ChemSearch Journal 12(2): 113 – 128, December, 2021
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 07/12/2021 Accepted: 23/12/2021
http://www.ajol.info/index.php/csj

Response Surface Optimization for Microwave-Assisted Alkaline Pretreatment of Plantain Pseudostem Biomass for Bioethanol Production

***[1]Oluyemisi Omotayo Omonije, [1,2]Egwim Evans Chidi, [1]Kabiru Yusuf Adamu and [3]Moses Aderemi Olutoye**
[1]Biochemistry Department, Federal University of Technology, Niger State PMB 65, Nigeria
[2]Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Niger State PMB 65, Nigeria
[3]Chemical Engineering department, Federal University of Technology, Niger State, PMB 65, Nigeria
***Correspondence Email:** yemisimyfavour@gmail.com

ABSTRACT

Bioethanol is a better alternative to gasoline because its combustion releases little or no dangerous gases to the environment and it is produced from renewable feedstock. However, the availability of bioethanol at commercial level is limited by factors such as the recalcitrant nature of the feedstock as well as the cost of the hydrolytic enzyme. In this study, microwave-assisted alkaline pretreatment was optimized via Box-Behnken experimental Design in Design expert software version 11 to effectively remove lignin from plantain pseudostem. The structure of plantain pseudostem pretreated at optimal conditions were characterized using FTIR, XRD, and SEM. Statistical and regression analysis on the experimental outcomes revealed that the lignin removal follows 2FI model with R^2 value of 0.9601, adjusted R^2 values of 0.9361 and predicted R^2 value of 0.8516; the cellulose content follows 2FI with R^2 value of 0.9616, adjusted R^2 values of 0.9386 and predicted R^2 value of 0.9094; the hemicellulose content followed quadratic model with R^2 value of 0.9591, adjusted R^2 values of 0.9065and predicted R^2 value of 0.8463. The high adequate precision values of cellulose (42.33), lignin removal (23.58) and hemicellulose (15.87) shows that the developed models are true representation of the experimental study. The XRD, FTIR and SEM results of the treated plantain demonstrated that microwave-assisted alkaline pretreatment is effective in removing the recalcitrant nature of lignin. This can be a relatively cheaper feedstock for the production of bioethanol.

Keywords: Bioethanol, Cellulose, Hemicellulose, Lignin, Optimization, Plantain

INTRODUCTION

The rapid growth and development of industries in the world result in a greater energy needs. In developing countries, most of the rural communities have less access to modern and clean energy sources and mostly depend on traditional fuel /biomass (woods, twigs, leaves, charcoal, animal dung and crop residue) for virtually all their energy requirements (Nnaji *et al.*, 2012). Fossil fuels account for majority of the global energy consumption and this has contributed to environmental pollution. The predicted depletion of the fossil oil feedstock and the negative impact of its combustion on the environment have led to a search for sustainable sources (Abas *et al.*, 2015).

Some of the alternative sources to fossil fuel are solar energy, nuclear energy, wave energy, hydroelectric energy, wind energy, biomass energy, natural gas as well as biofuels. The drawbacks to some of these alternative sources are heavy upfront cost of creation, complex process of maintenance, possibilities for contamination, and inconsistent supplies of feedstocks (Kline and Rosenberg, 2010). Biofuels such as bioethanol, biodiesel, biomethane (Kline and Rosenberg, 2010), biohydrogen and bio-butanol supplement the energy demand by reducing the usage of petroleum-based fuels. Bioethanol is considered a better alternative fuel because it has a higher-octane rating than gasoline and produces fewer emissions (Abas *et al.*, 2015). It reduces the dependence on oil-producing countries and supports rural economies by creating jobs and providing an additional source of income.

The production of bioethanol from starch and sugar-based feedstock is tagged first generation bioethanol. The world leading producers and users of first-generation bioethanol are US and Brazil. Though the technique of first-generation bioethanol production is well understood and its production is economically cost- effective, it has some major drawbacks such as consequent higher food prices, and destruction of primary forests resulting in negative impact on biodiversity (Burk, 2010). Also, studies have proved that they are not sufficient to replace a considerable portion of the one trillion gallons of fossil fuel presently consumed worldwide each year. Many of these problems can be addressed by the production of second-generation biofuels manufactured from Lignocellulose which are globally abundant Lignocellulose basically consists of cellulose, hemicellulose and lignin (Jonsson and Martin, 2016). Lignin which confers robustness and strength to the plant makes it recalcitrant to hydrolysis. Production of ethanol from lignocellulosic biomass therefore requires pretreatment to remove lignin, hydrolysis to generate fermentable sugars which are then converted to ethanol by microbial action (Wyman, 2007).

Production of bioethanol from lignocellulosic materials provides a clear link between access to energy services and poverty reduction as well as development. Apart from its contribution to poverty reduction, it is a path for social and economic development, as well as ensuring energy security (Londo *et al.,* 2016). Lignocellulosic bioethanol addresses the issue of energy production with zero/near zero emission of greenhouse gases due to its nobility of counterbalance capacity. In other words, biomass is utilized as raw material to produce biofuel and the emitted CO_2 during the consumption of this biofuel, is recycled in re-synthesizing biomass (Sims *et al.,* 2010).

Despite the promising window of lignocellulosic bioethanol production, it is characterized by several challenges. Such challenges include recalcitrance of lignocellulose feedstock to hydrolytic enzymes, cost of production of enzymes, and inability of native *Saccharomyces cerevisiae* to ferment all the available sugars to ethanol (Mu *et al*., 2010).

Nigeria is among the major producers of plantain in Africa and sixth in the world that produced 3,164,878 metric tonnes in 2017 (FAOSTAT, 2018). Though, most of the products are locally consumed due to the rapidly increasing urbanization and the great demand for easy and convenient foods by the non-farming urban populations. About 100 metric tons of banana stem is generated per hectare annually as waste after harvesting the edible fruit (Tripathi *et al.*, 2021) and this waste are presently not being used for any economic purpose but rather burnt or left to decay which constitute a menace to the environment. Plantain pseudo stem contains average amount of cellulose 47 %, hemicellulose 13 %, lignin 13.0 %, ash 8.2 %, extractives and others 3.05 % (Saraiva *et al*., 2012). Plantain pseudo-stem is potential bioethanol feed stocks because of its acceptable content of cellulose and low lignin content. In order to explore the huge potential of plantain for bioethanol production, this study was therefore focused on the optimization of the process variable for maximizing lignin removal and minimizing cellulose and hemicellulose loss via Microwave-Assisted Alkaline (MAA) pretreatment.

Materials and Methods

Materials

Plantain trunk biomass sample was collected from farms and agro- industry in Bosso, Minna, Niger state, Nigeria. The sodium hydroxide (NaOH) used in this study is of analytical grade.

Methods

Preparation of biomass

Plantain pseudostem samples was thoroughly washed, cut into pieces, and air dried until constant weight was attained. The dried samples were milled into fine particles and weighed (Sluiter *et al*., 2012).The samples were then stored in airtight containers and kept at room temperature until needed for further analysis.

Microwave Assisted Alkaline (MAA) Pretreatment of biomass

Each sample (10 % w/v) was first soaked in NaOH for 10 mins (Wen *et al.,* 2015) after which it was subjected to Microwave-Assisted Alkaline (MAA) pretreatment (Egwim *et al*., 2015; Singh *et al*., 2014)based on the experimental runs generated using Box-Behnken factorial design (BBD) of Design Expert software version 11 (Table 1). Three factors and three levels were used to evaluate the effect of three variables. The variables were concentration of sodium hydroxide (A), power (B), and treatment time (C) on the responses. The responses were percentages of lignin removal (Response 1), cellulose retained (Response 2) and hemicellulose retained (Response 3) after pretreatment of each agrowaste sample. The range of variables were: NaOH is 1 - 3 %, power 70–700W, and treatment time 1 - 5 min. The design matrix with 17 experimental processes were generated and these designs were experimented on the agrowaste samples in the lab. After this process, the mixture was then filtered and the residue was washed with clean water until a neutral pH was attained and afterwards dried in the oven at 80 ^{0}C to remove water and moisture, then weighed. The dried residue was kept for further analysis. Design expert software was used to determine the optimal conditions

Characterization

The actual lignin content (Table 2) was determined as the sum of acid insoluble lignin (or Klason lignin) and acid soluble lignin contents for the unpretreated and pretreated agrowaste as described by Sluiter *et al.* (2008). The composition of Cellulose and hemicellulose of unpretreated (Table 2) and pretreated agrowaste was determined by Chesson-Datta gravimetric method (Mahyati *et al.*, 2013). In order to further confirm the effectiveness of the treatment process, the untreated and treated plantain stem were subjected to XRD, FTIR and SEM analysis for comparison.

