Optimization study of bioethanol production from sponge gourd (*Luffa cylindrica*)

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ARTICLE INFO

ABSTRACT

Luffa cylindrica also known as sponge gourd (*SG*), is a non-edible feedstock that is readily available and under-utilized. In this study, *SG* as a potential source for production of ethanol was studied under statistically optimized conditions. *SG* was collected, peeled, dried, milled and sieved (1mm). Several pretreatment methods were employed on *SG* namely: steam explosion, alkaline, combination of alkaline and steam explosion, zinc chloride and sodium sulphite. Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to design and determine the optimum parameters for glucose yield as well as the fermentation for bioethanol production. The best pretreatment method for sponge gourd was investigated to be sodium sulphite pretreatment with a glucose yield of 6.65 kgm^{-3}. The sodium sulphite pretreated *SG* was modelled, optimized and validated with R^2 of 0.9974 at $p < 0.05$. Glucose production was optimal at conditions: sodium sulphite (9% w/w), temperature (100 °C) and reaction time (60 min) resulting in glucose yield of 6.673 kgm^{-3}. From the CCD, the factors that gave the highest ethanol concentration of 6.84kgm^{-3} were inoculum size (7.5 v/v), fermentation time (24 h) and nitrogen source (inorganic). The study concluded that sponge gourd could be a potential feedstock for bioethanol production and would prevent under-utilized agro-waste materials.

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List of symbols

Symbol	Definitions	S.I units
b_o	Intercept value	
b_i	First order model coefficient	
b_{ii}	Quadratic coefficient of Xi	
b_{ij}	Interaction effect	
E	Random error	
A	Sulphite concentration	v/v %
A^2	Quadratic term of sulphite concentration	
AB	Cross product of sulphite concentration and temperature	
AC	Cross product of sulphite concentration and reaction time	
B	Temperature	°C
B^2	Quadratic term of temperature	
C	Reaction time	S
C^2	Quadratic term of reaction time	s^2
F	Fisher	
P	Probability	
R	Coefficients of correlation	
R^2	Coefficients of determination	
X_i and X_j	Input variables	
Y	Response factor (yield)	kgm^{-3}

1. Introduction

Increased energy demands and the need to reduce global warming as a result of greenhouse gases emissions from petroleum derivatives have prompted the search for alternative fuels which are more environmentally friendly. Diverse options have been proposed which include bioethanol, biodiesel, methanol, hydrogen, boron, flammable gas, electricity, solar powers, liquefied petroleum gas (LPG), and Fisher-Tropsch fue. Biomass based fuels (Biofuels) are a renewable source of energy originating from agricultural products. It has presented many merits over Petroleum-based fuels [1]. The most generally utilized of the biofuel for transportation is bioethanol, which has low greenhouse emission effects and also reduces the release of carbon monoxide when utilized in cars as a fuel additive at the rate 5-10%. Bioethanol is along these lines considered as a vital renewable fuel to handle the present worldwide energy crisis and the worsening environment quality. The world production of bioethanol increased from 50 million m^3 in 2007 to more than 100 million m^3 in 2012 [2].

Biofuels can be sorted as primary and secondary biofuels. The natural and unprocessed biomass such as fuel-wood, wood chips, and pellets are alluded to as the primary biofuels. The secondary biofuels can be further divided into first, second and third generation biofuels based on crude materials and technology used for their production [3]. Fermentation of sugar-based raw material is referred to as 'first generation bioethanol', whereas the use of lignocellulose raw materials is usually called 'second generation bioethanol'. The 'third generations' of bioethanol take the upsides of uniquely design energy crops such as algae as its energy source. Subsequently, due to the numerous disadvantages of 1st and 3rd generations and evident points of interest of 2nd generation bioethanol, the ways to deal with fundamental usage of lignocellulosic biomass (LB) for sustainable development are more sensible. Lignocellulosic biomass is a complex polymer made up of three carbohydrates: cellulose, hemicellulose and lignin [4]. They are most times wastes from agricultural products, abundant in nature and can be utilized to address the difficulties of energy securities. These make them to stand out among other alternative energy sources. Annual production of LB is estimated to be 10-50 billion tons which is being examined by many researchers for bioethanol production (Joanne et al., 2010). Sponge gourd (SG) is a promising energy crop providing lignocellulosic biomass for biofuels production [5]. Sponge Gourd is a yearly herbaceous fibrous and climbing plant which can broaden the growth space in a vertical course to accomplished higher biomass yield. It is a plant from cucurbitaceous family and has a plant growth yield achieving 62,000 sponge gourd fruits/ha (20-25 fruits/plant), that is one mature sponge gourd will produce no less than 30 seeds or more.. The choice of sponge gourd among other LB is the fact that it grows well in Nigeria and other tropical nations with excellent adaptability to the atmosphere and barren soil [6], relatively available, cheap and underutilized.

Zaafouri et al. [5] reported the possible use of sponge gourd (*Luffa cylindrica*) a lignocellulosic biomass in possible production of bioethanol. Our study is the first of its kind where possible production of bioethanol from the sponge gourd was investigated using several pretreatment methods under statistically optimized conditions. The sponge gourd was milled and subjected to several pretreatments that allowed for optimum glucose yield. The fermentation of the glucose to ethanol by *Saccharomyces cerevisiae* offered a novel route to obtain ethanol from such cheap and biodegradable feedstock. Thus, a cheap, readily available and environmentally safe source for bioethanol production is envisaged with prospects adaptable for large scale production.

