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RESEARCH ARTICLE

Comparative Studies of Response Surface Methodology (RSM) and Artificial Neural Network (ANN) Predictive Capabilities on Enzymatic Hydrolysis Optimization of Sweet Potato Starch

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Abstract

The modeling and optimization efficiencies of artificial neural network (ANN) and response surface methodology (RSM) in a two-step enzymatic hydrolysis of sweet potato was investigated in this study. Optimization of the process was carried out using RSM and generic algorithm (GA) of ANN which were then compared. The optimum reducing sugar yields predicted were 190.034 g/l and 244.6 g/l for liquefaction and saccharification, respectively. These compared well to ANN validated yield of 190.877 g/l and 244.68 g/l for liquefaction and saccharification, respectively. The ANN model R^2 were 0.99998 and 0.99933 for both steps, respectively while 0.987 and 0.996 were obtained for both steps using RSM model. Also RMSE for ANN were found to be 0.1664 and 0.37922 while values for RSM were 3.19 and 0.58 for both steps. This showed that ANN had a higher predictive ability and was a better optimization tool than RSM on the hydrolysis of starch.

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Introduction

The world production of sweet potato tuber was 104.6 x 10^9 kg in 2008. China was the largest producer with an annual production of 78.8 x 10^9 kg followed by Nigeria (3.3 x 10^9 kg) having the largest output in Africa (FAO, 2008). In developing countries such as Nigeria, million tons of the tubers produced annually are being wasted due to lack of appropriate storage facilities. In solving the problem of wastage, value addition to these tubers to produce other useful products is imperative (Betiku and Adesina, 2013a). Sweet potato root is rich in starch which is composed of amylose and amylopectin. There are two commonest methods of hydrolysis of starch, first is the acid hydrolysis, which involves the use of dilute acid to hydrolyze the starch component. However, acid hydrolysis has some demerits as it requires the use of corrosion resistant materials, it also gives rise to coloured and saltish content. It needs more energy for heating and it is relatively difficult to control. It also gives a solution that needs to be neutralized before its use for fermentation purpose (Chaplin and Bucke, 1990). Acid hydrolysis is being largely replaced by enzymatic hydrolysis. In enzymatic hydrolysis, alpha-amylase (E.C.3.2.1.1) hydrolyzes starch by randomly cleaving the internal alpha-1,4glucosidic bonds while glucoamylases (E.C.3.2.1.3) are also able to hydrolyze the 1,6-linkages at the branching points of amylopectin which is then finally broken down into glucose. The obtained sugar syrups are employed by the food industry to make sweets, drinks, juices and products such as citric acid, gluconic acid and ethanol. It equally finds uses in paper and textile industry (Pandey, et al., 2000; Arzhar and Hamdy, 1981a).

Modelling and optimization has been noted to be the most important stages in biological process, this is because it leads to system improvement and increases the efficiency of the process without increasing the cost (Bas and Boyhaci, 2007). The classical unifactorial method of optimization is not only time-consuming and tedious but also does not depict the complete effects of the parameters in the process and ignores the combined interactions

between the physicochemical parameters (Ebrahimpour et al., 2008; Desai et al., 2008). This method can also lead to misinterpretation of results (Bas and Boyhaci, 2005; Fattah et al., 2005) and it seldom guarantees the determination of optimal conditions (Survase et al., 2006). These limitations of a single factor optimization process can be overcome by using empirical methods which are statistical-based approach and artificial intelligence-based black box based approach (Desai et al., 2008).

Response Surface Methodology (RSM) as a good example of Statistical-based approach is a comprehensive experimental design and mathematical modeling, through the partial regression fitting of the experimental factors (Wang et al., 2011). It has the advantage of reducing the number of experimental runs needed to give adequate information for statistically acceptable results. Many authors have reported on the use of RSM model for various biological media, such as citric acid (Imandi et al., 2008; Betiku and Adesina, 2013b), ethanol (Wang et al., 2011), Scleroglucan (Desai et al., 2008) and thermostable lipase (Ebrahimpour et al., 2008). Generally RSM model assesses the relationships between the response(s) and the independent variables (Chen et al., 2002), and defines the effect of the independent variables, alone or in combination, in processes (Ebrahimpour et al., 2008).

