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Effect of Acid mixtures on the Hydrolysis of Coconut Coir for Recovery of Fermentable Sugars

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ABSTRACT*:* In this study, coconut coir was hydrolysed to produce fermentable sugars using dilute nitric and acetic acid. The hydrolysis process was carried out according to a four variable Box-Behnken design which was used to develop a statistical model to describe the relationship between the concentration of fermentable sugars produced (dependent variable) and the independent variables (time, temperature, nitric acid concentration and acetic acid concentration). Results of analysis of variance (ANOVA) performed to determine the fit of the statistical model showed that the model was statistically significant ($p<0.0001$) with a low standard deviation (1.77) and non-significant lack of fit ($R^2=0.93$). The concentrations of nitric and acetic acid as well as the hydrolysis time and temperature all positively influenced the hydrolysis process as evident in the increase in the amount of fermentable sugars produced when the values of these variables were increased. When both acids were combined together, the amount of fermentable sugar produced was increased by as much 54%. Optimisation of the statistical model showed that the maximum sugar concentration was 32.7 g/L and this was obtained for coconut coir catalysed by 0.50 %w/v nitric acid, 0.40 %w/v acetic acid at 160 ^oC for 30 minutes. Validation of the statistical model showed that there was no significant difference between predicted and observed values. © JASEM

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KEYWORDS: Coconut coir, Lignocellulosic feedstock, Response Surface Methodology, Optimisation

The numerous environmental and energy security challenges associated with the use of conventional petroleum based liquid fuels for transportation has resulted in a rapid expansion in the market for bioethanol either as a blend with gasoline to enhance octane rating or as a primary fuel (Mohan and Reddy, 2012). Abundantly available lignocellulosic biomass has been considered as a cheap and sustainable feedstock for second generation bioethanol production (Vaithanomsat et al., 2010).

Nigeria is one of the countries that generate a substantial amount of lignocellulosic agricultural residues and wastes such as corn stover, wheat straw, cassava bagasse, sugar cane bagasse, coconut coir etc (Agbro and Ogie, 2012). Nigeria is the fifth largest producer of coconut in Africa with a net annual output of 260,000 metric tons (Uwubanmwen et al., 2011). Efforts have been made to create value from coconut wastes by utilising them as precursors for preparation of adsorbents, ropes, twines, mats, mulch for organic fertiliser etc (Anirudhan et al., 2008; Bilba et al., 2007; Namasivayam and Sangeetha, 2008). However, coconut coir has been reported to be an attractive feedstock for bioethanol production due to its high cellulose and hemicellulose content (Vaithanomsat et al., 2010). Hence more use can be found for this otherwise waste material.

The complex nature of the lignocellulose structure in coconut coir makes it necessary to take it through suitable pretreatment steps in order to recover fermentable sugars. Of all the pretreatment strategies identified in the literature, acid hydrolysis using dilute sulphuric acid is the most studied and most widely used (Satimanont et al., 2012). However, other acids such as phosphoric acid, acetic acid and nitric acid have also received some attention because these acids are less aggressive in action compared to sulphuric acid which enables them to produce a fermentable hydrolysate with lower concentration of microbial growth inhibitors such as furfural and hydroxyl methyl furfural (HMF) (Nantapipat et al., 2013).

To maximise the yield of fermentable sugars during hydrolysis, it is important to optimise the variables upon which the yield is dependent. Response surface methodology based on statistically designed experiments has been found to be very useful in optimising multivariable processes. According to Montgomery (2005), it is employed for multiple regression analysis of quantitative data obtained from statistically designed experiments. Hence, the aim of this study was to investigate the effect of combining dilute acetic and nitric acid on the hydrolysis of

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coconut coir to produce fermentable sugars. The hydrolysis process was optimised using response surface methodology to obtain maximum fermentable sugars.

MATERIALS AND METHODS

Lignocellulosic Feedstock: The coconut coir used in this study was obtained from the Faculty of Agriculture model farm in the University of Benin, Benin City, Edo State, Nigeria. It was sun dried for 24 hours to reduce moisture and prevent biodeterioration. The dried coir was milled to a particle size of about 1.5 mm, homogenised in a single lot and stored under dry conditions prior to use. The chemical composition of coconut coir has been reported previously by Fatmawati et al. (2013).