2. Material and methods

Fresh sponge gourds used in this study were obtained from a farm in Ipetumodu, Osun State, Nigeria. *Trichoderma reesei* cellulase was obtained from Sigma-Aldrich, USA. The yeast, *Saccharomyces cerevisiae,* was obtained from Biochemical Engi-

neering Laboratory of the Department of Chemical Engineering, Obafemi Awolowo University, Ile-Ife. All other reagents used in this study were used without further purification and were of analytical grade and were obtained either from Sigma-Aldrich, USA or British Drug House, Poole, England.

2.1. Pretreatment

The dried sponge gourd was milled and sieved using a sieve with a pore size of 1.0 mm. The dried, milled and sieved sponge gourd (*DMSG*) was then stored in sealed plastic bags at room temperature.

The *DMSG* were subjected to the following pretreatment methods

2.1.1. Alkaline pretreatment

The method of Ooi et al. [7] was modified and adopted for this pretreatment. Six grams of the *DMSG* was soaked in 25 % w/w sodium hydroxide for 30 min at room temperature. Ethanol of 10 % v/w was added as co-solvent for an additional 15 min. The sample was washed with warm distilled water until pH 7.0 was attained. The sample was then drained through a muslin cloth, oven dried to a constant weight and then stored in an airtight container to be used for subsequent hydrolysis.

2.1.2. Steam explosion

Six grams of *DMSG* was soaked in 100 ml of distilled water. The mixture was autoclaved at 121 °C for 15 min at 15 psi. The sample was then drained through muslin cloth, oven dried to a constant weight and then stored in an airtight container to be used for subsequent hydrolysis.

2.1.3. Combined alkaline and steam explosion

Alkaline pretreatment was carried out as described above, after which the mixture was autoclaved, washed with warm distilled water until pH 7.0 was attained and free from the alkali. The sample was then drained through muslin cloth, oven dried to a constant weight and stored in an airtight container to be used for subsequent hydrolysis.

2.1.4. Sodium sulphite pretreatment

The *DMSG* mixed in 6 % w/v sodium sulphite solution in a catalyst of 0.5% NaOH, at a temperature and reaction time of 100 °C and 100 min respectively. Pretreated solids were separated from the spent liquor by filtration and the solids were washed with distilled water and dried before undergoing saccharification [8].

2.1.5. Zinc chloride pretreatment

The method described by Awosusi *et al.* [9] was modified and used. Oven-dried zinc chloride salt was dissolved in deionized water at 71.6 % w/w to make $ZnCl_2.3H_2O$. Three hundred milligram (300 mg) of *DMSG* was added to 3.6 g of the zinc chloride hydrates in the Erlenmeyer flask and transferred into a shaking incubator at 70 °C for 60 min. After incubation, the sample was separated from the spent liquor by filtration and the biomass residue was washed and dried in an oven before undergoing saccharification.

The physical properties of the raw and pretreated substrate were observed. The weight of the sponge gourd before pretreatment was 6 g, while the color of Sponge Gourd was ash brown with a coarse texture.

2.2. Enzymatic hydrolysis

Two grams of *Trichoderma reesei* cellulase was dissolved in 100 mL of 0.05 M acetate buffer, pH 5.0 (Cellulase assay was determined using carboxymethyl cellulose as substrate and the glucose released was quantified using the dinitro salicylic (DNS) method [10]). Six grams of the *DMSG* was added to the solution prepared and were agitated (120 rpm at 50 °C for 72 h) in an incubator shaker. After 72 hours, the samples were centrifuged at 4000 x g for 20 min and the glucose released was quantified [10].

2.3. Optimization of hydrolysis (experimental design)

Central composite design of Response Surface Methodology (RSM) was used to determine the optimum parameters for the best pretreatment method for increased glucose yield. Three numerical factors were varied in 3 levels (3 factors-3-levels) plus and minus alpha factorial points, 1 replicate (6 runs), 1 axial point (4 runs) and 5 central points (5 runs). 15 runs were generated altogether. The three factors are: sulphite concentration (%w/w), temperature (°C), reaction time (h) were investigated as independent variables for the optimal enzymatic hydrolysis of the *DMSG* as shown in Table 1. The best combination of factors that produced the optimum yield was used for further fermentation to bioethanol.

2.4. Fermentation experimental design

A three –factors-three-levels Central composite Design was employed to generate 18 experimental runs by considering inoculum size (%v/v), fermentation time (h), and nitrogen source (Table 2).

Table 1
Experimental design for hydrolysis of pretreated sponge gourd fiber.

Factor	Name	Units	Type	Low Actual	High Actual	Low coded	High coded
A	Sulphite concentration	% w/v	Numeric	3	9	-1	1
B	Temperature	^{0}C	Numeric	60	100	-1	1
C	Time	Mins	Numeric	60	180	-1	1

Table 2
Experimental design for fermentation of the hydrolysates.

Factor	Name	Units	Type	Low Actual	High Actual	Levels
A	Time	Hours	Numerical	24	72	3
B	Innoculum size	v/v %	Numerical	5	10	3
C	Nitrogen source		Categorical	Organic	Inorganic	2

Organic source = Urea
Inorganic source = Ammonium sulphate

Table 3
Physical properties of the pretreated Sponge gourd.

