings of a one-stage concentrated acid hydrolysis process, which demonstrates the potential of Gadam sorghum stalks as suitable substrates for the production of fermentable sugars.

2. Materials and Methods

This section reports on experimental design, processing, and pretreatment of substrate, hydrolysis of substrate, and analysis of glucose.

2.1. Experimental Design. Central composite rotatable design (CCRD) was used to determine the optimum combination of hydrolysis conditions (temperature, time, and acid concentration) under constrained particle size and SLR. Design-Expert 13 (statistical software) was used to generate the CCRD matrix (Tables 1 and 2), plotting of graphs and subsequent analysis of data. Three-dimensional response surface methodology (RSM) plots were generated using Design-Expert 13 in order to evaluate the effects of the variables on the response. RSM and analysis of variance (ANOVA) were used to interpret the results. In order to fit the experimental data, a second-degree regression model

Table 1: CCRD matrix for actual and coded level of hydrolysis factors.

Independent variables	Symbol	Range and levels				
		Axial (-α)	Min (-1)	Center (0)	Max (+1)	Axial (+α)
Temperature, (°C)	X_1	26.4	40	60	80	93.6
Time (min)	X_2	9.5	30	60	90	110.5
Acid concentration (w/w, %)	X_3	16.36	30	50	70	83.64

Table 2: Experimental design matrix for the hydrolysis process: actual and coded levels of factors.

Std	Run	X_1 (temperature, °C)	X_2 (time, min)	X_3 (acid concentration, %, w/w)
8	1	26.4 (-1.682)	60 (0)	50 (0)
7	2	60 (0)	60 (0)	16.36 (-1.682)
20	3	80 (1)	30 (-1)	70 (1)
10	4	60 (0)	60 (0)	50 (0)
6	5	80 (1)	90 (1)	70 (1)
17	6	60 (0)	9.5 (-1.682)	50 (0)
11	7	80 (1)	30 (-1)	30 (-1)
2	8	40 (-1)	30 (-1)	30 (-1)
15	9	60 (0)	60 (0)	50 (0)
16	10	40 (-1)	90 (1)	30 (-1)
14	11	60 (0)	60 (0)	50 (0)
19	12	40 (-1)	30 (-1)	70 (1)
18	13	60 (0)	60 (0)	83.64 (1.682)
1	14	60 (0)	60 (0)	50 (0)
12	15	93.6 (1.682)	60 (0)	50 (0)
9	16	80 (1)	90 (1)	30 (-1)
4	17	60 (0)	60 (0)	50 (0)
3	18	60 (0)	110.5 (1.682)	50 (0)
13	19	40 (-1)	90 (1)	70 (1)
5	20	60 (0)	60 (0)	50 (0)

was obtained. The experimental data was subjected to regression analysis using RSM so as to establish the relationship between independent (temperature, time, and acid concentration) and dependent (glucose yield) variables. The model was used to establish the predicted value of glucose yield. In order to evaluate the fitness of the model equation on glucose yield, coefficient of determination (R^2) and lack of fit test using F-test were used.

2.2. Processing and Pretreatment of Substrate. Gadam sorghum stalks (substrate) were obtained from Bungoma, which is a County found in the Western part of Kenya. The substrate was cleaned using fresh tap water and dried under the sun for fourteen days. Further drying was done using a hot air oven set at 60°C for 24 hours [27]. The substrate was then subjected to physical pretreatment through milling and thereafter sieved to pass 0.8 mm screen. The chemical composition of substrate was established based on a study by [36]. The sieved substrate was collected and stored in sealed plastic containers prior to hydrolysis experiments. Gadam sorghum stalks used in this study are shown in Figure 1.

2.3. Hydrolysis of Substrate. 15 grams of substrate was soaked in sulphuric acid solution to form a SLR of 15.0% (w/v) in the hydrolysis reactor. The temperature, time, and concentration of acid was determined from the experimental design matrix (Tables 1 and 2) for each experimental run. The reactor was heated using a water bath to a temperature and for a period of time determined from the experimental design matrix. A total of 20 experimental runs were carried out based on the CCRD. At the end of each experimental run, the hydrolysate was separated from the spent solids through filtration. The spent solids were washed using distilled water in order to remove any adhering hydrolysate. The hydrolysate was then diluted using distilled water to form 1000 ml solution which was then treated with calcium hydroxide to a pH of 7.0 [17, 27]. Tables 1 and 2 show the actual and coded levels of hydrolysis factors that were investigated and the experimental design matrix, respectively.