Artificial neural network (ANN) is a highly simplified model of the structure of a biological network (Bas and Boyhaci, 2007). The fundamental processing element of ANN is an artificial neuron which receives inputs from other sources, combines them, generally performs a non-linear operation on the result, and then outputs the final result (Manshar and Diyakar, 2005). The ability of the ANNs to recognize and reproduce the cause-effect relationships through training for the multiple input-output systems makes them more efficient to represent even the most complex systems (Pareck et al., 2002). It has emerged as an attractive tool for non-linear multivariate modeling (Desai et al., 2004). It has also become the most popular artificial learning tool in biotechnology with a wide application range, including optimization of bioprocesses (Manshar and Diyakar, 2005). The power of ANN is that it is generic in structure and possesses the ability to learn from historical data. Advantages of ANN compared to RSM are: (i) ANN does not require a prior specification of suitable fitting function and (ii) ANN has universal approximation capability, i.e. it can approximate almost all kinds of non-linear functions including quadratic functions.(Desai et al., 2008).

Generic Algorithms(GAs) (Davis, 1991; Goldberg, 1989) is an artificial intelligence-based stochastic non-linear optimization formalism, which is used to optimize the input space of ANN model. This hybrid methodology is called ANN-GA. The GA makes use of principles of biological evolution namely, "survival-of-the-fittest" and "random exchange of data during propagation" followed by biologically evolving species. GA has been proved to be an ideal technique to solve diverse optimization problems in biochemical engineering (Sarkar and Modak, 2003; Nandi et al., 2002). Desai et al. (2008) compared the predictive capabilities of RSM and ANN on the fermentative production of scleroglucan while Ebrahimpour et al., (2008) worked on thermostable lipase production, but apparently, no existing report on such comparative analysis could be found on enzymatic hydrolysis of starchy materials.

The Objective of our work was modelling of enzymatic hydrolysis of starch using two empirical methods of ANN and RSM, optimization of the process variable as it affects hydrolysis of starch, comparison of prediction capability and optimization efficiencies of the methods.

Material and Methods

Sweet Potato Starch Preparation

Sweet potato tubers were obtained from a local market in Nigeria. The tubers were washed, peeled and milled with water. The slurry of sweet potato was mixed with water and later filtered with teflon cloth after which the filtrate was sun-dried and packed into container.

Enzymes

Enzymes used for the study were alpha-amylase (E.C.3.2.1.1) from bacterium source (*Bacillus licheniformis*) and *glucoamylase*(E.C.3.2.1.3) from *Aspergillusniger*. They were both obtained from the Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos, Nigeria.

Starch hydrolysis and Enzymatic Studies

25 % w/v slurry of sweet potato starch was made with water containing 40ppm Ca^{2+}. Ca^{2+} increases the activity of enzyme in the medium. The pH was adjusted to 6.5 with citrate-phosphate buffer. The slurried starch was gelatinized by heating the mixture to 100ºC for 20 min afterward, α-amylase (6.4 units/ml) was added according to BBD in Table 1 for liquefaction to take place. Enzyme activities were stopped by heating the mixture to boil and final mixtures were centrifuged at 12,000 rpm for 15 min. The supernatants were then analyzed for reducing sugar.

After the optimum condition for liquefaction had been established, it was then later subjected saccharification by addition of glucoamylase (789.6 units/ml) at pH of 4.5 according to BBD in Table 2.

Table 1: Coding of experiment factor and levels for liquefaction

Variable	Symbol	Coded variable levels		
		-1	0	+1
Temperature (^oC)	X_1	55	60	65
Time (min)	X_2	55	60	65
Enzyme dose (v/v)	X_3	0.5	0.75	1.0

Table 2: Coding of experiment factor and levels for saccharification

Variable	Symbol	Coded variable levels		
		-1	0	+1
Temperature (^oC)	X_1	40	50	60
Time (min)	X_2	20	40	60
Enzyme dose (v/v)	X_3	0.5	0.75	1.0

Analysis

Reducing sugar estimation

The reducing sugar produced from sweet potato starch was determined using dinitrosalicylic acid (DNS) method of Miller (1959). The procedure involves addition of 3 ml of the DNS to 1 ml of the supernatant in the test tube which was boiled for 15 min, cooled and diluted with water. Absorbance were later measured at a wavelength of 540 nm against blank using the UV-Visible Spectrophotometer (Libra 21 Model, UK) after which glucose calibration curve had been prepared.

