EVALUATION OF RESPONSE SURFACE METHODOLOGY, ARTIFICIAL NEURAL NETWORK AND ADAPTIVE NEURONS FUZZY INFERENCE SYSTEM FOR MODELLING AND OPTIMIZING OXALIC ACID PRODUCTION FROM PINEAPPLE WASTE

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: December 16, 2019 **Accepted:** March 12, 2020

Abstract: Oxalic acid is one of the important organic acids produced by fermentation and its production is affected by several factors. This study investigated the effect of three independent variables namely; potassium dihydrogen phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4$) and sodium nitrate ($NaNO_3$) and their mutual interactions on oxalic acid production from pineapple waste using Box Behnken Design (BBD). Modelling was carried out using Response Surface methodology (RSM), Artificial Neural Network (ANN) and Adaptive Neuro Fuzzy Inference System (ANFIS). A quadratic model was obtained to predict the concentration of oxalic acid as a function of the three independent variables. For ANN, Incremental Back Propagation (IBP) with hyperbolic tangent function (Tanh) was the best model for predicting oxalic acid production. For ANFIS, the Sugenoinference system combined with hybrid learning algorithm, Gaussian membership function was found suitable for the prediction of oxalic acid production. The developed RSM, ANN and ANFIS models described the fermentation with high accuracy as indicated by their high R^2 values (0.957, 0.9894 and 0.9893), low RMSE (1.0923, 0.5417 and 0.5422) and low AAD (7.8692, 1.1887 and 1.3130), respectively. RSM, ANN and ANFIS coupled with genetic algorithm were applied to optimize the process for best operating condition and ANN gave the maximum value of oxalic acid (20.73 g/L) with the best combination of the input variables (0.77 g/L of KH_2PO_4, 0.09 g/L of $MgSO_4$ and 1.78 g/L of $NaNO_3$). Based on the statistical indices used for evaluation, ANN performed slightly better than ANFIS-GA and both were better than RSM. RSM performed the least.

Keywords: Pineapple waste, oxalic acid, ANOVA, ANN, regression analysis

Introduction

Oxalic acid (OA), also referred to as ethanedioic acid is an organic acid with the molecular formula $C_2H_2O_4$. It is the simplest aliphatic dicarboxylic acid naturally present in leafy vegetables and vegetable products (Walaszczyk *et al*., 2017). It can sometimes be found in the uncombined state, although it is more commonly found a calcium salt. It can also combine with metals such as sodium, potassium, magnesium or iron to form less soluble salt (Emeko *et al*., 2015).

Oxalic acid has wide applications in pharmaceuticals, wastewater treatment, food industry, hydrometallurgy, printing, tanning, oil refining, dyes, explosives, metal and cloth cleaning etc (Adesina *et al*., 2014). Due to its high reducing nature, it can be used in the extraction of iron present in kaolin as impurity. This use in particular has become an area of interest in the field of hydrometallurgy where iron and other heavy metals are being extracted from their respective ores.

Presently, the majority of oxalic acid is produced via chemical methods. These methods include oxidation of olefins or alkenes and glycols, decomposition of formates followed by sulphuric acid (H_2SO_4) treatment, oxidation of carbohydrates with nitric acid (HNO_3), fusion of sawdust with caustic soda and radiation processing of carbonate solutions and molasses (Mandal and Banerjee, 2005). These chemical methods are known to have negative impacts on the environment and even may not be cost effective thus motivating the need for the biological production of this acid (Betiku *et al*., 2016; Walaszczyk *et al*., 2017).

Pineapple (*Ananascomosus*) is a tropical fruit and it is the leading edible member of the family *Bromeliaceae*. Pineapple accounts for over 20% of the world production of tropical fruits and as a crop is second only to bananas as the most relevant harvested fruit (Hossain, 2016). Pineapple is mainly processed commercially as canned fruits, juices, concentrates and jams (Upadhyay *et al*., 2013). The processing of pineapple into its various value added products results in massive waste generation especially the peels which account for about 10% w/w of the weight of the original fruit (Amenaghawon *et al*., 2014). These wastes are often improperly disposed, especially in a developing country like Nigeria, thereby causing environmental pollution (Amenaghawon *et al*., 2015).Due to the fact that these wastes are sources of carbohydrates, they could serve as important substrate in the production of useful products such as organic acids, ethanol etc. via biochemical means (Kareem *et al*., 2010). Oxalic acid is one of the organic acids that could be produced using pineapple waste as a substrate for fermentation.

Some of the microorganisms considered for oxalic acid production include *Burkhulderiaglumae*, *Paxillusinvolutus*, *Aspergillus niger*, *Penicillumoxalicum*, etc. (Emeko *et al*., 2015). Nevertheless, filamentous fungus, *Aspergillus niger* remains the microorganism of choice for oxalic acid production due to its easy handling, ability to ferment a variety of cheap raw materials, and high yields (Betiku *et al*., 2016).

In order to develop an economically viable fermentation process for industrial production of oxalic acid, it is pertinent to use cheap substrates, select the right fermentation technique and carry out optimization of the fermentation variables that are involved in the process (Santoro *et al*., 1999). Process parameters affecting oxalic acid yield such as pH, time, substrate concentration, medium composition (nitrogen, phosphorus etc.) are therefore optimized in order to maximize oxalic acid yield. In the optimization of these parameters, the one factor at a time approach has been used (Bohlmann *et al*., 1998; Mandal and Banerjee, 2005; André *et al*., 2010). This method is time consuming and it has been replaced with necessitating the need for more efficient methods. Response surface methodology (RSM), artificial neural network (ANN) and adaptive neuro fuzzy inference system (ANFIS) have been proposed as viable alternatives to the one factor at a time method. Both response surface methodology and artificial neural network have been employed in modelling and optimization of biochemical processes (Desai *et al*., 2008;

Youssefi *et al*., 2009; Elfghi, 2016; Amenaghawon and Amagbewan, 2017). Although adaptive neuro fuzzy inference system (ANFIS) has been applied in modeling of different processes, its application in the fermentation processes is relatively new.