2.4. Analysis of Glucose. In this research study, the concentration of glucose released during hydrolysis of substrate was determined spectrophotometrically [37, 38]. This procedure was based on Sigma glucose hexokinase (HK) assay kit [37]. The measurements and analysis were performed according to the instructions provided by the manufacturer of the assay kit [37]. The glucose assay method was based on a two-step enzymatic reaction, where glucose contained in the sample was first phosphorylated to glucose-6-phosphate (G6P) by adenosine triphosphate (ATP) in the presence of HK. G6P was then reacted with nicotinamide-adenine dinucleotide phosphate (NADP+) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to form gluconate-6-phosphate and reduced nicotinamide-adenine dinucleotide phosphate (NADPH). In order to estimate the concentration of glucose in the hydrolysis sample, the corresponding increase in NADPH was measured at 340 nm using a spectrophotometer [39, 40].

2.5. Reagents, Standards, and Equipment. The following reagents and standards were procured for use in the research: sodium hydroxide, sulphuric acid, calcium hydroxide, glucose standard, glucose hexokinase (HK) reagent, and ethanol. The major equipment were as follows: analytical balance (Mettler AE 160), oven (LabTech-LDO 150F),

Figure 1: Gadam sorghum stalk samples.

furnace (Carbolite Gero-ELF 11/14B), centrifuge machine (Elite), sieve and sieve shaker (Liya), pH meter (Labtech Digital meter), vacuum filtration unit (Rocker-Chemker 410), UV-Vis-spectrophotometer (Shimadzu), water bath (JOANLAB), and Soxhlet apparatus (Pyrex UK).

3. Results and Discussion

3.1. Composition of Gadam Sorghum Stalks. The composition of substrate established in the current study is shown in Table 3.

The carbohydrate (cellulose and hemicellulose) content in Gadam sorghum stalks is 51.0% by weight. This is a strong indication of its potential for use as a feedstock for the production of fermentable sugars which can be used to produce bioethanol [12, 41].

3.2. Glucose Yield. The actual and predicted yield of glucose obtained under various experimental conditions is shown in Table 4.

By applying multiple regression analysis on the experimental data, a second-degree polynomial was found to represent the relationship between glucose yield (response) and the independent variables (hydrolysis temperature, hydrolysis time, and acid concentration) adequately. The quadratic model (Equation (1)) was selected as suggested by Design-Expert 13 software. Prediction of glucose yield from concentrated sulphuric acid hydrolysis of Gadam sorghum stalks was done using Equation (1).

$$\begin{aligned} Y_{\mathrm{GSS}} = 86.22 &+ 1.39X_1 + 3.73X_2 + 9.66X_3 \\ &+ 1.85X_1X_2 - 3.43X_1X_3 + 1.18X_2X_3 \\ &- 3.97X_1^2 - 7.95X_2^2 - 7.91X_3^2, \end{aligned} \tag{1}$$

where

$$Y_{\mathrm{GSS}} = \text{predicted yield of glucose from Gadam sorghum stalks } (\%). \tag{2}$$

In order to test the significance of the model depicting the yield of glucose from Gadam sorghum stalks, Design-Expert 13 was used to perform ANOVA. The results are as shown in Table 5–7.

The results of ANOVA for testing the significance of the model equation representing the hydrolysis of Gadam sorghum stalks are shown in Table 5. From Table 5, the model representing the yield of glucose has an F value of 88.25. This indicates that the experimental data involving the yield of glucose from Gadam sorghum stalks is well evaluated by Equation (1). The model F value (88.25) implied that the model was significant, and there was negligible chance (0.01%) that an F value this large can occur because of noise. The implication of a higher F value is that there is a high likelihood that the results did not happen by chance. The lack of fit F value of 2.78 implies that the lack of fit is not significant relative to pure error. There is a 14.31% chance that a lack of fit F value this large could occur due to noise. In addition, from Table 6, the coefficient of determination (R^2) for the model was 0.9876 with an adjusted R^2 of 0.9764 and predicted R^2 of 0.9218 for the yield of glucose from Gadam sorghum stalks, which indicated that the model adequately represented the real relationship between hydrolysis time, hydrolysis temperature, and concentration of sulphuric acid. An R^2 value of 0.9876 means that 98.76% of the variability was explained by the model and only 1.24% was as a result of chance. The validity of the model is supported by the high coefficient of determination R^2. This suggests that the model obtained can be used for prediction and simulation of the dependency of the yield of glucose on hydrolysis time, hydrolysis temperature, and concentration of sulphuric acid within the range of investigation.

3.3. Model Reduction. In order to test the significance of the various regression coefficients that describe the yield of glucose from Gadam sorghum stalks in Equation (1), Design-Expert 13 was used during the analysis. From Table 5, P values lower than 0.05 indicate that the model terms are significant. In the present study, X_1, X_2, X_3, X_1X_2, X_1X_3, X_1^2,

Table 3: Gadam sorghum stalks: chemical composition.

