Hybridization of Selected Nigerian Lignocellulosic Biomass Feedstocks for Bioethanol Production: Modeling and Optimization of Pretreatment and Fermentation Process Parameters Using Response Surface Methodology

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Abstract: In this study, hybridized feedstocks (mixtures of biomass) of cassava peels plus yam peels, as well as corn cobs plus rice husks biomass, were optimized using the response surface methodology centered on the statistical design of experiments (DOE) of the Box-Behnken design (BBD), to produce bioethanol. The feedstocks were locally sourced, hybridized (mixed), pretreated, and fermented before being distilled in a UOP3CC continuous distillation column. The BBD was applied using a 3-level, 3-factor process variables using pH, time, and particle size, and indicated as X1, X2, and X3, respectively. The bioethanol yield from the two hybridized biomass feedstocks was predicted by the developed quadratic polynomial models from BBD. For the hybridized biomass mixture of cassava peels plus yam peels, the optimal condition was statistically predicted as pH 5.00, fermentation time of 120.00 hours, and particle size of 362.5 microns, the predicted bioethanol yield under the optimal condition was 115.75 mL per 1500 g of hybridized biomass and the average volume of bioethanol obtained was 125.00 mL per 1500 g of biomass, which is within the projected range of the model equation, same applies to rice husks plus corn cobs hybridized biomass, but with a better prospect for bioethanol production.

Key words: bioethanol, Box–Behnken design, pretreatment, modeling, optimization

1 Introduction

Lignocellulosic biomass obtained from agricultural residues has been a good raw material in the production of fuel bioethanol as they are readily available, cheaply acquired (as they are waste materials that would have been discarded), and most importantly, they are very rich in polysaccharide (cellulose, hemicellulose, and lignin). Nigeria is blessed with a large expanse of arable land for crop cultivation from which lignocellulosic biomasses can be obtained as wastes after processing. Till now, the economy of Nigeria is worryingly dependent on crude oil with the country occupying 8th position among the largest global oil producers and accounts for close to 3% of the entire world’s oil production. Crude oil being a fossil fuel is non-renewable and not environmentally friendly. The use of bioethanol produced from lignocellulosic biomasses such as cassava peels, yam peels, rice husks, corn cobs, among others as an alternative source of fuel for automobiles is a positive development and is currently being embraced by many countries of the world.

Bioethanol is an evaporative, colorless liquid that is produced by the fermentation and subsequent distillation of starchy food crops such as yam, corn, cassava, potatoes, and so on. The production of bioethanol meant for augmentation/replacement of the conventional automobile fuel (gasoline) from lignocellulosic biomass is a process that is vigorously being pursued by many researchers world over because of its environmental friendliness and renewability. In comparison with gasoline fuel, bioethanol is more beneficial because of its very high octane number, wider flammability limits, better heat of vaporization, and most importantly, the lesser release of acid gases such as carbon(iv)
oxide and Sulphur (iv) oxide.

Aside from its use as fuel, bioethanol obtained from lignocellulosic biomass can be used for other important purposes in the food industry such as for food preservation.

At present globally, the use of combined lignocellulosic biomass feedstock for bioethanol production is not being adequately harnessed. There is a dearth of information globally on the performance of hybridized (mixed) feedstocks in bioethanol production, however, the little information that could be gathered from previous researches shows that the use of combined feedstock can influence massive savings in the production process when compared to the use of single feedstocks as it ensures availability of enough feedstocks for bioethanol production. Also, the information available shows that the use of mixed feedstock in bioethanol production gives a higher yield of bioethanol than the single feedstocks.

The modeling and optimization of the bioethanol production process help in the improvement of the process design and operation of process units for improved production of bioethanol from some of the available lignocellulosic biomass available in Nigeria. The importance of bioethanol in the emerging renewable energy development in Nigeria makes the optimization of its production process a worthy venture to provide information that serves as a reference for future researchers and industrialists in this field of study. Numerous designs of experiments (DOEs) notably Box–Behnken design (BBD), central composite design (CCD), and face-centered composite design (F CCD) can be used for research purposes.