RESULTS AND DISCUSSION

Optimization of the lignin removal, cellulose and hemicellulose improvement of plantain Stem for bioethanol production via Box-Behnken Experimental design

The major components considered for bioethanol production from any biomass are lignin, hemicellulose and cellulose. High lignin content result in low bioethanol production due to the recalcitrance of lignocellulose feedstock to hydrolytic enzymes (Mu *et al*., 2010; Sahare *et al.*, 2012), while high hemicellulose and cellulose content enhances bioethanol production due high sugar content. Selective removal of lignin usually leads to increase in both hemicellulose and cellulose content of biomass (Song *et al.*, 2019). The numerical values of the lower and upper limits of the independent variables in Table 1 were chosen for the experimental design based on the preliminary experiment. This corresponds to range of independent values at which appreciable responses were obtained.

The dependent and independent variables for pretreated plantain stem are presented in Table 2. The actual responses correspond to the experimental outputs, while the predicted values correspond to the values obtained from the simulated experimental conditions by equation 1 to 3. The highest actual lignin removal is 76.58 %, this is not too far from the corresponding predicted value (73.12 %) of the lignin removal with only 4.52 % error. The lowest actual lignin removal is 34 % while the predicted value is 34.18 % with 0.53 % error; this implies the developed model equation is suitable for predicting the amount of lignin that can be removal via microwave-assisted pretreatment. The least actual cellulose (53 %) of run 16 is much close to the predicted cellulose of 53.57 % with 1.06 % error. Run 8 has the highest actual cellulose (65.7 %) which is much close to the predicted cellulose (65.43 %), this is an indication that the developed model in equation (2) is reliable for predicting the cellulose of microwave-assisted alkaline pretreated plantain stem. The least value of the actual hemicellulose (13.4 %) that was recorded in runs 8 is close to the 13.86 % predicted hemicellulose with only 3.43 %. Run 7 has the highest actual hemicellulose (25.54 %) which is approximately the same as the predicted value of 25.73 % with only 0.74 5 error, this satisfy the developed model to be good for predicting the hemicellulose from microwave-assisted alkaline pretreated plantain stem. The equation (1) was employed for the prediction of percentage lignin, cellulose and hemicellulose:

Lignin Removal (%) = +4.67212 + 15.46917 Conc. NaOH + 0.081087 Power + 3.02056 Time - 0.025952 Conc. NaOH * Power + 0.362500 Conc. NaOH * Time + 0.000135 Power * Time (1)

Cellulose Content (%) = +52.19530 + 3.92708 Conc. NaOH - 0.023552 Power + 4.28472Time + 0.010595 Conc. NaOH * Power - 2.00000 Conc. NaOH * Time + 0.000397Power * Time (2)

Hemicellulose (%) = +27.85197 + 0.304444 Conc. NaOH - 0.003296 Power - 3.52750Time - 0.009206 Conc. NaOH * Power + 0.625000 Conc. NaOH * Time + 0.000214 Power * Time +0.197500 Conc. NaOH^2 + 0.000016 Power^2 + 0.183125 Time^2 (3)

Table 1: Specifications of independent variables for generation of experimental conditions for plantain waste treatment

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Conc. NaOH	%	Numeric	1.0000	3.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.7071
B	Power	Watts	Numeric	70.00	700.00	-1 ↔ 70.00	+1 ↔ 700.00	385.00	222.74
C	Time	Minutes	Numeric	1.0000	5.00	-1 ↔ 1.00	+1 ↔ 5.00	3.00	1.41

Table 2: Dependent and Independent variables for plantain stemwaste treatment

Run	A: Conc. NaOH (%)	B: Power (Watts)	C: Time (Minutes)	Lignin Removal (%)		Cellulose (%)		Hemicellulose (%)	
				Actual	Predicted	Actual	Predicted	Actual	Predicted
1	2	70	5	53.61	56.42824	61	61.44669	21	21.075
2	1	385	5	59.43	58.54324	63	63.32169	16	16.3825
3	1	700	3	69	69.16824	55	54.74044	21	20.8075
4	3	70	3	60.91	63.65824	60	59.49044	25	25.1925
5	2	385	3	59.21	58.23824	62	60.45294	18	18.32
6	2	385	3	59.21	58.23824	61.45	60.45294	17	18.32
7	2	70	1	41	41.40824	60	60.19669	25.54	25.73
8	3	700	3	63.21	65.94824	65.7	65.42794	13.4	13.8575
9	2	700	1	59.46	59.87824	59	58.95919	20	19.925
10	1	70	3	34	34.17824	62.65	62.15294	21	20.5425
11	2	385	3	63	58.23824	59.9	60.45294	19	18.32
12	3	385	1	57.53	56.48324	65	65.58419	20	19.6175
13	2	385	3	59	58.23824	60	60.45294	17.6	18.32
14	2	700	5	72.41	75.23824	61	61.20919	16	15.81
15	3	385	5	76.58	73.12324	59	59.33419	18	17.7325
16	1	385	1	43.28	44.80324	53	53.57169	23	23.2675
17	2	385	3	59.21	58.23824	60	60.45294	20	18.32

Analysis of Variance for 2FI model of Lignin Removal, Cellulose and Hemicellulose Retained from Treated Plantain Stem waste

It can be clearly seen in Table 3 that the lignin removal best fit into the two factor (2FI) model with R^2 value of 0.9601, adjusted R^2 values of 0.9361 and predicted R^2 value of 0.8516. In table 4, the cellulose content also best fit into 2FI with R^2 value of 0.9616, adjusted R^2 values of 0.9386 and predicted R^2 value of 0.9094. In Table 5, the quadratic model is most preferred model hemicellulose content with R^2 value of 0.9591, adjusted R^2 values of 0.9065and predicted R^2 value of 0.8463. Cubic mode was not selected despite having high values of R^2 and adjusted R^2 because the mode is aliased.

Sequel to model fitness test, Analysis of Variance (ANOVA) was carried out on the selected model in order to ascertain it if it is significant or not at 95% confidence level. Probability value (P-Value) less than 0.0500 is consider significant, a significant model implies that the developed model is suitable for the prediction of the responses. It can be seen that the 2FI model for lignin removal and cellulose content are significant with probability value P-Value of < 0.0001 (Table 3) and < 0.0001 (Table 4) respectively, the quadratic model for the hemicellulose is also significant with P-value of 0.0005. The analysis of variance also helps to know the independent variable that has either significant or insignificant effect on the responses. The significant model terms in Table 6 on lignin removal are single effect of conc. NaOH (A), Power (B), Time (C), and interactive effect between conc. NaOH and Power (AB). The significant model terms in Table 5 on cellulose content are single effect of conc. NaOH (A), Time (C), and interactive effect between conc. NaOH and Power (AB) as well as between conc. NaOH (A) and Time (C). The significant model terms in Table 8 on hemicellulose are single effect of Power (B), Time (C), and interactive effect between conc. NaOH and Power (AB), conc. NaOH (A) and Time (C) as well as the square of the power (B^2). A non-significant Lack of Fit is usually preferred in model development, in all the model developed lignin removal, cellulose and hemicellulose all have a non-significant lack of fit value of 3.48, 0.35 and 0.24. Another way of testing the efficiency of any developed model is through the use of adequate precision measurement to noise ratio, value above is usually desired. The three developed models have adequate precision above 4, hence they are all sufficient enough for predicting the responses. However, cellulose has the highest adequate precision of 42.3332 followed by lignin removal with 23.5808, while hemicellulose has the least value of 15.8702.