Acid Hydrolysis: Dilute acid hydrolysis of the coconut coir was carried out in an autoclave using dilute nitric acid and acetic acid. The operating conditions of the hydrolysis process such as temperature, time and acid concentration were fixed by the experimental design. At the end of the hydrolysis process, the solid residue was separated by centrifugation and the pH of the resulting supernatant was adjusted to 10 using 2M $Ca(OH)_2$. The resulting precipitate was removed by centrifugation and the supernatant was adjusted to a pH of 6.5 using 10% H_2SO_4 (Amenaghawon et al., 2014a).

Analytical Methods: The fermentable sugars recovered from the coconut coir during hydrolysis were quantified by treating the hydrolysate with 3,5-dinitro-salicylic acid (DNSA) which is reduced to 3-amino-5-nitro-salicylic acid. The latter was quantified by measuring the absorbance at a wavelength of 540 nm using a UV-Vis spectrophotometer (PG Instruments model T70). The DNSA reagent consisted of 1 g DNS dissolved in 20 mL 2M NaOH and 50 mL distilled water. Thirty grams of Rochelle salt (potassium sodium tartarate tetrahydrate: $KO_2CCH(OH)CH(OH)CO_2Na•4H2O$) was added and distilled water was added to make up the volume to 100 mL. The reducing sugars were measured as follows: To a test tube were added the following; 0.2 mL reducing sugar solution, 1.8 mL distilled water and 2 mL DNSA reagent. The mixture in the test tube was boiled for 5 min in a water bath followed by cooling to room temperature and diluting to 24 mL. A standard curve was prepared using known concentrations of glucose from which the concentration of reducing sugar was determined.

Experimental Design: A four variable Box-Behnken design (BBD) for response surface methodology was used to develop a statistical model for the hydrolysis process. The range of the variables that were optimised is shown in Table 1. The Box-Behnken design is suitable for the exploration of quadratic response surfaces and this design can be used to develop a second degree polynomial model which can be utilised for optimisation purposes (Amenaghawon et al., 2013). The number of experimental run for this design is obtained from Equation (1). $N= k^2 + k + c_p$ (1) Where k is the number of factors and c_p is the number of replications at the center point. The experimental design was developed using Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA). The coded and actual values of the independent variables were calculated using Equation (2). $x_i = \frac{X_i - X_o}{\Delta X_i}$ (2) Where x_i and X_i are the coded and actual values of the independent variable respectively. X_o is the actual value of the independent variable at the center point and ΔX_i is the step change of X_i. The following generalised second order polynomial equation was used to estimate the response of the dependent variable.

$$Y_i = b_o + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad (3)$$

where Y_i is the dependent variable or predicted response, X_i and X_j are the independent variables, b_o is offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the error term. The Design Expert software was used for regression and graphical analysis of the experimental data. The goodness of fit of the model was evaluated by the coefficient of determination (R^2) and analysis of variance (ANOVA). The optimum values of the variables tested were obtained by numerical optimisation based on the criterion of desirability (Jargalsaikhan and Saracoğlu, 2008).

RESULTS AND DISCUSSION

Statistical Modelling: The coefficients of the regression model were calculated using Design Expert and the following regression model was obtained.

$$\begin{aligned} Y = {} & -76.09 + 2.59X_1 - 0.24X_2 - 162.67X_3 - 393.96X_4 \\ & - 0.018X_1X_2 + 4.55X_1X_3 - 0.085X_1X_4 + 2.64X_2X_4 \\ & + 28.36X_3X_4 - 0.032X_1^2 + 109.53X_2^2 + 7.54X_3^2 \end{aligned} \quad (4)$$

