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Comparative Evaluation of Artificial Neural Network Coupled Genetic Algorithm and Response Surface Methodology for Modeling and Optimization of Citric Acid Production by *Aspergillus Niger* MCBN297

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A comparative modeling and optimization of citric acid production from *Aspergillus niger* MCBN297 using Response Surface Methodology (RSM) and Artificial Neural Network (ANN) coupling Genetic Algorithm (GA) was carried out on seven process parameters.

A polynomial model was developed and RSM optimum process setpoints were determined. A multilayer ANN was structured, trained on experimental data, and served as fitness function for GA optimization.

Two ANN optimized media for citric acid production with predicted values of 4.69 g/L each, gave experimental productions of 6.65 and 6.68 g/L respectively, values higher than expected. Similarly, two RSM optimized media with predicted production of 7.19 and 7.04 g/L respectively, gave experimental values of 2.40 and 3.53 g/L respectively, exceedingly below RSM expectation. However, RSM provided good insight on parameters interactions. Both models can be developed using the same data pool.

1. Introduction

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is one of the most exploited fermentation product, produced almost exclusively by *Aspergillus niger*. It is used in many industrial areas such as the food, cosmetic, pharmaceutical, chemical, textile and electroplating industries (Lofty et al., 2007). The global production of citric acid has reached 1.7 million tonnes per year as estimated by Business Communications Co. (BCC, www.bccresearch.com) and is increasing at annual growth rate of 5 %. The demand for citric acid production is increasing faster than its production and hence more economical process models are needed.

Presently, fermentation process modeling and optimization is carried out using either the statistical Response Surface Methodology (RSM) with its associated designs such as Plackett, Box-behnken and Central composite (Kılıc et al., 2002; Bari et al., 2009;), or the Artificial Neural Network (ANN) modeling and Genetic Algorithm (GA) optimization approach (Prakasham et al., 2011). RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions (Kalil, 2000). With RSM, the experimental responses to design of

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experiments (DOEs) are fitted to a quadratic function (Imandi et al, 2008). These models assume that a second-order polynomial relation can reasonably approximate many of the fermentation system dynamics. ANN is a biologically inspired computational tool, simulating the connective behavior of natural neurons, and is used in modeling of various systems. Its power resides on its ability to learn from historical process data and to approximate linear and non linear functions. Genetic Algorithm, is a globalized optimization search technique that optimizes a given function over a particular range, and is based on the evolutionary methods of natural selection of the best individuals in a population (Goldberg, 1989). Both RSM and ANN strategies are suitable for process modeling, but differ in their extrapolation and interpolation capabilities on complex non linear fermentation processes, and thus potentially conflict in their predictive accuracy.

This paper explores and compares the capabilities of RSM and ANN in modelling the production of citric acid from *Aspergillus niger* MCBN297 on factors of sucrose, magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), potassium dihydrogen phosphate (KH_2PO_4), ammonium nitrate (NH_4NO_3), initial pH, temperature and process time. The optimized setpoints are further validated experimentally.

2. Materials and Methods

2.1 Response surface analysis and optimization

The RSM Box–Behnken design was employed to evaluate the interaction of various factors on citric acid production using *A. niger*. Seven factors, namely concentrations of ammonium nitrate (NH_4NO_3), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), potassium dihydrogen phosphate (KH_2PO_4), sucrose, pH, and temperature and process time were considered (Table1). According to this design, 62 experimental runs were generated. Each run represents a unique combination of factors levels. For each experiment the total amount of citric acid produced was determined. The optimization method described by Myers and Montgomery (2002) was used by RSM optimization.

Table 1: Independent variables associated with their coded and real values

Independent variables	Symbols	Actual values of coded -1	0	1
Ammonium nitrate (g/L	A	1	3	5
Magnesium sulphate heptahydrate (g/L)	B	0.1	0.55	1
pH	C	2	4	6
Potassium dihydrogen phosphate (g/L)	D	1	3	5
Process time (hr)	E	72	120	168
Sucrose (g/L)	F	100	190	280
Temperature ^oC	G	25	32.5	40

2.2 Inoculum development and fermentation process

A culture *of Aspergillus niger* MCBN297 was used. The spore suspension was prepared to inoculate 150 ml aliquots of the fermentation medium dispensed in 250 mL Erlenmeyer flasks. All the physico-chemical process parameters were set as specified in the Box-behnken design.

2.3 Determination of Citric acid

Citric acid concentration was estimated using pyridine–acetic anhydride method according to Marrier and Boulet (1958). The absorbance was measured on a spectrophotometer (420 nm) and citric acid contents of the sample were estimated with reference to the standard.