Table 3: Model Summary Statistics and ANOVA for 2FI model of Lignin Removal

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks		
Model	1770.62	6	295.10	40.08	< 0.0001	Significant	R²	0.9601
A-Conc. NaOH	344.79	1	344.79	46.83	< 0.0001	Significant	Adjusted R²	0.9361
B-Power	694.90	1	694.90	94.37	< 0.0001	Significant	Predicted R²	0.8516
C-Time	461.47	1	461.47	62.67	< 0.0001	Significant		
AB	267.32	1	267.32	36.30	0.0001	Significant		
AC	2.10	1	2.10	0.2855	0.6048	Not significant		
BC	0.0289	1	0.0289	0.0039	0.9513	Not significant		
Residual	73.63	10	7.36					
Lack of Fit	61.79	6	10.30	3.48	0.1240	Not significant		
Pure Error	11.84	4	2.96					
Cor Total	1844.25	16						

Adequate Precision:23.5808

Table 4: Model Summary Statistics and ANOVA for 2FI model of Cellulose Content

Source	Sum of Squares	df	Mean Square	F-value	p-value			
Model	148.22	6	24.70	41.74	< 0.0001	Significant	**R^2**	**0.9616**
A-Conc. NaOH	32.20	1	32.20	54.41	< 0.0001	Significant	**Adjusted R^2**	**0.9386**
B-Power	1.09	1	1.09	1.84	0.2050	Not Significant	**Predicted R^2**	**0.9094**
C-Time	6.12	1	6.12	10.35	0.0092	Significant		
AB	44.56	1	44.56	75.28	< 0.0001	Significant		
AC	64.00	1	64.00	108.13	< 0.0001	Significant		
BC	0.2500	1	0.2500	0.4224	0.5304	Not Significant		
Residual	5.92	10	0.5919					
Lack of Fit	2.05	6	0.3418	0.3534	0.8769	not significant		
Pure Error	3.87	4	0.9670					
Cor Total	154.14	16						

Adequate Precision: 24.3332

Table 5: Model summary statistics and ANOVA for Quadratic model of Hemicellulose

Source	Sum of Squares	df	Mean Square	F-value	p-value			
Model	156.14	9	17.35	18.24	0.0005	Significant	**R^2**	**0.9591**
A-Conc. NaOH	2.65	1	2.65	2.78	0.1394		**Adjusted R^2**	**0.9065**
B-Power	61.27	1	61.27	64.40	< 0.0001		**Predicted R^2**	**0.8463**
C-Time	38.46	1	38.46	40.42	0.0004			
AB	33.64	1	33.64	35.36	0.0006			
AC	6.25	1	6.25	6.57	0.0374			
BC	0.0729	1	0.0729	0.0766	0.7899			
A^2	0.1642	1	0.1642	0.1726	0.6902			
B^2	10.54	1	10.54	11.08	0.0126			
C^2	2.26	1	2.26	2.37	0.1672			
Residual	6.66	7	0.9514					
Lack of Fit	1.01	3	0.3373	0.2389	0.8654	not significant		
Pure Error	5.65	4	1.41					
Cor Total	162.80	16						

Adequate Precision: 15.8702

Single Effect of Conc. NaOH, Power and Time on Responses

The plot of individual effect of con. NaOH and time on lignin removal from platain stem is presented in presnted in Figure 1. The amout of lignin removevd shows an increase from 51.67 to 64.68 % when con. NaOH increases from 1 to 3 %, this implies that the increase in conc. NaOH has positive significant contribution on the lignin removal and is inline with the study by Kusmiyati Sukmaningtyas (2018) who reported decrease in lignin content of banana pseudo steam from 19.39 % to 11.4 % when pretreated for 2 % NaOH with 90 minutes in autoclave. Increase in conc. NaOH leads to increase bond breaking of the lignin from the main host. Increase in power from 70 to 700 Watts resulted in increase in lignin removal from 48.91 to 67.48 %, this could be attributed to increase in power which is directly proportional to increase in temperature and increase in temperature leads to increase in the kinetic energy of the lignin molecule thereby leading to increaase in ease of the lignin removal. Increase in treatment time from 1 to 5 minutes brought about increase in the lignin removal from 50.6 to 65.77 %, this implies that increase in the treatment time increases the duration of contacts between the reactant molecules which favours lignin removal.

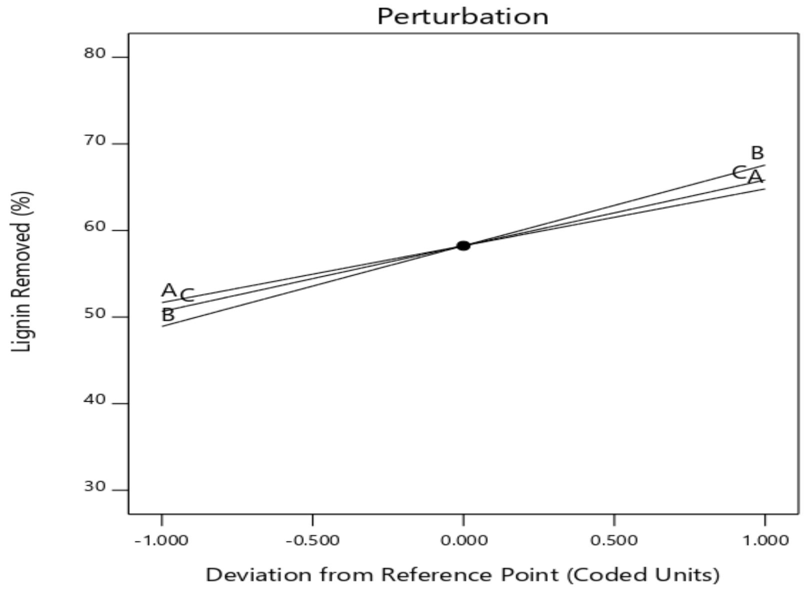

Figure 1: Single Effect of Con. NaOH, Power and Time on Lignin Removal from Platain Stem

The individual effect of the treament paraments on the hemicellulose is presented in Figure 2. It can be clearly seen that increase in con. NaOH from -1 (1 %) to +1 (3%) leads to slight decrease in the hemicellulose from 19.77 to 17.95 %, this implies a minimal lose of the hemicellulose due to degradation (Kusmiyati and Sukmaningtyas, 2018). Increase in power from -1 (70 Watts) to +1 (700) during treatment leads to decrease in hemicellulose from 22.68 % to 17.13 %. This implies that increase power has a negative effect on the hemicellulose leading to its loss. It can be seen that increase in treatment time from -1 (1 minute) to +1 (5 minutes) leads to decrease in the hemicellulose from 21.24 % to 16.87 %, this implies that significant amount of the hemicellulose required for bioethanol production was still retained even after the exposure of the biomass to alkaline treat mnet over a time frame (Wanitwattanarumlug *et al.* 2012).

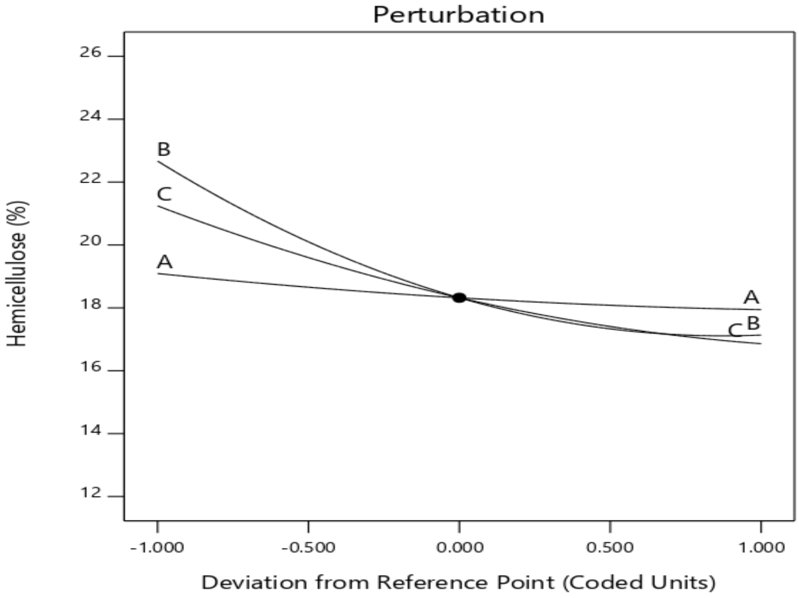

Figure 2: Single Effect of Con. NaOH, Power and Time on Hemicellulose from Platain Stem

The influence of individual factors from the independent parameters on the cellulose content of plantain stem is presented in Figure 3. Increase in conc. NaOH from -1 (1 %) to + (3 %) leads to increase in the cellulose content from 58.45 % to 62.45 %. This shows that increase in alkalinity has positive influence on the cellulose content of plantain stem as it promotes the loss of lignin content and subsequently leads to increase in the cellulose content (Kusmiyati and Sukmaningtyas, 2018). Increase in power from -1 (70 Watt) to +1 (700) leads to slight decrease in cellulose content from 60.82 % to 60.09 %, this implies that the effect of power is insignificant on the cellulose content of plantain stem. Increase in the treatment time from -1 (1 minute) to +1 (5 Minutes) only has a slight increase on the cellulose from 59.57 % to 61.32 %, this implies that the treatment time does not have much influence on the cellulose. Figure 1 to 3 clearly shows that the selected process parameters in this study are suitable for high lignin removal, minimum hemicellulose lose and increase in cellulose content of plantain stem.