Response surface methodology (RSM) is a compendium comprising of mathematical and statistical methods used for optimizing processes involving many process variables to give desirable responses and the major goal is to optimize the responses obtained. RSM expresses the impact of the identified process variables singly and/or their collaborative effects on the response of the process. From a few experiments, RSM develops a huge quantity of information that describes the characteristics of the system and develops a second-order polynomial projecting model that correlates the response of the process to the independent process variables.

Box–Behnken design (BBD) is a set of rotatable or almost rotatable second-order models centered on 3-level partial factorial models. The BBD is an effective design for response surface methodology as it evaluates factors of quadratic model, develops successive models, utilizes blocks, and identifies lack of fit of a model.

RSM comes with the advantages of requiring a much lower number of experimental runs required to assess numerous parameters (independent variables) and their relationships, also, the process can be easily modeled mathematically and is time and cost-saving. Other design methods such as artificial Neural network (ANN) has a major disadvantage of requiring numerous data points for the analysis.

Timung et al. worked on the comparative study of the optimization of dilute acid and hot water pretreatment of different lignocellulosic biomass. The major focus of their work involved the comparison of the total reducing sugars (TRS) after the acid and hot water pretreatment on the different lignocellulosic biomass and the results obtained showed that the maximum production of TRS was noticed at run order 8 in both the acid and hot water pretreatment for sugar cane bagasse biomass.

Chen et al. worked on understanding alkaline pretreatment parameters for corn stover enzymatic saccharification. The total number of experiments required to investigate the correlation between pretreatment parameters and compositional change of pretreated corn stover, as well as its glucan/xylan transformation was investigated using the central composite design to reduce the total number of experiments. The result of their work showed that the sodium hydroxide loading is the most prevailing variable for enzymatic digestibility.

The objective of this work is to optimize the production of bioethanol from hybridized i.e a mixture of cassava peels plus yam peels and corn cobs plus rice husks biomass using the Box–Behnken design (BBD) of the response surface methodology (RSM). Hybridization of the biomass feedstock is expected to impart some unique behaviors to the process which ultimately improves the bioethanol yield and purity of the produced bioethanol. Furthermore, there is little information about the potentials of yam peels biomass for bioethanol production in the literature. The hydridized feedstocks are more representative of commercial practice, as it would be difficult to operate at scale with single biomass sources. This is the particularly novel component of this study. The BBD was used to generate the experimental runs to acquire data which were then used for the modeling and optimization of the pretreatment and fermentation parameters namely; time, pH, and particle size.

The success of this investigation will help to determine the best approach for bioethanol production from hybridized (mixed) biomass of different particle sizes.

2. Experimental Section

2.1 Materials and biomass preparation and pretreatment

Materials and Biomass provision. The rice husks were obtained from a local rice mill in Ekperi, Etsako Central Local government Area of Edo State Nigeria, the corn cobs were obtained from Ogume, Ndokwa West Local Government Area of Delta State Nigeria, the cassava peels, yam peels, and sugar cane bagasse were all sourced from
Effurun, Uvwie Local Government Area of Delta State Nigeria. The biomass was afterward sundried for about seven days and then grounded in a mill after which they were sieved into two particle sizes of 300 and 425 microns respectively. Analytical grade chemical reagents such as sodium hydroxide pellets, hydrogen peroxide, and tetraoxosulphate (VI) acid were used. To study the effects of biomass combinations, 750 g each of 300 microns particle size cassava and yam peels biomass and corn cobs and rice husks biomass was measured and transferred into different vessels. The pretreatment and characterization of the biomass have been discussed in our previous work. Approximately 10 liters of each of the pretreated biomass were obtained and made up to 15 liters by the addition of water and transferred to the fermentation vessels.

2.2 Fermentation
Industrial grade S. cerevisiae Y-461159 yeast produced by Nike Chemical India was used for the fermentation of the pretreated hybridized biomass samples. The procedure used by Pratto et al. was followed in the activation of the S. cerevisiae yeast. The process was activated in an Erlenmeyer flask. 10 g/L yeast extract, 20 g/L peptones, and 40 g/L glucose were thoroughly mixed in an incubator at a temperature of 34°C for four hours before about 3 g/L dry cell weight each was introduced in the pretreated hybridized biomass after the pretreated samples were adjusted for pH in the range of 5.7 to 6.0. They were then left to ferment for 5 and 7 days at ambient temperature before being harvested for distillation.