Pretreatment method	Color	Texture	Weight after pretreatment (g)
Alkaline only	Light Brown	Coarse	4.62
Steam Explosion	Dark Brown	Coarse	4.14
Combined Alkaline and Steam explosin	Golden Brown	Fine	4.36
Zinc Chloride	Cartoon Brown	Coarse	4.79
Sodium Sulphite	Cream Brown	Fine	4.68

2.5. Estimation of ethanol concentration

The concentration of bioethanol in the fermentation sample was estimated using the sodium dichromate method. The absorbance of the green colored product was determined at a wavelength of 578 nm [11,12]

2.6. Statistical data analysis

The data obtained were analyzed statistically using response surface methodology. In order to fit the second-order mathematical model generated by the Design-Expert software version 10 (Stat Ease Inc., Minneapolis, USA), analysis of variance (ANOVA), a regression analysis and the plotting of response surface were carried out. From the mathematical model developed, the experimental data obtained from the established optimal condition were used as validating set and these were compared with the predicted values. The fitted second-order mathematical model is described below

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii}X_i^2 + \sum_{i<j}^{k} b_{ij}X_iX_j + e$$

3. Results and discussion

3.1. Effects of different pretreatment methods on Sponge Gourd Fiber

The physical properties of the raw and pretreated substrate used in this study were obtained for different pretreatment methods are shown Table 3. This swelling of the sample by acid and alkali led to an increase in the accessible surface area, partial depolymerization of the cellulose and separation of structural linkages between lignin and carbohydrate that led to the disruption of the lignin structure as reported by Fan et al. [13]. Among the five-pretreatment method evaluated at the initial screening stage, pretreatment with sodium sulphite gave the highest glucose concentration which was 6.65 g/L while alkaline only gave the lowest glucose concentration 4.85 g/L. The results show that the vulnerability of sponge gourd fiber to enzymatic hydrolysis depends on the type of pretreatment method employed. The combined effect of NaOH and sodium sulphite (Na$_2$SO$_3$) led to the removal of lignin and depolymerization of xylan without degrading the cellulose [8]. Thus, sodium sulphite pretreatment was established as the best pretreatment method for the downstream enzymatic hydrolysis of cellulose in sponge gourd fiber to glucose.

3.2. Influence of process parameters on glucose yield

The experimental results obtained above was inputted into the experimental design generated by RSM to obtain the analysis of variance (ANOVA) of the regression equation model displayed. A quadratic model was proposed as appeared in Table 4 and the model is significant as shown in Table 5. An F-value of 214.49 with a low probability value of less

Table 4
Test of Significance for every regression coefficient for the hydrolysis stage.

Model	Sum of squares	Df	Mean square	F-value	p-value
A-Sulphite Concentration	0.60	1	0.60	143.24	<0.0001
B-Temperature	0.16	1	0.16	37.03	0.0017
C- Reaction Time	1.43×10^{-3}	1	1.43×10^{-3}	0.34	0.5859
AB	1.55	1	1.55	367.18	<0.0001
AC	0.07	1	0.07	15.93	0.0104
BC	4.49×10^{-4}	1	4.49×10^{-4}	0.11	0.7574
A^2	0.03	1	0.03	8.06	0.0363
B^2	0.90	1	0.90	214.04	<0.0001
C^2	2.24	1	2.24	531.01	<0.0001

Table 5
Analysis of Variance (ANOVA) of the regression equation for Hydrolysis stage

Source	Sum of squares	Df	Mean square	F-value	p-value
Model	8.13	9	0.90	214.49	<0.0001
Residual	0.02	5	4.21×10^{-3}		
Lack of fit	0.01	1	0.01	7.61	0.0509
Pure error	7.25×10^{-3}	4	1.81×10^{-3}		
Cor Total	8.15	14			
			$R^2 = 99.74\%$		$R^2(adj)=99.28\%$

than 0.0001 implies that the model is significant. Combination of multiple coefficients of correlation (R) and coefficient of determination (R2) was utilized to survey the goodness of fit of the regression equation. R of 0.9987 of the models showed a good correlation between the observed and predicted values. While R2 of the model was 0.9974, which demonstrated that 99.74% sample variation for glucose produced is inferable from the independent factors and just 0.26% of the total variation are not depicted by the model. The adjusted R2 of 0.9928 demonstrated that the model was significant. It has been proposed that R2 ought to be between 80-100% for the good fit of a model [14]. The residuals between the observed and predicted values in this work revealed a good fit of the model (Table 6). This was likewise affirmed by both the bias factor (Bf =1.09), which is a proportion of the agreement between observed and predicted values [15] and correspondence plot. These perceptions inferred that the model developed for the hydrolysis stage appropriately depicted the actual relationship among the chose factors. The p-values of the model terms were significant at p < 0.05 (Table 4). Among the factors, the residence time was not significant (p>0.05). Additionally, the observed low p-value of 0.0001 and the comparing high F-value of 143.24 in the sulphite concentration demonstrated that the model acquired was significant. The contrast between negative and positive significant effects of each term in the model is not shown by both the F-value and p-value as reported by Betiku and Taiwo [16]. The data regarding coded factors relating the glucose yield (kgm−3) Y with independent variables were fitted using the following second-order polynomial equation.

$$Y = 5.32 + 0.39A + 0.20B + 0.019C + 0.88AB - 0.18AC + 0.015BC - 0.066A^2 + 0.34B^2 - 0.54C^2 \quad (1)$$

Where Y is the glucose concentration and A is sulphite concentration, B is Temperature and C is Time.