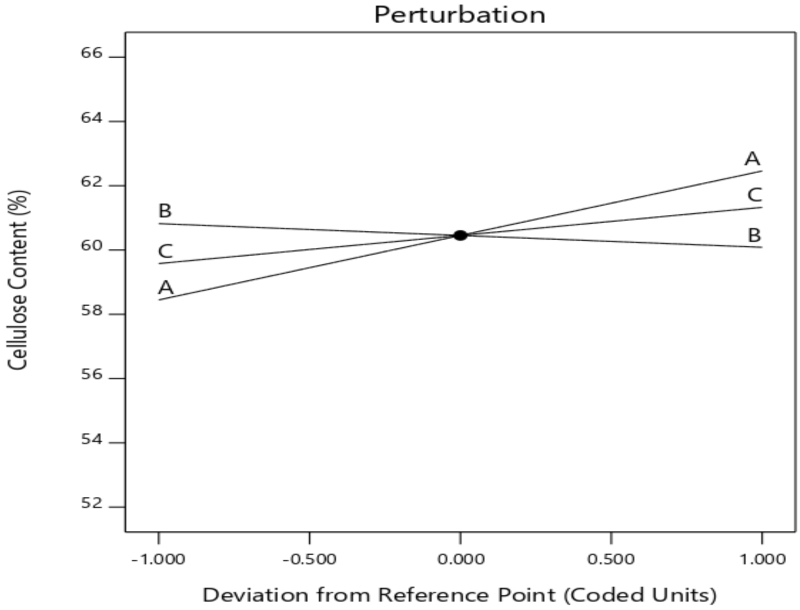

Figure 3: Single Effect of Con. NaOH, Power and Time on Cellulose from Platain Stem

Interactive Effect Between the Dependent and Independent Variables

Based on the outcome of the ANOVA, only the signifcant interactives (AC and BC) have meaningful contributions to the outcome of the experiment. Hence, the contours and three diamentional plots are presentented below.

Interactive Effect between Power and Conc. NaOH on the Responses

The graphical representation of the combined effect of power and con. NaOH on lignin removal, cellulose and hemicellulose of treated plantain stem is presented in Figure 4, and 6 respectively. The contour plots of the combined effects is presented in Figure 4a, Figure 5a and 6a, while the three diamentional views are presented in Figure 4b, 5b and 6b. The different colours represent the different proportions of the responses. The lignin removal clearly shows an increase from 40 to 50 % when power increased from 70 to 700 Watts, while con. NaOH increases from 1 to 3 %. This implies that as con. NaOH interacts with the plantain biomass to remove the lignin component, sufficient kinetic energy was also provided to make the lignin removal significant (Ethaib *et al.*, 2015). There reduction in the cellulose from 62 % to 56 %, while the hemicellulose also decreases from 22 to 17 % when the powr was increased from 70 to 700 Watt and conc. NaOH was increase from 1 to 3 %. This implies that the interactive effect of power and NaOH have and inverseeffect on both the cellulose and hemicellulose of the treated plantain stem.

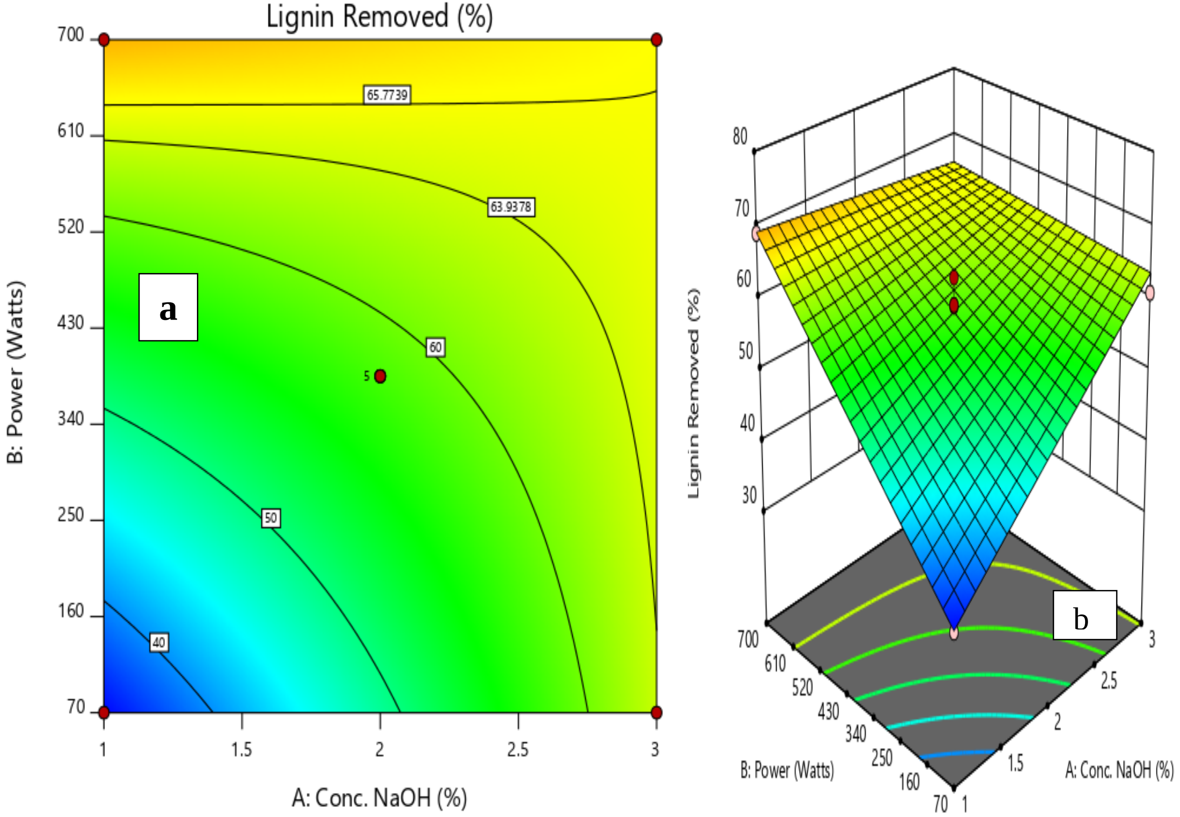

Figure 4: (a) Contour, (b) Three Diamentional: Plot of the interactive Effect between Power and Con. NaOH on Lignin Removal from Plantain Stem

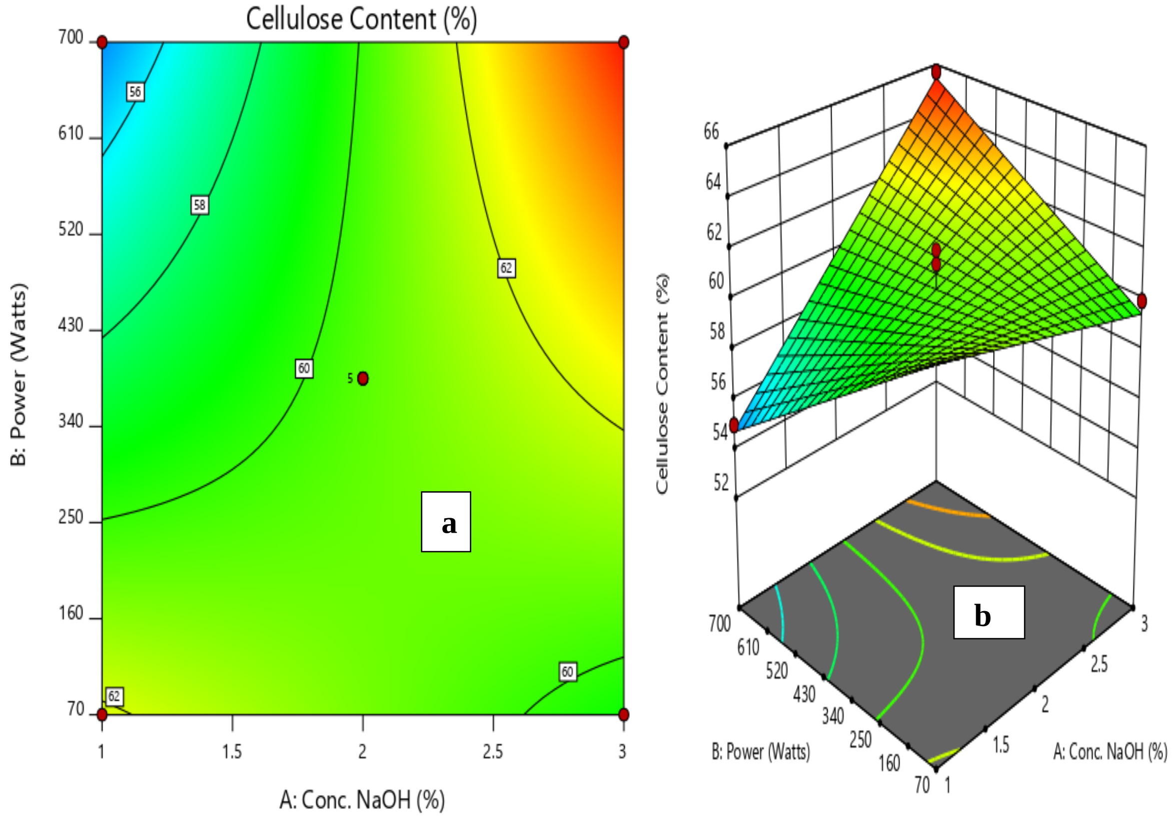

Figure 5: (a) Contour, (b) Three Diamentional: Plot of the Interactive Effect between Power and Con. NaOH on Cellulose from Plantain Stem