Component	Percentage (w/w)
Cellulose	27.49
Hemicellulose	23.51
Lignin	33.00
Ash	5.33
Moisture	9.98
Extractives	0.69
Total	100

${X_2}^2$, and ${X_3}^2$ are significant model terms. However, P values greater than 0.1 indicate that the model terms are not significant. If there are many insignificant model terms (not including those required to support model hierarchy), model reduction my improve the model. From ANOVA (Table 5), the interaction term X_2X_3 is not significant (P value is 0.1355). This term was removed, and the reduced model is shown as Equation (3).

$$
\begin{aligned}
Y_{\mathrm{GSSR}} = 86.22 + 1.39X_1 + 3.73X_2 + 9.66X_3 \\
+ 1.85X_1X_2 - 3.43X_1X_3 - 3.97X_1^2 \\
- 7.95X_2^2 - 7.91X_3^2,
\end{aligned}
\tag{3}
$$

where

$$
Y_{\mathrm{GSSR}} = \text{Predicted yield of glucose from Gadam sorghum stalks (\%)}. \tag{4}
$$

The results of ANOVA for testing the significance of the reduced model equation representing the hydrolysis of Gadam sorghum stalks are shown in Table 8. From Table 9, the reduced model representing the yield of glucose from Gadam sorghum stalks has an F value of 86.14. This indicates that the experimental data involving the yield of glucose from Gadam sorghum stalks is well evaluated by Equation (3). The model F value (86.14) implied that the reduced model was significant, and there was negligible chance (0.01%) that an F value this large can occur because of noise. The implication of a higher F value is that there is a high likelihood that the results did not happen by chance. The lack of fit F value of 3.15 implies that the lack of fit is not significant relative to pure error. There is a 11.46% chance that a lack of fit F value this large could occur due to noise. In addition, from Table 10, the coefficient of determination (R^2) for the reduced model was 0.9843 with an adjusted R^2 of 0.9729 and predicted R^2 of 0.9289 for the yield of glucose from Gadam sorghum stalks which indicated that the reduced model adequately represented the real relationship between hydrolysis time, hydrolysis temperature, and concentration of sulphuric acid.

3.4. Optimization of Concentrated Sulphuric Acid Hydrolysis of Gadam Sorghum Stalks. In order to optimize the variables that influence concentrated sulphuric acid hydrolysis of Gadam sorghum stalks, response surface, and contour plots were generated using Design-Expert 13. The response surface and contour plots (three-dimensional (3D) surface plots) were generated by keeping one variable constant at the center point (0) and varying the other two variables within the experimental range. The RSM plots were plotted in terms of coded factors as shown in Table 1. The resulting response surface and contour plots showed the effect of hydrolysis temperature (X_1), time (X_2), and acid concentration (X_3) on the yield of glucose from Gadam sorghum stalks.

Table 4: Gadam sorghum stalks: results of experimental and predicted glucose yield.

Std	Run	X_1	X_2	X_3	Y (glucose yield, w/w) Actual (%, w/w)	Predicted (%, w/w)
8	1	-1.682	0	0	70.56	72.65
7	2	0	0	-1.682	45.59	47.59
20	3	1	-1	1	66.68	67.25
10	4	0	0	0	84.38	86.22
6	5	1	1	1	81.47	80.77
17	6	0	-1.682	0	55.29	57.45
11	7	1	-1	-1	59.41	57.15
2	8	-1	-1	-1	52.13	51.21
15	9	0	0	0	87.05	86.22
16	10	-1	1	-1	54.8	52.6
14	11	0	0	0	84.38	86.22
19	12	-1	-1	1	77.35	75.02
18	13	0	0	1.682	79.78	80.08
1	14	0	0	0	87.54	86.22
12	15	1.682	0	0	77.11	77.33
9	16	1	1	-1	65.23	65.93
4	17	0	0	0	87.54	86.22
3	18	0	1.682	0	69.84	69.98
13	19	-1	1	1	80.5	81.14
5	20	0	0	0	86.81	86.22

Figure 2 shows the response surface and the corresponding contour plot for the yield of glucose (R_1) as a function of hydrolysis temperature (X_1) and hydrolysis time (X_2). The response surface and contour plot depict how the yield of glucose from Gadam sorghum stalks varied with hydrolysis temperature and hydrolysis time. From the plot, maximum glucose yield was 87.54% which was realized when hydrolysis temperature and hydrolysis time were 60°C and 60 minutes, respectively. From Table 1, the level of these factors (temperature and time) was at the central setting and corresponded to the smallest eclipse as illustrated by the contour plot. The experimental observations were compared with the results depicted by the response surface and contour plot. There was good agreement between experimental and predicted results which showed that optimum yield of glucose from Gadam sorghum stalks (87.54%) was realized at 60°C (temperature) and 60 minutes (hydrolysis time). An increase in hydrolysis temperature and time resulted in an increase in glucose yield until the optimum value of 87.54%. Any

Table 5: Gadam sorghum stalks: ANOVA for testing the significance of the model equation (Source: Design-Expert 13 Software).