Table 3 shows the concentration of fermentable sugars predicted by Equation (4) alongside the experimental data. To test the fit of the statistical model, the regression equation was subjected to analysis of variance (ANOVA) and the results are presented in Tables 3 and 4. Results presented in Table 3 showed that the statistical model was highly significant as evident from the Fisher's F-test with very low probability value ($p<0.0001$). The statistical significance of the coefficients of the model was determined by p values as shown in Table 3. The smaller the magnitude of the p value of a term, the more significant that model term is (Tanyildizi et al., 2005). The linear terms representing the effects of time and acetic acid concentration were the most significant. Furthermore, the interactive effect of time

and nitric acid concentration as well as temperature and acetic acid concentration were more significant than the others. Since linear and quadratic effects of time and nitric acid concentration were significant, it suggests that little variation in their levels could alter the rate of the hydrolysis process. Statistical information for ANOVA as presented in Table 4 shows that the model had a high coefficient of determination (R^2=0.93) indicating that the model explained 93% of the variability in the response. The high R^2 value coupled with the low standard deviation suggests that the model showed a good fit with the experimental data and as such it was able to adequately represent the relationship between the independent variables and the response (Montgomery 2005). An adequate precision of 15.60 indicates an adequate signal for the signal–noise ratio showing that the model can be used to navigate the design space (Cao et al., 2009). Furthermore, a relatively low coefficient of variation (CV=10.89) suggests that the experiments were carried out with a high degree of precision (Hou and Chen, 2008). A parity plot comparing experimental and model predicted results is shown in Figure 1. The Figure shows that there was an acceptable level of fit between the experimental and model predicted results as seen in the clustering of the data points around the 45^o diagonal.

Optimisation of Dilute Acid Hydrolysis of Coconut Coir: The 3D response surface plots which are graphical representations of the statistical model are shown in Figures 2 to 5. The main objective of the response surface method is to efficiently search for the optimum values of the response that is being maximised. Figure 2 shows that there was an increase in the total sugar concentration released during hydrolysis when the hydrolysis time was increased. This trend is indicative of the conversion of the cellulose and hemicellulose fractions of the feedstock to fermentable sugars (Amenaghawon et al., 2014b; Palmqvist and Hahn-Hagerdal, 2000). The hydrolysis process was positively influenced by temperature as shown in Figure 2. This could be attributed to the enhancement of the rate of reaction at elevated temperatures (Najafpour et al., 2007). When the temperature was maintained below 145 ^{o}C, the sugar yield was observed to drop sharply. Hence, in order to obtain high sugar yield, the temperature should be maintained at 145–160 ^{o}C. Though a positive trend was observed with respect to temperature, the implementation of high temperature hydrolysis is often discouraged as a result of the degradation of sugars to produce inhibitory products such as furfural (Palmqvist and Hahn-Hagerdal, 2000). Similar observations have been reported by previous researchers (Amenaghawon et al., 2014b; Lu and Mosier 2008; Zhang et al., 2012).

The total sugar concentration increased with increase in nitric acid concentration as shown in Figure 3. This shows that nitric acid had a significant overall positive effect on the recovery of sugars from coconut coir. Similarly, the concentration of acetic acid also had a positive influence on the hydrolysis process as shown in Figure 4. This observation could be attributed to the catalysing effect of the respective acids in cleaving the glycosidic bonds in the cellulosic material (Mosier et al., 2002). The hydrogen ions in solution are responsible for the catalytic activity of the acid. Since the catalytic activity of an acid is proportional to its hydrogen ion concentration, more hydrogen ions will be formed in solution when the concentration of the acid is increased. Consequently, the hydrolysis reaction will proceed rapidly because of the increase in the rate at which the glycosidic bonds are being cleaved to produce fermentable sugars (Kumar et al., 2009). Similar trends have been reported by previous researchers. Hu et al. (2010) investigated the acid hydrolysis of sugar maple wood extract at atmospheric pressure using dilute sulphuric acid. They observed that increasing the concentration of acid resulted in an increase in the concentration of fermentable sugars. This resulted in the conclusion that the acid acted as a catalyst in the cleavage of the β (1–4) glycosidic linkages in the xylooligomers to yield xylose monomers. Lenihan et al. (2010) also reported that increasing the concentration of acid at mild temperatures resulted in an increase in the rate of the hydrolysis reaction.

