

Appraisal of Artificial Neural Network and Response Surface Methodology in Modeling and Process Variable Optimization of Oxalic Acid Production from Cashew Apple Juice: A Case of Surface Fermentation

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This study assessed the effects and interactions of cashew apple juice (CAJ) concentration, pH, time, methanol concentration, and NaNO₃ concentration on oxalic acid fermentation in a central composite design. The efficacies of artificial neural network (ANN) and response surface methodology (RSM) in modeling and optimizing the process were evaluated using correlation coefficient (R), coefficient of determination (R^2), and absolute average deviation (AAD). The highest oxalic acid production observed was 120.66 g/L under optimum values of a CAJ concentration of 291 g/L, pH of 6.9, time of 10.82 days, methanol concentration of 2.91% (v/v), and NaNO₃ concentration of 1.05 g/L that were numerically predicted by the developed RSM quadratic model. Using the developed ANN model coupled with rotation inherit optimization, the highest oxalic acid production observed was 286.75 g/L under the following optimum values: CAJ of 291 g/L, pH of 6.5, time of 12.64 days, methanol concentration of 3.82% (v/v), and NaNO₃ concentration of 2.41 g/L. The results showed that the ANN model ($R = 0.9996$, $R^2 = 0.9999$, AAD = 0.21%) was better than the RSM model ($R = 0.9986$, $R^2 = 0.9973$, AAD = 1.00%) for optimizing oxalic acid fermentation. The use of the ANN model led to a 2.4-fold increase in oxalic acid yield over the RSM model.

Keywords: Cashew apple juice; Oxalic acid; Central composite design; Fungi; Modeling

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INTRODUCTION

Oxalic acid, also known as ethanedioic acid, is a dicarboxylic acid with some industrial applications such as bioleaching, an ingredient in food preservatives, and it is also used as a cleaning agent (Gürü *et al.* 2001). Oxalic acid combines with metals such as calcium, sodium, potassium, magnesium, or iron to form less soluble salts. As of 2007, the annual production of oxalic acid was 124,000 tons (Sauer *et al.* 2008), and the bulk of the production is done through chemical processes (Nakata and He 2010).

There have been reports on microbial production of oxalic acid using fermentative processes. This is an emerging development and is desirable because of its environmental friendliness. Some of the microorganisms considered for oxalic acid production include *Burkholderia glumae* (Nakata and He 2010), *Paxillus involutus* (Lapeyrie *et al.* 1987), *Aspergillus ficuum* (Strasser *et al.* 1994), *Penicillium oxalicum* (Ikotum 1984), *Gleophyllum trabeum* (Strasser *et al.* 1994), *Sclerotium rolfisii* (Chakrabarti and Samajpati 1980), and *Aspergillus niger* (Strasser *et al.* 1994; Ruijter *et al.* 1999; Santoro *et al.* 1999; Rymowicz

and Lenart 2004; Musiał *et al.* 2011). *A. niger*, however, remains the best microorganism for microbial oxalic acid production because of its ease of handling, its ability to utilize diverse, cheap, raw materials, and its high yields.

To develop an economically viable fermentation process for commercial oxalic acid production, it is necessary to use cheap substrates, select the right fermentation technique, and carry out optimization of the fermentation variables that are involved. Some carbon substrates that have been evaluated for oxalic acid production using *A. niger* include green syrup, lactose permeate (Strasser *et al.* 1994), lipids (Rymowicz and Lenart 2003), glucose (Ruijter *et al.* 1999; Mandel and Banerjee 2005), biodiesel-derived waste glycerol (Musiał *et al.* 2011), sugar beet molasses (Podgorski and Leśniak 2003), sweet potato peel (Ayodele 2012), and milk whey (Santoro *et al.* 1999). There is, however, a dearth of information on the use of cashew apple juice (CAJ) for oxalic acid production. This juice is rich in reducing sugars such as fructose, glucose, and sucrose, as well as vitamins, minerals, and some amino acids (Layokun *et al.* 1986). The prospect of using CAJ as the sole carbon source for fermentation processes such as the production of biosurfactants, lactic acid, wine, single cell proteins, and mannitol have been investigated in several studies (Layokun *et al.* 1986; Osho 1995; Rocha *et al.* 2006; Honorato *et al.* 2007).

Possible fermentation techniques available for oxalic acid production are solid-state fermentation, submerged fermentation, and surface fermentation. Most of the literature on microbial production of oxalic acid involves the use of submerged fermentation. Shierholt (1978) reported a detailed cost analysis study on citric acid production using *A. niger* under both surface and submerged fermentation processes. The author demonstrated several advantages of surface fermentation over submerged fermentation. Overall, surface fermentation was projected as a cheaper technique than submerged fermentation. In previous works, oxalic acid was produced using *A. niger* grown on CAJ under submerged fermentation. The process was modeled and optimized using RSM with a maximum oxalic acid concentration of 122.68 g/L (Emeko 2014). Thus, this present work employed surface fermentation for the microbial production of oxalic acid.

Optimization of fermentation variables in oxalic acid production using *A. niger* is critical to maximizing its productivity. Ruijter *et al.* (1999) reported that pH affects oxalic acid production in *A. niger* and maximum oxalic acid production is observed at pH 6 (Bohlmann *et al.* 1998). The maximum oxalic acid production in *A. niger* has been reported to occur on the 7th and the 9th day of fermentation (Strasser *et al.* 1994; Rymowicz and Lenart 2004; Mandal and Banerjee 2005). This demonstrates that time is an important variable in oxalic acid fermentation. Also, the use of methanol in enhancing oxalic acid production in *A. niger* has been demonstrated. Rymowicz and Lenart (2004) investigated the addition of methanol (1.5% w/v) to the fermentation medium for oxalic acid production using *A. niger* and reported a 1.4-fold increase in the amount of acid produced.

