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Bioconversion of Locally Made Cassava Wastewater for Bio-hydrogen Production and Its Statistical Analysis: A Case of Response Surface Methodology (RSM) and Artificial Neural Network (ANN)

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Abstract: This study is centered on the production of bio-hydrogen from cassava wastewater and its statistical analysis. Analysis of the sample was carried out by determining the physiochemical properties of the cassava wastewater for its suitability for industrial application. A broth medium was prepared, substrate preparation and inoculum pretreatment were carried out and the medium was cultivated following standard method. To optimize the process condition, response surface methodology (RSM) and artificial neural network (ANN) were engaged. An experimental design was carried out using RSM, three variable factors such as fermentation time (X_1) (days), pH effect (X_2) and substrate concentration (X_3) (mg/L) were considered and 17 experimental runs were created. Results showed the physicochemical properties of wastewater had an initial pH of 5.58 (low acidity), total diffuse solid (TDS) of 3.93 mg/l, chemical oxygen demand (COD) of 0.25 mg/l and biochemical oxygen demand (BOD) of 0.16 mg/l. The statistical analysis by RSM predicted bio-hydrogen yield (HY) of 4.011 ml at X_1 = -1, X_2 = -1 and X_3 = -0.043 variable conditions, this was validated in triplicate experiments, and the average HY was 3.98 ml. Similarly, ANN statistical software predicted HY of 4.221 ml at X_1 = - 1, X_2 = -1 and X_3 = -0.032 variable conditions, this was also validated in triplicate experiments, and the average HY was 4.002 ml. The coefficient of determination (R^2) and R-Sq. (adj.) for RSM (99.98% and 99.96%) and ANN (99.993% and 99.986%) indicate that the model fitted well for the acceptable representation of the relationship among the variables under consideration. The results of this experiment established that the use of both RSM and ANN with appropriate experimental design can give the optimum yield of bio-hydrogen, even though, ANN predict better than RSM in terms of yield of bio-hydrogen.

Keywords: Optimization, Response Surface Methodology, Artificial Neural Network, Physicochemical Properties, Bio-hydrogen Production, Coefficient of Determination

1. Introduction

The bioconversion of agricultural waste products to bio-hydrogen has been brought to limelight by several researchers and this is as a result of the importance of hydrogen as an energy carrier which is likely to phase out the long standing problem of greenhouse gas emission and the depleting of non-renewable energies [1]. Agricultural wastes which are often discarded, have been reported by various researchers to be a good source of bio-hydrogen because it consists of a large percentage of biodegradable solids that are volatile [2-4], often times, these wastes are not solely converted to bio-hydrogen, buffers and minerals are added to give an optimum yield [5]. Meanwhile, the demand for hydrogen is not streamlined to energy utilization, it also serves as a feedstock for the hydrogenation of fat and oils in food industry, production of chemicals and electronic devices, processing steel, desulfurization and re-formulation of gasoline in refineries [6], therefore a large quantity of hydrogen is needed for sustaining these industries and energy reserves, this has made researchers to delve into the search for various methods of hydrogen production.

Until recently, the major methods of production of hydrogen were the auto-thermal processes, electrolysis of water and steam reforming of hydrocarbons [7], these methods are now slowly being phased out and replaced with methods of productions from agricultural and industrial products or wastes [8] and the best method for this production is the dark fermentation method because it combines hydrogen generation with waste treatment thereby making it environmentally friendly [9]. Several research works have been carried out on the production of bio-hydrogen from agricultural and industrial wastes and they include; bio-hydrogen production by anaerobic fermentation of food waste [10], effect of food to microorganism ratio on bio-hydrogen production from food waste via anaerobic fermentation [11], bio-hydrogen production from molasses by anaerobic fermentation with a pilot scale bioreactor system [12], acidophilic bio-hydrogen production from rice slurry [13], bio-hydrogen production from starch in wastewater under thermophilic condition [14], bioaugmented hydrogen production from microcrystalline cellulose using co-culture– *Clostridium acetobutyricum* X9 and *Ethanoligenens harbinense* B49 [15], microbial hydrogen production from sweet potato starch residue [16], improvement of bio-hydrogen production and treatment efficiency on palm oil mill effluent with nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor [17], to mention but a few and it is important to know the optimum conditions in which bio-hydrogen can be produced from different waste and this can be achieved from different software's.