2.3 Distillation of produced bioethanol
The fermented supernatants were harvested on the 5th and 7th day and made to undergo distillation in a UOP3CC continuous distillation column supplied by Armfield. The UOP3CC is equipped with a matching computer, fully installed with Armfield software, to supply output information received from its sensors. The distillate received from the UOP3CC continuous distillation column was measured with a measuring cylinder and recorded.

2.4 Optimization process using response surface methodology (RSM)
The use of the conventional approach to determine the optimum levels of all the process parameters comes with some disadvantages such as; waste of time, more experimental runs which may not be reliable and makes the total process cost unnecessarily over-bloated. All these shortcomings can be mitigated by the optimization of all the process conditions using RSM, which is an example of the statistical experimental model. This approach is most notably an expense of time and resources as well as more reliability. The Design-Expert 8.0.3.1 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for the design, modeling, and optimization studies. Three different parameters were chosen as independent variables: pH, time, and Particle Size, and assigned as $X_1$, $X_2$, and $X_3$, respectively in Table 1. The low, middle, and high levels of each variable were coded as −1, 0, and +1, respectively. Box-Behnken was employed for the design of the experiment for the optimization studies involving Cassava peels plus yam peels biomass. In this study, Box-Behnken design (BBD), a class of rotatable or nearly rotatable second-order design based on three-level incomplete factorial designs, was selected for the experimental design. Box-Behnken can be applied as an effective method to develop the second-order response models. The Box-Behnken consists of three sections including the full or fractional factorial design points (where the factor levels were coded to the upper level to +1 and the lower level to −1 values), axial points (sometimes called “star” points), and the center point. A three-level-three-factor design was applied, which generated 17 experimental runs for bioethanol production as shown in Table 2. This included 12 factorial points and 5 central points to supply data involving the core of the experimental region. Response surface methodology (RSM) was used to optimize the bioethanol production process from the hybridized feedstocks and regression equation analysis was used to assess the response surface model. To relate the response variable to the independent factors, numerous regressions were used to match the coefficient of the polynomial model of the response. The property of the fit of the model was assessed using the test of significance and analysis of variance (ANOVA). The generalized response surface model for describing the variation in the response

| Variable | Unit | Symbols | Coded factors |
|----------|------|---------|---------------|
| pH       | X1   |         | -1 0 +1       |
| Time     | hr   | X2      | 5 7.5 10      |
| Particle Size | Microns | X3 | 120 144 168 |
|          |      |         | 300 362.5 425 |

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The response variable, $b_0$ is the intercept value, $b_i (i = 1, 2... k)$ is the first-order model coefficient, $b_{ij}$ is the interaction effect, and $b_{ii}$ represents the quadratic coefficients of $X_i, X_i$ and $X_i$ are the input variables that influence the response variable and $e$ represents the random error.

2.5 Analysis of the produced bioethanol

The produced bioethanol samples were then taken to Lighthouse Petroleum Engineering Company Limited, Effurun GRA, Delta State, Nigeria for characterization for physical and fuel properties using the test methods as shown in Table 3.

3 Results and Discussion

3.1 Regression model and statistical analysis for the produced bioethanol from the hybridized feedstocks

Tables 4a and 4b depict the data for actual, predicted and residual values gotten and the parity curve is shown in Fig. 1. Tables 5a and 5b show the results of BBD analysis of variance (ANOVA) for the response surface quadratic model. The outcomes revealed that the $p$-values of $X_1, X_2,$ and $X_1^2$ of the model terms were significant (i.e. $p < 0.05$) while others are non-significant for the two hybridized feedstocks. The high Fisher test F-value of 11.98 and 22.80 as shown in the analysis of Variance (ANOVA) for Regression Equation of Tables 6a and 6b for cassava plus yam peels and rice husks plus corn cobs respectively implies the model is significant which is also reflected in $X_1, X_2,$