Modelling and Optimization

RSM experimental design

The Design-Expert 8.03 software was used to generate the experimental runs and modeling of the experimental data. A total of 17 experimental runs were generated for each step of hydrolysis using BBD for each step of the hydrolysis. The same experimental design was used for both RSM and ANN analysis. The variables considered for both the liquefaction and saccharification steps of hydrolysis were temperature (X_1), time (X_2) and enzyme dose (X_3). The coded independent variables levels for both liquefaction and saccharification steps are shown in Table 1 and Table 2, respectively.

Optimization

Response surface methodology (RSM) was used to optimize the quadratic model. The generalized response surface model for describing the variation in response variable is given as equation (1)

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the predicted response by RSM, i and j are the linear and quadratic coefficients, respectively, β is the regression coefficient, k is the number of factors studied and optimized in the experiment, and ε represents the random error (Ghorbania, 2008).

Artificial neural network analysis

A commercial ANN software, NeuralPower version 2.5 (CPC-X Software) was employed in the study. The data were tested with multilayer normal feed forward and multilayer full feed forward neural networks. The networks were trained with different learning algorithms (incremental back propagation, IBP; batch back propagation, BBP; quickprop, QP; Levenberg-Marquardt algorithm, LM and Generic algorithm GA). The network architecture consisted of an input layer with three neurons, an output layer with one neuron, and 2 hidden layers. The inputs for the network included temperature, time and enzyme dose, while the output was the reducing sugar concentration. In order to determine the optimal number of nodes for ANN hydrolysis network, a series of topologies were used, in which the number of nodes were varied (Mansour and Mostafa, 2011). The transfer functions of hidden and output layers (sigmoid, hyperbolic tangent function, Gaussian, linear, threshold linear and bipolar linear) were iteratively determined by developing several networks. The ANN was trained using a default stopping criteria of 100000 (Betiku and Ajala, 2014). Experimental data was divided into training and testing data sets. Fourteen experimental data were used for training while three were used for testing for both each stage of hydrolysis.

Data verification

Decision on the optimum topology was based on the minimum error of testing (Mansour and Mostafa, 2011) and the maximum coefficient of determination (R^2) and each topology was repeated ten times. In quest for optimum condition, each network was trained until the network root of mean square error (RMSE) is near zero and coefficient of determination (R^2) is close to 1. RSME and R^2 are calculated by Eqs. (2) and (3) respectively.

$$\text{RMSE} = \left(\frac{1}{n}\sum_{i=1}^{n}(y_i - y_{di})^2\right)^{1/2} \quad (2)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_{di})^2}{\sum_{i=1}^{n}(y_i - y_m)^2} \quad (3)$$

where*n* is the number of points, y_i is the predicted value, y_{di} is the actual value, and y_m is the average of the actual values. (Mansour and Monstafa, 2011)

Other parameters for the network were chosen as the default values of the used software. At the start of the training, weights were initialized with random values and adjusted through a training process in order to minimize network error (Basri et.al, 2007).

