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The potential of artificial neural network (ANN) in optimizing media constituents of citric acid production by solid state bioconversion

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Abstract: This work aims at optimizing the media constituents for citric acid production from oil palm empty fruit bunches (EFB) as renewable resource using artificial neural networks (ANN) approach. The bioconversion process was done through solid state bioconversion using *Aspergillus niger*. ANN model was built using MATLAB software. A dataset consists of 20 runs from our previous work was used to develop ANN. The predictive and generalization ability of ANN and the results of RSM were compared. The determination coefficients (R^2-value) for ANN and RSM models were 0.997 and 0.985, respectively, indicating the superiority of ANN in capturing the non-linear behavior of the system. Validation process was done and the maximum citric acid production (147.74 g/kg-EFB) was achieved using the optimal solution from ANN which consists of 6.1% sucrose, 9.2% mineral solution and 15.0% inoculum.

Keywords: EFB, ANN, SSB, sucrose, mineral solution, inoculums, citric acid

Introduction

Citric acid has a lower toxicity compared to other acidulants which make it suitable for various applications especially in pharmaceutical and food industries. It was commonly produced by submerged fermentation. The annual demand of citric acid is increasing at a rate of around 3.5-4.0% of its consumption (Vandenberghe *et al*., 2000). The current world market also estimates upwards of 4.0 x 10^5 tones citric acid per year may be produced (Ali *et al*., 2002). The increasing demand requires a low cost raw materials and more efficient bioconversion process. The suitable low cost raw materials are abundantly produced by agricultural sector such as palm oil industry. The palm oil industry in Malaysia for example generates about 90 million tones of renewable biomass (trunks, fronds, shells, palm press fiber and empty fruit bunches) per year, including about 1.3 million tons of oil palm trunks, 8 million tons of pruned and felled fronds, and 2.4 million tons of oil palm empty fruit bunches (EFB) (MPOB, 2003). The oil palm biomass (OPB) is classified as lignocellulosic residues that typically contain 50% cellulose, 25% hemicellulose and 25% lignin in their cell wall (Ma *et al*., 1993).

EFB is one of agricultural waste that has high potential bioconversion into value added products because it is easily accessible and rich in lignocelluloses. The bioconversion process can reduce the environmental impacts caused by EFB. Therefore, there is increasing of interest and research on the use of EFB in a solid state bioconversion (SSB) into value added products such as compost, citric acid, and enzymes (Molla *et al*., 2004). The use of SSB with agro-industrial residues is economically important because it has lower volume equipment lower energy requirement and produce less wastewater, thus minimize environmental problems (Lu *et al*., 1997). When SSB is used for citric acid production, some important parameters must be optimized to increase the efficiency of the bioconversion process. Examples of the parameters are media constituents, temperature, pH and agitator speed (Yigitoglu, 1992). Optimization of media constituents is very important in any SSB because the media has high sensitivity to the yield of citric acid production (Bari *et al*., 2009). It also depends on the type of fungi used in the SSB. Different fungi will have different preferred media constituents.

The optimization of a bioconversion process has many challenges such as laborious, expensive, open ended and time consuming which involves many experiments (Panda *et al*., 2007). The performance of the bioconversion processes is affected by numerous factors and their effects are very complex with possible interactions among the factors, thus they are often characterized through experimentation (Yigitoglu, 1992). Response surface methodology (RSM) is the most common method used for optimization (Desai *et al*., 2008; Panda *et al*., 2007). Usually, RSM is used in designing the experiments and analyzing the results for optimizing the interactive influences of different factors and reducing the number of laborious experiments (Panda *et al*., 2007). RSM uses statistical experimental design to develop empirical models which relate a response to some factors. But, its main limitation is it assumes only quadratic non-

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linear correlation (Desai *et al*, 2008).