A variety of substrates have been investigated for oxalic acid fermentation using A. niger, which include lactose permeate, milk whey, molasses, post-refining fatty acids, lipids, glucose, biodiesel-derived waste glycerol and sweet potato starch hydrolysate (Adesina *et al*., 2014; Emeko *et al*., 2015; Betiku *et al*., 2016). There is, however, a dearth of information on the use of pineapple wastes for oxalic acid production (Amenaghawon *et al*., 2014).

Thus, the aim of this study is to evaluate the comparative performance of RSM, ANN and ANFIS as tools for optimising oxalic acid production from pineapple waste.

Materials and Methods

Pineapple juice extraction

The pineapple wastes used for this work were procured from a local market in Benin City, Edo State, Nigeria. The peels were washed with clean water to remove any adhering dirt after which they were blended to obtain a slurry. The juice used for fermentation was obtained by pressing the slurry through a filter cloth and this was autoclaved at 121°C for 20 min and then stored at −20°C before use (Emeko *et al*., 2015).

Microorganism and inoculum preparation

Aspergillus niger, obtained from Microbiology Department of the University of Benin, Benin City, Edo State, Nigeria was used throughout the study as the fermenting organism. *Aspergillusniger* spore were obtained from cultures grown on Sabouraud dextrose agar (SDA) for 5 to 7 days at 30ºC. This fungus was maintained on SDA plates at 4ºC and sub cultured regularly (Emeko *et al*., 2015).

Medium composition for oxalic acid production

The fermentation medium described by Betiku *et al*. (2016) was modified and used in this study. It was composed of pineapple juice as carbon source, 1.6 g/l of yeast extract, 0.025 g/l of KCl, 1.50 g/l $NaNO_3$, 0.50 g/l of KH_2PO_4, 0.025 g/l of $MgSO_4.7H_2O$. Twenty millilitres of universal pH indicator solution was then added per litre of medium to observe the culture pH which was maintained at 6.0 ± 0.5 with 4M NaOH solution during fermentation. All media were sterilized using an autoclave at 121ºC before use (Emeko *et al*., 2015).

Production of oxalic acid

For the inoculum, spores were transferred from the SDA plates aseptically into a 250 mL flask containing 100 mL of sterile distilled water. The inoculated flask was incubated on a controlled–environment incubator shaker at 30ºC for 1 h before it was used for fermentation. For the main experiment, 100 ml of the substrate were measured into a 250 mL pyrex flask and the nutrients were added. To this was added 5% (v/v) of the inoculum size aseptically and the flasks were then placed on a clean table for surface fermentation for 9 days (Emeko *et al*., 2015).

Analytical methods

The concentration of oxalic acid was determined using the catalytic spectrophotometric method (Zhi-Liang *et al*.,1996). This technique is based on the acid catalytic effect of the redox reaction between rhodamine B and dichromate at the maximum absorption wavelength of 555 nm in sulphuric acid. For the assay, 10 mL of the sample was withdrawn from the fermentation medium and filtered with Whatman No. 4 filter paper. Subsequently, to 1 mL from the filtrate was added 0.5 mL of 0.06 mol/L potassium dichromate ($K_2Cr_2O_7$), 0.20 mL of 0.25 mol/L sulphuric acids (H_2SO_4) and 0.1 mL of 3.28×10^{-4} mol/L rhodamine B in a 10 mL test tube and then diluted to the mark with water and mixed thoroughly. The mixture was placed in a water bath at 90ºC. After 8 min, the reaction was quenched by cooling with tap water and the absorbance of the mixture read at 555 nm against the blank solution. The quantity of oxalic acid produced was determined using a standard calibration curve prepared using oxalic acid (Betiku *et al*., 2014).

Design of experiment

A three-level, three-factor Box-Behnken design was employed for the experimental design in this work. The independent variables considered for this design include KH_2PO_4 (0.5-1.0 g/l), $NaNO_3$ (1-2 g/l), and $MgSO_4$ (0-0.5 g/l). The coded and actual levels of the independent variables are shown in Table 1.The independent variables used were coded according to Equation 1 as follows

$$x_i = \frac{X_i - X_o}{\Delta X_i} \qquad (1)$$

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 centre point and ΔX_i is the step change in the actual value of the independent variable. Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), a statistical software used to develop the experimental design.

Table 1: Coded and actual values of factors

Variables	Units	Symbols	Coded and actual levels		
			-1	0	+1
KH_2PO_4	g/l	X_1	0.50	0.75	1.00
$MgSO_4$	g/l	X_2	0.00	0.25	0.50
$NaNO_3$	g/l	X_3	1.00	1.50	2.00

To correlate the response variable to the independent variables, multiple regression was used to fit the polynomial model to the experimental data. The fitted quadratic response model used to estimate the response of the dependent variable (oxalic acid concentration) is given in Equation 2.

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

Where Y_i is the predicted response or dependent variable, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the experimental error term (Amenaghawon *et al*., 2014). The second order regression model is very flexible and it is rather easy to estimate the model parameters using the least squares method (i.e. minimizing the sum of squares of the errors). Moreover, a lot of practical experiences indicate that these types of models are suitable for representing most real-life response surface problems.