This equation can be used to make predictions of glucose yield under varying conditions of the stipulated factors of sulphite concentration, temperature and resiedence time.The model terms (A, B, AB, B^2, A^2, C,^{2} AC, BC) with positive coefficients show a good impact on sponge gourd hydrolysis while the model terms (C) with negative coefficient demonstrate an unfavourable fit on sponge gourd hydrolysis. Sulphite concentration × Temperature (cross product of the linear term) was the most positive significant model term on sponge gourd hydrolysis followed by sulphite concentration, Temperature^2 (quadratic term), and Temperature (linear term). This observation is authenticated by the p-values (Table 4) of these model terms (p >0.05). The outcome showed that the cross product of linear term of Temperature and Reaction time contributed the least to the hydrolysis of Sponge gourd.

The low estimation of the standard error of the intercept and every model term revealed that the regression model fits the data well and the prediction was great. The variance inflation factor (VIF) obtained in this work revealed that the centre points are orthogonal to every other factor in the model. This is in accordance with the study that Betiku and Taiwo [16] conducted on sweet potato.

As a matter of fact, the high levels of the factors are coded as +1 and low levels of the factors are coded -1. The coded equation is valuable for recognizing the relative effect of the factors by looking at the coefficients of the factors. However, in terms of actual factors, the levels must be specified in the original units for each of the factors. The equation in terms of actual factors cannot be utilized to decide the relative contribution of each factor because the coefficients are scaled to suits the units of each factor and intercept is not at the centre of the design space. The last generated polynomial equation as far as actual factors is:

$$\text{Glucose yield} = +13.19036 - 0.83251A - 0.21631B + 0.04131C + 0.01466AB - 0.001017AC + 1.24821 \times 10^{(-5)}BC$$

$$-0.00737A^2 + 8.5466 \times 10^{(-4)}B^2 - 1.49573 \times 10^{(-5)}C^2 \quad (2)$$

Table 6
Effect of different process parameters on glucose concentration at the end of enzymatic hydrolysis.

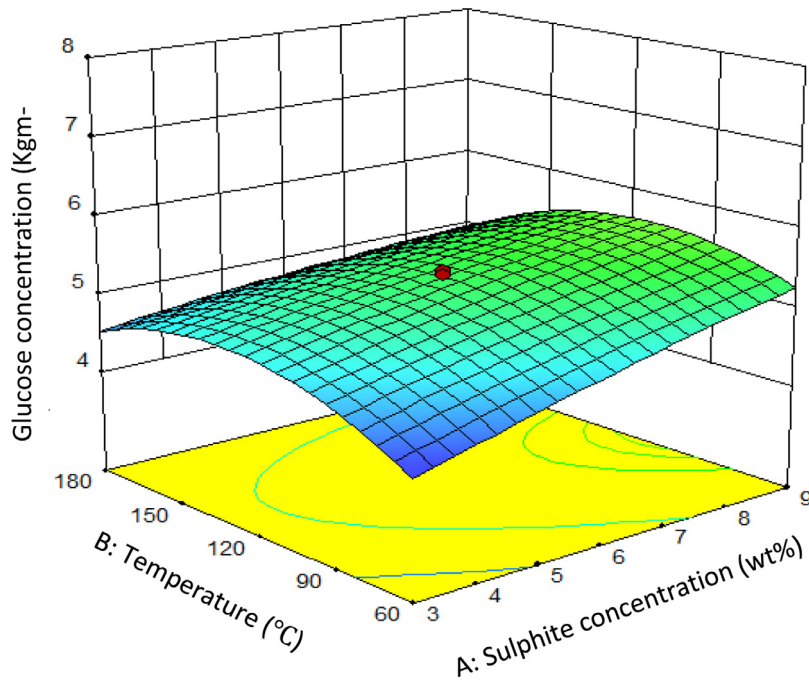| Run	Sulphite Concentration (%w/w)	Temperature (°C)	Time (H)	Actual Glucose Yield (kgm^{-3})	Predicted Glucose Yield (kgm^{-3})	Residual
1	10.2	80	120	5.70	5.74	-0.036
2	1.8	80	120	4.60	4.64	-0.036
3	6.0	80	35	4.18	4.22	-0.036
4	6.0	52	120	5.69	5.73	-0.036
5	3.0	100	180	4.24	4.21	0.036
6	6.0	108	120	6.25	6.28	-0.036
7	6.0	80	120	5.32	5.32	-0.003
8	6.0	80	120	5.40	5.32	0.076
9	6.0	80	120	5.34	5.32	0.015
10	3.0	60	60	5.20	5.17	0.036
11	9.0	60	180	4.23	4.19	0.036
12	6.0	80	205	4.24	4.27	-0.036
13	9.0	100	60	6.71	6.67	0.036
14	6.0	80	120	5.35	5.32	0.025
15	6.0	80	120	5.28	5.32	-0.041
control	0.0	0	0	1.22		