Nowadays, many researchers have investigated artificial neural networks (ANN) as the artificial learning tool in a wide range of biotechnology applications including optimization of bioprocesses and enzyme production from microorganisms. ANN is biologically inspired and mimics human brain. They are consisting of a large number of simple processing elements named neurons. These neurons are connected with connection link. Each link has a weight that multiplied with transmitted signal in network. Each neuron has an activation function to determine the output. There are many kind of activation function. Usually nonlinear activation functions such as sigmoid and step functions are used. ANNs are trained by experience, when applied a new input to the network it can generalize from past experiences and produce a new result (Hanbay *et al*., 2008, Haykin, 1994). The simple structure of ANN normally consists of an input layer, a hidden layer and an output layer (Chayjan *et al*., 2007; Desai *et al*, 2008; Haykin, 1994; Krogh, 2008). By applying algorithms that mimic the processes of real neurons, the network can learn to solve many types of problems.

From the perspectives of process modeling, ANN has been applied to solve complex engineering problems where it is difficult to develop models from the fundamental principles, particularly when dealing with non-linear systems which also exist in bioconversion process. It provides a mathematical alternative to the quadratic polynomial for representing data derived from statistically designed experiments. ANN is also able to handle a large amount of data to approximate functions to any desired degree of accuracy, thus make it attractive as empirical model (Panda *et al*., 2007).

Therefore, this study use ANN for re-optimizing the earlier work done by Bari *et al*. (2009) who used RSM to optimize the media constituents of citric acid production. Three main factors (sucrose, minerals solutions and inoculums) were selected for the optimization. In this study, the same dataset from Bari *et al*. (2009) was used for the optimization of ANN and comparative analysis between ANN and RSM was conducted.

Materials and Methods

Data collection

The dataset was tabulated in Table 1. It consists of the design of experiments (inputs), experimental output and predicted output by RSM from Bari *et al*. (2009). The data of inputs and experimental outputs were used to developed ANN model. The dataset in Table 1 also includes the predicted output obtained from ANN model.

Artificial Neural Network modelling

The structure and topology of ANN model used in this study is shown in Figure 1. The model neuron in ANN is referred to as a threshold unit and its function is illustrated in Figure 2. It receives input from a number of other units or external sources, weighs each input and adds them up. If the total input is above a threshold, the output of the unit is one; otherwise it is zero (Krogh, 2008). The threshold unit receives input from N other units or external sources, numbered from 1 to N. Input i is called x_i and the associated weight is called w_i. The total input to a unit is the weighted sum over all inputs as in the following equation (Krogh, 2008).

$$\sum_{i=1}^{N} w_i x_i = w_1 x_1 + w_2 x_2 + \dots + w_N x_N$$

If this was below a threshold t, the output of the unit would be 1 and 0 otherwise. Thus, the output can be expressed as in the following equation (Krogh, 2008).

$$g \sum_{i=1}^{N} w_i x_i - t$$

where g is the step function, which is 0 when the argument is negative and 1 when the argument is nonnegative. The so-called transfer function, g, can also be a continuous sigmoid as illustrated in Figure 2.

The components of input layers consist of three parameters which were sucrose, mineral solution and inoculum as in Figure 1. The inputs were made into 3 x 20 matrix. Then, the yield of citric acid production was defined as output in this model. Data is subdivided into three groups; training, validating, testing, in the default ratio of 3:1:1, respectively. MATLAB software was used for modeling the ANN. For this study, Network/Data Manager in MATLAB was used.

Feedforward backpropagation neural network (BPNN)

A dynamic type of neural network used in this study was the feed forward backpropagation neural network (BPNN). This network consists of backpropagation algorithm which was developed by Rumerlhart in 1986 (Jain *et al*., 1996). It is the easiest algorithm to be understood (Abassi, 2008). The BPNN is a generalization of the delta rule used for training feed forward neural networks with non-linear units. This simple gradient descent method

Table 1. Experimental design using Central Composite Design (CCD) showing coded and actual values along with the experimental and predicted values of citric acid production (using RSM and ANN)

