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# Application of Artificial Neural Network for Yield Prediction of Lipase-Catalyzed Synthesis of Dioctyl Adipate

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**Abstract** In this study, an artificial neural network (ANN) trained by backpropagation algorithm, Levenberg–Marquadart, was applied to predict the yield of enzymatic synthesis of dioctyl adipate. Immobilized *Candida antarctica* lipase B was used as a biocatalyst for the reaction. Temperature, time, amount of enzyme, and substrate molar ratio were the four input variables. After evaluating various ANN configurations, the best network was composed of seven hidden nodes using a hyperbolic tangent sigmoid transfer function. The correlation coefficient ($R^2$) and mean absolute error (MAE) values between the actual and predicted responses were determined as 0.9998 and 0.0966 for training set and 0.9241 and 1.9439 for validating dataset. A simulation test with a testing dataset showed that the MAE was low and $R^2$ was close to 1. These results imply the good generalization of the developed model and its capability to predict the reaction yield. Comparison of the performance of radial basis network with the developed models showed that radial basis function was more accurate but its performance was poor when tested with unseen data. In further part of the study, the feedforward backpropagation model was used for prediction of the ester yield within the given range of the main parameters.

**Keywords** Esterification · Lipase · Adipate ester · Neural network · Modeling · Prediction

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## Introduction

Dioctyl adipate (DOA) is a light-colored oily liquid generally used as a plasticizer for polyvinyl chloride. It features flexibility at low temperatures, good electrical properties, good resistance to weathering, and good stability to heat. DOA is also used to produce clear films for food packaging applications. In addition, it is compatible with nitrocellulose, ethyl cellulose, and most synthetic rubbers.

Adipates are presently synthesized by esterification using chemical catalysts. Synthesis of DOA has been reported using different catalysts such as magnetic nanometer-sized solid superacids [1], composite solid acid [2], and SnO loaded on activated carbon fiber [3]. However, the chemical method involves some problems such as the high reaction temperature, corrosive acid catalysts, complex and expensive reaction setup, large amounts of raw materials due to the unselectiveness of the process and high waste generation [4]. Hence, in recent years, several enzymatic procedures for the preparation of adipate esters have been developed to overcome such shortcomings of chemical method [5, 6]. However, synthesis of dioctyl adipate using enzyme as catalyst has not been reported before. Furthermore, for future upscale enzymatic production of adipate esters, it would be beneficial to simplify the reaction conditions as much as possible.

Due to nonlinear behavior, uncertainties, and complicated structure of biotechnological processes, it is always more difficult to predict the effects of independent variables on the product and rate of reaction. In addition, the lack of a deterministic mathematical model for the enzymatic reactions causes the finding of optimal conditions in order to increase the efficiency of bioprocesses to become almost impossible. Sensitive structure of enzymes to variables such as temperature, substrate molar ratio, reaction time, and activator or inhibitor concentrations may potentially increase the complexity of models. In this case, low-order empirical polynomial models are no longer sufficient. In many simplified models, the effects of some independent parameters are not taken into account and this causes an increase in the error value [7].

In recent years, utilizing artificial neural networks (ANN) is investigated as an effective approach in modeling and optimization of many bioprocess-engineering problems. Neural networks generally consist of a number of interconnected processing elements or neurons, which can handle multiple independent and dependent variables simultaneously and have elasticity in order to be updated with new data [7, 8]. In this regards, many applications of the ANN for estimating and real-time prediction of the biotechnological processes are reported in the literatures [9–12].

Manohar and Divakar [11] have presented an application of feedforward neural network model for analysis of porcine-pancreas-lipase-catalyzed esterification of anthranilic acid with methanol, where substrate concentration, enzyme amount, period of incubation, buffer volume, and log $P$ values were the five important parameters of developed models. The network was trained by using backpropagation algorithm and logistic function as activation function based on experimental data. ANN analysis showed good correspondence between experimental and predicted values. An application of feedforward backpropagation neural networks with one hidden layer for controlling of enzyme production process has been also presented by Linko et al. [13]. Online state estimation and multistep ahead prediction of enzyme activity with a great degree of satisfactorily have been reported in this work. Such models can be used as the software sensors for monitoring of key process and quality variables in control systems.

Employing neural network models would lead to saving time and money by predicting the results of chemical reactions so that the most promising conditions can then be verified

in the laboratory, rather than performing a large number of experiments to gain the same information.

The present study is aimed at examining the possibility of artificial neural network application to predict the percentage yield of lipase-catalyzed esterification between *n*-octyl alcohol and adipic acid to produce dioctyl adipate. In this regard, the related parameters of developed model are determined by applying neural computing techniques on the experimental data. The effects of four reaction parameters (temperature, reaction time, substrate molar ratio, and amount of enzyme) on the degree of esterification are also evaluated.