Fig. 1. Response surface plots of 3D for Sponge Gourd hydrolysis for interaction between sulphite concentration and temperature.
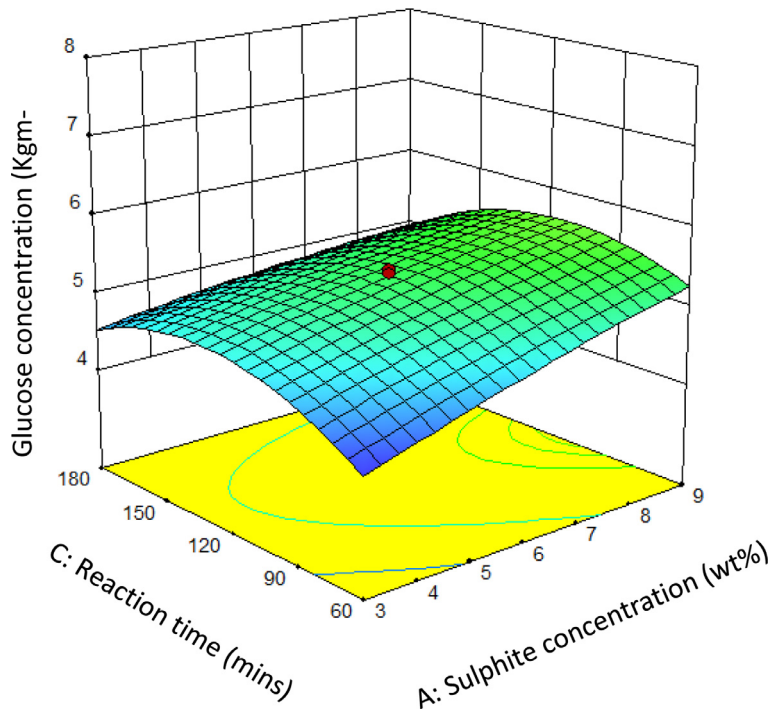

Fig. 2. Response surface plots for Sponge Gourd hydrolysis for interaction between sulphite concentration and reaction time.

3.3. Interaction between temperature, sulphite concentration and time during hydrolysis

Fig. 1 demonstrates the surface response plots for the optimization of the hydrolysis stage for the glucose yield. The visualization of the predicted model equation can be obtained by the surface response plot [17]. The curvature nature of the 3-dimensional surfaces in Fig. 1 indicates significant interactions among the factors varied in this study. Fig. 2 show
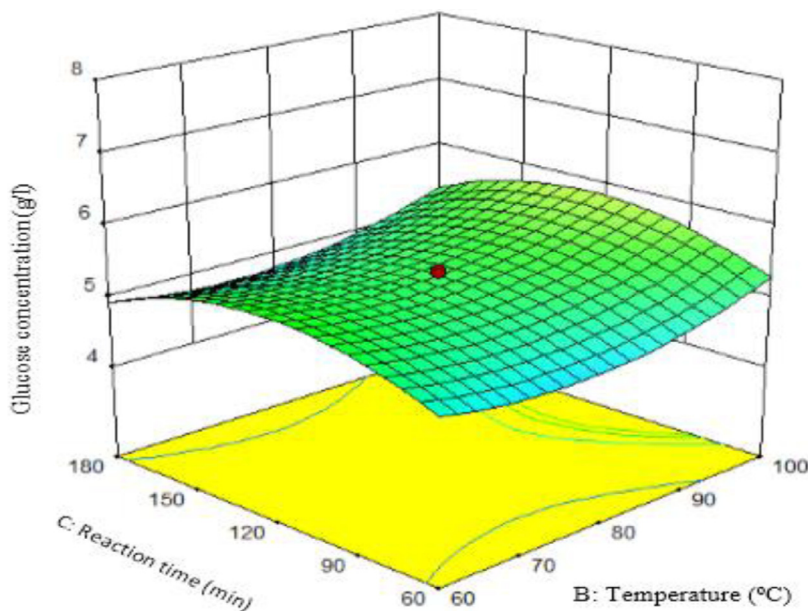
Fig. 3. Response surface plots for Sponge Gourd hydrolysis for interaction between temperature and reaction time

the 3D surface plot of interaction between sulphite concentration and reaction time while keeping constant temperatures at 80 °C. From Fig. 2, the optimum glucose concentration of 5.65 kgm^{-3} was obtained when the sulphite concentration was 9 %w/w and the reaction time was 120 min. The reaction time has no significant effect on the glucose yield, whereas an increase in sulphite concentration increases the glucose yield. The combined effect is that at any time (t), increasing sulphite concentration increases the glucose concentration. Similarly, Idrees et al.[8] observed that enzymatic hydrolysis of wheat husk was favoured by the high concentration of sodium sulphite and lower pretreatment time.

Fig. 3 show the 3D response surface plot of the interaction between temperature and reaction time while keeping the sulphite concentration constant at 6 %(w/w). From Fig. 3, the optimum glucose concentration of 5.85 kgm^{-3} was obtained when the temperature was 100 °C and the reaction time was 120 min. The temperature has a more significant effect on the glucose concentration than the reaction time. These results strongly agree with the work of Idress *et al.* [8] who investigated that time affected the hydrolysis yield at a lower temperature but at high temperature, the difference was not significant.

3.4. Process optimization during hydrolysis

The hydrolysis process was optimized by solving Eq. (2) using Design – Expert V10. The optimum combination of parameters at the hydrolysis stage; constraints of sulphite concentration, Temperature and reaction time were set. Sulphite concentration was set to be in the range because the reagent is readily available and can be used in large amounts with a lower limit and the upper limit set at 3 % w/w and 9 % w/w respectively as indicated in the original design. The temperature was also set in the range whereas the reaction time was minimized. The objective is to maximize the glucose concentration with varied parameters in highest desirability. The optimal condition for this step was established as sulphite concentration at 9 % w/w, Temperature at 100 °C and reaction time of 60 min. The predicted glucose concentration under the above set of conditions was 6.673 kgm^{-3}. To verify the prediction of the model, the optimal condition was applied to two independent replicates and the average glucose concentration obtained was 6.632 kgm^{-3} which is well within the predicted value of the model equation. The results of this work have proven that response surface methodology could be used to optimize the enzymatic hydrolysis of sponge gourd sugar. The results obtained from this study favorably compared with the previous work on saccharification of Sponge gourd carried out by Enzyme AP2 (Pectinase; Cellulases and Hemicellulase activities: 54000 unit g^{-1}) as reported by Zaafouri et al. [5], where they obtained a glucose concentration of about 59.4 g kg^{-1}.