Figure 5 shows the effect of the interaction between acetic and nitric acid concentrations on the recovery of sugars during hydrolysis. When nitric acid was used as a standalone hydrolysing agent (i.e. with 0%w/v acetic acid), the maximum sugar concentration recorded was about 15 g/L. When nitric acid was then combined with acetic acid, it was observed that the sugar concentration increased to a maximum of 32.7 g/L at an acetic acid concentration of 0.4%w/v which corresponds to an increase of about 54%. This could be attributed to an increase in the rate of xylan hydrolysis to produce fermentable sugars. From Figure 5, it is evident that the combined use of 0.5% (w/v) of nitric acid and 0.4% (w/v) of acetic acid is optimal for obtaining the maximum sugar yield. These results indicate that the combined use of nitric and acetic acid has a synergistic effect in improving sugar yield compared to using nitric alone (Zhang et al., 2012).

Based on the statistical model, numerical optimisation was carried out with the help of Design Expert considering each value of response and the optimisation results showed that the maximum sugar concentration of 32.7 g/L was obtained for coconut coir catalysed by 0.50 %w/v nitric acid, 0.40 %w/v acetic acid at 160 ^{o}C for 30 minutes. To confirm these results, hydrolysis runs were carried out in triplicate under optimised conditions and the results indicate that there was no significant difference between

observed and predicted values. The results obtained are similar to earlier findings by other researchers. Sun and Cheng (2005) studied the dilute H_2SO_4 (0.6-2.2% w/w) hydrolysis of rye straw and Bermuda grass and found that the release of fermentable sugars was over 30 g/L when treated with 1.5% H_2SO_4 for 90 min at 121°C. In another study, Tellez-Luis et al. (2002) investigated the dilute H_2SO_4 hydrolysis of sorghum straw and found that the release of fermentable sugar was 24.9 g/L when treated with 2.0% H_2SO_4 for 71 min at 122°C. Zhang et al. (2012) reported improvements in the yield of fermentable sugars when dilute sulphuric acid was combined with phosphoric acid to catalyse the hydrolysis of oil palm empty fruit bunch for high yield production of xylose.

Conclusion: The hydrolysis of coconut coir was catalysed using dilute nitric and acetic acid to produce fermentable sugars. The hydrolysis process was carried out according to a four variable Box-Behnken design and the process was optimised using response surface. Increasing the hydrolysis time had an overall positive effect on the hydrolysis process. The same trend was observed for temperature as well as the concentration of nitric and acetic acid. When applied individually, both acids positively influence the recovery of fermentable sugars from coconut coir. When combined together, the amount of fermentable sugar produced was increased by as much 54%. The maximum sugar concentration of 32.7 g/L was obtained for coconut coir catalysed by 0.50 %w/v nitric acid, 0.40 %w/v acetic acid at 160 ^oC for 30 minutes.

Table 1: Experimental range and levels of independent variables

Independent Variables	Symbols	Coded and actual levels		
		-1	0	+1
Time (min)	X_1	10	20	30
Temperature (^oC)	X_2	140	150	160
Nitric acid conc. (%w/v)	X_3	0.25	0.38	0.5
Acetic acid conc. (%w/v)	X_4	0	0.2	0.4