Source	Sum of squares	df	Mean square	*F* value	*P* value	
Model	3368.12	9	374.24	88.25	<0.0001	Significant
X_1	26.47	1	26.47	6.24	0.0315	
X_2	189.7	1	189.7	44.73	<0.0001	
X_3	1274.75	1	1274.75	300.6	<0.0001	
X_1X_2	27.35	1	27.35	6.45	0.0294	
X_1X_3	93.85	1	93.85	22.13	0.0008	
X_2X_3	11.18	1	11.18	2.64	0.1355	
X_1^2	227.11	1	227.11	53.55	<0.0001	
X_2^2	912.2	1	912.2	215.11	<0.0001	
X_3^2	902.4	1	902.4	212.79	<0.0001	
Residual	42.41	10	4.24			
Lack of fit	31.19	5	6.24	2.78	0.1431	Not significant
Pure error	11.22	5	2.24			
Total	3410.53	19				

Table 6: Model fit statistics (Source: Design-Expert 13 Software).

Parameter	Value
R^2	0.9876
Adjusted R^2	0.9764
Predicted R^2	0.9218

Table 7: Model coefficients (Source: Design-Expert 13 Software).

Factor	Coefficient
Intercept	86.22
X_1	1.39
X_2	3.73
X_3	9.66
X_1X_2	1.85
X_1X_3	-3.43
X_2X_3	1.18
X_1^2	-3.97
X_2^2	-7.95
X_3^2	-7.91

further increase in hydrolysis temperature and time was found to be unfavourable for the yield of glucose as explained by the decreasing trend observed. This could be attributed to the degradation of glucose to fermentation inhibitors due to increased hydrolysis temperature and time.

Kanchanalai et al. [17] carried out kinetic studies of concentrated acid hydrolysis of Avicel, which is a pure form of cellulose and xylan, the major component in hemicellulose. The concentration of sulphuric acid used was varied (10% to 50%, *w*/*w*), while temperature was varied (80 to 100°C). The authors reported that there was a decline in glucose concentration as the hydrolysis time increased past 120 minutes, mainly due to the decomposition of glucose. On the other hand, there was a notable decrease in glucose concentration at constant acid concentration (40%, *w*/*w*) as temperature and time increased. The current research study deals with real LGB unlike the study reported by Kanchanalai et al. [17], which deals with pure form of cellulose. The study establishes optimum conditions involved in concentrated sulphuric acid hydrolysis of Gadam sorghum stalk.

Wijaya et al. [24] did a study on the effect of acid concentration (65-80%, *w*/*w*), hydrolysis temperature (80°C-100°C), and time (2 hours) on sugar recovery for different biomass species (oak wood, pine wood, and empty fruit bunch (EFB) of palm oil). Under optimized conditions, the range of theoretically extractable sugars was 78-96% for the three biomass species. The authors further reported that the hydrolysis reaction time affected the concentration of sugar at higher temperature (100°C) because extending the hydrolysis reaction time decreased the overall sugar yield at this temperature. In the current research study, the main hydrolysis parameters (temperature, time, and acid concentration) were optimized in a single-stage hydrolysis process.

Sarrouh et al. [27] did a study on hydrolysis of sugarcane bagasse using 70% (*w*/*w*) sulphuric acid solution, at a temperature of between 30°C and 70°C and for a time interval of between 10 and 90 minutes. The authors reported that the yield of fermentable sugars increased as temperature increased up to 50°C. The maximum reported yield of fermentable sugars was 87.6% which was obtained at 60 minutes and a temperature of 50°C. This was followed by a decrease in the yield of fermentable sugars (to 86.4%) when the reaction was carried on after one hour.

The effect of hydrolysis temperature (X_1) and acid concentration (X_3) on the yield of glucose (R_1) is shown in Figure 3. An increase in hydrolysis temperature along with

Table 8: Gadam sorghum stalks: ANOVA for testing the significance of the reduced model equation (Source: Design-Expert 13 Software).