| Std Run | $X_1$ (hr) | $X_2$ (microns) |
|---------|------------|-----------------|
| 1       | -1 (5)     | 0 (362.5)       |
| 2       | 1 (5)      | 0 (362.5)       |
| 3       | -1 (5)     | 1 (168)         |
| 4       | 1 (10)     | 1 (168)         |
| 5       | -1 (5)     | 0 (144)         |
| 6       | 1 (10)     | 0 (144)         |
| 7       | -1 (5)     | 0 (144)         |
| 8       | 1 (10)     | 0 (144)         |
| 9       | 0 (7.5)    | -1 (120)        |
| 10      | 0 (7.5)    | 1 (168)         |
| 11      | 0 (7.5)    | -1 (120)        |
| 12      | 0 (7.5)    | 1 (168)         |
| 13      | 0 (7.5)    | 0 (144)         |
| 14      | 0 (7.5)    | 0 (144)         |
| 15      | 0 (7.5)    | 0 (144)         |
| 16      | 0 (7.5)    | 0 (144)         |
| 17      | 0 (7.5)    | 0 (144)         |

\[ Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + e \] (2)

Where $Y$ is the response variable, $b_0$ is the intercept value, $b_i (i = 1, 2... k)$ is the first-order model coefficient, $b_{ij}$ is the interaction effect, and $b_{ii}$ represents the quadratic coefficients of $X_i, X_i$ and $X_i$ are the input variables that influence the response variable and $e$ represents the random error.

Table 2 Box Behnken for three independent factors for bioethanol production showing coded and actual value (in bracket).

Table 3 Test methods used for characterization of the produced bioethanol.
Table 4a  Experimental data for observed yield, predicted yield, and residual values for bioethanol production from cassava peels plus yam peels.

| Std | Observed Volume (mL/1500 g biomass) | Predicted Volume (mL/1500 g biomass) | Residual Values |
|-----|-------------------------------------|--------------------------------------|-----------------|
| 1   | 125.00                              | 115.75                               | 9.25            |
| 2   | 0.000                               | 12.75                                | -12.75          |
| 3   | 86.00                               | 73.25                                | 12.75           |
| 4   | 30.00                               | 39.25                                | -9.25           |
| 5   | 60.00                               | 76.00                                | -16.00          |
| 6   | 23.00                               | 17.00                                | 6.00            |
| 7   | 86.00                               | 92.00                                | -6.00           |
| 8   | 30.00                               | 14.00                                | 16.00           |
| 9   | 24.00                               | 17.25                                | 6.75            |
| 10  | 32.00                               | 28.75                                | 3.25            |
| 11  | 40.00                               | 43.25                                | -3.25           |
| 12  | 9.00                                | 15.75                                | -6.75           |
| 13  | 9.00                                | 9.00                                 | 0.000           |
| 14  | 9.00                                | 9.00                                 | 0.000           |
| 15  | 9.00                                | 9.00                                 | 0.000           |
| 16  | 9.00                                | 9.00                                 | 0.000           |
| 17  | 9.00                                | 9.00                                 | 0.000           |

Table 4b  Experimental data for observed yield, predicted yield, and residual values for bioethanol production from rice husks plus corn cobs biomass.

| Std | Observed volume (mL/1500 g biomass) | Predicted volume (mL/1500 g biomass) | Residual value |
|-----|-------------------------------------|--------------------------------------|----------------|
| 1   | 160.00                              | 150.00                               | 10.00          |
| 2   | 100.00                              | 105.00                               | -5.00          |
| 3   | 56.00                               | 51.00                                | 5.00           |
| 4   | 32.00                               | 42.00                                | -10.00         |
| 5   | 52.00                               | 51.00                                | 1.00           |
| 6   | 52.00                               | 36.00                                | 16.00          |
| 7   | 56.00                               | 72.00                                | -16.00         |
| 8   | 32.00                               | 33.00                                | -1.00          |
| 9   | 76.00                               | 87.00                                | -11.00         |
| 10  | 68.00                               | 74.00                                | -6.00          |
| 11  | 170.00                              | 164.00                               | 6.00           |
| 12  | 26.00                               | 15.00                                | 11.00          |
| 13  | 26.00                               | 26.00                                | 0.000          |
| 14  | 26.00                               | 26.00                                | 0.000          |
| 15  | 26.00                               | 26.00                                | 0.000          |
| 16  | 26.00                               | 26.00                                | 0.000          |
| 17  | 26.00                               | 26.00                                | 0.000          |