## Materials and Methods

### Materials

Novozym 435, *Candida antarctica* lipase B immobilized on a macroporous acrylic resin (10,000 propyl laurate units per gram), was purchased from NOVO Nordisk A/S (Bagsværd, Denmark). Adipic acid and *n*-octyl alcohol were purchased from Sigma-Aldrich. All other chemicals and solvents used in this study were of analytical grade.

### Lipase-catalyzed Esterification

Different molar ratios of adipic acid and *n*-octyl alcohol were mixed corresponding to the different substrate molar ratios generated by central composite rotatable design (CCRD), in screw-capped vials. Five milliliters of hexane were added as solvent. Different amounts of lipase, which were generated by CCRD, were subsequently added. The reaction was performed in a horizontal water bath at 150 rpm at different temperatures and for different periods as indicated in Table 1. Selection of hexane (log $P$=3.5) as solvent and Novozym 435 as catalyst was based on prior studies, in which several lipases including Novozym 435, Lipozyme RM IM, Lipozyme TL IM, and layered-double-hydroxide-immobilized *Candida rugosa* in hexane, heptane, ethyl acetate, butanol, and acetonitrile were screened for activity via lipase-catalyzed esterification of adipic acid and different alcohols [6].

### Analysis and Characterization

The reaction was terminated by dilution with 5 ml of ethanol/acetone (50:50 *v*/*v*). The lipase was removed by filtration and the remaining free acid in the reaction mixture was determined by titration with 0.1 M NaOH using an Autotitrator (808 Titrando System, Metrohm) to an end point of pH 10. The moles of acid reacted were calculated from the values obtained for the blank (without enzyme) and the test samples. The ester formed has been expressed as equivalent to conversion of the acid. Product was also monitored by thin-layer chromatography using chloroform/hexane (95:5) solvent system.

## Model Development: Artificial Neural Network Analysis

A mathematical plant model can be developed based on data obtained from experimental design. Developing a test-data-based model from input–output data includes four main

**Table 1** Experimental data of training, validating, and testing of artificial neural network.

| Temperature (°C) | Time (min) | Enzyme amount (mg) | Substrate molar ratio | Actual yield (%) |
|---|---|---|---|---|
| Training data | | | | |
| 42.5 | 112.5 | 100.75 | 3.25 | 30.9 |
| 57.5 | 112.5 | 100.75 | 3.25 | 61.2 |
| 42.5 | 277.5 | 100.75 | 3.25 | 62.5 |
| 57.5 | 277.5 | 100.75 | 3.25 | 80.2 |
| 42.5 | 112.5 | 300.25 | 3.25 | 64.1 |
| 57.5 | 112.5 | 300.25 | 3.25 | 80.3 |
| 42.5 | 277.5 | 300.25 | 3.25 | 78.6 |
| 57.5 | 277.5 | 300.25 | 3.25 | 85.4 |
| 42.5 | 112.5 | 100.75 | 7.75 | 40.5 |
| 57.5 | 112.5 | 100.75 | 7.75 | 68.8 |
| 42.5 | 277.5 | 100.75 | 7.75 | 72.9 |
| 57.5 | 277.5 | 100.75 | 7.75 | 89.9 |
| 42.5 | 112.5 | 300.25 | 7.75 | 73.8 |
| 57.5 | 112.5 | 300.25 | 7.75 | 92.0 |
| 42.5 | 277.5 | 300.25 | 7.75 | 91.9 |
| 57.5 | 277.5 | 300.25 | 7.75 | 97.0 |
| 35.0 | 195.0 | 200.5 | 5.50 | 63.2 |
| 65.0 | 195.0 | 200.5 | 5.50 | 98.8 |
| 50.0 | 30.0 | 200.5 | 5.50 | 57.4 |
| 50.0 | 360.0 | 200.5 | 5.50 | 95.0 |
| 50.0 | 195.0 | 1.00 | 5.50 | 26.9 |
| 50.0 | 195.0 | 400.00 | 5.50 | 65.8 |
| 50.0 | 195.0 | 200.50 | 1.00 | 76.0 |
| 50.0 | 195.0 | 200.50 | 10.00 | 97.6 |
| 50.0 | 195.0 | 200.50 | 5.50 | 94.1 |
| Validating data | | | | |
| 52.5 | 277.5 | 250.0 | 7.75 | 96.0 |
| 52.5 | 112.5 | 250.0 | 3.25 | 68.1 |
| 37.5 | 277.5 | 250.0 | 3.25 | 68.9 |
| 37.5 | 112.5 | 250.0 | 3.25 | 68.9 |
| 37.5 | 112.5 | 250.0 | 7.75 | 52.9 |
| 45.0 | 60.0 | 75.0 | 5.0 | 35.5 |
| Testing data | | | | |
| 59.8 | 285.9 | 97.18 | 7.83 | 92. 2 |
| 58.7 | 255.8 | 172.75 | 6.82 | 93.8 |
| 60.9 | 246.4 | 120.00 | 8.74 | 95.1 |
| 59.1 | 269.3 | 146.20 | 9.98 | 98.6 |

steps: choosing a model structure, design of experiments to obtain data, estimation of model parameters, and model validation [14].