ANN model development

Commercial ANN Software, Neural Power, version 2.5 (C.P.C-X Software USA) was used to model and optimize oxalic acid production via fermentation. Oxalic acid yield was predicted using the multilayer full feed forward (MFFF) and the multilayer normal feed forward (MNFF) neural networks. These networks were trained using different learning algorithms including incremental back propagation (IBP), batch back propagation (BBP), quick propagation (QP), generic algorithm (GA), and Levenberg-Marquadt algorithm (LM). The ANN architecture was made up of an input layer with three neurons, a hidden layer and an output layer with one neuron. The optimal network topology was determined using only one hidden layer while the number of neurons in this layer and the transfer function of the input and output layer were determined iteratively by developing several neural networks with different transfer functions (Sigmoid, Hyperbolic-tangent, Gaussian, Linear, Threshold, Linear and Bipolar Linear). Each of the network was trained using a stopping criteria of 100,000 iterations (Ajala and Betiku, 2015). The learning algorithms employed 70% of the

experimental data as training set, 15% as validating set and the remaining 15% as testing set. This was to evaluate the predictive ability of the model with respect to the hidden data which were not used for training and to appraise the generalization capacity of the ANN.

In designing the network topology i.e. number of hidden layers and number of neurons, it is also not possible to know the number of neurons apriori. Too many neurons lead to over-fitting problem which implies that a trained ANN has weak generalization capability. However, if the number of neurons is not sufficient, it leads to under-fitting problems which means that the trained ANN is too simple to have the capability of representing the relationship between the input and output variables. For this work, the chosen network topology contained a single hidden layer.

ANFIS model development

ANFIS, which has a feed forward neural network structure, was used to develop a fuzzy model for the estimation of oxalic acid concentration in a fermentation process with three input variables (KH_2PO_4, $NaNO_3$ and $MgSO_4$) and one output variable (oxalic acid concentration). The proposed ANFIS model included five distinct layers which are fuzzification, product, normalization, defuzzification and overall summation layers (Jang, 1993). The first order Sugeno model with three input variables was used for this work. With the assumption of two input (x,y) and one output (z), the main fuzzy rule set with two IF-THEN rules can be described as follows:

Rule 1: if x is A_1 and y is B_1 then $z_1 = p_1 * x + q_1 * y + r_1$ (3)

Rule 2: if x is A_2 and y is B_2 then $z_2 = p_2 * x + q_2 * y + r_2$ (4)

Where p_i,q_i and r_i (i=1 or 2) are called linear parameters or consequent parameters of the first order Sugeno model. A_1,A_2, B_1 and B_2 are the fuzzy sets.

Layer 1: This layer consist of three variables and all the nodes in this layer are adaptive. Each node *i* in this layer is defined by a node function:

$$O_{1,i} = \mu_{A_i}(x) \quad (5)$$

Where x is the input variable to node *i* and $O_{1,i}$ is the membership grade of a fuzzy set A_i and it specifies the level to which the input variable, x satisfies A. μ_{A_i}depicts the membership fuction.

A Gaussian membership function (MF) was chosen for this work. Every node in this layer denotes a membership value to a linguistic term as a generalized MF, which has a mean as depicted in Equation (6).

$$\mu_{A_i}(x) = a_i exp\left[-\left(\frac{x-a_i}{a_i}\right)^2\right] \quad (6)$$

Where: a_i and c_i are the premise parameters of the Gaussian membership function. The value of the Gaussian MF ranges between 0 and 1.

Layer 2: This layer is the product of all inward- bound signals and it used to check the weight of each membership function. The firing strength of the weight is shown by the output of each node. The function is given by:

$$w_i = \mu_{A_i}(x) \times \mu_{B_i}(y) \quad (7)$$

Where: w_i is the weight or firing strength of the rule

Layer 3: This layer calculates the relative weight which is the ratio of the ith rule firing strength to the sum of all rule's firing strength.

$$\overline{w}_i = \frac{w_i}{w_1+w_2} \quad (8)$$

The output of this layer can be called normalized firing strength. Each node implements the required matching of the fuzzy rules by calculating the activation level of every rule.

Layer 4: Also known as the rules layer and it is obtained by the multiplication of the normalised firing strength by first order of Sugeno fuzzy rule. This layer (defuzzification) defuzzifies the MFs to get the output.

$$O_{4,i} = \overline{w}_i \cdot z_i = \overline{w}_i(p_1 * x + q_1 * y + r_1) \quad (9)$$

Where $\overline{w}_i$ is the output from layer 3 and (p_i,q_i, r_i) are consequent parameters.

Layer 5: The single node computes the overall output through summation of all incoming signals.

$$O_{5,i} = \sum_i \overline{w}_i z_i = \frac{\sum_i w_i z_i}{\sum_i w_i} \quad (10)$$

In this work, the model uses a hybrid-learning algorithm, that combines the gradient method and the least squares estimate to learn parameters. Fuzzy logic toolbox of MATLAB R2015a (Mathworks Inc., USA) was used forthe implementation of the ANFIS modeling.

Optimization studies of the fermentation process

To optimize the objective function (oxalic acid concentration), RSM and GA optimization techniques were used to find the best combination of the process variables examined. For RSM optimization, the oxalic acid concentration was set at maximum while the process variables were set in the ranges investigated. ANN optimization was carried out using the genetic algorithm embedded in the software. For ANFIS optimization, the developed ANFIS model was integrated with the algorithm, which was then used as the fitness functions (Pathak *et al.*, 2015). The GA sought stochastically, the optimum condition that gave the maximum oxalic acid concentration. The characteristics of the GA used are shown in Table 2.