Response surface methodology is a statistical method used in building models, designing experiments and analyzing the effect of process variables/parameters to responses and these responses are fitted to quadratic functions [18]. This method consists of various designs such as Box Behnken, Central Composite and Plackett [19-21]. Artificial Neural Network on the other hand, is a biological system that is based on computational techniques that simulates neurological processing ability that can effectively relate or quantify non-linear relationship between variables and responses [22-23]. Recently, researchers [19, 22, 24-26] have taken the plunge and are using various software's in the modelling and optimization of production, fermentation and other processes and this is as a result of the drawback in using single software and the prevalence of one software over the other [27].

Hence, this paper explicitly explores and compares the capabilities of Response Surface Methodology (RSM) and Artificial Neural Network (ANN) in the production of bio hydrogen synthesized from agro waste.

2. Material and Methods

2.1. Broth Medium

The broth medium was prepared by dissolving 1.1 g of the broth powder in a litre of distilled water, and it was thoroughly mixed and sterilized in an autoclave at 120 ◦C for 120 min. This medium was kept in a refrigerator for a day after which the cultured *Clostridium coliforme* was added to the sample to form inoculum. The inoculum was pretreated by placing in an incubator for 24 h without shaking.

2.2. Substrate Preparation

The substrate used for this work was cassava wastewater and this was obtained from a cassava processing plant in Ikot Akpaden, Mkpat Enin L. G. A., Akwa-Ibom State, Nigeria. The testing sample was formed by mixing 7 ml of cassava wastewater and 8 ml of distilled water in a test tube and this was used in determining the compositions. A blank (distilled water) was used in calibration before the sample was tested.

2.3. Inoculum Pretreatment and Cultivation Medium

Clostridium coliforme was used in forming an inoculum in this work because it is known to be a high forming bacteria. Heat-shock treatment was used in pre-treating the broth medium where already cultured microorganisms were added to the nutrient broths to form the inoculum, this was autoclaved for 20 min at 121 ^{o}C and the pH of the inoculum was adjusted using acid pre-treatment. The aim of this pretreatment was to allow the growth of the hydrogen forming bacteria and inhibit the growth of methanogenic bacteria in the inoculum. A turbid solution was gotten after incubating the inoculum for 24 h at 37 ^{o}C which showed the growth of the microorganisms.

2.4. Physicochemical Properties

The physicochemical properties of cassava waste water such as total alkalinity, ammonia nitrogen, total phosphate, total solids, aluminum, potassium, copper, iron, magnesium, calcium, zinc, COD (Chemical Oxygen Demand) were analysed using the ELE International Photometer while the analysis on organic carbon, total kjedahl nitrogen, ash content, conductivity test were carried out using Walkley-black titration method, Kjeltex auto-distillation apparatus model, Muffle furnace, Electrochemical analyser (Consort C6020), respectively. Meanwhile, volatile solids content was calculated from the ash content value.

2.5. Experimental Design

The experiment was carried out under an anaerobic condition and a dark fermentation process. The Response Surface Methodology and Artificial Neural Network (ANN) was used in the experimental design and modelling. For the RSM, Box-Behnken three-level-three-factors (for three variable) design was applied and 17 experimental runs were generated. The variables considered were fermentation time (X_1) (days), pH effect (X_2) and substrate concentration (X_3) (mg/L). The quadratic equation derived from anova analysis was used in determining the optimum conditions of hydrogen yield. The same design used in RSM was also used in optimizing and obtaining a set of experimental data for ANN with force approach and the results were compared.

$$\gamma = \beta_0 +. \sum_{i-1}^{k} \beta_i X_i + \sum_{i-1}^{k} \beta_i X_i^2 + \sum_{i-1}^{k} \beta_{ii} X_j^2 + \sum_{i-1}^{k} \beta_{iii} X_k^2 + \sum_{i-1}^{k} \sum_{j-1}^{k} \beta_{ij} X_i X_j + \in \quad (1)$$

3. Results and Discussion

3.1. Physicochemical Properties of Cassava Wastewater

Table 1 shows the results of physicochemical properties of cassava wastewater used for this experiment. Observation from the table showed the wastewater had an initial pH of 5.58 (low acidity), total diffuse solid (TDS) of 3.93 mg/l, chemical oxygen demand (COD) of 0.25 mg/l and biochemical oxygen demand (BOD) of 0.16 mg/l.