Result and Discussion

RSM Modeling and Optimization

A quadratic model was used to describe both steps of hydrolysis stages of sweet potato starch. Results of the analysis of variance (Tables 3 and 4) show the F-value of 11909.01 and 2183.31 for liquefaction and saccharification, respectively with ($p < 0.0001$). This indicates that the model obtained is significant. The data obtained fit well to a quadratic model and it exhibits low standard deviation (Betiku and Adesina, 2013a). It has high coefficient of determination (R^2) of 0.987 and 0.996 for the liquefaction and saccharification, respectively. This demonstrates that the model proves adequate for the representation of the actual relationship among the selected factors (Betiku and Adesina, 2013b). The p-values were used as a tool to check the significance of each of the coefficients, which in turn, are necessary to understand the pattern of the mutual interactions between the test variables (Ebrahimpour et al., 2008). In the case of liquefaction step of the hydrolysis, $p < 0.05$ for each of the three linear terms (X_1, X_2, X_3), three cross-products (X_1X_2, X_2X_3,X_1X_3) and the three quadratic terms (X_1^2, X_2^2 and X_3^2), these indicated that the model terms were significant. For the saccharification step, the results showed that the two linear terms (X_1, X_3), three cross products (X_1X_2, X_1X_3, X_2X_3) and the three quadratic terms (X_1^2, X_2^2 and X_3^2) weresignificant model terms at 95% confidence level , i.e $p < 0.05$, also the interaction between X_1 X_2, X_1 X_3, and X_2, X_3were remarkably significant model terms at 95% confidence level. Therefore, the quadratic model obtained in this study for both liquefaction and saccharification stages of hydrolysis are represented in Eq.4 and 5, respectively. The final equation in terms of coded factors for the BBD response surface quadratic model is expressed in Y (reducing sugar concentration in g/l).

$$Y = 111.79 - 10.22X_1 + 23.43X_2 + 26.25X_3 - 0.58X_1X_2 - 9.03X_1X_3 + 13.86X_2X_3 + 2.61X_1^2 + 2.41X_2^2 + 1.88X_3^2 \quad \ldots\ldots(4)$$

$$Y = 210.41 + 1.71X_1 + 9.88X_2 + 4.13X_3 + 3.32X_2 - 2.75X_1X_3 + 9.77X_2X_3 - 9.24X_1^2 + 10.1 - 2.83X_3^2 \quad \ldots\ldots(5)$$

The optimization tool of RSM was used to optimize the reducing sugar yield of both steps of the hydrolysis. The optimum conditions predicted for the liquefaction were: temperature (64.9 ^oC), time (58 min) and enzyme dose (1% v/v) and corresponding to a predicted response of 190.034 g/l of reducing sugar concentration. This was validated as average yield of 189.03 g/l with four replicates. In the case of saccharification step, the optimum conditions predicted were temperature (42.84 ^oC), time (59 min), and enzyme dose (0.999 % v/v), while the response value was 242.95 % . It was also validated as 243.6 g/l with four replicates. This confirmed the efficacy of the quadratic mathematical model used for the hydrolysis (Betiku and Ajala, 2014).

ANN Modeling

The neural network architectures and topologies of ANN were selected and tested for estimation and prediction of reducing sugar yield of hydrolysis of sweet potato starch (Betiku and Ajala, 2014). The effect of learning algorithm and transfer function were studied by successful training of neural network model employing the different learning

algorithms and transfer functions of ANN (Ebrahimpour et al., 2008). After a series of testing it was found that quick propagation (QP) was the best algorithm with hyperbolic tangent function as hidden and sigmoid transfer functions for output layer for liquefaction stage, while in the case of saccharification stage the best algorithm was tahn as hidden and output transfer functions. Various topologies (from 1 to 20 hidden neurons) were also examined using QP algorithm. After repeated trials, it was found that a network with 16 hidden neurons produced the best performance for liquefaction step of hydrolysis and 15 hidden neurons for saccharification step of hydrolysis as depicted inFigure 1(a-b) whichillustrates the performance of the network for testing data *versus* the number of neurons in the hidden layer. ANN optimum configuration was achieved using multilayer normal feed forward quick prop network, the configuration model was 3-16-1 and 3-15-1 for liquefaction and saccharification, respectively. Also the best ANN model was observed to be Multilayer Normal Feed Forward (MNFF) with R^2 and RSME for training set as 0.99998 and 0.1664, respectively while for testing set are 0.9993 and 0.1764, respectively for liquefaction. For saccharification, R^2 and RSME for training set were 0.99947 and 0.37922, respectively while corresponding value for testing set were 0.9979 and 0.3924, respectively (Table 5a-b). Figure 2(a-c) and Figure 3(a-c) depict the surface plots for the hydrolysis of sweet potato starch. The curvature nature of the curves showed that there was an interaction between the response and the individual variables, in the case of liquefaction stage of the hydrolysis, it showed an increase in reducing sugar as the temperature increased until 60.3 ^oC mark, after which subsequent increase in the temperature led to reverse in trend. However on the effect of enzyme dosage, increase in enzyme dosage within the investigated value showed increase in the yield of reducing sugar. Meanwhile as the contact time increases, a maximum in yield of reducing sugar at time 61.7 min was observed. In the case of saccharification stage, an increase in reducing sugar was observed within the first 26 min, this was followed a sudden decrease, which however picked up subsequently. More so, increase in enzyme dosage increased the yield of reducing sugar linearly. On temperature effect, maximum yield was observed at 40.7 ^oC mark after which there was a decline in the yield of the reducing sugar. Figures 4(a-b) depict the importance of the level of the variables considered on the yield of reducing sugar. For both stages of hydrolysis, temperature was the most important variable, this was followed by enzyme dosage and time, being the least