### 3.5. Sponge Gourd as potential substrate for bioethanol production

This study verified the possible use of sponge gourd as a major carbon source for the production of bioethanol using yeast under surface fermentation. Fig. 4a-c show the profile of bioethanol yield against fermentation time with nitrogen source. The results show that the yeast was able to metabolize the sponge gourd hydrolysate without stress. The reaction dynamics were observed for different inoculum sizes (5, 7.5 and 10%v/v), and the trend of ethanol concentration with time was investigated for the organic and inorganic nitrogen source. For Fig. 4a the organic and inorganic nitrogen sources increase the ethanol concentration from 0 h till 24 h, after which there was a decline in the bioethanol yield. This may be as a
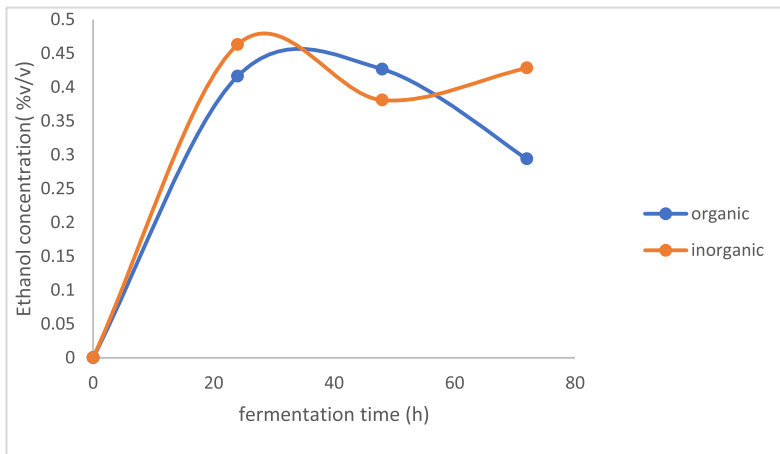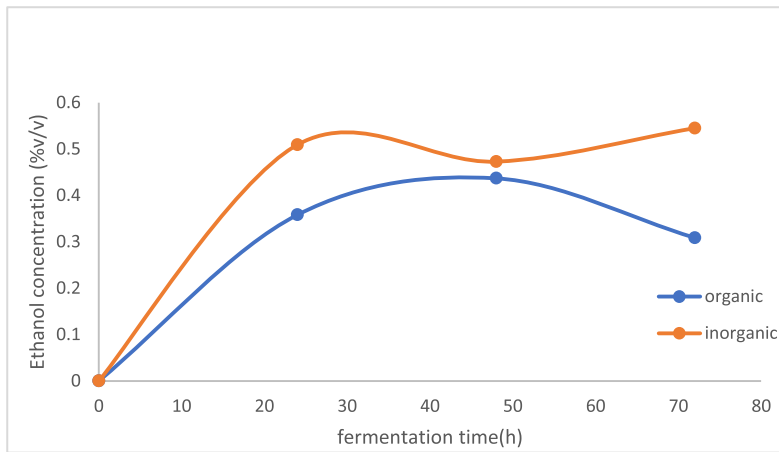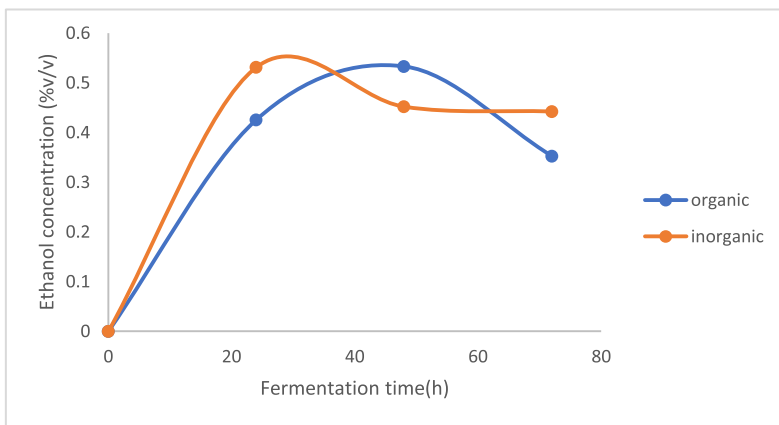
Fig. 4. a A plot of ethanol concentration against fermentation time for 5 % v/v inoculum Size. b: A plot of ethanol concentration against fermentation time for 7.5 % v/v inoculum size. c: A plot of ethanol concentration against fermentation time for 10 % v/v inoculum size

Table 7
CCD of three independent factor for Bioethanol production.