Typically, optimization of processes is carried out by one-factor-at-a-time approach, but this method is time-consuming and cumbersome and has been replaced with artificial neural networks (ANNs) and response surface methodology (RSM). For example, Betiku and Taiwo (2015) evaluated the performances of ANN and RSM in modeling and optimization studies on ethanol production from breadfruit starch hydrolyzate. Also, both ANN and RSM were applied to the extraction of seed oil from yellow oleander seeds (Ajala and Betiku 2014). The authors reported that ANN was better than RSM with respect to data fitting and prediction capability.

In this study, an attempt was made to apply ANN and RSM in the modeling and optimization of the essential variables of CAJ concentration, pH, time, methanol concentration, and NaNO_3 concentration in oxalic acid production using the filamentous fungus *A. niger* under surface fermentation. The models developed were evaluated for their effectiveness in predicting oxalic acid production as well as in data fitting by determining multiple coefficients of correlation (R), coefficients of determination (R^2), and absolute average deviation (AAD) from the experimental data. The oxalic acid concentrations predicted by the models were validated experimentally. Additionally, a mathematical model was developed by RSM to describe the oxalic acid fermentation process.

EXPERIMENTAL

Materials and Methods

Microorganism

The strain *Aspergillus niger* used in this work was obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. *A. niger* spores were produced on Sabouraud dextrose agar (SDA) for 5 to 7 days at 30 °C. The fungus was maintained on SDA plates at 4 °C and sub-cultured regularly.

Cashew apple juice (CAJ) preparation

Freshly harvested ripe cashew apple fruits were obtained from the market in Ile-Ife, Nigeria. The foreign materials and dirt in the fruits were removed by washing with water. CAJ was obtained mechanically by pressing the apples. The CAJ was characterized physically and chemically and centrifuged at 10,000 g for 10 min; the supernatant was decanted and sterilized using an autoclave and stored at -20 °C until further use. The characterization of the CAJ is reported elsewhere (Emeko 2014).

Medium composition for oxalic acid production

The fermentation medium described by Strasser *et al.* (1994) employed in this study was composed of (g/L) CAJ as carbon source; NaNO_3 , 1.5; KH_2PO_4 , 0.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025; KCl, 0.025; and yeast extract, 1.6. The medium pH was adjusted manually to 6.0 with 4 M NaOH solution before sterilization. Twenty milliliters of universal pH indicator solution (Burgoyne Burbidges and Co, India) was then added per liter of medium (*i.e.*, for each 50 mL of medium, 1 mL of universal pH indicator solution was added) to observe the culture pH, which was maintained at 6.0 ± 0.5 with 4 M NaOH solution during fermentation. All media were sterilized using an autoclave at 121 °C for 15 min before use.

Inoculum preparation and oxalic acid production by surface fermentation

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 (New Brunswick Scientific Co USA) at 3.3 Hz and 30 °C for 1 h before it was used for the fermentation (Emeko 2014). For the main experiment, 50 mL of CAJ was measured into a 250-mL Pyrex flask and the nutrients were added. The pH of the media was adjusted to 6.0 ± 0.5 using with 4 M NaOH solution. Then, 5% (v/v) of inoculum size was added aseptically to the flasks, which were

placed on a clean table for surface fermentation. To monitor the culture pH, 1 mL of universal indicator solution was added per 50 mL of the medium while 4 M NaOH solution was used to maintain the culture pH at the set points described by the central composite design (CCD). CAJ consumption and oxalic acid production rates were monitored during the course of fermentation.

Experimental design

CAJ concentration, pH, time, methanol concentration, and NaNO₃ concentration were chosen as independent variables and designated as X_1 , X_2 , X_3 , X_4 , and X_5 , respectively (Table 1). It has been shown that these variables affect oxalic acid production under submerged fermentation (Emeko 2014). A central composite design (CCD) that is suitable for fitting complex surfaces when a second-order model is assumed was chosen to determine the experimental conditions used in this study. A three-level, five-factor fractional factorial design was applied, which produced 26 runs that included 11 factorial points, 10 axial points, and 5 central points to provide information regarding the interior of the experimental region. The center point was repeated five times to determine the repeatability of the method (Montgomery 2001). The range of experimental conditions with their coded and uncoded levels ($-\alpha$, -1, 0, 1, α ; $\alpha = 1.821$) is shown in Table 2. The experiments were designed using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA).

Table 1. Codes and Actual Levels of Independent Variables for OA Fermentation

Factor	Unit	Symbols	Coded factors				
			Axial ($-\alpha$)	-1	0	1	Axial ($+\alpha$)
CAJ	g/L	X_1	109	150	200	250	291
pH	-	X_2	4.18	5	6	7	7.82
Time	day	X_3	5.36	7	9	11	12.64
Methanol	% (v/v)	X_4	0.18	1	2	3	3.82
NaNO ₃	g/L	X_5	0.59	1	1.5	2	2.41

A quadratic mathematical equation, including all interaction terms, was used to calculate the response,

$$Y = \gamma_0 + \gamma_1 X_1 + \gamma_2 X_2 + \gamma_3 X_3 + \gamma_4 X_4 + \gamma_5 X_5 + \gamma_{12} X_1 X_2 + \gamma_{13} X_1 X_3 + \gamma_{14} X_1 X_4 + \gamma_{15} X_1 X_5 + \gamma_{23} X_2 X_3 + \gamma_{24} X_2 X_4 + \gamma_{25} X_2 X_5 + \gamma_{34} X_3 X_4 + \gamma_{35} X_3 X_5 + \gamma_{45} X_4 X_5 + \gamma_{11} X_1^2 + \gamma_{22} X_2^2 + \gamma_{33} X_3^2 + \gamma_{44} X_4^2 + \gamma_{55} X_5^2 \quad (1)$$

where Y is the oxalic acid concentration expressed in g/L, γ_0 is the intercept term, γ_1 , γ_2 , γ_3 , γ_4 , and γ_5 are the linear coefficients, γ_{12} , γ_{13} , γ_{14} , and γ_{15} are the interaction coefficients, γ_{11} , γ_{22} , γ_{33} , γ_{44} , and γ_{55} are the quadratic coefficients, and X_1 , X_2 , X_3 , X_4 , and X_5 are the coded independent variables. The statistical analysis was performed using Statistica 12 software (Statsoft Inc., Tulsa, OK, USA).