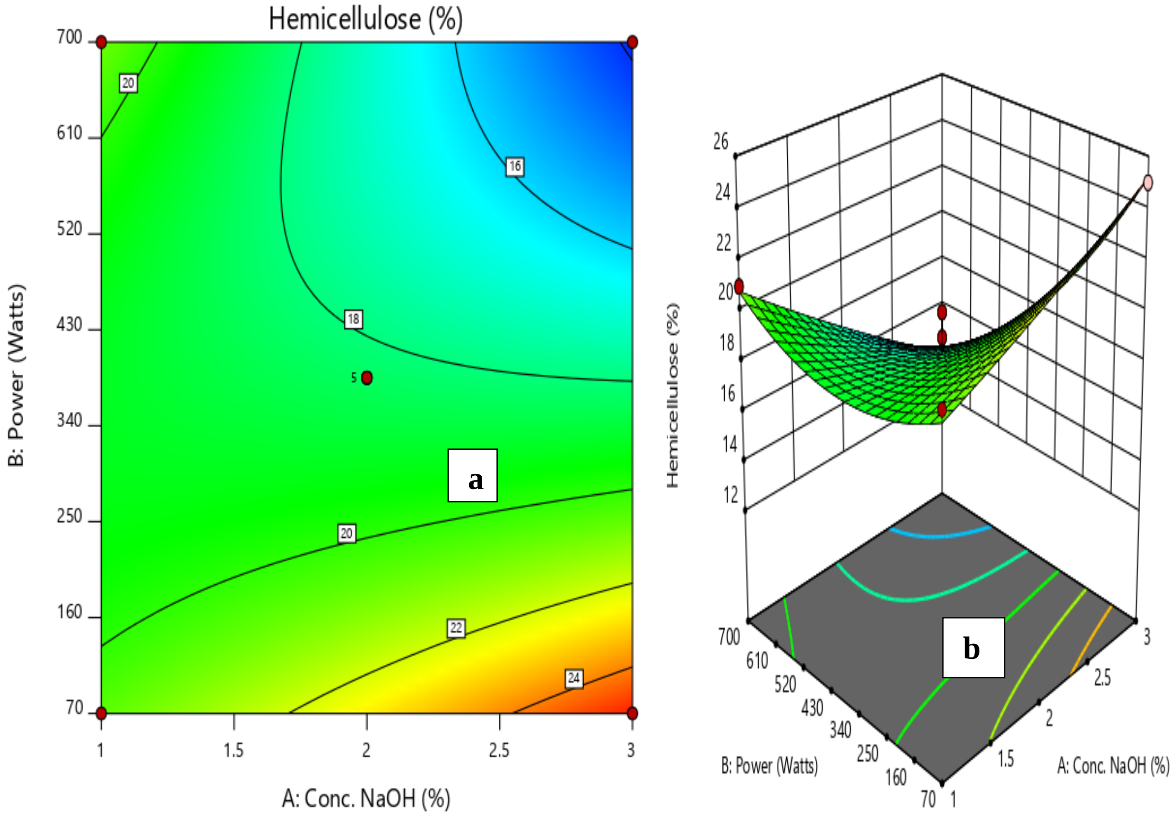

Figure 6: (a) Contour, (b) Three Diamentional: Plot of the Interactive Effect between Power and Con. NaOH on Hemiellulose from Plantain Stem

Interactive Effect between Time and Conc. NaOH on the Responses

The graphical representation of the interactive effect of time and con. NaOH on lignin removal, cellulose and hemicellulose retained of treated plantain stem is presented in Figure 7 and 8 respectively. The contour plots of the combined effects is presented in Figure 7a and 8a, while the three diamentional views are presented in Figure 7b and 8b. The different colours represent the different proportions of the responses. It can be clearly seen that the cellulose increases from 54 % to 60 % when the time and con. NaOH increases simutaneously from 1 to 3.64 minutes and 1 to 2.07 % respectively. This implies an inverse relationship between the interactive factors and the cellulose. It also implies that adequate time was provided during the treatment for the coc. NaOH to remove the lignin content in order to have improvred cellulose content. Further more, the cellulose increases from 62 to 64 % when con. NaOH increases from 2.4 % to 3 %, while the time decreases from 3.27 Minutes to 1 minues. This implies that to achieve higher cellulose content, there is need for increase in con. NaOH and reduction in the treatment time so as to prevent the lose of the celulose. The interactive plots in Figure 8 clearly shows that the hemicellulose decreases from 22 to 17.46 % when the time increases from 1 to 5 minutes and the con. NaOH also increases from 1 to 3 %. This implies a negative influnce of the interacive effects on the hemicellulose which could be attributed to the loss of the hemicellulose due to prolong exposure of the plantain biomass to the con. NaOH.

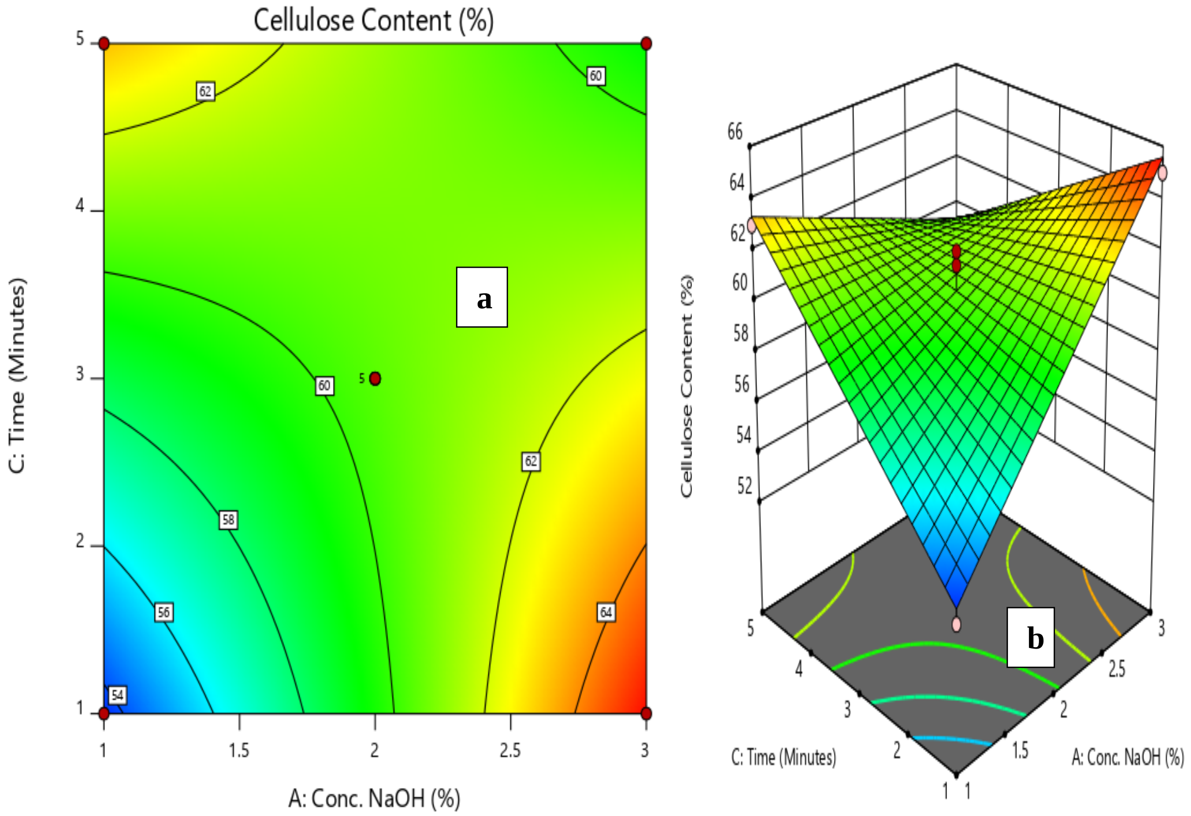

Figure 7: (a) Contour, (b) Three Diamentional: Plot of the Interactive Effect between Time and Con. NaOH on Cellulose from Plantain Stem