Table 1. *Physicochemical properties of cassava wastewater.*

S/N	Parameters	Value
1	Temperature (°C)	24. 3
2	pH	5.58
3	Total diffused solids (TDS) mg/l	3.93
4	Total hardness (mg/l)	185
5	Conductivity (ms/cm)	0.786
6	COD (mg/l)	0.25
7	BOD (mg/l)	0.16
8	Dissolved oxygen (mg/l)	1.44
9	Alkalinity (mg/l) $CaCO_3$	310
10	Ammonium (mg/l)	0.47
11	Nitrate (mg/l)	2.08
12	Relation (ammonia/nitrogen) (mg/l)	0.36
13	Phosphate (mg/l) (PO_4)	101.1
14	Potassium (mg/l)	4.1
15	Calcium (mg/l)	38
16	Magnesium (mg/l)	50
17	Sulfate (mg/l)	73
18	Iron (mg/l)	4.40
19	Zinc (mg/l)	19.5
20	Nickel (mg/l)	13.4
21	Chlorine (mg/l)	2.02
22	Aluminum (mg/l)	0.46
23	Fluoride (mg/l)	1.45
24	Nitrite (mg/l)	0.54

3.2. Optimization of Bio-hydrogen Production

Shown in Table 2 and 3 is the coded independent variables factor, the predicted values and the residual values by response surface methodology and artificial neural network, respectively. Design Expert 10.0.3.1 and NeuralPower 21356 software's were employed to determine the coefficients of independent variables in a full regression model equation. For ANN, the input layers were three (3), with four (4) hidden layers. For the optimization, force approach was used, Table 4 showed the results of test of significance for all regression coefficient. Observation from the results showed that the X_2, X_3, X_1^2, X_2^2 and X_3^2 were remarkable with $p<0.0001$ at 95% confidence level. However, X_1, X_1X_2 and X_2X_3 were also significant since the $p<0.05$, with an exception of the interaction of X_2X_3 with $p>0.05$ that is not significant. To minimize the error, all the coefficients in the design were randomly considered.

Table 2. *Variables factors considered for bio-hydrogen production.*

Variable	Symbol	Coded factor levels		
		-1	0	+1
Time (days)	X_1	1	2	3
pH effects	X_2	5.50	5.85	6.20
SC (mg/l)	X_3	3.70	3.98	4.25

Table 3. *Experimental data for experimental bio-hydrogen, predicted (RSM &ANN) and residue values (RSM & ANN).*

Std. run	X_1	X_2	X_3	HY (ml)	Predicted		Residual	
					RSM	ANN	RSM	ANN
1	-1	-1	0	4.04	4.05	4.0133	-0.012	0.0067419
2	1	-1	0	4.30	4.29	3.9593	0.02	0.010663
3	-1	1	0	4.20	4.20	4.0493	0.000	0.0092589
4	1	1	0	3.80	3.80	4.0813	0.000	0.0012514
5	-1	0	-1	3.75	3.74	3.7426	0.013	0.0074447
6	1	0	-1	3.90	3.90	3.8101	0.000	0.010074
7	-1	0	1	3.90	3.90	4.2990	0.000	0.0009966
8	1	0	1	3.90	3.90	4.1881	0.000	0.0081047
9	0	-1	-1	3.80	3.80	3.7402	-0.0025	0.010248
10	0	1	-1	3.80	4.30	3.7883	0.0025	0.011701
11	0	-1	1	3.90	3.90	4.2104	0.000	0.010391