2.4 Artificial Neural network modeling

The multi-layered perceptron (MLP) architecture of ANN approximates non-linear relationships existing between multiple causal (input) process variables and the corresponding dependent (output) variables (Nandi et al., 2001). Once an ANN-based process model with good generalization capability is constructed, its input space can be optimized to secure the optimal values of process variables. In the MLP architecture, data flow from input layer to the output layer, through the hidden layer. The input layer introduces scaled input data to the hidden layers via the weights and bias which are the network numerical parameters. The hidden layer sums up the weighted inputs and bias as follows:

$$sum = \sum_{i=1}^{n} X_i W_i + \Omega \tag{1}$$

w_i are the connections weights, x_i are the input parameters, Ω is the bias. The weighted inputs are passed through the activated function to the output layer as:

$$f(sum) = \frac{1}{1 + e(-sum)} \tag{2}$$

The neurons in the output layer produce an output based on a similar procedure as the hidden layer. In the training phase, an error value is produced based on the difference between the predicted network output and the experimental value.

Experimental data generated from Box–Behnken design were used to construct the ANN module. The idea was to use data that are statistically well distributed in the input search window. A total number of 62 experimental data were divided into two sets, 52 for training and 10 for validation. A feedforward multiplayer perceptron was structured on easyNN software with 7 inputs, 1 hidden layer and 1 output layer given a topology of 7:5:1 (Figure.2) which refers to the number of inputs, neurons in the hidden and output layers. The input vector was made up of concentrations of ammonium nitrate (NH_4NO_3), magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), potassium dihydrogen phosphate (KH_2PO_4), sucrose, pH, temperature and process time, while citric acid concentration was the output. The log-sigmoid and linear transfer functions were used for the hidden and output layers respectively.

3. 3. Results and Discussion

3.1 Response Surface Methodology (RSM) modeling

The obtained regression model was tested for statistical significance and adequacy using the Analysis of Variance (ANOVA). The mathematical relations used to determine the estimators of ANOVA have been described in literature of DoE and RSM by Myers and Montgomery (2002).

The F-value is obtained as ratio of the mean square regression and mean square residual. The Model F-value of 2.00 implies the model is significant. This implies that this model could be used to navigate the optimization search space for citric acid with regard to the factors under consideration.

The ANOVA coefficient of determination R^2 is about 0.7290, thus indicating that 72.9 % of the observed variation in citric acid production can be accounted for by the model. The adjusted R^2 is 0.3642.

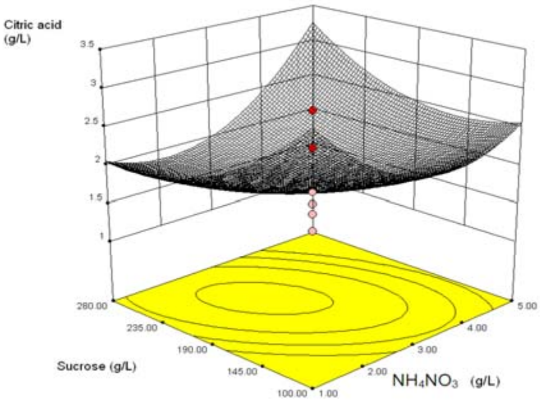
The "Lack of Fit F-value" of 1.28 implies that, the Lack of Fit is not significant relative to the pure error. A non-significant lack of fit is desirable, since a fitting model is being sought for. A high value of the correlation coefficient (R=0.851) suggests an acceptable correlation between the predicted values and the experimental results.

For the coefficient terms of the model, the values of "Prob > F" less than 0.0500 indicate that the model terms are significant. In this case A^2, B^2, C^2, E^2, F^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not too significant. Thus a possible reduced quadratic model, including terms required to support the hierarchy could be:

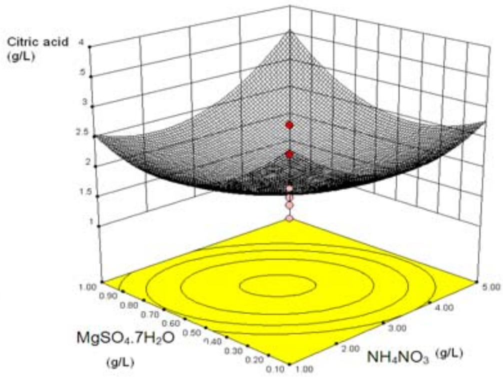
$$\text{Citric acid} = 1.77 + 0.12° + 0.012B - 0.26C + 0.036D + 0.076E\ 0.13F + 0.12G + 0.63A^2 + 0.67B^2 + 0.50C^2 + 0.99E^2 + 0.39F^2 \tag{3}$$

With regard to the linear effect of variables, the low probability values of the coefficient of pH (0.06) makes its first order effect very significant, meaning that the production of citric acid would be affected directly by the change in pH value in the environment according to this model. In the decreasing order, is NH_4NO_3, temperature, sucrose, process time, KH_2PO_4 and $MgSO_4{\cdot}7H_2O$.