Table 3: Analysis of Variance of Regression Equation for Liquefaction

Source	Sum of Squares	df	Mean Square	F-Value	p-value
Model	11909.01	9	1323.22	10577.56	< 0.0001
X_1	835.18	1	835.18	6676.24	< 0.0001
X_2	4390.78	1	4390.78	35098.98	< 0.0001
X_3	5511.45	1	5511.45	44057.36	< 0.0001
X_1X_2	1.33	1	1.33	10.66	0.0138
X_1X_3	325.98	1	325.98	2605.84	< 0.0001
X_2X_3	768.68	1	768.68	6144.63	< 0.0001
X_1^2	28.58	1	28.58	228.45	< 0.0001
X_2^2	24.36	1	24.36	194.72	< 0.0001
X_3^2	14.89	1	14.89	118.99	< 0.0001
Residual	0.88	7	0.13		
Lack of Fit	0.43	3	0.14	1.30	0.3898
Pure Error	0.44	4	3.19		
Cor Total	11909.88	16			

Table 4: Analysis of Variance of Regression Equation for Saccharification

Source	Sum of Squares	Df	Mean Squares	F-Value	p-value
Model	2183.31	9	242.59	517.35	< 0.0001
X_1	23.46	1	23.46	50.03	0.0002

X_2	81.51	1	781.51	1666.64	< 0.0001
X_3	136.37	1	136.37	290.83	< 0.0001
X_1X_2	44.22	1	44.22	94.31	< 0.0001
X_1X_3	30.25	1	30.25	64.51	< 0.0001
X_2X_3	381.62	1	381.62	813.83	< 0.0001
X_1^2	359.15	1	359.15	765.93	< 0.0001
X_2^2	433.5	1	433.5	924.48	< 0.0001
X_3^2	33.68	1	33.68	71.83	< 0.0001
Residual	3.28	7	0.47		
Lack of Fit	0.98	3	0.33	0.57	0.6646
Pure Error	2.30	4	0.58		
Cor Total	2186.60	16			

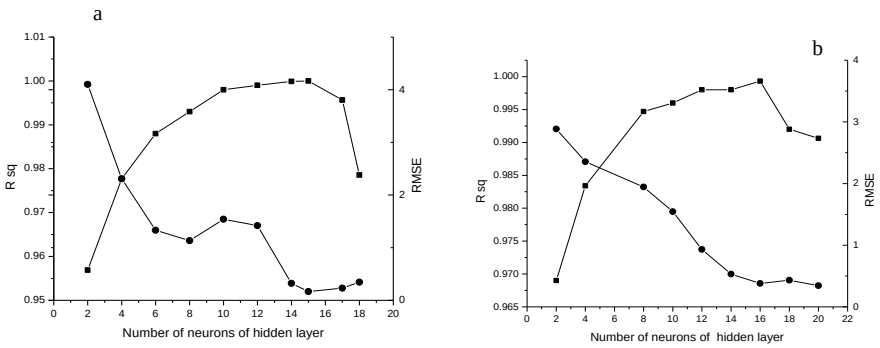

Figure 1(a-b):Performance of the network for testing data *versus* the number of neurons in the hidden layer. a. Liquefaction b. Saccharification