Modeling by artificial neural network (ANN)

ANN is a learning system based on a computational technique that can simulate the neurological processing ability of the human brain. NeuralPower, version 2.5 (CPC-X Software), was employed throughout this work.

Table 2. CCD for the OA Production with Five Independent Factors

Run	X ₁ (g/L)	X ₂	X ₃ (day)	X ₄ % (v/v)	X ₅ (g/L)	Observed OA (g/L)	ANN Predicted OA (g/L)	RSM Predicted OA (g/L)
1	200 (0)	6 (0)	9 (0)	0.18 (-α)	1.5 (0)	64.08	64.25	64.93
2	150 (-1)	7 (1)	7 (-1)	3 (1)	2 (1)	56.58	56.58	56.11
3	150 (-1)	5 (-1)	7 (-1)	1 (-1)	1 (-1)	51.42	51.40	50.49
4	150 (-1)	5 (-1)	11 (1)	3 (1)	2 (1)	57.75	57.77	57.28
5	200 (0)	6 (0)	9 (0)	2 (0)	0.59 (-α)	70.42	70.43	71.27
6	200 (0)	6 (0)	9 (0)	2 (0)	1.5 (0)	94.72	92.94	92.37
7	150 (-1)	7 (1)	11 (1)	3 (1)	1 (-1)	60.17	60.17	59.70
8	200 (0)	6 (0)	9 (0)	2 (0)	1.5 (0)	93.00	92.94	92.37
9	200 (0)	6 (0)	5.36 (-α)	2 (0)	1.5 (0)	50.25	50.25	51.10
10	108.94 (-α)	6 (0)	9 (0)	2 (0)	1.5 (0)	84.36	84.36	85.21
11	200 (0)	4.18 (-α)	9 (0)	2 (0)	1.5 (0)	63.17	62.98	64.02
12	150 (-1)	7 (1)	11 (1)	1 (-1)	2 (1)	63.92	63.91	63.45
13	250 (1)	7 (1)	11 (1)	1 (-1)	1 (-1)	77.08	77.06	76.61
14	200 (0)	6 (0)	12.64 (α)	2 (0)	1.5 (0)	60.00	60.00	60.85
15	291.06 (α)	6 (0)	9 (0)	2 (0)	1.5 (0)	107.42	107.43	108.27
16	250 (1)	5 (-1)	11 (1)	3 (1)	1 (-1)	85.58	85.60	85.11
17	200 (0)	6 (0)	9 (0)	2 (0)	1.5 (0)	92.00	92.94	92.37
18	200 (0)	6 (0)	9 (0)	2 (0)	1.5 (0)	93.00	92.94	92.37
19	200 (0)	6 (0)	9 (0)	3.82 (α)	1.5 (0)	80.00	79.99	80.85
20	250 (1)	7 (1)	7 (-1)	1 (-1)	2 (1)	63.08	62.89	62.61
21	250 (1)	5 (-1)	7 (-1)	3 (1)	2 (1)	62.00	62.24	61.53
22	200 (0)	6 (0)	9 (0)	2 (0)	1.5 (0)	92.00	92.94	92.37
23	200 (0)	7.82 (α)	9 (0)	2 (0)	1.5 (0)	61.00	61.01	61.85
24	200 (0)	6 (0)	9 (0)	2 (0)	2.41 (α)	81.03	81.03	81.88
25	250 (1)	5 (-1)	11 (1)	1 (-1)	2 (1)	67.00	67.00	66.53
26	250 (1)	7 (1)	7 (-1)	3 (1)	1 (-1)	68.42	68.41	67.95

Bold numbers indicate ANN testing set; normal numbers indicate ANN training set; α denotes the axial point with a coded level of 1.821

Multilayer normal feedforward and multilayer full feedforward neural networks were used to predict the oxalic acid production. Networks were trained by different learning algorithms, *i.e.*, incremental back propagation (IBP), batch back propagation (BBP), quickprob (QP), and genetic algorithms. The ANN architecture included an input layer with five neurons, an output layer with one neuron, and a hidden layer. To determine the optimal network topology, only one hidden layer was used and the number of hidden and output layers (sigmoid, hyperbolic tangent (Tanh), Gaussian, linear, threshold linear,

and bipolar linear) were iteratively determined by developing several networks (Betiku and Taiwo 2015). A total of 17 of the experimental data sets were used to train the network, and the remaining data were used for testing (Table 2).

Verification of estimated data

The estimation capabilities of the ANN and RSM models were assessed. To do this, a way in which the model output error between the experimental and predicted values that could be evaluated is required (Baş and Boyacı 2007). The multiple coefficient of correlation (R), coefficient of determination (R^2), and absolute average deviation (AAD) were determined, and their values were used together to assess RSM and ANN models by comparing the evaluated values for the models. The accuracies of the models were determined by evaluating R , R^2 , and AAD values. R shows the correlation between the predicted and experimental values while R^2 depicts the degree of fit for the mathematical model. AAD describes the level of accuracy of a model prediction.