Run number	Sucrose *a* (% w/w)	Mineral Solution *B* (% w/w)	Inoculum *c* (% w/w)	Citric acid yield (g/kg-dry EFB)		
				Experimental	*Predicted (RSM)*	*Predicted (ANN)*
1	8	12	20	259.23	258.10	260.19
2	6	2	16	267.47	268.15	267.31
3	4	12	20	256.52	242.52	261.04
4	6	8	16	334.68	332.81	333.57
5	6	8	16	334.23	332.81	333.57
6	4	4	20	208.69	213.22	211.73
7	4	4	12	236.65	231.32	236.48
8	6	8	16	332.44	332.81	333.57
9	8	4	12	259.22	266.75	259.19
10	8	4	20	260.65	247.19	260.13
11	4	12	12	250.02	257.02	250.12
12	6	8	16	333.88	332.81	333.57
13	6	8	16	333.08	332.81	333.57
14	6	14	16	292.42	300.18	292.22
15	6	8	16	334.14	332.81	333.57
16	3	8	16	247.46	249.8	247.16
17	6	8	10	257.79	256.12	264.39
18	8	12	12	285.06	274.06	283.45
19	9	8	16	278.90	288.06	278.04
20	6	8	22	217.41	230.58	213.16

a: 1% (w/w) = 33.3 g/kg-EFB
b: 1% (v/w) = Zn, 3; Cu, 3.3; Mn, 13.3 and Mg, 166.7 mg/kg-EFB
c: 1% (v/w) = 6.7 x 10^10 spores/kg-EFB

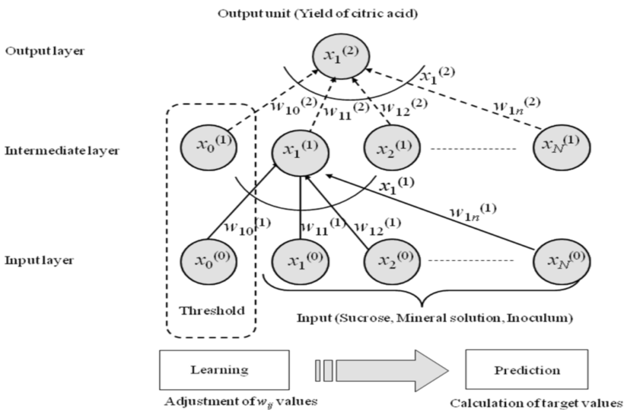

Figure 1. The topology of the artificial neural network model with an input layer, one intermediate (hidden) layer and an output layer

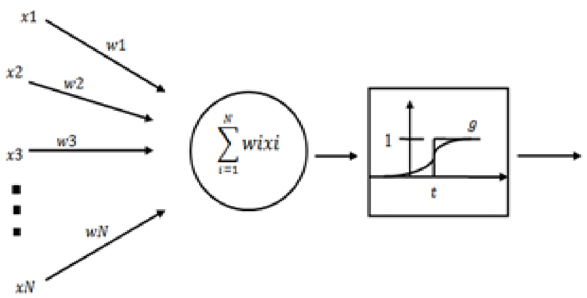

Figure 2. Graphical representation of the McCulloch-Pitts model neuron or threshold unit (Krogh, 2008)

designed to minimize the total error or mean error of the output computed by the network (Lee and Park, 2008). Since the initial configuration of ANN is arbitrary, the result of presenting a pattern to the ANN is likely to produce incorrect output. The errors for all input patterns are propagated backwards, from the output layer towards the input layer. The corrections to the weights are selected to minimize the residual error between actual and desired outputs. Thus, the system will ensure that the output is correctly within the specified range (Sencan and Kalogirou, 2005).

Determination of hidden layer

The neural network model in this study used the default algorithm that exists in the GUI of MATLAB. In MATLAB, the most common network used with backpropagation is the two-layer feed-forward network. The default function only considered hidden layer and output layer as the significant layers for the performances. The input is not considered as the layer (Demuth *et al*., 2009). However, networks that are trained using backpropagation can still have more than one hidden layers, which can make learning of complex relationships easier for the network. Other architectures add more connections to help networks to learn (Haykin, 1994; Demuth *et al*., 2009). The use of more than one hidden layer is depending on the type and the size of the data. Because of the Network/Data Manager of MATLAB uses the default algorithm and not capable for varying the number of hidden layer, thus advanced study of neural network architecture using command-line function can be used (Demuth *et al*., 2009). However, in this study, the default of single hidden layer and single output layer were used for all ANN models.

Determination of hidden neurons (nodes)

In Network/Data Manager, only the number of nodes in hidden layer (layer 1) can be specified while the number of node of output layer (layer 2) depends on the number of target output data. For current study 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 nodes of hidden layer were tested. The best number of nodes was selected from the model that gave the best performance with highest correlation coefficient (R-valte).