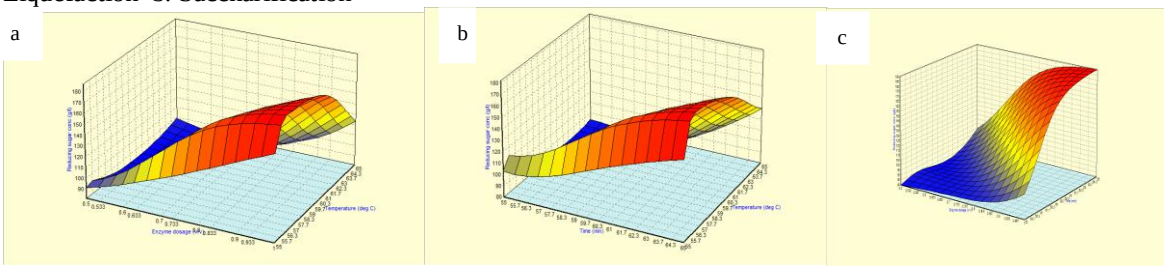

Figure 2(a-c): Surface plots for liquefaction of sweet potato starch.

a. effect of enzyme dose, temperature and their reciprocal interaction on glucose yield
b. effect of enzyme dose, time and their reciprocal interaction on glucose yield.
c. effect of time, temperature and their reciprocal interaction on glucose yield

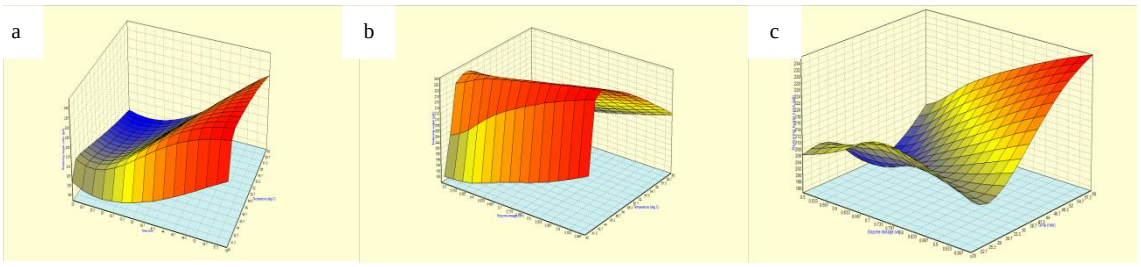

Figure 3(a-c): Surface plots for Saccharification of sweet potato starch.

a. effect of enzyme dose, temperature and their reciprocal interaction on glucose yield
b. effect of enzyme dose, time and their reciprocal interaction on glucose yield.
c. effect of time, temperature and their reciprocal interaction on glucose yield

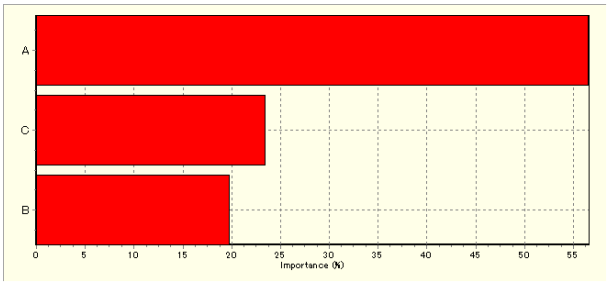

Figure 4a: level of Importance of process variable on the yield of reducing sugar for liquefaction
A: Temperature
B: Time
C: Enzyme dosage

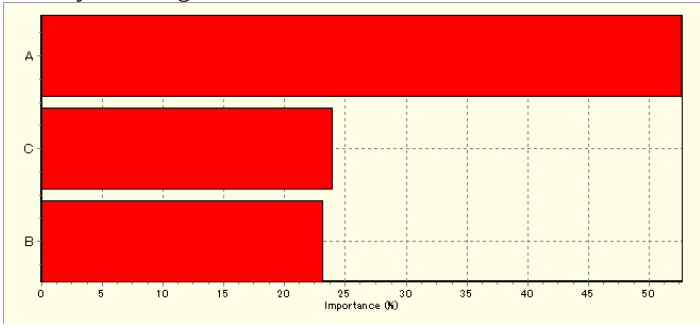

Figure 4b: level of Importance of process variable on the yield of reducing sugar for saccharification
A: Temperature
B: Time
C: Enzyme dosage