AAD is defined in Eq. (2) as,

$$\text{AAD (\%)} = \left(\frac{1}{n} \sum_{i=1}^n \left(\frac{y_{\text{pred}} - y_{\text{exp}}}{y_{\text{exp}}} \right) \right) \times 100 \quad (2)$$

where y_{pred} and y_{exp} are the predicted and observed responses, respectively, and n is the number of the experimental data. The model with the lowest AAD and highest R^2 is considered best (Betiku and Taiwo 2015).

Reducing sugar analysis

The method of Saqib and Whitney (2011), which was modified from the dinitrosalicylic acid (DNS) method of Miller (1959), was used to determine the CAJ concentration, expressed as sucrose. Three milliliters of the DNS solution was added to 1 mL of the supernatant in a test tube and was boiled for 15 min, cooled, and diluted appropriately. Afterwards the absorbance was measured at a wavelength of 540 nm using a UV-visible spectrophotometer (Libra 21 Model, UK).

Determination of oxalic acid concentration

A catalytic kinetic spectrophotometric method was used for the determination of oxalic acid produced in this study (Jiang *et al.* 1996). It is based on the acid catalytic effect on the redox reaction between dichromate and rhodamine B, measured at the maximum absorption wavelength of 555 nm in sulfuric acid (H_2SO_4). For the assay, 10 mL of sample was withdrawn from the culture medium and filtered with Whatman No. 1 filter paper. Subsequently, 1 mL of the filtrate was added to 0.50 mL of 0.06 M potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$). Then, 0.20 mL of 2.5 M H_2SO_4 and 0.10 mL of 3.28×10^{-4} M rhodamine B were combined and brought up to 10 mL in a test-tube and mixed thoroughly. The mixture was placed in a water bath at 90 °C for 8 min. The reaction was quenched by cooling under tap water. The absorbance of the mixture was read at 555 nm against the blank solution. The oxalic acid produced was quantitated using a standard curve prepared with synthetic oxalic acid.

RESULTS AND DISCUSSION

Modeling and Optimization by RSM

Five independent variables known to influence the microbial production of oxalic acid using *A. niger* were studied for their individual and reciprocal effects using RSM. A total of 26 experimental runs were generated and subsequently carried out. The results of these experiments are presented in Table 2; they showed stochastic variations in the observed responses, indicating the impact of the different variables on the oxalic acid fermentation. The data were fitted to the following CCD response surface quadratic model:

$$\begin{aligned}
 Y = & -325.04 - 0.73X_1 + 68.69X_2 + 25.93X_3 + 45.16X_4 + 128.77X_5 + 0.07X_1X_2 \\
 & + 0.05X_1X_3 + 0.01X_1X_4 - 0.17X_1X_5 + 2.59X_2X_3 - 0.52X_2X_4 \\
 & + 1.08X_2X_5 + 0.39X_3X_4 - 1.46X_3X_5 - 12.48X_4X_5 + 0.001X_1^2 - 8.89X_2^2 \\
 & - 2.75X_3^2 - 5.88X_4^2 - 19.08X_5^2 \quad (3)
 \end{aligned}$$

where Y is the oxalic acid concentration in g/L as a function of CAJ concentration (X_1), pH (X_2), time (X_3), methanol concentration (X_4), and NaNO_3 concentration (X_5).

The accuracy of the regression equation was evaluated using the multiple coefficient of correlation (R) and coefficient of determination (R^2). Plots of predicted vs. observed values are shown in Fig. 1. The value of R (0.9986) of the model showed agreement between the experimental and predicted values. R^2 for the model was 0.9973, which indicated that a sample variation of 99.73% for oxalic acid produced is attributed to the independent variables and only 0.27% of the total variation was not explained by the model (Betiku and Taiwo 2015). The adjusted coefficient of determination ($R^2_{\text{adj}} = 0.9862$) was high enough to suggest the significance of the model. For a good model fit, R^2 should be at least 0.80 (Joglekar and May 1987).

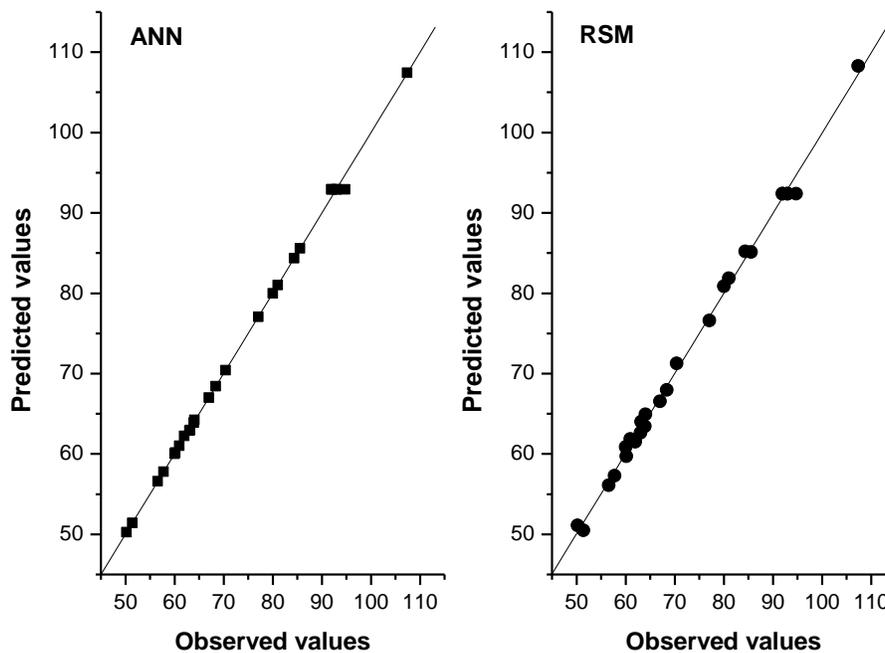


Fig. 1. Parity plots of predicted values versus observed values for both ANN and RSM models

The residuals between the experimental values and predicted values give an assessment of the model. The correspondence plot of predicted values *versus* observed values indicated the accuracy of the model (Fig. 1). Also, the F -value of 90.62 with a corresponding low p -value ($p < 0.0001$) suggested that the model developed was significant.