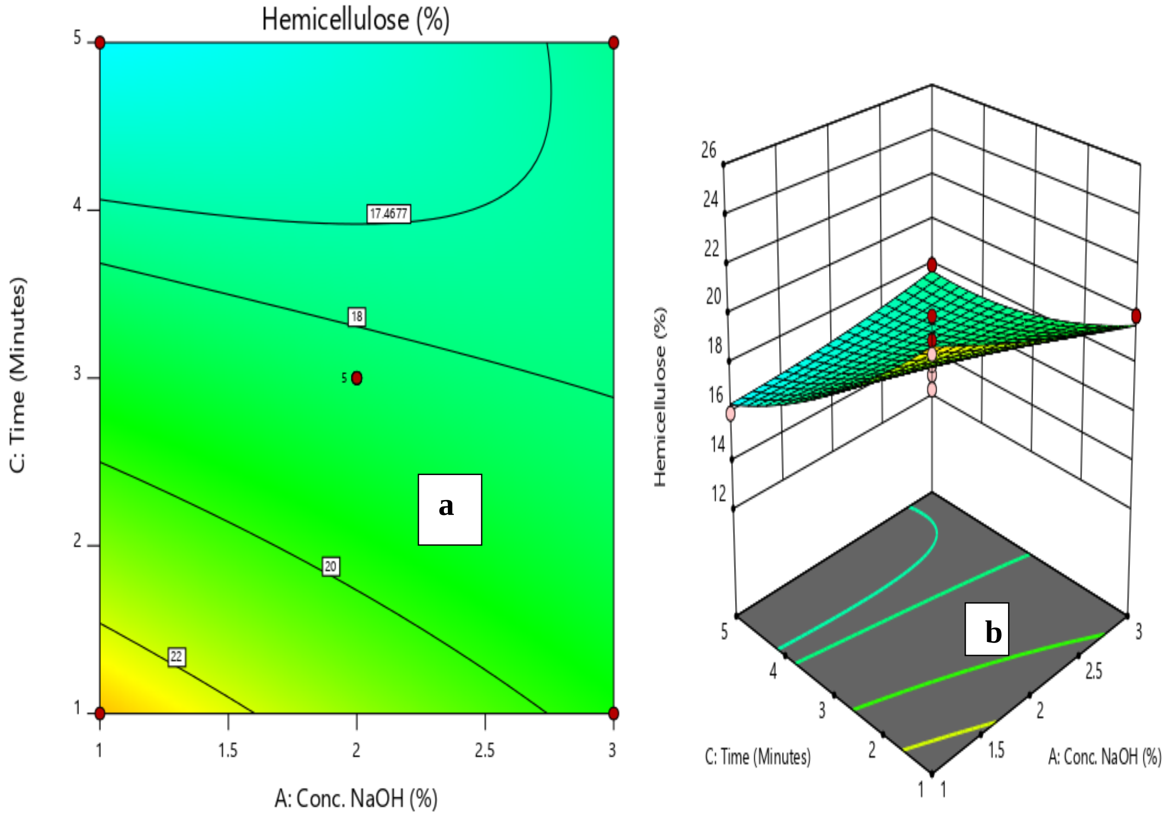

Figure 8: (a) Contour, (b) Three Diamentional: Plot of the Interactive Effect between Time and Con. NaOH on Hemicellulose from Plantain Stem

Optimization Constarinst for Lignin Removal, Cellulose and Hemicellulose from Treated Plantain

The optimization constraint for the optimum point prediction is presented in Table 9. The choice of the goal of optimization of the indpendent variables were based on the ANOVA while the goal of the dependent variable were based on the requirement of biomass for bioethanol production. Numerical optimization method was used for the optimium point predictions of lignin removal (56.90 31 %), cellulose (61.2829 %), hemicellulose (21.185 %) at optimum process conditions of con. NaOH (2.03071 %), power (70 watts) and time (5 minutes) with desirability of 0.681 (Figure 9). Comfirmatory test on the predicted optimum process paraments gave a close value of lignin removal (60.23 %), cellulose (60.67 %) and hemicellulose (22.33 %). In a similar study, Sahare *et al.*,(2012)reported 50.4 % cellulose, 31.7 % hemicellulose and 50 % lignin removal via alkaline pretreated corn corb at treatment time of 4 hours, 1% NaOH, and temperature of 50 ^{o}C. Luz *et al.* (2021), reported 44.5 % cellulose, 37.3 % hemicellulose and 41 % lignin removal via suberosa enzyme treatment of sugar cane residues at 40 ^{o}C and 23 houre. In another study by Kusmiyati and Sukmaningtyas (2018), optimum cellulose of 51.66 %, hemicellulose of 23.29 % and 41 % lignin removal was achieved at 2 % NaOH and 90 minutes autoclaving of pretreated banana pseudo steam. The reduced pretretment time demostarted in this study to achieved high delignificantion without compromise on the cellulose and hemicellulose content of biomas compard to several hours of dlignification time from previous studies can significantly contribute to the reduction in the cost of bioethanol production which can result from prolong equipment shell life and reduce high energy consumption during the production process.

Table 9: Numerical Factors for the Optimum Point Prediction of the Dependent and Independent Variables

Name	Goal	Lower Limit	Upper Limit
A:Conc. NaOH	maximize	1	3
B:Power	minimize	70	700
C:Time	maximize	1	5
Lignin Removal	minimize	34	76.58
Cellulose Content	maximize	53	65.7
Hemicellulose	maximize	13.4	25.54

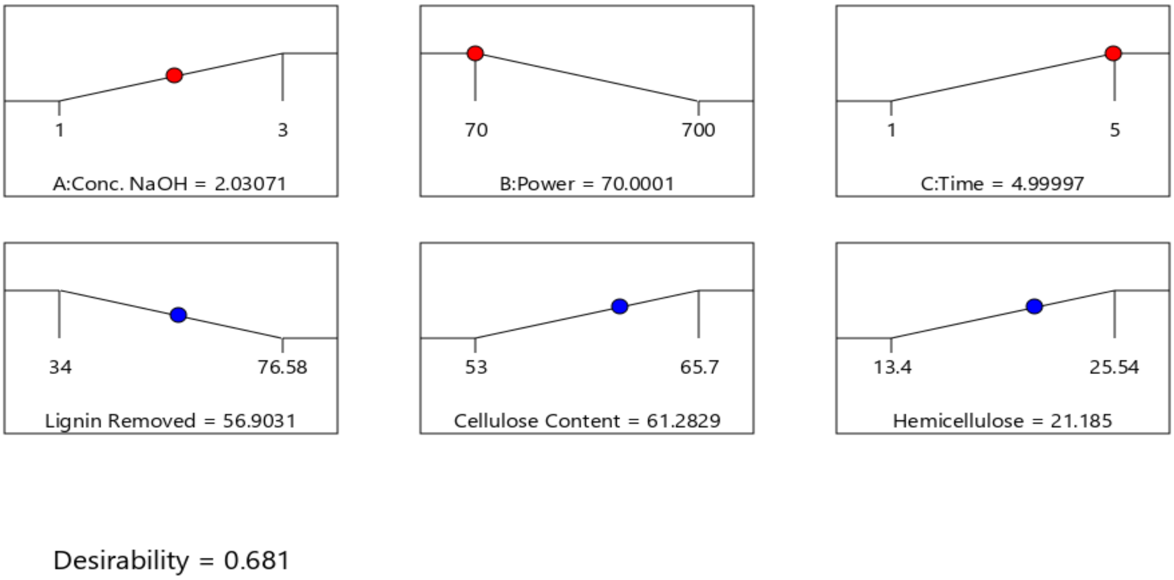

Figure 9: Optimum Predicted Points for Lignin Removal, Cellulose and Hemicellulose Retained from Plantain Stem

Characterization of Treated and Untreated Biomass

The crystallinity index (C. I) was evaluated from the XRD profile of both untreated and treated plantain. The more the lignin and hemicellulose in a lignocellulos material, the lower the crystallinity index of the material (Herverton *et al.*, 2015). C.I was determined from the minimum intensity (I_{Min}) and maximum intensity (I_{Max}) in Figure 10. According to Hevertone *et al.* (2015), the diffraction angle of 16^0, 22^0 and 35^0 are characteristics of cellulose. Paavo *et al.* (2006), reported that transition of cellulose I to cellulose II start at room temperature in 12 % NaOH and completed in 15 % NaOH. However, there is no transition of cellulose I to cellulose II in this study due to treatment with 3 % NaOH. Figure 10 present the XRD of both untreated and treated plantain. The increase in the C. I. of the treated sample shows that that the alkali treatment using NaOH enhances the removal of both lignin and the hemicellulose contented of the plantain. The diffraction angle of untreated plantain stem (18.62^0, 22.29^0 and 35.7^0) are much close to the diffraction angle of pretreated plantain (18.87^0, 22.5^0 and 35.2^0) which correspond to the lattice plane of $[\bar{1}01]$, $[\bar{1}11]$ and [002] respectively. This confirmed that there is no transition from cellulose I to cellulose II. The C.I of the untreated plantain in this study is 35.66 %, this is below the 41.41 % C.I reported for sugar cane residue by Luz *et al.* (2019) while that of the C.I of the treated plantain stem in this study is 58.36 %, this is above the 46.76 % C.I reported for pretreated sugar cane residue using suberoza enzyme by Luz *et al.* (2019). This implies that the treated plantain stem has lower lignin content and will therefore be suitable for bioethanol production.