Determination of training and learning function

There are several numbers of training functions and learning functions provided in Network/Data Manager for feedforward BPNN (Demuth *et al*., 2009). For this case, other default parameters in the Network/Data manager were kept constant. All training functions and learning functions were tested. The best training function and learning function were selected from the model that gave the best performance with highest R-value.

Determination of transfer function

In an ANN model, the output of the neuron largely depends on the transfer functions (TF) (Pratihar, 2008). TF is also called as activation function for the ANN model (Engelbretch, 2007). There are three TFs exist in the Network/Data Manager for the BPNN network. For this study, TF were varied for the hidden layer and the output layer. The best combination of TFs for hidden layer and output layer was selected from the model that gave the best performance with highest R value.

Training and model verification

After training the neural network model using BPNN, the best model was selected according to the R-value and determination coefficient (R^2-value) from the relationship between the target output (experimental output) and the network output (predicted output). R^2-value is a statistical indicator that compares the accuracy of the model to the accuracy of a trivial benchmark model wherein the prediction is just the mean of all the samples. The R^2 is mathematically described as in following equation (Ahmed and Daniel, 2001)

$$R^2 = 1 - \frac{\sum_{i=1}^{1-n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{1-n}(y_i - y)^2}$$

where y_i is the actual output value, $\hat{y}_i$ is the output value predicted by the network, y is the mean of y values, and n is the total number of data records. A perfect fit would result in an R^2 value close to *'1'* while R^2 value close to *'0'* means very poor fit.

The MSE values of each simulation were also checked. Only the results having the best MSE value at epoch 1 or larger epoch were accepted. Sometimes, the best MSE occurred at epoch 0 due to the instability of the network. So, the networks need to be trained again until preferable result was obtained. For each cases and parameters, ten accepted simulation and results were taken. Then, the average and maximum of R-value and R^2-value were calculated.

The values of MSE were used to indicate the performance of the neural network. MSE is under the perceptron learning rule, the least mean square error (LMS) algorithm which is an example of supervised training, in which the learning rule is provided with a set of examples of desired network behavior (Demuth *et al*., 2009)

As each input is applied to the network, the network output is compared to the target. The error is calculated as the difference between the target output and the network output. The goal is to minimize the average of the sum of these errors. The LMS algorithm adjusts the weights and biases of the network according to following equation so as to minimize the MSE (Demuth *et al*., 2009).

$$mse = \frac{1}{Q}\sum_{k=1}^{Q} e(k)^2 = \frac{1}{Q}\sum_{k=1}^{Q}(t(k) - a(k))^2$$

Q is the number of data, e is the error, t is the target output while a is the network output.

Sensitivity analysis of input parameters

Sensitivity analysis was carried out by the MATLAB using the linear correlation coefficient function (coreff) in command-line function. The result of sensitivity analysis shows the most significant input to the neural network. The value of the correlation coefficient (r) ranges from -1 to 1. The r-value close to 1 suggest that there is a positive linear relationship between the data columns while the r-value close to -1 suggest that one column of data has a negative linear relationship to another column of data (anticorrelation) and finally the r-values close to or equal to 0 suggest there is no linear relationship between the data columns. This command function will also give the p-value of each relationship. Each p-value is the probability of getting a correlation as large as the observed value by random chance when the true correlation is zero. If p is small and less than 0.05, the correlation or r-value is significant (Demuth *et al*., 2009). The sensitivity analysis was done for optimization factors which are sucrose, minerals solution and inoculum.