Std. run	X_1	X_2	X_3	HY (ml)	Predicted		Residual	
					RSM	ANN	RSM	ANN
12	0	1	1	3.97	3.96	4.2722	0.013	0.027816
13	0	0	0	3.90	3.90	3.9011	0.000	0.0010966
14	0	0	0	3.73	3.74	3.9011	-0.020	0.0010966
15	0	0	0	4.18	4.19	3.9011	-0.013	0.0010966
16	0	0	0	4.08	4.08	3.9011	0.0025	0.0010966
17	0	0	0	4.02	4.02	3.9011	-0.0025	0.0010966

Table 4. *Test of Significance for Every Regression Coefficient.*

Source	Sum of squares	df	Mean Square	F-value	p-value
X_1	8.000E-004	1	8.000E-004	6.59	0.0372
X_2	0.011	1	0.011	92.65	< 0.0001
X_3	0.45	1	0.45	3716.18	< 0.0001
X_1X_2	2.025E-003	1	2.025E-003	16.68	0.0047
X_1X_3	7.225E-003	1	7.225E-003	59.50	0.0001
X_2X_3	2.250E-004	1	2.250E-004	1.85	0.2156
X_1^2	0.017	1	0.017	140.92	< 0.0001
X_2^2	0.017	1	0.017	140.92	< 0.0001
X_3^2	8.059E-003	1	8.059E-003	66.37	< 0.0001

The model coefficients with probability value, the residual, the lack of fit, the pure error and core total are shown in analysis of variance of regression equation called ANOVA (Table 5). The model F-value of 0.52 ($p<0.0001$) inferred the model was significant. The lack-of-fit has no value, which revealed that the model was significant for the bio-hydrogen yield. However, the data obtained was tested with quadratic model, and it fitted best to a quadratic model. It exhibited low standard deviation (0.011: RSM; 1.283: ANN) and low Mean 3.98 and -0.4934 for RSM and ANN, respectively. The reasonable agreement between the coefficient of determination (R^2) and R-Sq. (adj.) for RSM (99.98% and 99.96%) and ANN (99.993% and 99.986%) indicate that the model is perfectly suitable for the acceptable representation of the relationship among the variables under consideration. The low values of standard error (0.004928) observed in the intercept, the linear variables (0.003896), the interactions (0.00551) and the quadratic (0.00537) terms shows that the regression model fits the statistical data with a good prediction (Table 6). The final model equation in terms of coded factors considered for this is expressed in Eqn. (2).

Table 5. *Analysis of Variance (ANOVA) of Regression Equation.*

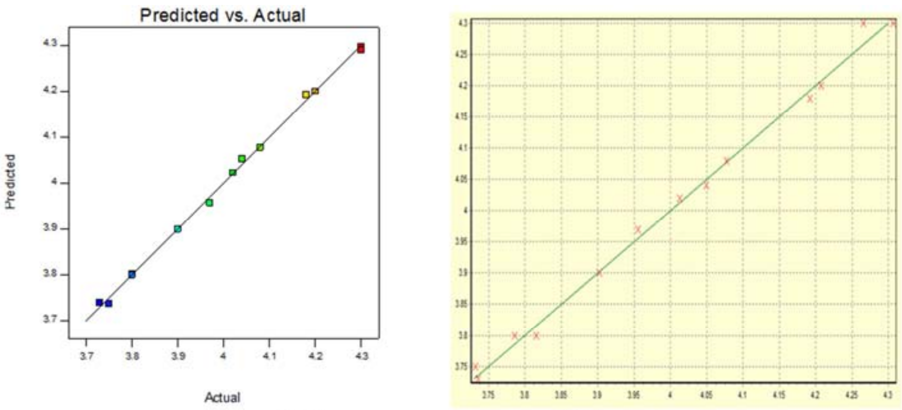
Source	Sum of squares	df	Mean Square	F-value	p-value
Model	0.52	9	0.058	475.67	< 0.0001
Residual	0.00085	7	0.0001214		
Lack of Fit	0.00085	3	0.0002833		
Pure Error	0.000	4	0.000		
Cor. Total	0.52	16			
RSM: S.D =0.011;	Mean = 3.98;	R-Sq. = 99.984%,		R-Sq.(adj.) = 99.630%	
ANN: S.D =1.283;	Mean = -0.4934;	R-Sq.= 99.850%,		R-Sq.(adj.) = 99.693%,	