Table 2: Box-Behnken design matrix for the hydrolysis of coconut coir

Run No	Factors								Response	
	Coded levels				Actual values				Total sugar conc. (g/L)	
	X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	Observed	Predicted
1	0	0	1	-1	20	150	0.50	0.0	20.16	20.44
2	0	1	0	1	20	160	0.38	0.4	17.45	15.66
3	-1	0	-1	0	10	150	0.25	0.2	13.37	12.95
4	-1	0	0	1	10	150	0.38	0.4	14.16	14.54
5	0	0	1	1	20	150	0.50	0.4	15.75	15.66
6	1	1	0	0	30	160	0.38	0.2	8.24	6.85
7	-1	-1	0	0	10	140	0.38	0.2	14.00	15.66
8	0	-1	-1	0	20	140	0.25	0.2	5.63	5.98
9	-1	0	1	0	10	150	0.50	0.2	8.56	8.17
10	0	0	0	0	20	150	0.38	0.2	14.00	15.66
11	0	0	-1	1	20	150	0.25	0.4	15.46	15.61
12	1	0	-1	0	30	150	0.25	0.2	14.71	13.58
13	-1	0	0	-1	10	150	0.38	0.0	15.32	14.23
14	-1	1	0	0	10	160	0.38	0.2	15.99	15.68
15	0	-1	0	1	20	140	0.38	0.4	17.53	15.66
16	0	0	0	0	20	150	0.38	0.2	17.08	18.25
17	1	0	0	1	30	150	0.38	0.4	11.95	12.84
18	0	-1	0	-1	20	140	0.38	0.0	15.62	15.70
19	0	-1	1	0	20	140	0.50	0.2	15.62	17.08
20	0	0	0	0	20	150	0.38	0.2	20.05	19.10
21	1	0	0	-1	30	150	0.38	0.0	4.79	4.48
22	1	0	1	0	30	150	0.50	0.2	22.84	21.66
23	0	0	0	0	20	150	0.38	0.2	12.24	13.72
24	0	0	-1	-1	20	150	0.25	0.0	20.99	19.68
25	0	1	-1	0	20	160	0.25	0.2	11.08	10.73
26	0	1	0	-1	20	160	0.38	0.0	11.92	12.23
27	1	-1	0	0	30	140	0.38	0.2	8.46	9.86
28	0	1	1	0	20	160	0.50	0.2	16.04	17.10
29	0	0	0	0	20	150	0.38	0.2	17.42	17.71

Table 3: Analysis of variance for statistical model

Sources	Sum of Squares	df	Mean Squares	F value	p value
Model	641.48	12	53.46	16.99	< 0.0001
X_1	115.58	1	115.58	36.74	< 0.0001
X_2	5.64	1	5.64	1.79	0.1995
X_3	49.13	1	49.13	15.62	0.0011
X_4	110.23	1	110.23	35.04	< 0.0001
X_1X_2	12.95	1	12.95	4.12	0.0595
X_1X_3	129.52	1	129.52	41.17	< 0.0001
X_1X_4	0.11	1	0.11	0.036	0.8512
X_2X_4	112.17	1	112.17	35.65	< 0.0001
X_3X_4	2.01	1	2.01	0.64	0.4358
X_1^2	68.39	1	68.39	21.74	0.0003
X_3^2	19.70	1	19.70	6.26	0.0236
X_4^2	0.61	1	0.61	0.19	0.6653
Residual	50.34	16	3.15		
Lack of Fit	40.41	12	3.37	1.36	0.4157
Pure Error	9.93	4	2.48		
Cor Total	691.81	28			

Table 4: Statistical information for ANOVA

Parameter	Response Value
R^2	0.93
Adjusted R^2	0.87
Mean	16.29
Standard	1.77
CV %	10.89
Adeq. Precision	15.60