Table 2: Genetic algorithm optimization parameters

Property	Value/Comment
Population size	5-20
Crossover	1/Scattered
Mutation rate	0.01/Uniform
Generation	15-50
Selection	Stochastic uniform
Creation function	Constrain dependent

Comparison of RSM, ANN and ANFIS models

The efficiency of the developed mathematical models namely RSM, ANN and ANFIS in their prediction capabilities of the response were evaluated extensively for the fermentation process. Statistical indicators which include correlation coefficient (R), coefficient of determination (R^2), adjusted R^2, mean square error (MSE), root mean square error (RMSE), standard error of prediction (SEP), mean absolute error (MAE) and average absolute deviation (AAD) were employed for this purpose. Equations 11-18 were used to compute these statistical indices and the results obtained were then compared to determine the best optimization tool.

$$R = \frac{\sum_{i=1}^{n}(x_{p,i}-x_{p,ave}).(x_{a,i}-x_{a,ave})}{\sqrt{\left[\sum_{i=1}^{n}(x_{p,i}-x_{p,ave})^2\right]\left[\sum_{i=1}^{n}(x_{a,i}-x_{a,ave})^2\right]}} \quad (11)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_{a,i}-x_{p,i})^2}{\sum_{i=1}^{n}(x_{p,i}-x_{a,ave})^2} \quad (12)$$

$$Adjusted\ R^2 = 1 - \left[(1-R^2) \times \frac{n-1}{n-k-1}\right] \quad (13)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(x_{p,i} - x_{a,i})^2 \quad (14)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_{p,i} - x_{a,i})^2} \quad (15)$$

$$SEP = \frac{RMSE}{x_{a,ave}} \times 100 \quad (16)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|(x_{a,i} - x_{p,i})\right| \quad (17)$$

$$AAD = \frac{1}{n}\left(\sum_{i=1}^{n}\left(\frac{\left|(x_{a,i}-x_{p,i})\right|}{x_{a,i}}\right)\right) \times 100 \quad (18)$$

Where: n is the number of experimental data,$x_{p,i}$ is the estimated values, $x_{a,i}$ is the experimental values, $x_{a,ave}$ is the average experimental values, $x_{p,ave}$ is the average estimated

values and k is the number of input variables (Ogaga *et al.*, 2017; Betiku *et al.*, 2018).

The coefficient of determination (R^2) gives an indication of consistency between the experimental values and predicted value. The closer the R^2 value is to 1, the better the model fits the actual data. The value of R^2 should be at least 0.8 for a good fit of a model (Guan and Yao, 2008). R^2 is a measure of the amount of the reduction in the variability of the response by using the repressor variables in the model while RMSE and AAD are direct methods for describing deviations. The RMSE, AAD and MAE between predicted and experimental values must be as small as possible (Amenaghawon and Amagbewan, 2017).

Results and Discussion

Analysis of the RSM model

The results of the fermentation process are presented in Table 3 which include the experimental and predicted values. Regression analysis was performed to fit the response. The model developed represents oxalic acid concentration (Y) as a function of KH_2PO_4 (X_1), $MgSO_4$ (X_2) and $NaNO_3$ (X_3). The model is given by Equation 19 in terms of the actual factors.

$$Y = -99.40042 + 164.97610X_1 + 60.93220X_2 + 57.22605X_3 + 10.00000X_1X_2 - 2.37800X_1X_3 - 27.84000X_2X_3 - 104.06440{X_1}^2 - 54.92440{X_2}^2 - 14.35410{X_3}^2 \quad (19)$$

The results of test of significance for every regression coefficient and ANOVA as shown in Table 4. The results showed that the model was significant because of the low p-value (< 0.05). Apart from indicating whether a model is significant or not, the p-value tells whether a term in a model is significant or not and the Fisher test (F value) shows the level of significance for the model terms but do not differentiate between the positive and negative effect of the model terms (Betiku *et al*., 2014). It was observed that model terms X_1, X_3, X_2X_3, ${X_1}^2$, ${X_2}^2$ and ${X_3}^2$were significant because of low pvalues ($p<0.05$) while the termsX_2, X_1X_2 and X_1X_3were not significant. The lack of fit of the model was 0.1036, which is insignificant. It is desirable because we want the model to fit.

Table 3: Comparison of experimental data and RSM predictions

Run No	Coded value of factors			Actual value of factors			Responses (g/l)	
	X_1	X_2	X_3	X_1	X_2	X_3	Actual value	RSM predicted
1	0	0	0	0.75	0.25	1.5	19.57	19.90
2	-1	0	-1	0.50	0.25	1.0	6.50	4.85
3	0	0	0	0.75	0.25	1.5	21.57	19.90
4	-1	1	0	0.50	0.50	1.5	6.73	7.19
5	1	-1	0	1.00	0.00	1.5	11.95	11.49
6	0	0	0	0.75	0.25	1.5	19.57	19.90
7	1	0	1	1.00	0.25	2.0	12.51	14.17
8	0	-1	1	0.75	0.00	2.0	20.46	19.26
9	-1	0	1	0.50	0.25	2.0	11.38	10.99
10	0	0	0	0.75	0.25	1.5	18.54	19.90
11	0	1	-1	0.75	0.50	1.0	12.25	13.45
12	1	1	0	1.00	0.50	1.5	14.06	12.34
13	1	0	-1	1.00	0.25	1.0	8.82	9.35
14	0	-1	-1	0.75	0.00	1.0	6.95	6.88
15	-1	-1	0	0.5	0.00	1.5	7.11	8.83
16	0	0	0	0.75	0.25	1.5	20.25	19.90
17	0	1	1	0.75	0.5	2.0	11.85	11.91

Table 4: Analysis of variance for response surface quadratic model

Source	Sum of squares	df	Mean square	F-value	p-value
Model	450.84	9	50.09	17.29	0.0005
X_1-KH_2PO_4	30.52	1	30.52	10.53	0.0141
X_2-$MgSO_4$	0.31	1	0.31	0.11	0.7524
X_3-$NaNO_3$	58.76	1	58.76	20.28	0.0028
X_1X_2	1.56	1	1.56	0.54	0.4866
X_1X_3	0.35	1	0.35	0.12	0.7372
X_2X_3	48.44	1	48.44	16.72	0.0046
${X_1}^2$	178.12	1	178.12	61.47	0.0001
${X_2}^2$	49.62	1	49.62	17.12	0.0044
${X_3}^2$	54.22	1	54.22	18.71	0.0035
Residual	20.28	7	2.90		
Lack of Fit	15.29	3	5.10	4.09	0.1036
Pure Error	4.99	4	1.25		
Cor Total	471.13	16			

The RSM model was validated by comparing the RSM predicted results with those obtained from the actual experiments and the results are shown in Tables 3. The values predicted by the RSM model were very similar to those of the experiments indicating validity and reliability of the RSM model.