## Neural Network Architecture

Enzymatic esterification reactions do not depend on any single parameter alone and a combination of parameters affects the catalytic activity of enzyme. Artificial neural network is a powerful tool for analyzing this dependency.

In this study, a multilayer feedforward neural network is employed in order to characterize the essential behavior of enzymatic production of adipate ester. The proposed ANN consists of three layers including input layer that comprises four nodes or independent variables, which are time, temperature, substrate molar ratio, and amount of enzyme, and one hidden layer consisting of several nodes, which are changed to obtain the best model and the output layer that has one output node (which is the ester yield). The structure of proposed ANN used for prediction of esterification yield is shown in Fig. 1.

The performance of neural networks is affected by several factors such as selection of input variables, the number of neurons in the hidden layer, and the type of the transfer function [13]. The best transfer functions may often be selected by performing a process of trial and error. In this study, activation functions in the hidden layer are considered as the hyperbolic tangent function, which can generate nonlinear behavior of the process. These functions are very smooth and it is easy to calculate their derivatives, which are very important for backpropagation algorithm.

It is noted that the shape of hyperbolic tangent function is the same as sigmoidal function and therefore their computational costs are similar. In addition, it is shown that the converging performance of the error function during training is much better when hyperbolic tangent transfer function is employed in hidden layer [13, 15]. Neural networks based on these functions are also good universal approximators. The output layer contains linear transfer function in order to simulate functions without discontinuities.

## Experiments and Models Training

The performance of a network is obviously dependant on the number and quality of data provided for system training. Too many data can extremely slow down the learning process and too few data provide insufficient information about localized featuring and cause the

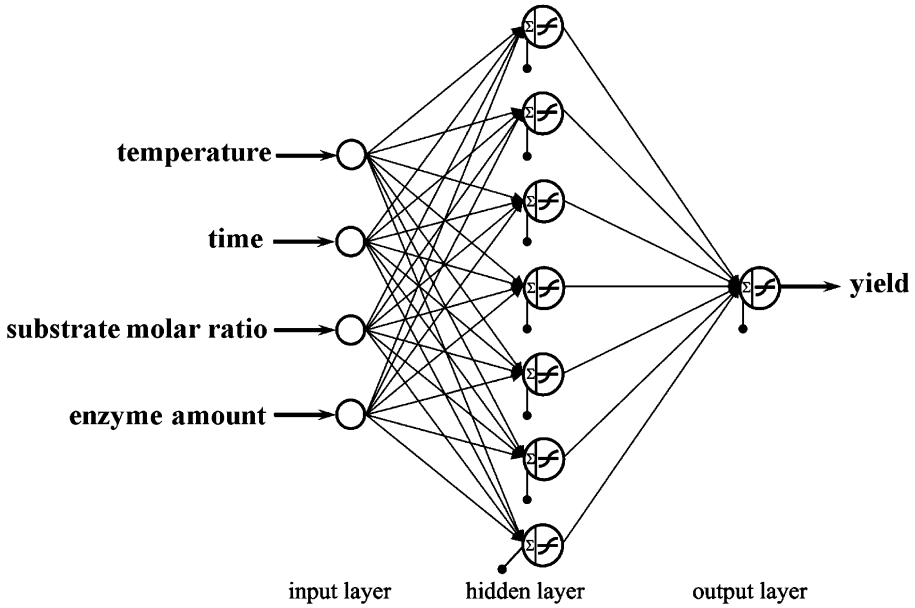


**Fig. 1** Structural organization of the neural network used for the estimation of adipate ester yield

network to have a poor performance. Therefore, an optimized number of input data must be used for training of the network [16]. According to Jenkins [17], using a hypercube design for the selection of input data is sufficient for appropriate training of the network. In this study, a five-level four-factor CCRD was employed for experimental design to represent the training data. CCRD is an efficient statistical design with a hypercube geometry region which is generally one of the best designs for decreasing the number of experiments while maintaining statistical significance [18]. In order to define the parameters of ANN model, a batch training approach based on a set of experimental input–output data is preformed. In batch training, weights and biases are only updated after all the inputs and targets are presented. Once the training is over, ANN is capable of predicting the output when any input similar to the pattern that it has learned is fed. A second set of data is used to evaluate the quality of the network during training. In addition, the performance of the trained network is estimated based on the accuracy of the network on the test data. This set consists of data unseen by the ANN during training. The training process is carried on until a minimum of the error is reached in the second (validating) set. The values of the experimental yield of adipate ester, which have been used for training, validating, and testing the network are shown in Table 1.