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A similar result (F-value of 11.08) was obtained by Maurya et al. in their work on bioethanol production sugar-cane bagasse. F-value is a statistically acceptable degree of how perfectly the factors depict the variation in the data about its mean. The data obtained for both feedstocks fitted best to a linear and quadratic mathematical model and exhibited a low standard deviation. The values of the coefficient of determination ($R^2$) in both cases indicate uniformity between the experimental values and the predicted values. Guan and Yao recommended that $R^2$ should be at least 0.80 for a reliable fit of a model. In this case, $R^2$ of the models obtained were 0.9390 and 0.9670 for the cassava peels plus yam peels biomass and rice husks plus corn cobs biomass respectively, which indicated that the sample vari-

Table 5a  Analysis of variance (ANOVA) for response surface quadratic model (Cassava peels plus yam peels).

| Source      | Sum of Squares | Df | Mean Square | F-value | p-value |
|-------------|----------------|----|-------------|---------|---------|
| $X_1$       | 9384.50        | 1  | 9384.50     | 55.09   | 0.0001  |
| $X_2$       | 128.00         | 1  | 128.00      | 0.75    | 0.4148  |
| $X_3$       | 84.50          | 1  | 84.50       | 0.50    | 0.5040  |
| $X_1X_2$    | 1190.25        | 1  | 1190.25     | 6.99    | 0.0333  |
| $X_1X_3$    | 90.25          | 1  | 90.25       | 0.53    | 0.4903  |
| $X_2X_3$    | 380.25         | 1  | 380.25      | 2.33    | 0.1788  |
| $X_1^2$     | 5881.64        | 1  | 5881.64     | 34.53   | 0.0006  |
| $X_2^2$     | 810.59         | 1  | 810.59      | 4.76    | 0.0655  |
| $X_3^2$     | 49.96          | 1  | 49.96       | 0.28    | 0.6121  |

Table 5b  Analysis of variance (ANOVA) for response surface quadratic model (Rice husks plus corn cobs).

| Source      | Sum of Squares | Df | Mean Square | F-value | p-value |
|-------------|----------------|----|-------------|---------|---------|
| $X_1$       | 1458.00        | 1  | 1458.00     | 9.47    | 0.0179  |
| $X_2$       | 13122.00       | 1  | 13122.00    | 85.21   | <0.0001 |
| $X_3$       | 162.00         | 1  | 162.00      | 1.05    | 0.3392  |
| $X_1X_2$    | 324.00         | 1  | 324.00      | 2.10    | 0.1902  |
| $X_1X_3$    | 144.00         | 1  | 144.00      | 0.94    | 0.3658  |
| $X_2X_3$    | 4624.00        | 1  | 4624.00     | 30.03   | 0.0009  |
| $X_1^2$     | 606.32         | 1  | 606.32      | 3.94    | 0.0876  |
| $X_2^2$     | 10109.47       | 1  | 10109.47    | 65.65   | <0.0001 |
| $X_3^2$     | 421.05         | 1  | 421.05      | 2.73    | 0.1422  |

and $X_1^2$. A similar result (F-value of 11.08) was obtained by Maurya et al. in their work on bioethanol production sugar-cane bagasse. F-value is a statistically acceptable degree of how perfectly the factors depict the variation in the data about its mean. The data obtained for both feedstocks fitted best to a linear and quadratic mathematical model and exhibited a low standard deviation. The values of the coefficient of determination ($R^2$) in both cases indicate uniformity between the experimental values and the predicted values. Guan and Yao recommended that $R^2$ should be at least 0.80 for a reliable fit of a model. In this case, $R^2$ of the models obtained were 0.9390 and 0.9670 for the cassava peels plus yam peels biomass and rice husks plus corn cobs biomass respectively, which indicated that the sample vari-
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Table 6a Analysis of variance (ANOVA) for regression equation (cassava peels plus yam peels biomass).