Table 6. *Regression Coefficients and Significance of Response Surface Quadratic.*

Factor	Coefficient Estimate	df	Standard Error	95%CI Low	95%CI High	VIF
Intercept	3.90	1	0.004928	3.89	3.91	-
X_1	-0.01	1	0.003896	-0.019	-0.0007875	1.00
X_2	0.038	1	0.003896	0.028	0.047	1.00
X_3	0.24	1	0.003896	0.23	0.25	1.00
X_1X_2	0.022	1	0.00551	0.009472	0.036	1.00
X_1X_3	-0.042	1	0.00551	-0.056	-0.029	1.00
X_2X_3	0.0075	1	0.00551	-0.005528	0.021	1.00
X_1^2	0.064	1	0.00537	0.051	0.076	1.01
X_2^2	0.064	1	0.00537	0.051	0.076	1.01
X_3^2	0.044	1	0.00537	0.031	0.056	1.01

$$HY\ (ml) = 3.90 - 0.01X_1 + 0.038X_2 + 0.24X_3 - 0.042X_1X_2 + 0.0075X_1X_3 - 0.042X_2X_3 + 0.064X_1^2 + 0.064X_2^2 + 0.044\,X_3^2 \quad (2)$$

Fig. 1 shows the plots of predicted (HY) against the actual by both RSM and ANN software's. It was observed that plots (RSM and ANN) properly fits but there was slight negligible deviation between the actual values and the predicted values. Fig. 2 shows the 3D's plots among the selected variables on bio-hydrogen yield (HY). Observation from the graphs shows there was a perfect interaction between the pH (X_1) and the fermentation time (X_2), and mutual interaction X_1X_3 and X_2X_3, respectively.