Run	Inoculum size (% v/v)	Fermentation time (h)	Nitrogen source	Actual value	Predicted value	Residual value	Leverage
1	5	48	Inorganic	5.08	5.11	-0.029	0.417
2	7.5	48	Inorganic	6.35	6.32	0.026	0.333
3	5	72	Inorganic	5.23	5.25	-0.015	0.625
4	7.5	72	Inorganic	6.47	6.43	0.042	0.417
5	10	24	Inorganic	6.39	6.25	0.130	0.625
6	10	72	Organic	4.35	4.40	-0.046	0.625
7	5	72	Organic	4.62	4.84	-0.22	0.625
8	10	72	Inorganic	5.69	5.78	-0.094	0.625
9	7.5	48	Organic	5.61	5.46	0.15	0.333
10	10	24	Organic	5.13	4.94	0.19	0.625
11	10	48	Inorganic	5.84	5.70	0.14	0.417
12	7.5	24	Organic	5.88	6.01	-0.13	0.417
13	10	48	Organic	4.03	4.35	-0.32	0.417
14	5	24	Inorganic	5.82	5.60	0.22	0.625
15	5	48	Organic	4.78	4.74	0.036	0.417
16	7.5	24	Inorganic	6.42	6.84	-0.42	0.417
17	7.5	72	Organic	5.87	5.53	0.33	0.417
18	5	24	Organic	5.28	5.27	0.010	0.625

result of the depletion in the glucose concentration. From the graph, it can be seen that the inorganic source influences the ethanol production more than the organic nitrogen source. For Fig. 4b at 7.5 %v/v inoculum size, the ethanol concentration increases with time from 0 h to 34 h before it started decreasing and later start rising at 50 h for the inorganic nitrogen source. Also, for the organic nitrogen source, the ethanol concentration increases with time for the first 50 hours before decreasing. From the graph, it can be seen that the inorganic nitrogen source increases the ethanol concentration than the organic nitrogen source. From Fig. 4c, the ethanol concentration increased with time from 0 h to 36 h of the inorganic nitrogen source before the gradual decrease. While the inorganic source increases the ethanol concentration from 0 h till 48 h before the decline in the yield of bioethanol. From the graph it can also be seen that inorganic nitrogen source has a better influence on ethanol yield. Generally, a rapid increase in ethanol concentration was observed from 0 h to 30 h, corresponding to the exponential stage. This is normal, as ethanol is a primary metabolite and is therefore produced during the exponential phase of cell growth. This corresponds to what was observed by Rorke and Gueguim [18].

3.6. Effect of varying inoculum size, nitrogen source and fermentation time on bioethanol yield

The optimum conditions obtained during the pretreatment and hydrolysis of sponge gourd core fiber above was used to pretreat and hydrolyze the raw sponge gourd. After centrifuging, the hydrolysate was analysed for glucose concentration which was 6.53 kgm^{-3}. This is not too far from the optimum value 6.67 kgm^{-3}. Therefore 6.53 kgm^{-3} was the initial glucose concentration of all the experimental runs at the fermentation stage. The yield of ethanol obtained by varying the factors such as fermentation time, inoculum size and nitrogen source is shown in Table 7. Experimental run 4 (fermentation time,72 h; inoculum size,7.5 % and nitrogen source, inorganic) has the highest yield of ethanol with concentration of 6.4682 kgm^{-3}, while run 13 (fermentation time = 48 h, inoculum size= 10 %, nitrogen source =organic) has the lowest ethanol concentration of 4.03 kgm^{-3}. These yields are in agreement with those reported by Ballesteros et al [19].

3.7. Assessment of the RSM model performance

The RSM in conjunction with CCD was used to generate the results in Table 7 where the experimental results obtained were inputted into the experimental design. A quadratic model was selected and the model was significant. The model Fisher F-test of 15.25 with low probability value [($p_{model} > F$) =0.0002] demonstrate a high significance for the regression model [20]. There was only a 0.02 % chance that an F-value this large was not significant, which could occur due to noise. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable, the adequate precision is 13.395 which indicates an adequate signal. This model can be used to navigate the design space.

The results showed that the p-values of the model terms were significant, i.e., p<0.05. The smaller the magnitude of P-values, the more significant is the corresponding coefficient [20]. In this case, the two linear terms (B, C), the quadratic term (A^2, B^2, AC) are significant model terms at 95% confidence level.

The goodness of fit was checked by the coefficient of determination (R^2). For this stage, the R^2 was at 93.13 % and the adjusted R^2 was found to be 87.02 %, the reasonable agreement between the R^2 and adjusted R^2 implied that in the model investigated, that the selected variables are suitable for the model. The value of the R^2 obtained from this work showed a high consistency between the observed values and the predicted values. These values indicated that the regression is statistically significant; only 0.02 % of the total variation was not explained by this regression model.