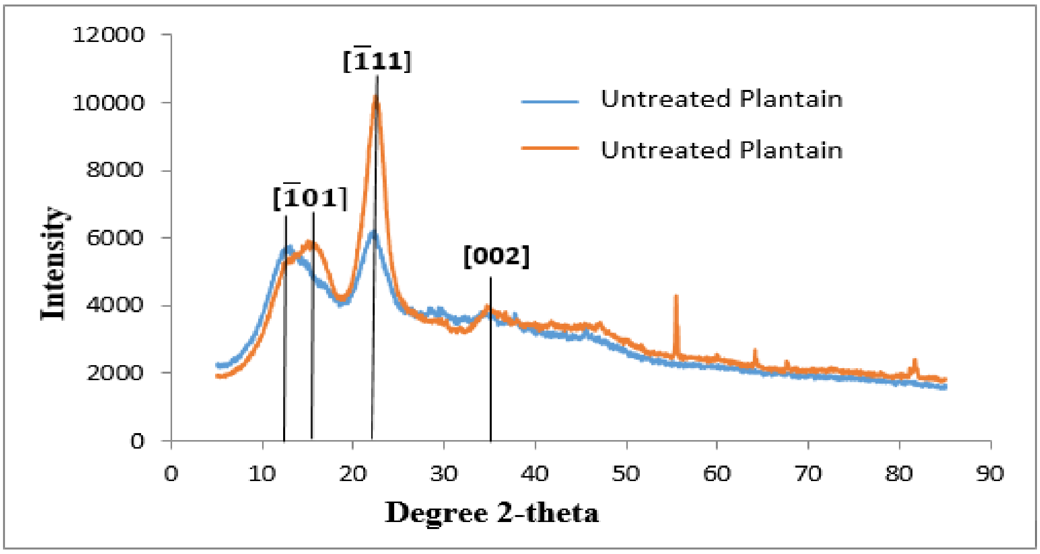

Figure 10: X-Ray Diffraction Pattern of the untreated and treated plantain

Infrared Spectra of the untreated and treated plantain is presented in Figure 11. The functional groups of the untreated plantain shows wider wavelength ranging from 711.5 to 3954.2 cm^{-1} while the treated plantain has shorter wavelength between 1571 and 3954.2 cm^{-1}. The OH-vibration (acid and methanol) is observed between 2995 cm^{-1} to 4000 cm^{1}. This functional group is present in cellulose, hemicellulose and lignin. The treated plantain has a higher absorbance intensity from 2995 $cm^{-1}$to 3401.54 cm^{-1}, but the absorbance intensity decreases as it approaches 4000 cm^{-1}.The change is caused by the variation of binding energy of hydrogen in the system of internal and intermolecular interactions. The decline in the intensity at 3401.54 $cm^{-1}$is an indication that lignin and hemicellulose had experienced bond loss due to the change in the hydrogen bond. A strong broad band with H-C-H functional group exists between 2995CM^{-1} to 2208 cm^{-1}. This stands for alkyl and aliphatic compounds. The H-C-H group is present in cellulose, hemicellulose, and lignin, but symmetric and asymmetric methyl and methylene cellulose groups are stretched (Anggono *et al.*, 2019).The treated plantain has higher absorbance intensity within this region than the untreated plantain. This is due to loss of lignin during pretreatments as it leads to the removal of the aromatic structure of lignin (Luz *et al.*, 2019). The band between 1765 to 1715 cm^{-1}corresponds to the C=O functional group. It stands for the ketone and carbonyl compound and it is only present in hemicellulose. The band at 1646 cm^{-1}corresponds to the fiber-OH group which stands for the bending vibration of the absorbed water and it is only present in cellulose. It has a more prominent absorbance peak in the treated plantain than the untreated as a result of the delignification. The noticeable difference between untreated and treated plantain stem is the disappearance of the band between 1536cm^{-1}to 711.5 cm^{-1}in the treated sample; this corresponds to the syringyl ring in the structure of lignin. This is a clear indication of the elimination of lignin within the streaking band (Luz *et al.*, 2019; Anggono *et al.*, 2019).

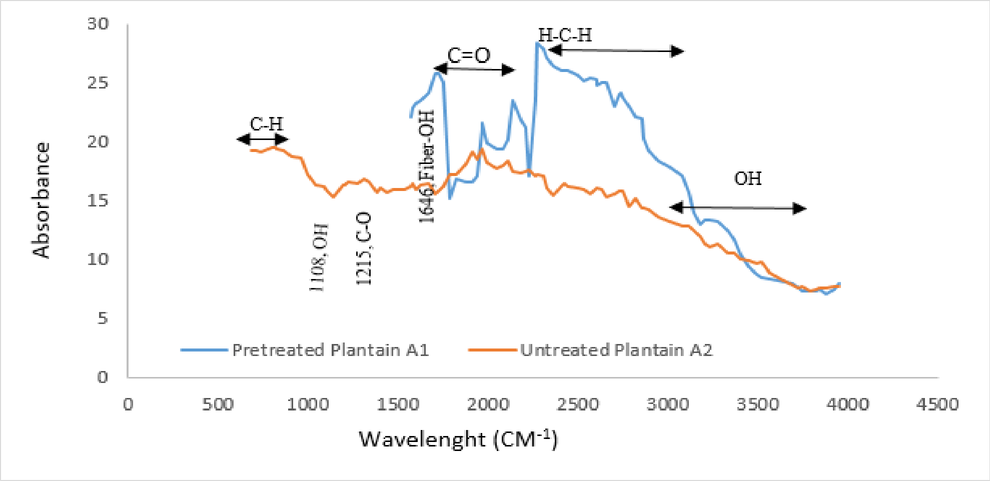

Figure 11: Infrared Spectra of the untreated and treated plantain

The scanning electron microscope (SEM) image of both the treated and the untreated plantain is presented in Figure 12. The untreated plantain stem have clearer images due to higher lignin and hemicellulose compared to the treated plantain. This implies that untreated plantain have lesser pore compared to the treated. The increase in the porosity of the treated plantain is as a result of the alkaline treatment with NaOH which causes degradation of the lignin and the hemicellulose content of the plantain.

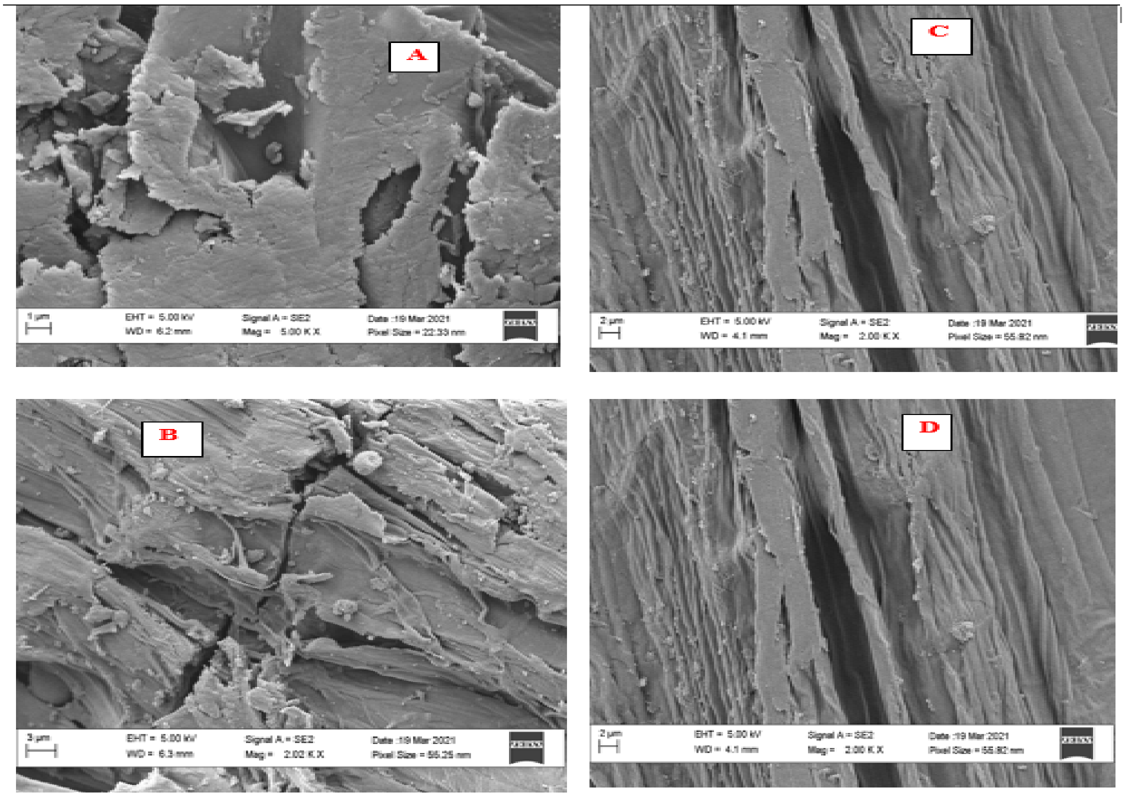

Figure 12: SEM Micrograph of untreated (A and B) and treated (C and D) plantain at Different Magnifications