Source	Sum of squares	df	Mean square	*F* value	*P* value	
Model	3356.95	8	419.62	86.14	<0.0001	Significant
X_1	26.47	1	26.47	5.43	0.0398	
X_2	189.7	1	189.7	38.94	<0.0001	
X_3	1274.75	1	1274.75	261.68	<0.0001	
X_1X_2	27.35	1	27.35	5.61	0.0372	
X_1X_3	93.85	1	93.85	19.27	0.0011	
${X_1}^2$	227.11	1	227.11	46.62	<0.0001	
${X_2}^2$	912.2	1	912.2	187.25	<0.0001	
${X_3}^2$	902.4	1	902.4	185.24	<0.0001	
Residual	53.59	11	4.87			
Lack of fit	42.37	6	7.06	3.15	0.1146	Not significant
Pure error	11.22	5	2.24			
Total	3410.53	19				

Table 9: Model coefficients for the reduced model (Source: Design-Expert 13 Software).

Factor	Coefficient
Intercept	86.22
X_1	1.39
X_2	3.73
X_3	9.66
X_1X_2	1.85
X_1X_3	-3.43
${X_1}^2$	-3.97
${X_2}^2$	-7.95
${X_3}^2$	-7.91

Table 10: Model fit statistics for the reduced model (Source: Design-Expert 13 Software).

Parameter	Value
R^2	0.9843
Adjusted R^2	0.9729
Predicted R^2	0.9289

a steady increase in acid concentration resulted in an increase in glucose yield until an optimum value of 87.54%. This was realized at 60°C hydrolysis temperature and 50.0% (*w*/*w*) acid concentration. Under conditions of high acid concentration, the crystalline fraction of cellulose found in LGB is disintegrated and more glucose is produced [15, 16]. Since the acid acts as a catalyst, high concentration of acid increases the rate of the hydrolysis reaction by attacking and degrading the crystalline portion of cellulose, resulting in high glucose yield [15]. Further increase in hydrolysis temperature and acid concentration beyond the optimum levels resulted in a decrease in the yield of glucose.

Janga et al. [13] did a study of the influence of acid concentration, temperature, and time on decrystallization of cellulose in a two-stage concentrated sulphuric acid hydrolysis of pine wood and aspen wood. The optimum predicted yield of total sugars obtained during the study were 74% (*w*/*w*) and 91% (*w*/*w*) for aspen wood and pine wood, respectively. The authors reported that the most influential variables on total sugar yield were acid concentration and temperature. The authors further reported that the formation of sugar degradation products such as HMF was mostly influenced by the reaction temperature.

According to Tizazu and Moholkar [16], glucose mainly forms from hemicellulose heteropolymers and amorphous fraction of cellulose during hydrolysis of LGB at low acid concentration. However, at relatively higher acid concentration, the crystalline fraction of cellulose also undergoes hydrolysis, thus resulting into higher glucose yields.

Kanchanalai et al. [17] reported that the concentration of glucose increased when the acid concentration increased from 20% to 40% (*w*/*w*). At 50% (*w*/*w*) acid concentration, the concentration of glucose increased with increase in reaction time up to 120 minutes and thereafter started to decline due to decomposition of glucose into fermentation inhibitors.

Wijaya et al. [24] reported that the production of glucose increased as the concentration of sulphuric acid increased from 70% to 75% (*w*/*w*). However, when the concentration of acid was increased to 80% (*w*/*w*), the concentration of fermentable sugars decreased. In addition, the authors reported that within the range of acid concentration (70% to 80% (*w*/*w*)) for oak wood, pine wood and EFB, a reduction in glucose yield was observed when the temperature was increased. This was attributed to degradation of sugars at high temperature (100°C). In the current research study, the use of moderate hydrolysis conditions which were obtained after optimization gave rise to high yield of fermentable sugars.