RSM ANN

Fig. 1. *Plots of predicted against the actual.*

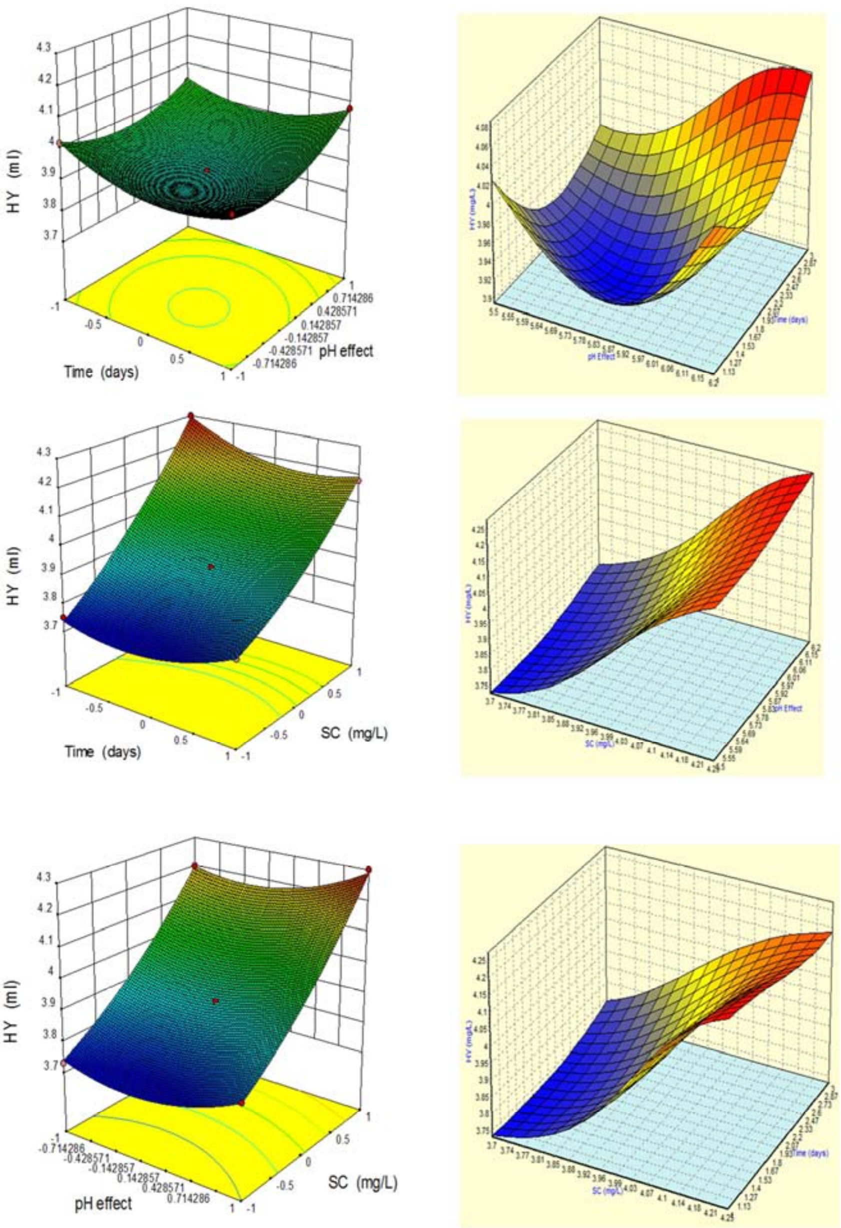


Fig. 2. *RSM and ANN 3-D's plots.*

Fig. 3 shows the level of important contribution among the selected independent variables plotted by ANN, observation showed that all the variables considered were very important and contributed actively to the response (Hy). The statistical analysis by RSM predicted Hy of 4.011 ml at X_1 = -1, X_2 = -1 and X_3 = -0.043 variable conditions, this was validated in triplicate experiments, and the average Hy was 3.98 ml. Also, ANN statistical software predicted Hy of 4.221 ml at X_1 = -1, X_2 = -1 and X_3 = -0.032 variable conditions, this was validated in triplicate experiments, and the average Hy was 4.002 ml.

The results of this experimental study established that the use of both RSM and ANN with appropriate experimental design can give the optimum yield of bio-hydrogen, even though, ANN predict better than RSM in terms of yield of bio-hydrogen.

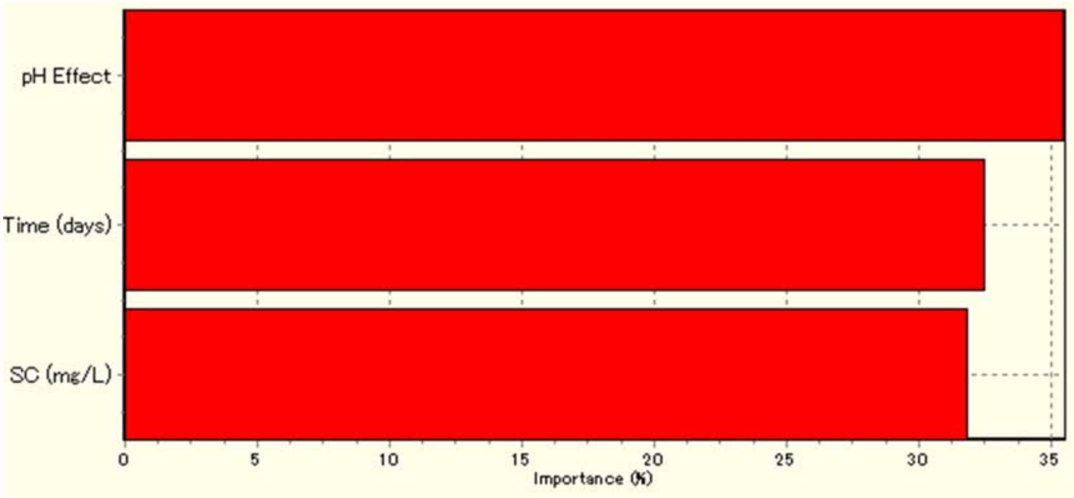


Fig. 3. *Level of importance contribution.*

4. Conclusion

In this study, both ANN and RSM perfectly suited the prediction for bio-hydrogen production. The variable factor considered for experimental design showed a remarkable significant contribution to HY. Physicochemical properties of the cassava wastewater showed that it could serve as a good stock for bio-hydrogen production.

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