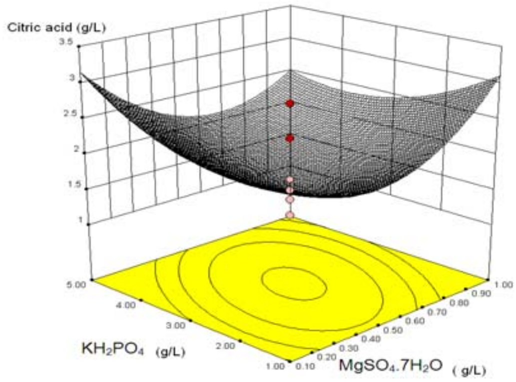
The quadratic main effects of process time, $MgSO_4{\cdot}7H_2O$, NH_4NO_3, pH, Sucrose and KH_2PO_4 with p values < 0.0001, 0.0011, 0.0018, 0.0106, 0.036 and 0.05 respectively are more pronounced than those of linear effects of the parameters considered. These data suggest that any minor change in these variables from their median level (coded) values may cause a second order positive or negative shift in the production of citric acid. Thus, a change in setpoint values of these variables with a relatively higher magnitude must be monitored towards ensuring an increase in citric acid production.
The following interaction effects were considered based on P-values. These are sucrose and NH_4NO_3, $MgSO_4{\cdot}7H_2O$ and NH_4NO_3, KH_2PO_4 and $MgSO_4{\cdot}7H_2O$ then KH_2PO_4 and NH_4NO_3 with P-values of 0.05, 0.11, 0.16 and 0.29 respectively. The three dimensional response surface and contour lines-maps computed by means of response surface model are shown in Figures 1.a-d, revealing the predicted effect of factors interaction on the production. Figure 1.a shows the influence of sucrose and NH_4NO_3 on citric acid production while maintaining other parameters at their median values. It reveals that with a concentration of NH_4NO_3 (>4g/L), an increase in sucrose concentration from 100 to 280 g/L leads to an increase in citric acid production. The interaction between $MgSO_4.7H_2O$ and NH_4NO_3, in Figure 1.b shows a high production of citric acid at NH_4NO_3 concentration >3 g/L and $MgSO_4.{\cdot}7H_2O$ concentration > 0.7 g/L. The response surface of the interaction between KH_2PO_4 and $MgSO_4{\cdot}7H_2O$ shows that at high values of $MgSO_4{\cdot}7H_2O$ (> 0.6) and low values of KH_2PO_4 (< 2), or low values of $MgSO_4{\cdot}7H_2O$ (< 0.4) and high concentration of KH_2PO_4 (> 3.5) there is an increase in citric acid production (Figure 1.c). In Figure 1.d, there is high positive interaction effect when KH_2PO_4 and NH_4NO_3 concentrations exceed 4g/L for both factors.



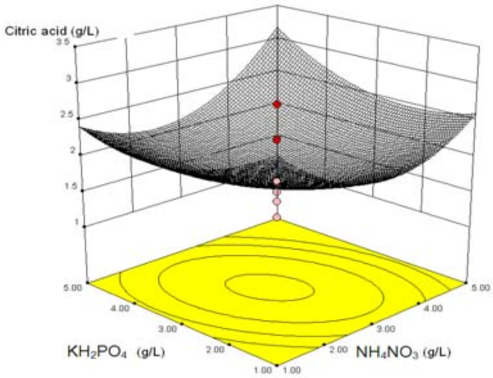
a) Interaction of sucrose and NH_4NO_3,



b) Interaction of $MgSO_4.7H_2O$ and NH_4NO_3



c) Interaction of KH_2PO_4 and $MgSO_4.7H_2O$



d) Interaction of KH_2PO_4 and NH_4NO_3

Figure 1: Three dimensional response surfaces and contour lines-maps showing parameter interactions

3.2 Artificial Neural Network model

The Network was trained using the implemented Levenberg-Marquardt algorithm or backpropagation method (Costa, 2007) (Figure.3). The learning rate was set at 0.6 and the momentum at 0.8. With this algorithm, the training was carried out by adjusting the weight connections between neurons with the aim of reducing the Mean Square Error (MSE) between the predicted and the experimental outputs below an acceptable threshold, thus minimizing the performance of MSE function. A successful training was achieved after 57,000 cycles with MSE validating error of 0.05, a MSE training error of 0.0039 and 5 of 10 validating data were correct when rounded and 5 were within 10 % range.