The low values of standard error observed in the intercept and all the model terms showed that regression model fits the data properly and that the prediction was good. The variance inflation factor (VIF) shows that the center points are orthog-
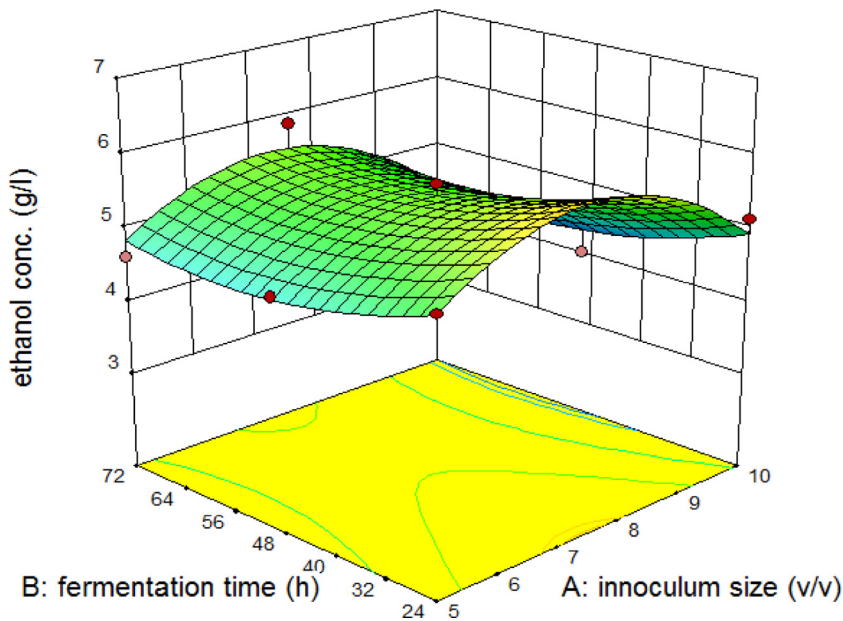
Fig. 5. 3D Response Surface plots for ethanol production for interaction between fermentation time and inoculum size.

onal to all other factors. Table 6 shows the experimental conditions determined together with the observed and predicted values. The data were fitted using the following second-order polynomial equation.

$$Y = 5.89 + 0.051A - 0.22B + 0.43C - 0.030AB + 0.25AC + 0.017BC - 0.91A^2 + 0.31B^2 \qquad (3)$$

Where Y is the bioethanol produced in kgm^{-3} and A is inoculum size in % v/v, B is fermentation time, C is nitrogen source. This equation can be used to make predictions of ethanol yield under varying conditions of the stipulated factors of fermentation time, inoculum size and nitrogen source. By default, the high levels of the factors are coded as +1 and low levels of the factors are coded -1. The coded equation is useful for identifying the relative impact of the factors by comparing the coefficients of the factors. RSM has proven to be an advantageous tool for optimizing culture conditions and culture media composition [21].

3.8. Interaction between inoculum size, fermentation time and organic nitrogen source for bioethanol production

The effect of the interactions of the fermentation parameters on the yield of bioethanol with respect to two variables were studied by plotting 3D response surface while keeping the other variable constant. Fig. 5 shows the interaction between inoculum size and fermentation time while keeping the organic nitrogen source constant. The curve nature of the plot shows there is a significant and moderate interactions among the variables observed for bioethanol production. According to the plot, the maximum ethanol concentration was 6 kgm^{-3}, a fermentation time of 24 h and the inoculum size of 7.5 % v/v while the minimum ethanol concentration of 4.3 kgm^{-3} was obtained when the fermentation time was 55 h and the inoculum size was 10 % v/v. it can be observed that the higher the fermentation time and the inoculum size, the higher the ethanol produced. But as the time prolonged the ethanol produces diminished at higher inoculum size [13]. Also, the interaction between inoculum size and nitrogen source while keeping the fermentation time constant was studied. It can be observed that the maximum ethanol yield of 6.34 kgm^{-3} was obtained when the inoculum size is 7.59 %v/v and nitrogen source is inorganic while the minimum ethanol yield of 4.03 kgm^{-3} was obtained when the inoculum size is 10 %v/v and nitrogen source is organic for all inoculum size. This implies that inorganic nitrogen source (Ammonium sulphate) is better than organic source (Urea) for bioethanol production using *Saccharomyces cerevisiae*. Moreover, the interaction between fermentation time and Nitrogen source while keeping inoculum size constant at 7.5 % were also consider. The maximum ethanol yield of 6.84 kgm^{-3} was obtained at a fermentation time of 24.4 h and inorganic nitrogen source, while the minimum yield of 5.413 $kgm^{-3}$was obtained at 58.8 h and inorganic nitrogen source. Both the inorganic nitrogen source and organic nitrogen source, the ethanol yield decreased up to the mid-range concentration, followed by a further increase in the yield. Also, from the plot bioethanol yield for the inorganic nitrogen source is higher than when the nitrogen source is organic for all fermentation time.

3.9. Process optimization at fermentation stage

The optimal values of the independent variables selected for the fermentation step were also obtained by solving Eq. (3) using the Design –expert software. The optimal condition was investigated to be A= 7.500, B=24.000 and C =inorganic. The ethanol concentration predicted under the above set of condition was 6.840 kgm^{-3}. In order to verify the prediction of the model, the optimal condition was run on two independent replicates and the average ethanol concentration was 6.632 kgm^{-3}, which is well within the predicted value of the model equation. The results of this work show that the response surface methodology could be used to optimize ethanol production yield from sponge gourd. The results obtained in this research for bioethanol production from sponge gourd are therefore promising.

4. Conclusion

Response surface methodology was successfully used to optimize the enzymatic hydrolysis and fermentation stages. The combinations that gave the optimum glucose yield during the hydrolysis are Sodium sulphite Concentration (9 % v/v), Temperature (100 °C) and Reaction Time (60 mins) with a glucose concentration of 6.673 kgm^{-3}. For the fermentation step, the optimal condition was investigated as 7.5 % v/v inoculum size; fermentation time of 24 h; and inorganic Nitrogen source using *Saccharomyces cerevisiae* with an ethanol concentration 0f 6.84 kgm^{-3}. It can be concluded from this study that sponge gourd (a lignocellulosic material) can be a good feedstock for bioethanol production and could prevent wastage of under-utilized agro material.

Declaration of Competing Interest

We declare no conflict of interest.

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