The computed *R*, R^2, and adjusted R^2 for the quadratic regression model were 0.97824, 0.957 and 0.902, respectively (Table 5). These set of high values suggest a good correlation of the experimental and predicted values, and goodness of fit of the model. Adequate precision measures the signal to noise ratio, the value of 11.53 shows an adequate signal for the model. This model can be used to navigate the design space. The coefficient of variance (CV) was 12.58% for the model. The low value of CV observed is indicative of a good model fit.

Table 5: Goodness of fit statistics for RSM model

Parameter	Value
R	0.978
R^2	0.957
Adjusted R^2	0.902
Standard deviation	1.700
Mean	13.530
C.V. %	12.580
PRESS	252.500
Adeq	11.530

Table 6: R^2 and RMSE values of MNFF and MFFF using different training algorithms

Network architecture	Learning algorithm	RMSE	R^2
MNFF	IBP	0.7653	0.9801
	BBP	0.6181	0.9870
	QP	2.8434	0.7254
	GA	1.0945	0.9593
	LM	10.1490	-3.0703
MFFF	**IBP**	**0.5584***	**0.9894***
	BBP	0.5990	0.9878
	QP	1.6397	0.9087
	GA	2.2085	0.8344
	LM	10.1490	-2.4983

Analysis of the ANN model

Two neural network architectures (multilayer normal feed forward and multilayer full feed forward) and topology of the ANN (number of neurons in the hidden layer, transfer functions for both the hidden and output layers) were selected, tested and used for the prediction of oxalic acid concentration. Five training algorithms, incremental back propagation, quick propagation, genetic algorithm, batch back propagation and Levenberg-Marquadt algorithm were all used to train the experimental data. The R^2 and RMSE values obtained from these trainings are shown in Table 6.

From Table 6, it is observed that the IBP was the best training algorithm to predict the oxalic acid concentration. The network was chosen because it gave the least RMSE value (0.5584) and the highest R^2 value (0.9894). The best ANN model obtained in this present study is thus the MFFF incremental back propagation network with Hyperbolic-Tangent as the transfer function for both the hidden and output layers.

Different neural networks with different number of neurons were investigated and the results are shown in Fig. 1. As can be seen from the results obtained, the R^2 value increased as the number of neurons was increased from 1 to 3. This is an indication that the predictive capability of the network was increasing. However, increasing the number of neurons beyond 3 did not result in any change in the R^2 value showing that the predictive capability of the network was not enhanced beyond 3 neurons. Thus, the optimum number of neurons for the network was chosen as 3. Hence, for a neural network with three input factors, four neurons in the hidden layer and 1 factor in the output layer, the corresponding optimum neural networks will be 3-3-1 (Fig. 2). This network topology was used for further studies to predict the concentration of oxalic acid produced. For the data set, the value of R, R^2 and AAD were 0.99469, 0.98941 and 1.1887 (Table 7). The value of R and R^2 shows that there was a good agreement between the experimental and predicted values.

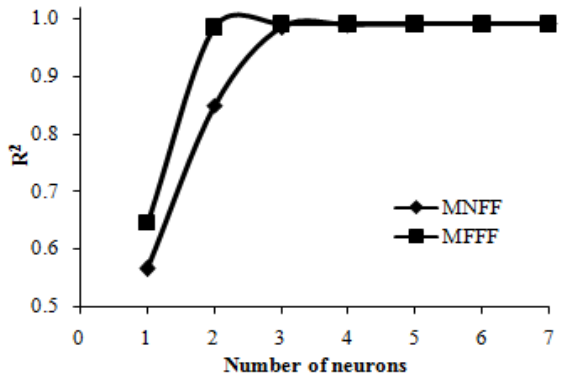

Fig. 1: Determination of optimum number of neurons

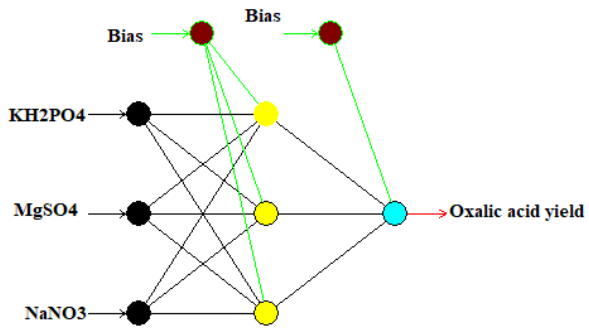

Fig. 2: Architecture of final ANN model

Table 7: Goodness of fit statistics for ANN model

Parameter	Value
R	0.9947
R^2	0.9894
Adjusted R^2	0.9868
MSE	0.2934
RMSE	0.5417
SEP	4.0028
MAE	0.2379
AAD	1.1887