Table 5a: Effect of ANN architecture and topologies on R^2 and RSME obtained in training and testing data set for liquefaction

Configuration	Algorithm	Model	Output transfer function	Input transfer function	Training set R^2	Testing set R^2	Training set RSME	Testing set RSME
3-16-1	QP	MNFF	Tanh	Sigmoid	0.99998	0.9993	0.1664	0.1764
3-16-1	QP	MNFF	Tanh	Linear	0.9274	0.9019	0.3520	0.4921
3-16-1	BBP	MNFF	Linear	Sigmoid	0.9937	0.9919	0.4978	0.5432
3-16-1	IBP	MNFF	Tanh	Linear	0.95841	0.9184	0.60673	0.7334
3-15-1	QP	MNFF	Tanh	Gaussian	0.91902	0.9097	0.6002	0.6231

Table 5b: Effect of ANN architecture and topologies on R^2 and RSME obtained in training and testing data set for saccharification.

Configuration	Algorithm	Model	Output transfer function	Input transfer function	Training set R^2	Testing set R^2	Training set RSME	Testing set RSME
3-15-1	QP	MNFF	Tanh	Tanh	0.99947	0.9979	0.37922	0.3924
3-15-1	QP	MNFF	Tanh	Linear	0.9294	0.9099	1.4720	1.5231
3-15-1	BBP	MNFF	Linear	Sigmoid	0.99909	0.9989	0.4978	0.5432
3-15-1	IBP	MNFF	Tanh	Linear	0.87841	0.8284	1.6173	1.7345
3-15-1	QP	MNFF	Tanh	Gaussian	0.99902	0.9917	0.3902	0.4231

Optimization using ANN-GA

The input vector comprising of input variables (temperature, time and enzyme dosage) was optimized using GA. For liquefaction, ANN-GA predicted reducing sugar yield of 190.034 g/l at optimized conditions of temperature 64.9 ^{o}C, time 57.5 min and enzyme dosage of 0.99 % v/v, the predicted yield was validated in triplicate as 190.877 g/l. In the case of saccharification, 244.6 g/l was predicted at optimum conditions of temperature, 42.8 ^{o}C, time, 59.99 min and enzyme dosage of 0.99 % v/v, which was validated as 244.68 g/l of reducing sugar concentration.

ANN and RSM Comparison

Tables 6 and 7 showed Box-Behnken design (BBD) of the process variables and the experimental, predicted responses for RSM model and ANN model for liquefaction and Saccharification, respectively. In comparing values predicted by both models, it was observed that ANN model predictions are more accurate than RSM. This confirmed the superiority in prediction capability of ANN over RSM. Also in comparing the R^2 and RSME for both models, R^2 and RSME for ANN model were 0.99998 and 0.1664, respectively for liquefaction while that of RSM model were 0.987 and 3.19, respectively. In the case of saccharification, R^2 and RSME for ANN were 0.99947 and 0.37922 while that of RSM model were 0.996 and 0.58, respectively. This further showed that ANN was more accurate in data fitting than RSM as it is shown in Figures 5 and 6.

Conclusion.

This work focused on comparison of ANN and RSM models for their predictive capability and optimization efficiency in starch hydrolysis. A box Behnken design was used to design the experiment, ANN showed higher accuracy in finding optimum condition and predicting yield. Thus, artificial intelligence-based method performed better than RSM for data fitting, optimization and estimation capabilities.

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Table 6: Box-Behnken design of three variables and the experimentally determined, RSM model predicted and ANN model predicted for liquefaction.