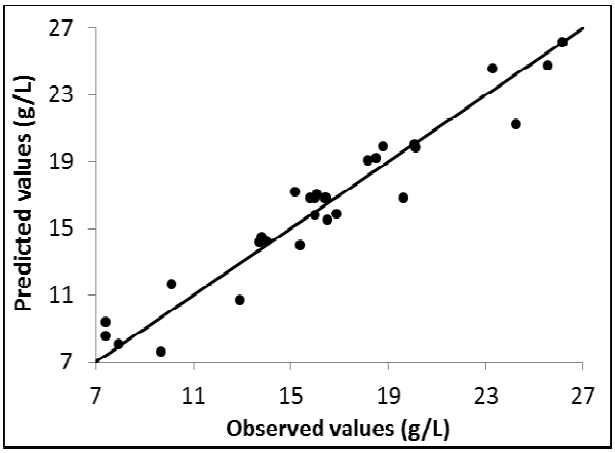

Fig 1: Parity plot showing comparison between experimental and predicted values

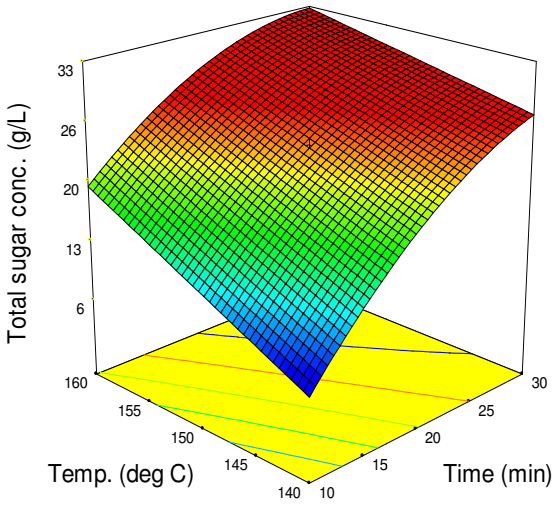

Fig 2: Effect of time and temperature on total sugar concentration

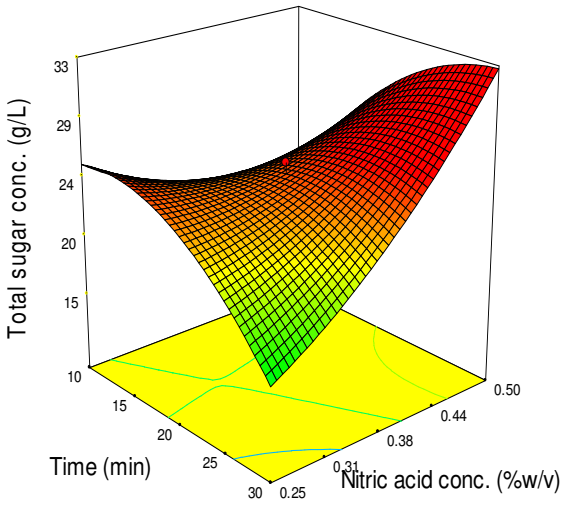

Fig 3: Effect of time and nitric acid concentration on total sugar concentration

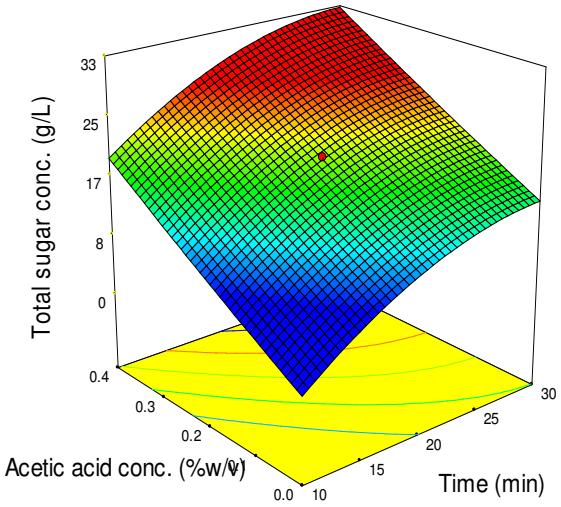

Fig 4: Effect of time and acetic acid concentration on total sugar concentration

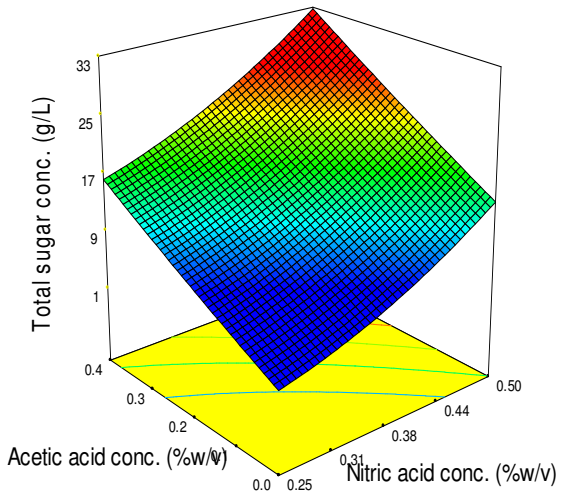

Fig 5: Effect of acetic and nitric acid concentration on total sugar concentration

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