| Source      | Sum of Squares | Df | Mean Square | F-value | p-value |
|-------------|----------------|----|-------------|---------|---------|
| Model       | 18363.03       | 9  | 2040.34     | 11.98   | 0.0018  |
| Pure Error  | 0.0000         | 4  | 0.0000      |         |         |
| Cor Total   | 19555.53       | 16 |             |         |         |

R² = 0.9390, Adj.R² = 0.8606, Adequate Precision = 10.664

Table 6b Analysis of variance (ANOVA) for regression equation (rice husks plus corn cobs).

| Source      | Sum of Squares | Df | Mean Square | F-value | p-value |
|-------------|----------------|----|-------------|---------|---------|
| Model       | 31600.12       | 9  | 3511.12     | 22.80   | 0.0002  |
| Pure Error  | 0.0000         | 4  | 0.0000      |         |         |
| Cor Total   | 32678.12       | 16 |             |         |         |

R-Squared = 0.9670, Adj R² = 0.9246, Adequate Precision = 15.655

atation of 93.90% and 96.70% for bioethanol volume was attributed to the independent variables. The result of R² obtained in this study conforms with that obtained by Chen et al. in their work on corn and rice straw which was approximately 93%. This observation implies that the models have proven fit for the suitable representation of the definite relationship among the selected factors as corroborated by Jeya et al. Similarly, the adjusted determination of coefficient, R²_adj of 0.8606 and 0.9246 proves that the model has a high significance (Tables 6a and 6b). A large difference between R² and R²_adj indicates that non-significant terms are involved in the model. Adequate precision evaluates the signal to noise ratio, a ratio greater than 4 is desired. Hence, the adequate precision of 10.664 and 15.655 indicates an adequate signal, and these models can be used to navigate the design space. Therefore, the quadratic mathematical models obtained in this study could be used in the theoretical prediction of bioethanol production from a liquid extract containing a mixture of cassava plus yam peels biomass as well as rice husks plus corn cobs biomass. The final equation in terms of coded factors for the Box-Behnken design response surface quadratic model for cassava peels plus yam peels biomass can be expressed as:

\[ Y = 9.00 - 34.25X_1 - 4.00X_2 + 3.25X_3 + 17.25X_1X_2 - 4.75X_1X_3 - 9.75X_2X_3 + 37.38X_1^2 + 13.87X_2^2 + 3.3X_3^2 \]  

Where \( Y \) is the bioethanol volume yield, \( X_1 \) is pH, \( X_2 \) is time and \( X_3 \) is particle size. The low standard error observed in the intercept and all the model terms in Tables 6a and 6b prove that the regression model suits the data favorably and the prediction is okay. The Variance Inflation Factor (VIF) gotten in this work indicated that the center points are orthogonal to all other factors in the models.

3.2 Effects of process parameters on bioethanol production

The three-dimensional response surface plots were obtained by plotting the response (bioethanol yield) on the z-axis against two variables on the x- and y-axis.

3.2.1 Interactive effect of time and pH on hybridized biomass

The response surface plot of the interaction of time and pH on bioethanol production is presented in Fig. 2. For the acid pretreated biomass mixtures, the maximum bioethanol of 125 mL/1500 g of hybridized cassava plus yam peels biomass was obtained at a time of 120 hours and a pH of 5.0 while that of rice husks plus corn cobs biomass was 160 mL/1500 g at a time of 120 hours and a pH of 5.0. The plots show that increasing the time of fermentation with a corresponding increase in the pH above 6.0 in an acidic pretreatment condition is unfavorable to bioethanol production from the hybridized mixture of yam peels plus cassava peels as well as rice husks plus corn cobs biomass. Besides, in a basic pretreatment condition, for hybridized cassava peels plus yam peels biomass, maximum bioethanol yield is 30 mL/1500 g biomass at a pH of 10.0 and a time of 168 hours while that of rice husks plus corn cobs biomass was 52 mL/1500 g biomass at a pH of 10.0 and a time of 144 hours. However, in a hot water hydrolysis condition near-neutral pH, maximum bioethanol yield is 40 mL/1500 g...
biomass at a pH of 7.5 and a time of 120 hours for cassava peels plus yam peel biomass, while that of rice husks plus corn cobs biomass was 170 mL/1500 g biomass mixture at a pH of 7.5 and time of 120 hours. This indicates that maximum bioethanol production for cassava peels plus yam peels biomass is dependent on the time and pH, which should be between 120 hours and 136 hours and 5.0 and 6.0 for time and pH respectively, while for rice husks plus corn cobs biomass, there is greater flexibility in the optimum pH for bioethanol production from the hybridized biomass, ranging between 5.0 and 7.5.