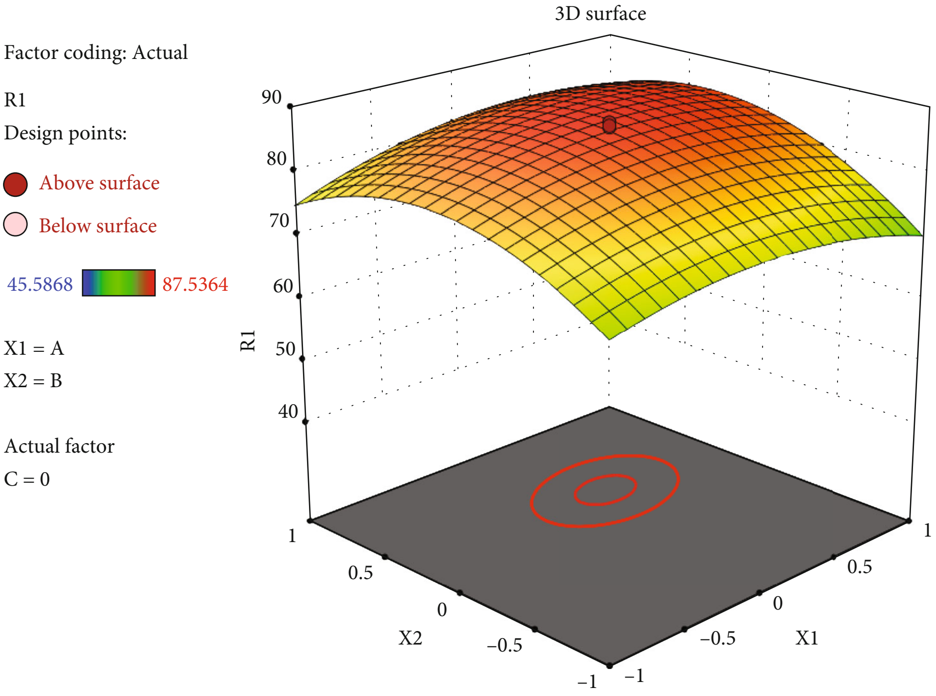

Figure 2: RSM plot: effect of temperature (X_1) and time (X_2) on glucose yield (R_1).

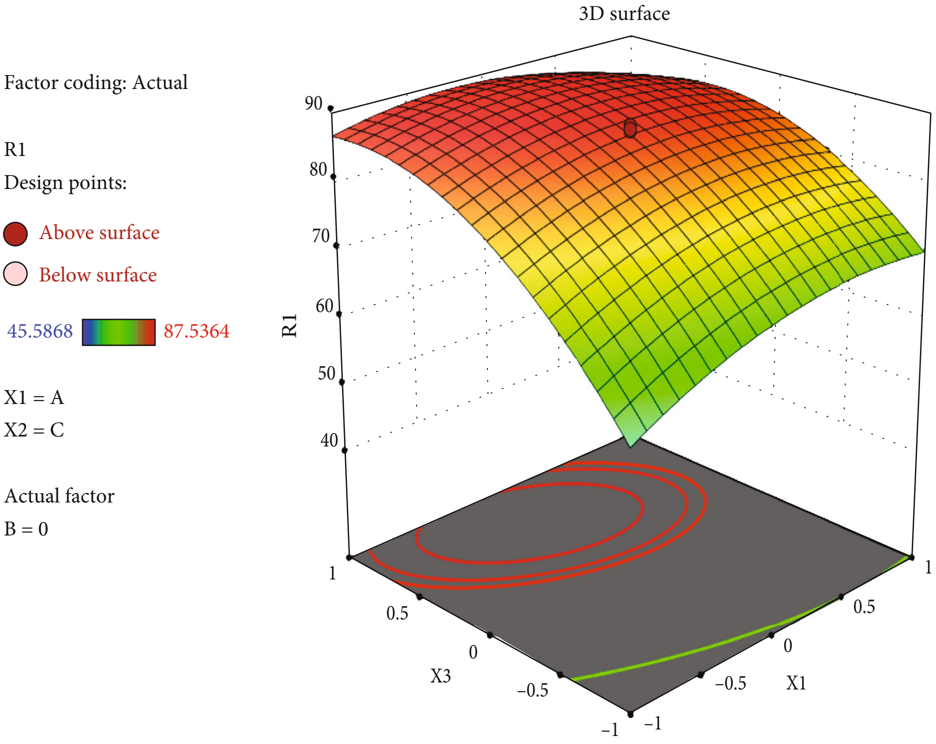

Figure 3: RSM plot: effect of temperature (X_1) and acid concentration (X_3) on glucose yield (R_1).

According to Chandel et al. [25], most researchers have reported that mild temperature leads to substantial recovery of fermentable sugars during acid hydrolysis of LGB, while higher temperatures cause more sugar degradation, contributing to the formation of fermentation inhibitors which leads to lower bioethanol yields. In the current research study, moderate hydrolysis reaction conditions (60°C, 60 minutes hydrolysis time and 50.0% (*w*/*w*) acid concentration) were established which gave rise to high yield of fermentable sugars.

Kolo et al. [42] reported that the concentration of reducing sugars obtained during microwave-assisted sulphuric acid hydrolysis of elephant grass increased as the concentration of acid increased. In addition, the authors reported that as the temperature and microwave irradiation power increased, the concentration of reducing sugars decreased as a result of degradation of sugars to fermentation inhibitors such as HMF.

Figure 4 shows the effect of hydrolysis time (X_2) and acid concentration (X_3) on the yield of glucose (R_1). The center point of Figure 4 reveals the optimal values of hydrolysis time and acid concentration that may be combined to obtain optimal yield of glucose from Gadam sorghum stalks. This was revealed to be 60 minutes hydrolysis time and 50.0% (w/w) acid concentration. At time levels higher than 60 minutes (central setting), the yield of glucose started to decrease slightly. This could be attributed to the degradation of glucose into fermentation inhibitors due to prolonged hydrolysis period.