Std run	X_1 (deg)	X_2 (min)	X_3 (v/v)%	Experimental value	RSM Predicted	ANN Predicted
1	-1	-1	0	103.00	103.03	103
2	1	-1	0	83.48	83.50	83.48
3	-1	1	0	150.92	150.93	150.92
4	1	1	0	129.45	129.43	127.45
5	1	0	-1	91.50	91.28	91.5
6	1	0	-1	89.00	88.81	89
7	-1	0	1	161.61	161.81	161.61
8	1	0	1	123.00	123.23	123
9	0	-1	-1	80.00	80.20	80
10	0	1`	-1	99.22	99.44	99.22
11	0	-1	1	105.21	104.99	105.21
12	0	1	1	179.80	179.60	179.80
13	0	0	0	112.00	111.79	111.79
14	0	0	0	111.43	111.79	111.79
15	0	0	0	112.10	111.79	111.79
16	0	0	0	111.43	111.79	111.79
17	0	0	0	112.00	111.79	111.79

Table 7: Box-Behnken design of three variables and the experimentally determined, RSM model predicted and ANN model predicted for Saccharification.

Std. Run	X_1 (deg)	X_2 (min)	X_3 %(v/v)	Experimental value	RSM Predicted	ANN Predicted
1	-1	-1	0	203.3	203.05	203.3
2	1	-1	0	200	199.83	200
3	-1	1	0	216	216.17	216
4	1	1	0	226	226.25	226
5	-1	0	-1	190	189.76	190
6	1	0	-1	199	198.68	199
7	-1	0	1	203.2	203.52	203.2
8	1	0	1	201.2	201.44	201.2
9	0	-1	-1	213	213.49	213
10	0	1	-1	213.65	213.72	213.65
11	0	-1	1	202.28	202.21	202.28
12	0	1	1	242	241.51	242
13	0	0	0	211	210.41	210.41
14	0	0	0	209.52	210.41	210.41
15	0	0	0	209.65	210.41	210.41
16	0	0	0	210.95	210.41	210.41
17	0	0	0	210.95	210.41	210.41

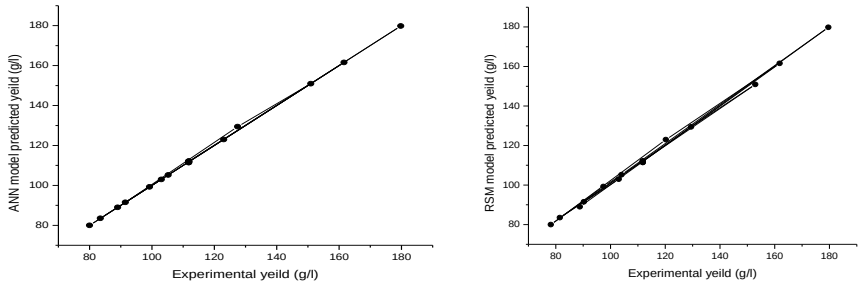

Figure 5(a):ANN model predicted yield *versus* experimental yield for liquefaction

(b):RSM model predicted yield *versus* experimental yield for liquefaction

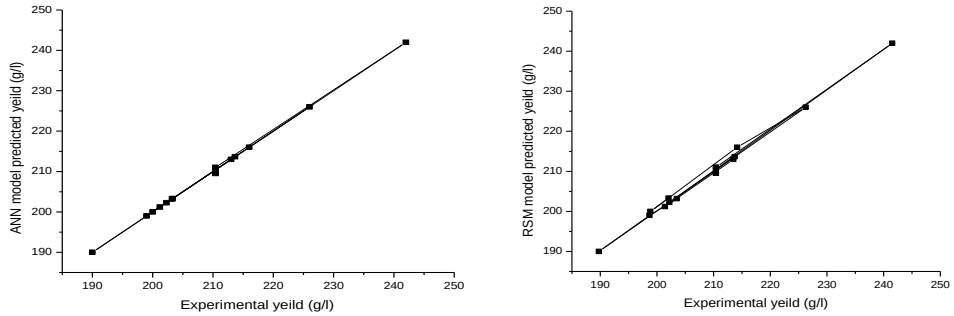

Figure 6(a):ANN model predicted yield *versus* experimental yield for Saccharification
(b): RSM predicted yield *versus* experimental yield for Saccharification

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