3.2.2 Interactive effect of particle size and pH on bioethanol production from hybridized biomass

The response surface plot of the interaction of particle size and pH on bioethanol production is presented in Fig. 3. The maximum bioethanol yield of 125 mL/1500 g from hybridized cassava plus yam peels biomass was obtained at a particle size of 362.50 microns and a pH of 5.0, while that of rice husks plus corn cobs biomass was 160 mL/1500 g. For both hybridized biomass samples, the plots show that increasing the particle size during pretreatment with a corresponding increase in the pH above 6.0 in an acidic pretreatment is unfavorable to optimum bioethanol yield. Besides, in a basic pretreatment condition maximum bioethanol yield is 30 mL/1500 g cassava plus yam peels biomass at a pH of 10.0 and a particle size of 362.50 microns whereas rice husks plus corn cobs hybridized biomass gave a bioethanol yield of 68 mL/1500 g biomass. However, in a hot water hydrolysis condition near neutral pH for cassava peels plus yam peel biomass, maximum bioethanol yield is 40 mL/1500 g biomass at a pH of 7.5 and a particle size of 425 microns whereas the rice husks plus corn cobs biomass gave a maximum yield of 170 mL/1500 g biomass. It can therefore be deduced that increasing the particle size with a decrease in the pH will result in optimum bioethanol yield for both hybridized biomass.

| Table 7a | Regression coefficients and significant of response surface quadratic model for cassava peels plus yam peels. |
| --- | --- |
| Factor | Coefficient Estimate | Df | Standard Error | 95% CI Low | 95% CI High | VIF |
| Intercept | 9.00 | 1 | 5.84 | -4.80 | 22.80 | - |
| $X_1$ | -34.25 | 1 | 4.61 | -45.16 | -23.34 | 1.00 |
| $X_2$ | -4.00 | 1 | 4.61 | -14.91 | 6.91 | 1.00 |
| $X_3$ | 3.25 | 1 | 4.61 | -7.66 | 14.16 | 1.00 |
| $X_1X_2$ | 17.25 | 1 | 6.53 | 1.82 | 32.68 | 1.00 |
| $X_1X_3$ | -4.75 | 1 | 6.53 | -20.18 | 10.68 | 1.00 |
| $X_2X_3$ | -9.75 | 1 | 6.53 | -25.18 | 5.68 | 1.00 |
| $X_1^2$ | 37.38 | 1 | 6.36 | 22.33 | 52.42 | 1.01 |
| $X_2^2$ | 13.87 | 1 | 6.36 | -1.17 | 28.92 | 1.01 |
| $X_3^2$ | 3.37 | 1 | 6.36 | -11.67 | 18.42 | 1.01 |

| Table 7b | Regression coefficients and significant of response surface quadratic model for rice husks plus corn cobs. |
| --- | --- |
| Factor | Coefficient Estimate | Df | Standard Error | 95% CI Low | 95% CI High | VIF |
| Intercept | 26.00 | 1 | 5.55 | 12.88 | 39.12 | - |
| $X_1$ | -13.50 | 1 | 4.39 | -23.87 | -3.13 | 1.00 |
| $X_2$ | -40.50 | 1 | 4.39 | -50.87 | -30.13 | 1.00 |
| $X_3$ | 4.50 | 1 | 4.39 | -5.87 | 14.87 | 1.00 |
| $X_1X_2$ | 9.00 | 1 | 6.20 | -5.67 | 23.67 | 1.00 |
| $X_1X_3$ | -6.00 | 1 | 6.20 | -20.67 | 8.67 | 1.00 |
| $X_2X_3$ | -34.00 | 1 | 6.20 | -48.67 | -19.33 | 1.00 |
| $X_1^2$ | 12.00 | 1 | 6.05 | -2.30 | 26.30 | 1.01 |
| $X_2^2$ | 49.00 | 1 | 6.05 | 34.70 | 63.30 | 1.01 |
| $X_3^2$ | 10.00 | 1 | 6.05 | -4.30 | 24.30 | 1.01 |
Singh et al.\textsuperscript{39} in their review on delignification of lignocellulosic biomass to enhance their capability for bioethanol production showed that a good yield of bioethanol (0.47 g/g glucose) was attained from cashew apple bagasse pretreated with dilute H\textsubscript{2}SO\textsubscript{4} at a temperature of 121.1°C for 15 minutes.