Table 3 shows the results of the significance test and ANOVA of the regression equation model. The p -values of the model terms were significant ($p < 0.05$), except for pH and the interactions of CAJ concentration with methanol concentration, pH with methanol concentration, pH with NaNO_3 concentration, time with methanol concentration, and time with NaNO_3 concentration (Table 3).

Table 3. Test of Significance for Every Regression Coefficient and ANOVA

Factor	Sum of squares	df	Mean square	F -value	p -value
X_1 (CAJ)	265.88	1	265.88	79.21	0.0003
X_1^2	35.85	1	35.85	10.68	0.0222
X_2 (pH)	2.35	1	2.35	0.70	0.4405
X_2^2	1634.19	1	1634.19	486.84	<0.0001
X_3 (Time)	47.53	1	47.53	14.16	0.0131
X_3^2	2498.15	1	2498.15	744.22	<0.0001
X_4 (Methanol)	126.72	1	126.72	37.75	0.0017
X_4^2	715.91	1	715.91	213.28	<0.0001
X_5 (NaNO_3)	56.29	1	56.29	16.77	0.0094
X_5^2	470.75	1	470.75	140.24	<0.0001
X_1X_2	34.80	1	34.80	10.37	0.0235
X_1X_3	88.11	1	88.11	26.25	0.0037
X_1X_4	0.21	1	0.21	0.06	0.8135
X_1X_5	55.44	1	55.44	16.52	0.0097
X_2X_3	81.81	1	81.81	24.37	0.0043
X_2X_4	0.83	1	0.83	0.25	0.6411
X_2X_5	0.90	1	0.90	0.27	0.6275
X_3X_4	1.90	1	1.90	0.57	0.4860
X_3X_5	6.52	1	6.52	1.94	0.2223
X_4X_5	118.96	1	118.96	35.44	0.0019
ANOVA					
Model	6083.79	20	304.19	90.62	<0.0001
Error	16.78	5	3.36		
Total sum of squares	6100.58	25			

$$R^2 = 0.9973, \quad \text{Adjusted } R^2 = 0.9862$$

Because p -value and F -value do not distinguish between a positive and a negative significance effect on a model (Oyeniran *et al.* 2013; Betiku and Taiwo 2015), a Pareto chart was prepared to examine the standardized effects and interactions of CAJ concentration, pH, time, methanol concentration, and NaNO_3 concentration on oxalic acid production (Fig. 2). The positive coefficients for the model terms (CAJ, methanol, NaNO_3 , time, CAJ x time, pH x time, CAJ x CAJ, and CAJ x pH) showed a favorable or cooperative interaction effect on oxalic acid produced, but the negative coefficients for the model terms (pH x pH, time x time, methanol x methanol, NaNO_3 x NaNO_3 , methanol x NaNO_3 , and CAJ x NaNO_3) showed an unfavorable effect on oxalic acid production (Oyeniran *et al.* 2013; Papagora *et al.* 2013; Shanmugaparakash *et al.* 2014; Betiku and Taiwo 2015). The linear effect of CAJ concentration was the most positive significant model term for oxalic

acid production, followed by methanol concentration, interactions of CAJ concentration with time, pH with time, linear effect of NaNO_3 , interaction of CAJ with NaNO_3 concentration, and linear effect of time (Fig. 2). The linear term of pH as well as the interactions of time with NaNO_3 , time with methanol, pH with NaNO_3 , pH with methanol, and CAJ with methanol remained inside the reference line (Fig. 2). These terms remaining inside the reference line corroborate a lack of significance as indicated by $p > 0.05$ in Table 3 (Oyeniran *et al.* 2013; Papagora *et al.* 2013; Shanmugaprakash *et al.* 2014; Betiku and Taiwo 2015).