Table 8: Comparison of experimental data and ANN predictions

Run No	Coded value of factors			Actual value of factors			Responses (g/l)	
	X_1	X_2	X_3	X_1	X_2	X_3	Actual value	ANN predicted
1	0	0	0	0.75	0.25	1.5	19.57	19.90
2	-1	0	-1	0.50	0.25	1.0	6.50	6.50
3	0	0	0	0.75	0.25	1.5	21.57	19.90
4	-1	1	0	0.50	0.50	1.5	6.73	6.73
5	1	-1	0	1.00	0.00	1.5	11.95	11.95
6	0	0	0	0.75	0.25	1.5	19.57	19.90
7	1	0	1	1.00	0.25	2.0	12.51	12.51
8	0	-1	1	0.75	0.00	2.0	20.46	20.46
9	-1	0	1	0.50	0.25	2.0	11.38	11.38
10	0	0	0	0.75	0.25	1.5	18.54	19.90
11	0	1	-1	0.75	0.50	1.0	12.25	12.25
12	1	1	0	1.00	0.50	1.5	14.06	14.06
13	1	0	-1	1.00	0.25	1.0	8.82	8.82
14	0	-1	-1	0.75	0.00	1.0	6.95	6.95
15	-1	-1	0	0.5	0.00	1.5	7.11	7.11
16	0	0	0	0.75	0.25	1.5	20.25	19.90
17	0	1	1	0.75	0.5	2.0	11.85	11.85

The ANN model was validated by comparing the ANN predicted results with those obtained from the actual experiments and the results are shown in Tables 8. The values predicted by the ANN model were also very similar to those of the experiments indicating validity and reliability of the ANN model.

Analysis of the ANFIS model

The developed ANFIS model was subjected to neuro fuzzy algorithm which was used to train the appropriate set of training data. For this study, the data was trained to identify the parameters of the Sugeno-type fuzzy inference system (FIS) based on the hybrid learning algorithm which combines gradient descent method and least squares estimate to learn parameters. The best number of membership function for each input was determined as 3 and Gaussian membership function was chosen for the membership grades, while the output of each rule was obtained using a constant defuzzifier formula. The characteristics of the ANFIS model are shown in Table 9 while the architecture of the ANFIS model is shown in Fig. 3. The calculated values of *R*, R^2 and adjusted R^2 were 0.9947, 0.9893 and 0.9868, respectively (Table 10). Also, the value of R^2 shows that 99.47% of the variation between the experimental and predicted values can be described by the model, indicating that only 0.53% could not be explained by the model. The value of *R*, which is ~1 indicates good correlation between the experimental and predicted values. Additionally, R^2 and adjusted R^2> 0.8 are indicative of the goodness of fit of the model (Joglekar and May, 1987).

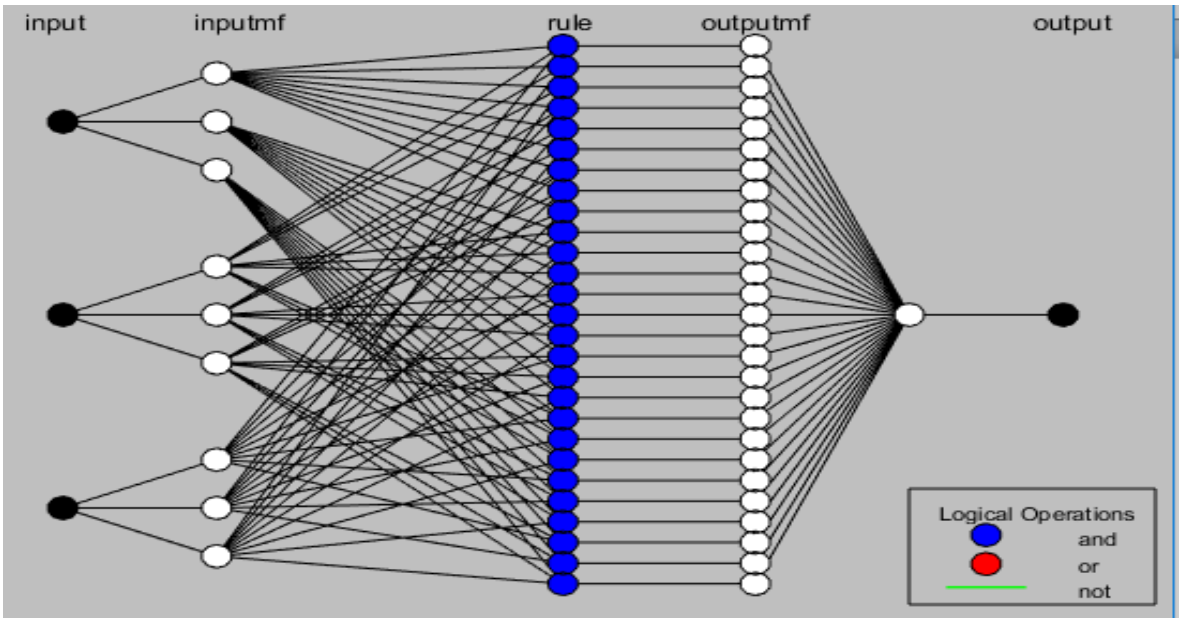

Fig. 3: Architecture of final ANFIS model

Table 9: ANFIS characteristics

Characteristic	Value
Epoch	10
Training error	0.5427
Testing error	0.5427
Number of nodes	78
Number of linear parameters	27
Number of nonlinear parameters	27
Number of fuzzy rules	27

Table 10: Goodness of fit statistics for ANN model

Parameter	Value
R	0.9947
R^2	0.9894
Adjusted R^2	0.9868
MSE	0.2940
RMSE	0.5422
SEP	4.0065
MAE	0.2534
AAD	1.3130

The ANFIS model was validated by comparing the ANFIS predicted results with those obtained from the actual experiments and the results are shown in Tables 11. The values predicted by the ANFIS model were also very similar to those of the experiments indicating validity and reliability of the ANFIS model.