The result from this study indicates that maximum bioethanol formation is also a function of the particle size and pH, which should be between 362.50 micros and 425 micros and 5.0 and 7.5 for particle sizes and pH respectively. Therefore, maximum bioethanol yield is achieved in an acid pretreated condition than basic and hot water pretreatment conditions.

3.2.3 Interactive effect of particle size and time on bioethanol yield from hybridized feedstocks

The response surface plot of the interaction of particle size and pH on bioethanol production.

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size and time on bioethanol production is presented in Fig. 4. Bioethanol yield of 40 mL/1500 g biomass was obtained at the highest particle size of 425 microns and at a time of 120 hours for cassava plus yam peels biomass while for that of rice husks plus corn cobs was 170 mL/1500 g biomass. The plot shows that maintaining a range of particle sizes between 350 microns and 375 microns with a corresponding increase in fermentation time results in a decrease in bioethanol yield which is unfavorable. However, increasing the particle sizes beyond the stipulated range or a decrease beyond the stated range with a corresponding decrease in fermentation time results in an increase in bioethanol yield. This indicates that maximum bioethanol formation is dependent on the particle size and time especially for the rice husks and corn cobs hybridized biomass which has been corroborated by Ojewumi et al. in their work on the bio-
conversion of sweet potato peel waste to bioethanol with the aid of *Saccharomyces cerevisiae*. The result from the work shows that optimum bioethanol yield is dependent on time. Longer fermentation time results in a decline in the yield of bioethanol as the yeast cells get used up as fermentation progresses.

### 3.3 Characterization of the produced bioethanol

Table 8 shows the fuel properties of bioethanol produced via different pretreatment processes from the hybridized mixture of cassava peels plus yam peels biomass as well as corn cobs plus rice husks biomass. The values obtained for different parameters analyzed in this study compare favorably to the normal ethanol and gasoline values in the literature as shown in Table 9.

### 4 Conclusions

The optimal values of the independent factors selected for the fermentation process of the hybridized biomass using RSM were obtained by analyzing the regression equations from the RSM technique. For the rice husks plus corn cobs biomass, the optimal condition was statistically predicted as pH 7.5, fermentation time of 120 hours, and particle size of 425 microns. The predicted bioethanol yield under the optimal condition was 164 mL/1500 g biomass. To verify the prediction of the model, the optimal condition
values were applied to three independent replicates and the highest bioethanol yield obtained was 170 mL/1500 g biomass, which was well within the estimated value of the model equation.

For cassava peel plus yam peel hybridized biomass, the optimal condition was statistically predicted as pH 5.00, fermentation time of 120.00 hours, and particle size of 362.50 microns. The predicted bioethanol yield under the optimal condition was 115.75 mL/1500 g biomass. To verify the prediction of the model, the optimal condition values were applied to three independent replicates and the average bioethanol volume obtained was 125.00 mL/1500 g biomass, which was well within the estimated value of the model equation.

The results of this research showed that RSM with appropriate experimental design can be effectively applied to...
the optimization of the process variables in bioethanol production using biomass mixture of corn cob plus rice husk as well as cassava peels plus yam peels as feedstocks for the process. This may provide useful information regarding the development of an economic and efficient fermentation process as well as information on the behavior of hybridized feedstocks for industrial bioethanol production.

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Author Contribution

A.A.A and D.L conceptualized the research, A.A.A carried out the experimental work and developed the manuscript, D.L corrected and updated the manuscript.

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