Bioconversion process for validation

The major substrate, the oil palm empty fruit bunches (EFB), was collected from the Seri Ulu Langat palm Oil Mill in Dengkil, Selangor, Malaysia and stored in a cold room at 4^{o}C to avoid the unwanted bio-degradation by the microorganisms. The EFB sample was milled to 0.5 mm down grade particle size by grinding after washing vigorously with tap water and drying at 105^{o}C for 24 h. The ground EFB was dried at 60^{o}C for 48 h to obtain constant dry weight for the experimental study. The characterization of EFB was carried out as 518 g/kg-EFB of cellulose, 224 g/kg-EFB of hemicelluloses and 219 g/kg-EFB of lignin. A report shows that it contains 4.4 g/kg-EFB of nitrogen, 1.44 g/kg-EFB of phosphorus, 22.4

g/kg-EFB of potassium, 3.6 g/kg-EFB of magnesium and 3.6 g/kg-EFB of calcium (Menon *et al*., 2003)

The culture of *Aspergillus niger* were grown on PDA at 32^{o}C for 4 days and washed with 25 ml sterilized distilled water to prepare the inoculum. Spore suspension was collected in a 250 ml Erlenmeyer flask by filtering with Whatman no. 1 filter paper. 20 g of total fermentation media on wet basis with required percentage of major substrate (EFB) to maintain 30% solid content was prepared with different levels of sucrose, mineral solution and inoculum. The moisture content of 70% (v/w) was adjusted with mineral solution, inoculum, methanol and distilled water. Methanol at 2% (v/w) was added after sterilization of media by autoclaving at 121^{o}C for 15 min. Methanol is important because it is the simulator for citric acid production (Bari *et al*., 2009).

The bioconversion experiment was carried out in 250 ml Erlenmeyer flask. The culture media was incubated for 6 days at 32^{o}C. The initial pH of the substrate recorded varied from 5.5 to 5.8 but was not adjusted during the bioconversion process. Harvesting and extraction of citric acid were carried out after 6 days of bioconversion. Fifty milliliters (50 ml) of distilled water was added to the fermented substrate and mixed with a spatula thoroughly for homogeneity in the dilute. Fermented media was shaken at 150 rpm for 1 h at room temperature (28 ± 1^{o}C) in a rotary shaker. The supernatant was collected by filtering with Whatman no. 1 filter paper and allowed to pass through a 0.2 µm filter and immediately analyzed by measuring the absorbance to determine the content of citric acid.

The concentration of citric acid in extract was determined by measuring the absorbance of the supernatant collected. A standard curve for citric acid was prepared. The solvents used are pyridine and acetic anhydride. The production of citric acid was expressed as g/kg-EFB.

Results and Discussions

The best number for hidden layer was found to be one hidden layer. Feed-forward neural networks with one hidden layer containing a sufficiently large number of hidden neuron is already capable of providing accurate approximations to any continuous nonlinear function.

The determination of hidden neurons gave the result as in Figure 3. From the graph, it shows that ten hidden neuron is already sufficient for the neural network to give the best performance with the highest R value. When the number of hidden neurons became larger than ten, the R value decreased and increased showing the neural network become unstable. The only requirement is that the hidden layer must have enough neurons. More hidden neurons are needed as the function being fitted increases in complexity. Larger numbers of neurons in the hidden layer give the network more flexibility because the network has more parameters it can optimize but if the number is too large, problem of under-characterized might occur and it can affect the stability of the network (Demuth *et al*., 2009).

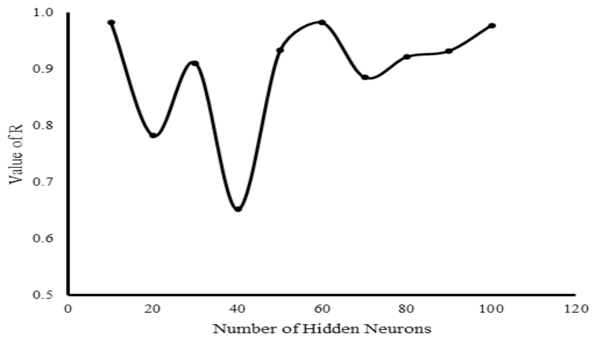

Figure 3. Graph of R value versus the number of hidden neurons

The Gradient descent with momentum weight/bias learning function (learngdm) was found to be the best learning function for the neural network. This learning function is used as the default learning function in Network/Data Manager and it is recommended for most training functions (Demuth *et al*., 2009). The log-sigmoid (logsig) was found to be the best TF for the hidden layer while the tan-sigmoid (tansig) was the best TF for the output layer. Logsig and tansig are the general and most frequent TF used in BPNN (Pratihar, 2008). The best ANN model gave the determination coefficient (R^2-value) of 0.997 and MSE obtained was 4.9767 as in Figure 4.