Wijaya et al. [24] reported that at lower acid concentration (65–70% (w/w)) and 100°C, the concentration of glucose increased over time while at higher acid concentration (80% (w/w)) and 100°C, glucose concentration significantly decreased over time. High concentration of acid was capable of hydrolyzing cellulose into glucose within a shorter time, unlike low acid concentration which required longer reaction time to hydrolyze as much cellulose into glucose.

Ioelovich [43] reported that treating cellulose with 50%-60% (w/v) sulphuric acid solution at room temperature gave rise to a reduction in crystallinity of cellulose by 25%-30%. The solubility of the resulting cellulose increased while the polymerization degree decreased. The study indicated that sulphuric acid has the potential of hydrolyzing LGB into fermentable sugars. This is demonstrated in the current research study which resulted in high yield of fermentable sugars during single-stage concentrated sulphuric acid hydrolysis of Gadam sorghum stalks. In order to select the optimum conditions and their respective levels, the model was analysed. The maximum response predicted from the model was 86.22% glucose yield. There was no significant difference between experimental and predicted values of glucose yield as shown in Table 4. The final optimized hydrolysis conditions obtained with RSM were 60°C (temperature), 60 minutes (hydrolysis time), and 50.0% (w/w) (acid concentration).

3.5. Overall Discussion. In the current research study, the yield of glucose realized was 87.54% (w/w). This was achieved at 60 minutes hydrolysis time, 60°C hydrolysis temperature, and 50.0% (w/w) concentration of sulphuric acid. Gadam sorghum stalks were subjected to physical/mechanical pretreatment through milling in order to enhance the penetration of concentrated sulphuric acid within the substrate particles for effective hydrolysis. Legodi et al. [15] reported that physical pretreatment (ball milling) reduces the crystallinity and degree of polymerization of polymers found in corn stover. This enhances the conversion of cellulose to glucose during hydrolysis [15].

A study by Kim [9] obtained 90.9% yield efficiency for glucose from soybean straw pretreated using sodium hydroxide and hydrolyzed using enzymes. In the current study, the yield of fermentable sugars is 87.54%, which is obtained in a single-stage hydrolysis process. The number of process steps is reduced in the current study which has the potential of reducing the capital costs during large-scale hydrolysis of LGB to fermentable sugars, thus contributing to a reduction in 2GBE production cost [4].

In a study by Legodi et al. [15], sodium hydroxide pretreated banana pseudostem produced the highest amount of glucose (43.5 g/l) upon enzymatic hydrolysis while liquid hot water (LHW) pretreated banana pseudostem had the highest saccharification efficiency (86.7%, w/w). Kolo et al. [42] did a study of microwave-assisted sodium hydroxide pretreatment and sulphuric acid hydrolysis of elephant grass (biomass) and obtained a saccharification efficiency of 66.57%. The hydrolysis method used in the current study has minimum chemical requirements compared to what is reported in literature [4, 9, 15, 42].

Chu et al. [44] did a study on kinetics of cotton cellulose hydrolysis using concentrated sulphuric acid. The range of acid and solid concentration was 45–60% (v/v) and 30-70 g/l, respectively. In addition, the reaction temperature was set at 27-50°C. The authors reported that there was partial hydrolysis of cotton cellulose into fermentable sugars when the concentration of sulphuric acid was below 53%. In addition, only 8.8% cotton cellulose was hydrolyzed when the concentration of sulphuric acid was 45%. Cotton cellulose dissolved completely when the concentration of sulphuric acid was above 55% (v/v) [44]. The authors further reported that at a reaction temperature of 40°C, 90 minutes reaction time, 55% (v/v) concentration of sulphuric acid and initial cotton cellulose concentration of 30-70 g/l, the yield of reducing sugars varied between 64.3 to 73.9% (w/w). Most studies reported in literature deal with multi-stage hydrolysis of LGB [11, 13, 24]. In addition, few studies report on optimization of hydrolysis conditions. The current study reports on the findings of single-stage hydrolysis process that establishes optimum and moderate conditions that results in high yield of fermentable sugars. The results obtained in the current study for glucose yield are within the range reported in literature. The difference between the results from the current study and those in literature can be attributed to the type of substrate (structure and composition) and process conditions.

4. Conclusions

Large-scale production of 2GBE and subsequent blending with gasoline for use in the transportation sector represents one way of reducing the dependency on fossil fuels which contribute to environmental pollution. Despite the existence of an elaborate policy on biofuels, large-scale production of 2GBE in Kenya has been stagnant. The main drawbacks include the lack of suitable, low cost, and sustainable substrates. In addition, the lack of sufficient data on optimum process conditions involved in 2GBE production impedes the growth of the industry.