The input sensitivity shows how much citric acid production changes when the inputs are varied within the experimental range. The inputs were all set to the median values and then each in turn was varied from the lowest to the highest value. In decreasing order of sensitivity was, sucrose, ammonium nitrate, temperature, pH, potassium dihydrogen phosphate, magnesium sulphate heptahydrate and process time with a sensitivity of 0.84, 0.36, 0.29,0.25,0.24,0.11 and 0.09 respectively. These observations imply that citric acid production by *Aspergillus niger* will be greatly influenced by sucrose, ammonium nitrate concentrations and process time. Papagianni et al. (2005) reported that in the production of citric acid from inhibition of glycolytic pathway, ammonium was highly appositive effectors that prevent the feedback repression caused by fructokinase gene on the complete oxidation of sugars in *A. niger.*

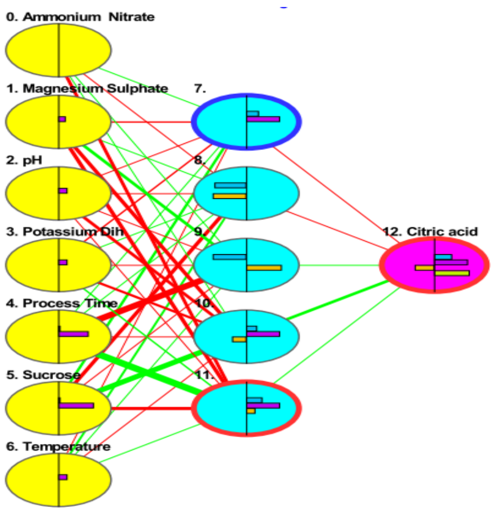


Figure 2: Artificial Neural Network topology of 7:5:1

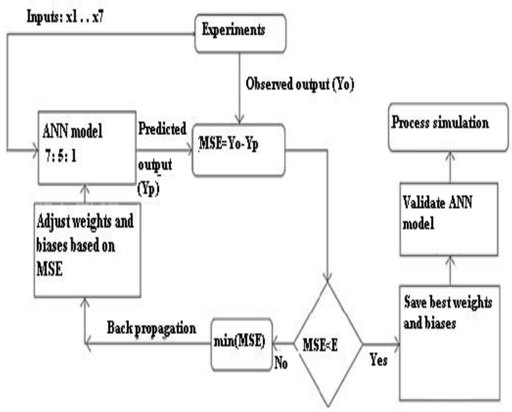


Figure 3: ANN training flowchart.

3.3 Optimization using Genetic algorithm and artificial neural network

To this end, each medium profile was referred to as (chromosome). The elements of the medium were the concentrations of ammonium nitrate, magnesium sulphate, pH, potassium dihydrogen, process time, sucrose and temperature, all refer to as genes. The search range was bound within the same range used for RSM design for all parameters. The generation size, parent size, mutation rate and crossover rate were set at 100, 20 %, 20 % and 60 % respectively. With these settings, 100 different media profiles were produced for generation G1. The performance value of each of these media for citric acid production was determined using the developed ANN model. To produce the next generation (G2), few best substrate profiles from G1 were selected, and then genetic operations were performed to produce 100 different substrate profiles, which were in turn evaluated using ANN. The performance value increased from one generation to another until the stopping criterium was met.

3.4 Comparative validation of optimum process conditions as determined by ANN and RSM

Experimental validation was carried out on four optimized media for citric acid production, two from each model. These evaluations yielded exciting insights.

For two ANN optimized media with predicted production of 4.69 g/L each, an experimental evaluation of these media showed 6.65 and 6.68 g/L respectively, values higher than expected. Similarly, for two RSM optimized media, with predicted production of 7.19 and 7.04 g/L, experimental evaluation showed 2.40 and 3.53 g/L respectively, exceedingly below RSM expectation. Thus ANN predicted optimum media emerged with highest observed experimental citric acid production, with values above expectation. It should be noted that the experimenter did not have prior knowledge of models predictions. These observations raise the suggestion that ANN derived models are more accurate in approximating the dynamics of microbial fermentation processes.The relatively low predictive accuracy exhibited by the RSM model in this work, encapsulates the inability of this modeling strategy (although mostly used) to approximate the non linear dynamic nature of fermentation processes, being limited by its second-order polynomial structure. The excellent predictive accuracy of ANN is accounted by the fact that this class of models uses transfer functions in the hidden and output layers to approximate complex non-linearities in systems, thus capturing the non linear behavior in bioprocess dynamics, whereas the RSM relies on the quadratic polynomial function. ANN combined to GA are more efficient in navigating the optimization search space for fermentation research and development

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