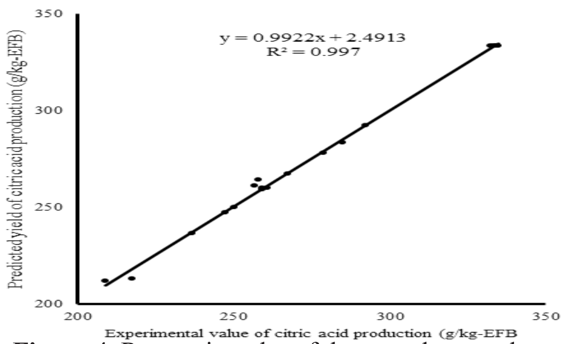

Figure 4. Regression plot of the neural network

For the sensitivity analysis, the results were shown in Figure 5. The results indicate that the positive effect of sucrose is the most influential parameter followed by mineral solution and inoculum. Sucrose is the most significant carbon source. The mineral solution consists of trace elements (Zn, Cu, Mn and Mg). These trace elements are necessary for citric acid accumulation (Bari *et al*., 2009).

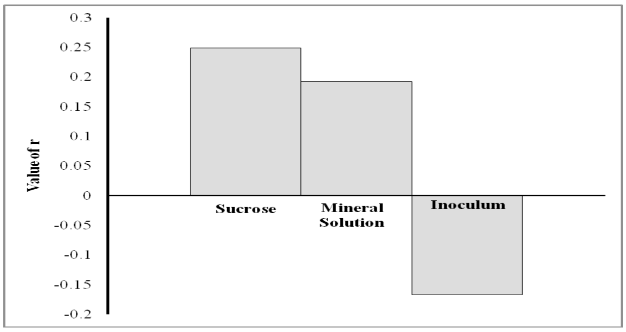

Figure 5. The sensitivity analaysis of input parameters

The three inputs used in the networks gave six combinations of the optimal solution. Table 2 shows the results of validation experiments of each combination of optimal solution. From Table 2, the optimal inputs were different between the RSM and ANN which then gave the different maximum citric acid yield. The validation result shows that the optimal solution ANN 4 gave the highest yield of citric acid production which is 147.74 g/kg-EFB. Although, the difference of citric acid yield between the optimal solution ANN 4 and RSM was not really significant, but the optimal solution of ANN 4 gave lower amount of sucrose and inoculum, thus it was more cost effective than the amount of them predicted by RSM. The value of experimental citric acid yield for all optimal solutions in Table 2 were lower than the experimental citric acid yield in Table 1 because different filtration technique was applied during the harvesting process as compared to the filtration techniques used by Bari *et al*. (2009). Since the aim of validation experiment was to determine the best optimal solution, therefore the results obtained were relevant to achieve the aim.

Table 2. Validation results for optimal solutions

Model	Sucrose *a* (% w/w)	Mineral Solutions *b* (% w/w)	Inoculum *c* (% w/w)	Experimental value of Citric Acid Production (g/kg-EFB)
RSM	6.4	9.0	15.5	143.94
ANN 1	5.4	8.3	15.0	140.93
ANN 2	5.4	8.6	15.1	139.55
ANN 3	5.8	9.2	15.0	144.33
ANN 4	6.1	9.2	15.0	147.74
ANN 5	5.2	8.4	15.2	142.43
ANN 6	6.0	9.6	15.2	147.07

Conclusions

The combination of Levenberg-Marquardt backpropagation training function, gradient descent with momentum weight/bias learning function, a single hidden layer, ten hidden neurons and LOGSIG-TANSIG transfer functions was found to give the best performance of the neural network. Higher R^2-value (0.997) was obtained compared to RSM (0.985) from the data obtained from previous research, thus making ANN more efficient than RSM. ANN is found to be applicable to analyze complex, non-linear, and dynamic data with multiple inputs. These make ANN valid as a tool to study biological process. ANN model developed in this study can be used to optimize and predict the performance of citric acid production by solid state bioconversion. This study shows that sucrose is the most significant media constituent for citric acid production from EFB. Besides that, mineral solution with trace elements (Zn, Cu, Mn and Mg) is also important for the citric acid production. EFB is also found to be a suitable recyclable waste for bioconversion into value added products such as citric acid thus can reduce the environmental problems caused by EFB and add more beneficial uses to palm oil industry.