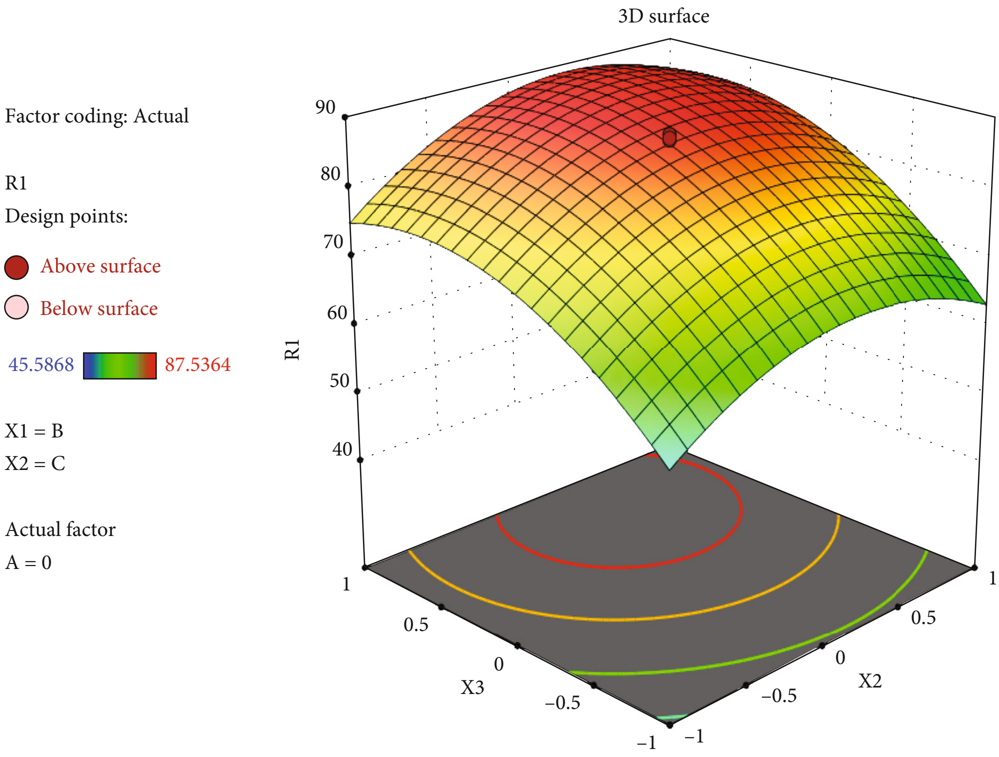

Figure 4: RSM plot: effect of time (X_2) and acid concentration (X_3) on glucose yield (R_1).

The objective of this research study was to optimize hydrolysis conditions involved in concentrated sulphuric acid hydrolysis of Gadam sorghum stalks found in Kenya. During hydrolysis, the parameters that were varied included temperature (40°C–80°C), time (30-90 minutes), and concentration of acid (30%-70%, *w*/*w*). CCRD was used to establish optimum levels of hydrolysis conditions. RSM and ANOVA were used to interpret the results.

The results from the study indicate that the maximum glucose yield (87.54%, *w*/*w*) from Gadam sorghum stalks was realized at 60 minutes hydrolysis time, 60°C hydrolysis temperature, and 50.0% (*w*/*w*) concentration of sulphuric acid. The minimum yield of glucose (45.59%, *w*/*w*) was realized at 60 minutes hydrolysis time, 60°C hydrolysis temperature, and 16.36% (*w*/*w*) acid concentration. The yield of glucose realized from concentrated sulphuric acid hydrolysis of Gadam sorghum stalks is of sufficient quantity that can be used to produce bioethanol. The quadratic regression model equation developed from this study can be used to predict the yield of glucose during concentrated sulphuric acid hydrolysis of Gadam sorghum stalks.

This research study contributes knowledge regarding the use of Gadam sorghum stalks as substrate for the production of fermentable sugars in Kenya. The use of Gadam sorghum stalks as substrate for the production of fermentable sugars will not affect food production since no additional land will be required for their production. In addition, since Gadam sorghum stalks are agricultural residues, they are available at low cost, they are sustainable and have the potential of reducing the cost of producing bioethanol in Kenya. The authors recommend further study on the technoeconomic viability of a large-scale process of hydrolyzing Gadam sorghum stalks using concentrated sulphuric acid.

Data Availability

The article contains all the relevant data. The corresponding author would provide any additional data upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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