CONCLUSIONS

The optimization of the compositional structure of plantain via Box-Benkhen experimental Design was carried out in this study. Statistical and regression analysis on the experimental outcome revealed that the lignin removal and the cellulose content are described by 2FI model, while the quadratic model is the most preferred model for hemicellulose content. The high adequate precision values for cellulose (42.33), lignin removal (23.58) and hemicellulose (15.87) further confirms the high predictive efficiency of the developed models equation. Conc. NaOH (A), Power (B), and Time (C) are all single independent variables that have significant effect on the amount of lignin removal, while conc. NaOH and Power (AB) is the only interactive independent variable that significantly influenced the amount of lignin removal. The amount of cellulose retained after treatment was significantly influence by conc. NaOH (A), Time (C), and the interactive effect between conc. NaOH and Power (AB) as well as between conc. NaOH (A) and Time (C). The change in the hemicellulose content was due to single effect of Power (B), Time (C), and interactive effect between conc. NaOH and Power (AB), conc. NaOH (A) and Time (C) as well as the square of the power (B^2). The lignin removal (56.90 31 %), cellulose (61.2829 %), hemicellulose (21.185 %) was predicted at optimum process conditions of con. NaOH (2.03071 %), power (70 watts) and time (5 Minutes) with desirability of 0.681. The alkali treatment using NaOH enhanced the removal of lignin, this was confirmed by XRD analysis which shows an increase in the C. I of 58.36 % for the treated plantain stem compared to the C. I of 35.66 % for the untreated plantain stem. The result of FTIR analysis of both the retreated and untreated plantain stem confirms the presence of different functional groups common to cellulose, hemicellulose and lignin. However, the disappearance of the syringyl ring band in lignin in the treated plantain stem further confirms the elimination of lignin within that streaking band. The higher degradation noticed on the SEM image of treated plantain was largely due to lignin removal and part of the hemicellulose. Hence, the treated plantain is a good potential feedstock for the production of bioethanol.

ACKNOWLEDGMENT

The authors' acknowledgment goes to the Petroleum Technology Development Fund (PTDF) of Nigeria for the grant (PTDF/ED/LSS/PHD/000/0284/19) to finance this research.

REFERENCES

Abas N., Kalair, A., and Khan, N. (2015). Review of fossil fuels and future energy technologies. *Futures*, *69*, 31-49.

Anggono, J., Purwaningsih, H., Sugondo, S., Henrico, S., Sewucipto, S., and Patel, J. (2019). *Structural Evaluation on Sugarcane Bagasse Treated Using Sodium and Calcium Hydroxide* (Doctoral dissertation, Petra Christian University).

Burk M. J. (2010). Sustainable production of industrial chemicals from sugars. *International Sugar Journal.* 112, 30-35.

Egwim, E. C., Oluwatosin, S. K., and Deborah, K. (2015). Microwave-alkaline assisted pretreatment of banana trunk for bioethanol production. *Journal of Energy and Power Engineering*, *9*, 705-13.

Ethaib, S., Omar, R., Kamal, S. M., and Biak, D. A. (2015). Microwave-assisted pretreatment of lignocellulosic biomass: A review. *Journal of Engineering Science and Technology*, *10*, 97-109.

Food and Agricultural Organization Organisation of the United Nations (FAOSTAT). (2018) (Accessed 4 August 2019 via www.factfish.com/Nigeria/plantain/quantity).

Jonsson, L. J., and Martín, C. (2016). Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource technology*, *199*, 103-112.

Kline, S. J., and Rosenberg, N. (2010). An overview of innovation. *Studies on science and the innovation process: Selected works of Nathan Rosenberg*, 173-203.

Londo, H. M., Deurwaarder, E. P., and Van Thuijl, E. (2006). Review of EU Biofuels Directive Public consultation exercise-Summary of the responses. *Energy Research Centre of the Netherlands, Amsterdam*.

Luz M. F. P., L. M., Salcedo Mendoza, J. G., and LópezGalán, J. E. (2019). Influence of pretreatments on crystallinity and enzymatic hydrolysis in sugar cane residues. *Brazilian Journal of Chemical Engineering*, *36*:131-141.

Mahyati, M., Abdul, R., Muhammad, N., and Paulina, T. (2013). Biodegradation of lignin from corn cob by using a mixture of *Phanerochaetechrysosporium*, *Lentinusedodes* and *Pleurotusostreatus*. *International Journal of Scientific and Technology Research, 2*(11): 79-82.

Mu, D., Seager, T., Rao, P. S., and Zhao, F. (2010). Comparative life cycle assessment of lignocellulosic ethanol production: biochemical versus thermochemical conversion. Environmental management, 46(4): 565-578.

Nnaji, C. E., Ukwueze, E. R., and Chukwu, J. O. (2012). Determinants of household energy choices for cooking in rural areas: evidence from Enugu state. *Continental Journal of Social Sciences*, *1*(5): 1-11.

Paavo M. k., Manu L., and Kari R. (2006). The conversion from cellulose I to cellulose II in NaOH mercerization performed in alcohol–water systems: An X-ray powder diffraction study. *Carbohydrate Polymers*. 68, 35–43.

Sahare, P., Singh, R., Laxman, R. S., and Rao, M. (2012). Effect of alkali pretreatment on the structural properties and enzymatic hydrolysis of corn cob. *Applied biochemistry and biotechnology*, *168*(7): 1806-1819.

Saraiva, A. B., Pacheco, E. A. V., Visconte, L. L. Y., Bispo, E. P., Escócio, V. A., de Sousa, A. F.,and BRITO, G. D. C. (2012). Potentials for utilization of post-fiber extraction waste from tropical fruit production in Brazil-the example of banana pseudo-stem. *Embrapa Agroindústria de Alimentos-Artigoemperiódicoindexado (ALICE)*.

Sims, R. E., Mabee, W., Saddler, J. N., and Taylor, M. (2010). An overview of second generation biofuel technologies. *Bioresource technology*, *101*(6), 1570-1580.

Sluiter. A., Hames, B., Ruiz, R ., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). Determination of Structural Carbohydrates and Lignin in Biomass, *National Renewable Energy Laboratory*

Song, K., Chu, Q., Hu, J., Bu, Q., Li, F., Chen, X., and Shi, A. (2019). Two-stage alkali-oxygen pretreatment capable of improving biomass saccharification for bioethanol production and enabling lignin valorization via adsorbents for heavy metal ions under the biorefinery concept. *Bioresource technology*, *276*, 161-169.

Sukmaningtyas, R. P. (2018). Pretreated of banana pseudo-stem as raw material for enzymatic hydrolysis and bioethanol production. In *MATEC Web of Conferences* (Vol. 154, p. 01035). EDP Sciences.

Tripathi, S. K., Kaur, D., Bhardwaj, N. K., Pathak, P., and Kumar, S. (2021). Improving Biogas Production by Co-digestion of Banana Stem Juice with Agro-Based Material Washings and Digestate Along with Microbial Culture. *Waste and Biomass Valorization*, *12*, 1385-1393.

Wanitwattanarumlug, B., Luengnaruemitchai, A., and Wongkasemjit, S. (2012). Characterization of corn cobs from microwave and potassium hydroxide pretreatment. *Int. Sch. Sci. Res. Innov*, *6*, 528-532.

Wen, J. L., Sun, S. L., Yuan, T. Q., and Sun, R. C. (2015). Structural elucidation of whole lignin from Eucalyptus based on preswelling and enzymatic hydrolysis. *Green Chemistry*, *17*(3), 1589-1596.

Wyman, C. E. (2007). What is (and is not) vital to advancing cellulosic ethanol. *TRENDS in Biotechnology*, *25*(4):153-157.