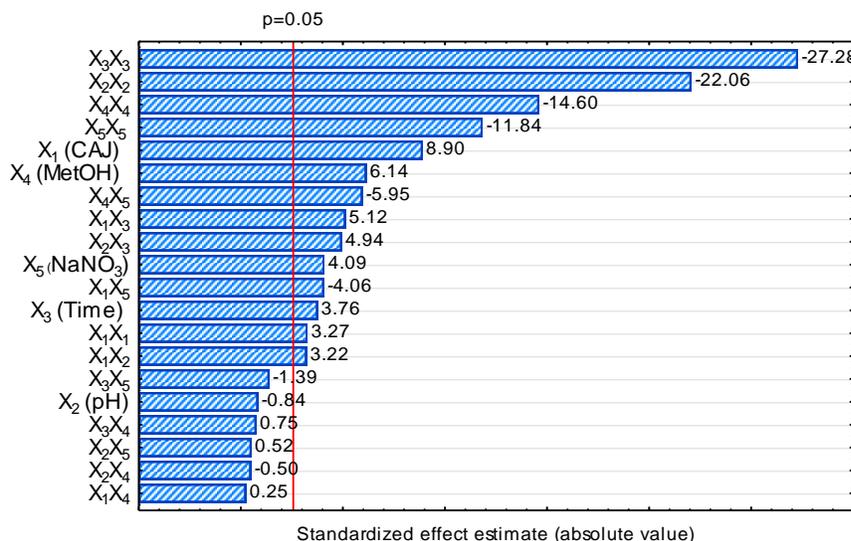


Fig. 2. Pareto chart of standardized effects for oxalic acid fermentation

The optimum values of the independent variables selected for the fermentation process were obtained by applying regression analysis to Eq. (3) using Statistica 12 software. The optimum levels were numerically predicted as CAJ concentration of 291 g/L, pH of 6.9, time of 10.82 days, methanol concentration of 2.91% (v/v), and NaNO_3 concentration of 1.05 g/L with a corresponding oxalic acid concentration of 121 g/L. The model was validated by applying the optimum values to three independent experimental replicates, and the average value of oxalic acid concentration obtained was 120.66 g/L (equivalent yield of 0.415 g/g).

The effects of pH on oxalic acid production in *A. niger* have been reported, and the highest yield of oxalic acid was observed at pH 6 (Bohlmann *et al.* 1998; Ruijter *et al.* 1999; Mandal and Banerjee 2005). The optimum pH value predicted in this study was within the range earlier reported. The maximum oxalic acid production in *A. niger* has been reported to occur on the 9th day of fermentation (Strasser *et al.* 1994; Rymowicz and Lenart 2004). The optimum time predicted in this study was 10.82 days, which is close to the time reported by earlier works. Similarly, as demonstrated by Rymowicz and Lenart (2004), the addition of methanol to the fermentation medium improves oxalic acid production in *A. niger*, which was also confirmed by the present study.

In previous reports on RSM optimization of oxalic acid in a submerged fermentation of CAJ using *A. niger*, the maximum oxalic acid production observed was 122.68 g/L, which, in terms of yield, is 0.818 g/g (Emeko 2014). These results showed that the oxalic acid yield from submerged fermentation is double the yield obtained from surface fermentation employed in the present work, suggesting the superiority of submerged fermentation over surface fermentation.

Relationship between the Selected Variables for the Oxalic Acid Fermentation

Visual observations of the relationship among the selected variables for the oxalic acid fermentation were determined using Statistica 12 software (Statsoft Inc., Tulsa, OK, USA). Figure 3 shows the contour and surface response plots for the optimization of fermentation variables for oxalic acid production.