Table 11: Comparison of experimental data and ANFIS predictions

Run No	Coded value of factors			Actual value of factors			Responses (g/l)	
	X_1	X_2	X_3	X_1	X_2	X_3	Actual value	ANFIS predicted
1	0	0	0	0.75	0.25	1.5	19.57	19.90
2	-1	0	-1	0.50	0.25	1.0	6.50	6.50
3	0	0	0	0.75	0.25	1.5	21.57	19.90
4	-1	1	0	0.50	0.50	1.5	6.73	6.72
5	1	-1	0	1.00	0.00	1.5	11.95	11.90
6	0	0	0	0.75	0.25	1.5	19.57	19.90
7	1	0	1	1.00	0.25	2.0	12.51	12.50
8	0	-1	1	0.75	0.00	2.0	20.46	20.50
9	-1	0	1	0.50	0.25	2.0	11.38	11.40
10	0	0	0	0.75	0.25	1.5	18.54	19.90
11	0	1	-1	0.75	0.50	1.0	12.25	12.20
12	1	1	0	1.00	0.50	1.5	14.06	14.10
13	1	0	-1	1.00	0.25	1.0	8.82	8.82
14	0	-1	-1	0.75	0.00	1.0	6.95	6.95
15	-1	-1	0	0.5	0.00	1.5	7.11	7.11
16	0	0	0	0.75	0.25	1.5	20.25	19.90
17	0	1	1	0.75	0.5	2.0	11.85	11.80

Table 12: Performance evaluation of RSM, ANN and ANFIS models

Parameter	RSM	ANN	ANFIS
R	0.9782	0.9947	0.9947
R^2	0.9570	0.9894	0.9893
Adjusted R^2	0.902	0.9868	0.9868
MSE	1.1931	0.2934	0.2940
RMSE	1.0923	0.5417	0.5422
SEP (%)	8.0714	4.0028	4.0065
MAE	0.8999	0.2379	0.2534
AAD (%)	7.8692	1.1887	1.3130

Performance evaluation of RSM, ANN and ANFIS models

The efficiency of the developed RSM, ANN and ANFIS models to predict the oxalic acid concentration for the fermentation process was statistically assessed by evaluating their R, R^2, adjusted R^2, mean square error (MSE), root mean square error (RMSE), standard error of prediction (SEP), mean absolute error (MAE) and average absolute deviation (AAD) as shown in Table 12.

The value of R should be close to 1 for good correlation between experimental and predicted values. Also, the three models had high values of R^2 indicating good fit of the models. For good correlation between experimented and predicted values, the value of R should be at least 0.8 (Guan and Yao, 2008). The adjusted R^2 was employed in checking overestimation of R^2, and they were also high for the three models, demonstrating model significance (Betiku *et al.*, 2018). MSE, a measure of closeness of a fitted line to data points, was determined for the three models. The RMSE, which is the square root of the MSE, was also calculated for the models. The values obtained for both MSE and RMSE were all low, supporting good fit of the models. SEP, MAE and AAD measure the accuracy and precision of a model. The lower the values of these statistical indices, the better the performance of the model (Sarve *et al*., 2015). These were all determined and their associated values are presented in Table 12. From the result of the statistical indicators, ANN and ANFIS were both superior to RSM, although comparing the result of ANN and ANFIS, it was seen that ANN was slightly better than ANFIS in predictive capability. This is seen from the high values of R, R^2 and adjusted R^2 for both ANN and ANFIS compared to that of RSM and their (ANN and ANFIS) very low values of MSE, RMSE, SEP, MAE and AAD.

The superiority of ANN to RSM has been reported in many reports (Betiku *et al*., 2014; Ajala and Betiku, 2015; Sarve, Sarve *et al*., 2015) while the reports for the performance comparison between ANN and ANFIS have been mixed. In some literature, ANN in terms of its predictive capability is superior to ANFIS (Kiran and Rajput, 2011; Betiku *et al.*, 2016) while ANFIS outperformed ANN in other reports (Sedighi *et al*., 2011; Rahmanian *et al.*, 2012; Ramzi *et al.*, 2015). In this work, while the results obtained from ANN and ANFIS were close, ANN was slightly better than ANFIS.

Effect of factors on oxalic acid production

Three-dimensional response surface plots were used to investigate the influence of the factor interactions on oxalic acid concentration (Figs. 4 to 6). The resulting response surface shows the effect of KH_2PO_4, $NaNO_3$ and $MgSO_4$ on oxalic acid produced. The contour plot may be rising ridges, saddle point, elliptical or circular plot. The shape of the 3D plots shows there are significant interactions among factors considered in this work. The dome shape of the plots (Figs. 4 and 5) indicate that there are shared interactions among factors. Other shapes of the plot indicated a striking significant interactions among factors considered (Betiku *et al*., 2016).

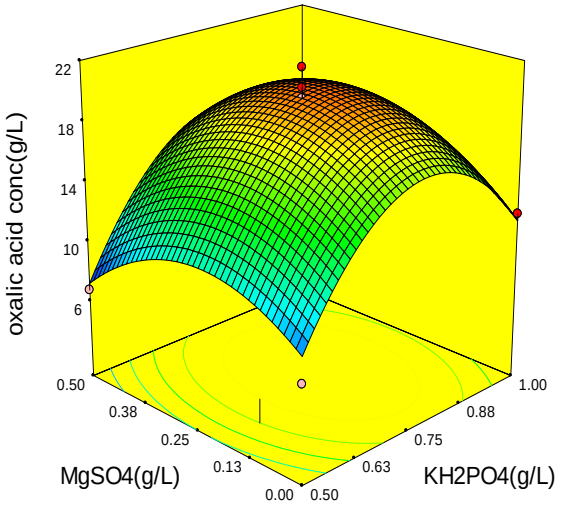

Fig. 4: Response surface showing the effect of $MgSO_4$ and KH_2PO_4 on oxalic acid concentration