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References

Abbasi, B. 2008. A neural network applied to estimate process capability of non-normal processes. Expert Systems with Applications 36(2): 3093-3100.

Ahmed, G. E.-D. and Daniel, W. S. 2001. A neural network model to predict the wastewater inflow incorporating rainfall events. Water Research 36: 1115–1126.

Ali, S., Ikram, U. H., Qadeer, M.A. and Javed, I. 2002. Production of citric acid by *Aspergillus niger* using cane molases in a stirred fermenter. Electronic Journal of Biotechnology 5(3): 258-271.

Bari Md, N., Md. Zahangir, A., Suleyman, A. M., Parveen, J. and Abdullah, A. M. 2009. Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. Bioresource Technology 100: 3113–3120

Chayjan, R. A., Montazer, G. A., Hashjin, T. T., Mohammad, H. K. and Ghobadian, B.. 2007. Prediction of Pistachio Thermal Conductivity Using Artificial Neural Network Approach." International Journal of Agriculture and Biology 9(6): 816-820.

Demuth, H., Beale, M. and Hagan, M. 2009. Neural Network Toolbox™ 6 User's Guide. The MathWorks Inc.

Desai, K. M., Survase, S. A., Saudagar, P. S., Lele, S.S. and Singhal, R. S. 2008. Comparison of artificial neural network and response surface methodology in fermentation media optimization: Case study of fermentative production of scleroglucan". Biotechnology Engineering Journal 41: 266-273.

Engelbretch, A. P. 2007. Computational Intelligence: An Introduction", John Wiley & Sons, Ltd, p. 1-578

Hanbay, D., Ibrahim, T. and Yakup, D. 2008. An expert system based on wavelet decomposition and neural network for modeling Chua's circuit. Expert Systems with Applications 34: 2278–2283

Haykin, S. 1994. Neural Networks: A Comprehensive Foundation". Macmillan College Publishing Company. p. 696.

Jain, A. K., Mao, J. and Mohiuddin, K.M. 1996. Artificial Neural Networks: A Tutorial. IEEE Computer, issue March: 31-44.

Krogh, A. 2008. What are artificial neural networks?. Nature Biotechnology 26(2):195-197.

Lu, M., Brooks, J. D. and Maddox, I. S. 1997. Citric acid production by solid-state fermentation in a packed-bed reactor using *Aspergillus niger*. Enzyme and Microbial Technology 21: 392-397.

Ma, A.N, S.A. Cheah, S.A. and Chow, M.C. 1993. Current Development and Commercializationof Oil Palm Biomass at MPOB: FromWaste toWealth. Malaysian Palm Oil Board.

Menon, N.R., Rahman, Z.A. and Bakar, N.A. 2003. Empty fruit bunches evaluation: mulch in plantation vs fuel for electricity generation. Oil Palm Industry Economy Journal 3(2): 15–20.

Molla, A.H., Fakhrul-Razi, A and. Alam, M. Z. 2004. Evaluation of solid-state bioconversion of domestic wastewater sludge as promising environmental friendly technique. Water Research 38: 4143–4152.

MPOB-Malaysian Palm Oil Board. 2003. Latest development and commercialization of oil palm biomass. MPOB-FMM Seminar on Business Opportunity in Oil Palm Biomass, 21 August 2003, Selangor, Malaysia.

Panda, B.P, Ali, M. and Javed, S. 2007. Ferrmentation Process Optimization. Research Journal of Microbiology 2: 201-208.

Pratihar, D. K. 2008. Soft Computing. Alpha Science International Ltd. p. 132-166.

Sencan, A. and Kalogirou, S. A. 1992. A new approach using artificial neural networks for determination of the thermodynamic properties of fluid couples". Energy Conversion and Management 46: 2405–2418

Yigitoglu, M. 1992. Production of Citric acid by Fungi. Journal of Islamic Academy of Sciences 5(2): 100-106.