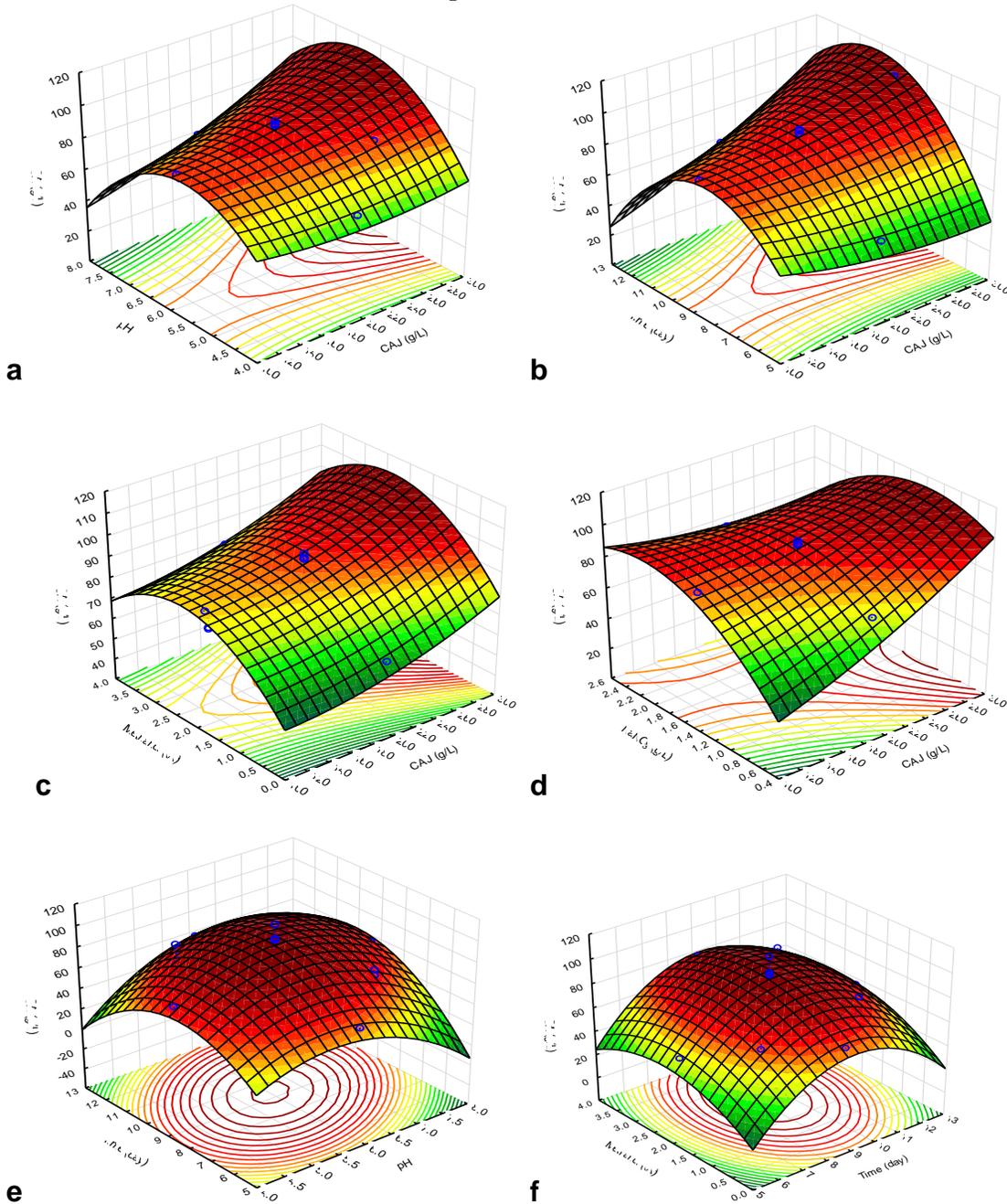


Fig. 3 (a-f). Response surface plots for oxalic acid fermentation

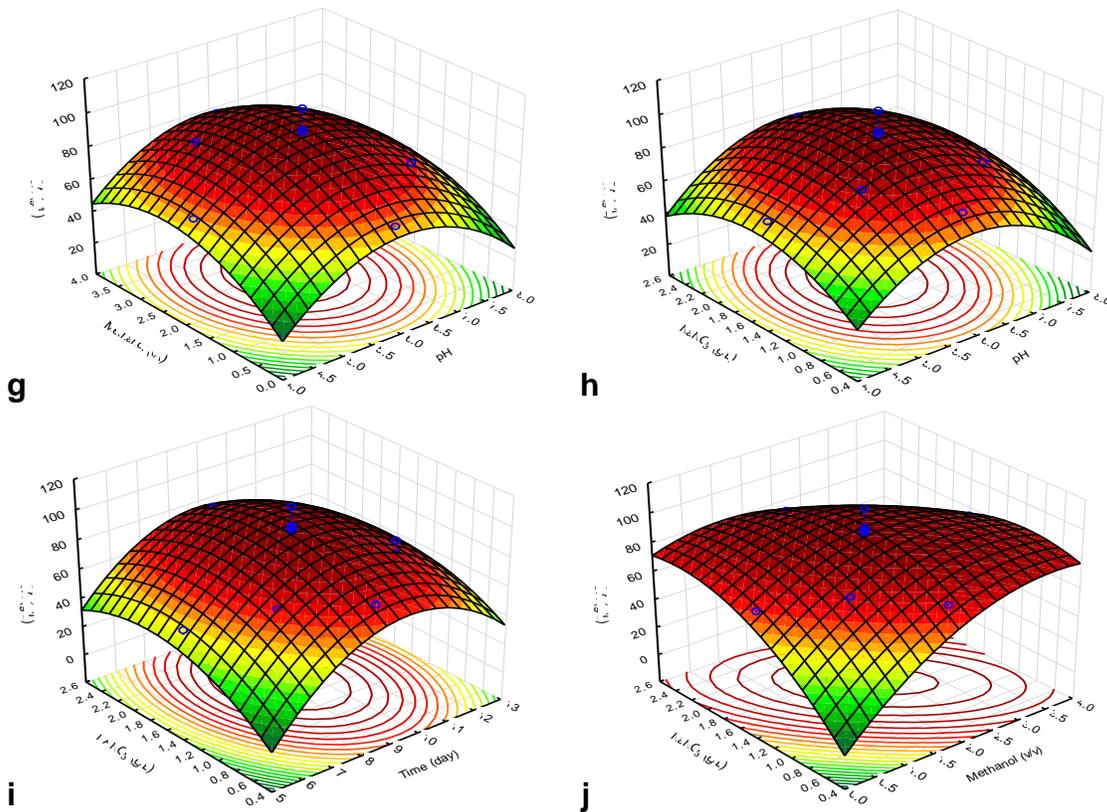


Fig. 3 (g-j). Response surface plots for oxalic acid fermentation

Each contour curve represents an unlimited number of combinations of the two variables. The elliptical contour plots in Fig. 3 (a-d) suggest significant interactions between CAJ concentration and pH (a), CAJ concentration and time (b), CAJ and methanol concentrations (c), and NaNO_3 and CAJ concentrations (d). The circular contour plots of the response surfaces in Fig. 3 (e-j) indicate that the interactions among the variables investigated were negligible *i.e.* between time and pH (e), methanol and time (f), methanol and pH (g), NaNO_3 concentration and pH (h), NaNO_3 concentration and time (i), and NaNO_3 and methanol concentrations (j). The nature of the curvature of the 3-D surfaces in Fig. 3 (e-j) indicates mutual interactions among the variables evaluated in this work. There were, however, marked interactions among the variables in the response surfaces in Fig. 3 (a-d).

Modeling and Optimization by ANN

ANNs are computer programs that are designed to model the relationship between independent and dependent variables and are capable of modeling complex, non-linear relationships directly from the raw data (Masoumi *et al.* 2011). An ANN was applied to model and optimize oxalic acid fermentation using *A. niger*. Several neural network architectures and topologies were tested for the estimation and prediction of oxalic acid concentration. This is because selection of an optimal neural network architecture and topology is crucial for a successful ANN application. Also, there are many different learning algorithm types reported in the current literature and it is difficult to know in advance which of the learning algorithms will be more efficient for a given problem (Saracoglu 2008). Additionally, the transfer function types employed affect the neural

network learning rate and help its performance (Baş and Boyacı 2007). Thus, several ANN learning algorithms and transfer functions effects were studied by successful training of the neural network models. The results obtained showed that IBP was the most effective learning algorithm for the oxalic acid fermentation process.