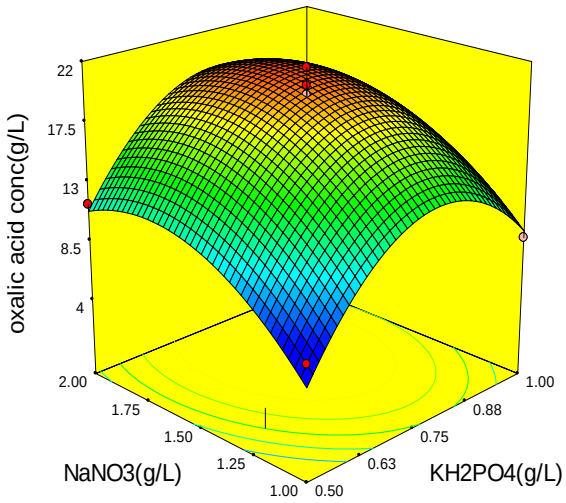

Fig. 5: Response surface showing the effect of $NaNO_3$ and KH_2PO_4 on oxalic acid concentration

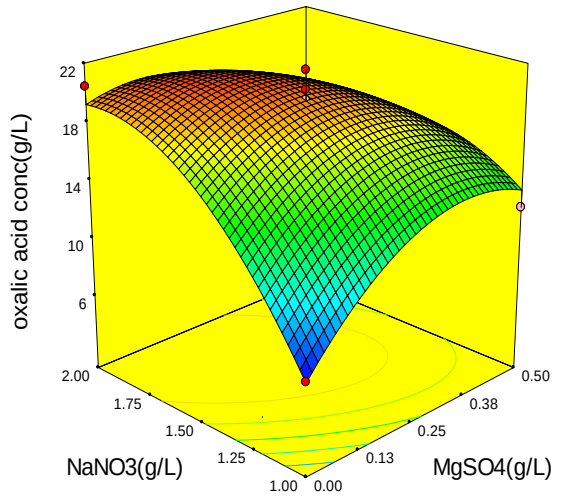

Fig. 6: Response surface showing the effect of $NaNO_3$ and $MgSO_4$ on oxalic acid concentration

From Fig. 4, it can be seen that intermediate values of $MgSO_4$ and KH_2PO_4 gave optimum value of oxalic acid concentration as a result of the parabolic shape of the 3D plot. Generally, the presence of these salts in the fermentation medium enhanced oxalic acid production. For instance, the increase in oxalic acid yield due to the addition of magnesium sulphate could be attributed to the fact that magnesium is essential for the growth and metabolic activity of *Aspergillus niger* in addition to its role in various enzymatic reactions within the microbial cell (Shankaranand and Lonsane, 1994). Previous reports have also indicated the positive influence of phosphorus (KH_2PO_4) during fermentation (Amenaghawon *et al*., 2015). An increase in $NaNO_3$ also increased oxalic acid production (Fig. 6) and similar observations have also been reported (Emeko *et al*., 2015).

Optimization of process variables by RSM, ANN and ANFIS

The maximum oxalic acid concentration was determined using the RSM, ANN and ANFIS models with genetic algorithm. Optimization of the RSM model was done using the Design Expert software while for the ANN and ANFIS models, both were genetic algorithm was adopted in Neural Power and MATLAB R2015a, respectively.

Table 13: Optimized conditions

Model	KH_2PO_4 (g/L)	$MgSO_4$ (g/L)	$NaNO_3$ (g/L)	Oxalic acid (g/L)	R^2
RSM	0.78	0.18	1.75	20.73	0.9570
ANN-GA	0.77	0.09	1.78	20.73	0.9894
ANFIS-GA	0.78	0.19	1.71	20.80	0.9893

From the results of optimized conditions for the three models (Table 13), it was observed that ANFIS gave the highest concentration of oxalic acid. However, this value was not far from what RSM and ANN gave. Based on the combination of the optimized conditions of the input variables, ANN proved to be the best as it utilizes a very small amount of $MgSO_4$ to produce 20.73 g/L of oxalic acid with the values of the other two variables being approximately equivalent to the values of the other models (RSM and ANFIS). ANFIS was better when compared to RSM as the combination of their optimized input variables were almost the same with ANFIS giving a higher yield. A closer look at the R^2 value indicated that ANN was the best modeling tool because of its high R^2 value although this value (0.9894) was slightly higher than that for ANFIS (0.9893) making both significantly superior to RSM.

Conclusion

This study compared the performance of RSM, ANN and ANFIS as modeling and optimization tools for the production of oxalic acid from pineapple juice. Oxalic acid production was enhanced by the presence of all three medium components. Based on statistical indices evaluated, the performance of the models follows the order: ANN>ANFIS>RSM. ANN is considered to be superior to both RSM and ANFIS because of it R^2 being the highest RMSE being the lowest. Maximum concentration of oxalic acid obtained was 20.732, 20.725 and 20.8 g/L for RSM, ANN-GA and ANFIS-GA, respectively. ANN-GA was considered to be more efficient because it gave the best combination of input and output variables. The best combination of the optimum level of process factors was thus 0.77 g/L of KH_2PO_4, 0.09 g/L of $MgSO_4$ and 1.78 g/L of $NaNO_3$ resulting to a maximum oxalic acid concentration of 20.73 g/L. Thus, this work has demonstrated the choice of modeling and optimization techniques employed in oxalic acid production via fermentation.

Conflict of Interest

Authors declare there is no conflict of interest related to this study.

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