Several topologies were investigated to determine the optimum number of neurons in the hidden layer, and these values varied from 1 to 7. R^2 and AAD were used as a measure of the predictability of the networks. The best topology (5-4-1), which had five inputs, four neurons as the optimum, and one output, was selected. The best ANN model for the oxalic acid fermentation process was an MNFF connection type and IBP network with linear as the hidden function and Tanh as the output layer function (Fig. 4). The values of R , R^2 , and AAD for the training data set were 0.9996, 0.9992, and 0.24%, respectively, while the values for the testing data set were 0.9998, 1.0000, and 0.15%, respectively. For the whole data set, the values were $R = 0.9996$, $R^2 = 0.9999$, and $AAAD = 0.21\%$. Joglekar and May (1987) suggested that R^2 should be at least 0.80 for an accurate model. The parity plot of predicted values against observed values showed a good fit for the model (Fig. 1).

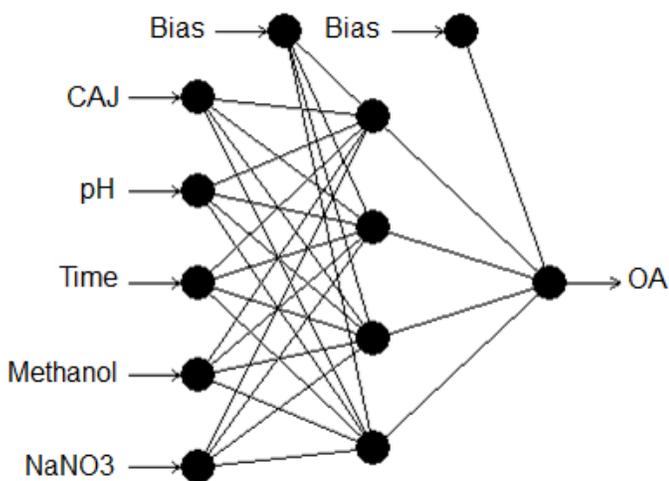


Fig. 4. Multilayer normal feed-forward neural network architecture

The optimum levels predicted by the ANN model coupled with rotation inherit optimization were a CAJ concentration of 291 g/L, pH of 6.5, time of 12.64 days, methanol concentration of 3.82% (v/v), and NaNO_3 concentration of 2.41 g/L with a corresponding oxalic acid concentration of 286.01 g/L. The model was validated by applying the optimum values to three independent experimental replicates, and the average value of the oxalic acid concentration obtained was 286.75 g/L, equivalent to a yield of 0.985 g/g.

Assessment of ANN and RSM Models

The accuracies of the models obtained from RSM and ANN were evaluated by comparing the values of R , R^2 , and AAD (Table 4). The results showed that the two optimization tools gave good predictions, but the ANN model with higher R and R^2 values and a lower AAD value was better than the RSM model (Table 4). This observation was also supported by the parity plots between the predicted values and experimental values (Fig. 1). Although the RSM model prediction for CAJ concentration was the same as the ANN model and the pH predicted by the two models was within the same range, the ANN model predicted higher values of time, methanol concentration, and NaNO_3 concentration

than the RSM model. The oxalic acid yield obtained using the optimal condition predicted by the ANN model was 2.4 times higher than that of the RSM model. Thus, the ANN model is superior for both data fitting and prediction capabilities in comparison to the RSM model. ANN models have been steadily shown to be superior to RSM models (Baş and Boyacı 2007; Ajala and Betiku 2014). Betiku and Taiwo (2015) demonstrated that the ANN model is better than the RSM model in bioethanol production prediction. The same trend was also reported in the modeling and optimization of the extraction process of yellow oleander oil (Ajala and Betiku 2014).

Table 4. Assessment of ANN and RSM Models

Variable	ANN	RSM
CAJ concentration (g/L)	291	291
pH	6.5	6.9
Time (days)	12.64	10.82
Methanol concentration %(v/v)	3.82	2.91
NaNO ₃ concentration (g/L)	2.41	1.05
Oxalic acid concentration (g/L)	286.75	120.66
Oxalic acid yield (g/g)	0.985	0.415
<i>R</i>	0.9996	0.9986
<i>R</i> ²	0.9999	0.9973
AAD (%)	0.21	1.00

CONCLUSIONS

1. Cashew apple juice was successfully employed as the sole carbon source for production of oxalic acid using the filamentous fungus *A. niger* under surface fermentation.
2. The best RSM model to describe the oxalic acid fermentation process was a quadratic polynomial with an *R*² of 0.9973. Modeling with an ANN showed that incremental batch propagation (IBP) with a linear hidden function and a hyperbolic tangent as the output layer function was the most successful learning algorithm for the oxalic acid fermentation.
3. The Pareto chart showed that CAJ concentration had the most positive significant effect on oxalic acid production, followed by methanol concentration, NaNO₃ concentration, and time. The pH was not a significant variable for oxalic acid fermentation.
4. Statistical optimization of the fermentation process by RSM gave optimum values for the five independent variables as follows: CAJ of 291 g/L, pH of 6.9, time of 10.82 days, methanol concentration of 2.91% (v/v), and NaNO₃ concentration of 1.05 g/L, with a predicted oxalic acid concentration of 121 g/L, which was validated at 120.66 g/L. The ANN model coupled with rotation inherit optimization predicted the optimum levels to be a CAJ of 291 g/L, pH of 6.5, time of 12.64 days, methanol concentration of 3.82% (v/v), and NaNO₃ concentration of 2.41 g/L, with a predicted oxalic acid concentration of 286.01 g/L, which was verified at 286.75 g/L. The ANN model led to a 2.4-fold increase in oxalic acid yield over that of the RSM model.
5. The results showed that the ANN model (*R* = 0.9996, *R*² = 0.9999, AAD = 0.21%) was better than the RSM model (*R* = 0.9986, *R*² = 0.9973, AAD = 1.00%) with respect to modeling and optimization of the oxalic acid fermentation process.

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