The performance of the ANN models are measured by mean absolute error (MAE) and correlation coefficient ($R^2$) between the predicted values of the network and the experimental values, which are calculated by Eqs. 1 and 2, respectively.

$$\mathrm{MAE} = \frac{1}{n}\sum_{i=1}^{n}\left|y_i^* - y_p^{(i)}\right| \tag{1}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(y_i^* - y_p^{(i)}\right)^2}{\sum_{i=1}^{n}\left(y_i^* - \overline{y}\right)^2} \tag{2}$$

where $\overline{y}$ is the average of $y$ over the $n$ samples, and $y_i^*$ and $y_p^{(i)}$ are the $i$th target and predicted esterification yield, respectively.

The network backpropagation is an effective approach for defining ANN parameters, which is widely used for current types of neural network systems to develop the prediction model because of its ability to model any function. Many different types of the backpropagation algorithm are presented in this regard [19]. It is shown that a great improvement on realization performance can be captured by employing second-order approaches such as conjugate gradient or the Levenberg–Marquardt (LM) optimization techniques [20, 21]. In this regard, the LM algorithm is introduced as the most efficient optimization method in terms of speed and accuracy. The LM algorithm appears to be the fastest method for training moderate-sized feedforward neural networks, where the training rate is ten to 100 times faster than the usual gradient descent backpropagation method [22]. For a multi-input–single-output neural network, the objective function or performance index is considered as the error function ($e$) given by Eq. 3.

$$e = \sum_{i=1}^{P}\left(y_i^* - y_i\right)^2 \tag{3}$$

where $y_i$ is the actual output for the $i$th pattern and $y_i^*$ is desired output. $P$ is the total number of training patterns. The algorithm for parameter updating ($w$) is presented by Eq. 4.

$$w_{k+1} = w_k - \left[J^T J + \mu I\right]^{-1} J^{\mathrm{T}} E \tag{4}$$

where $J$ is the Jacobian matrix; $I$ is the identity matrix and $\mu$ is the training parameter. Here, $J^T J = H$ is referred as the Hessian matrix. For $\mu=0$, the LM algorithm turns to Gauss–Newton method and for very large $\mu$ it turns to the steepest decent algorithm. In this study, the initial value for $\mu$ was considered equal to 0.01. The error vector $E$ is calculated as follows,

$$E = \left[\, e_1\, e_2 \ldots e_P \,\right]^{\mathrm{T}} \tag{5}$$

where $T$ outside the bracket stands for "transpose." The Jacobian matrix, $J$, is obtained as

$$J = \begin{bmatrix} \frac{\partial e_1}{\partial w_1} & \frac{\partial e_1}{\partial w_2} & \cdots & \frac{\partial e_1}{\partial w_N} \\ \frac{\partial e_2}{\partial w_1} & \frac{\partial e_2}{\partial w_2} & \cdots & \frac{\partial e_2}{\partial w_N} \\ \cdot & \cdot & \cdots & \cdot \\ \cdot & \cdot & \cdots & \cdot \\ \frac{\partial e_P}{\partial w_1} & \frac{\partial e_P}{\partial w_1} & \cdots & \frac{\partial e_P}{\partial w_N} \end{bmatrix} \tag{6}$$

It should be noted that $J$ is a $P$-by-$N$ matrix, where $N$ is the number of adjustable parameters. It is required that the Jacobian matrix $J$ and the inversion of $J^{\mathrm{T}} J$ (square matrix $N \times N$) be computed at each iteration step. For this reason, the LM algorithm is not practical for large-sized neural networks. There are modified versions of LM algorithms that can be employed in such conditions, which require less memory and computational operations [23]. It should be noted that an ill-conditioned Hessian matrix could make the minimization problem much harder to solve. It is reported that in such cases the Levenberg–Marquardt algorithm is often a better choice [24].

In this paper, the standard LM algorithm is employed for tainting of *NN* models. The model's training process is performed by using MATLAB Neural Network toolbox version 7.4. During the training process, the training performance is calculated with respect to training, validating, and testing datasets for different configuration of NN models, which are shown in Fig. 2. The average relative error of validating data decreased to 1%, which can be considered to suspend the model training.

In order to achieve fast convergence to minimal error, the input and output data are normalized within the range of [0, 1], which can increase the ability of the network to learn the association between inputs and output [15, 16].

## Results and Discussion

Finding the optimal number of neurons in hidden layers is an essential factor for the accuracy of developed models. However, some general approximation problems for a number of neurons in feedforward neural networks are presented, but the optimal number of neurons is usually determined by trial and error [25]. In this study, different neural networks with different possible configurations are made and one of them, which provides the best compromise between bias and variance and gives a good generalization, is selected.

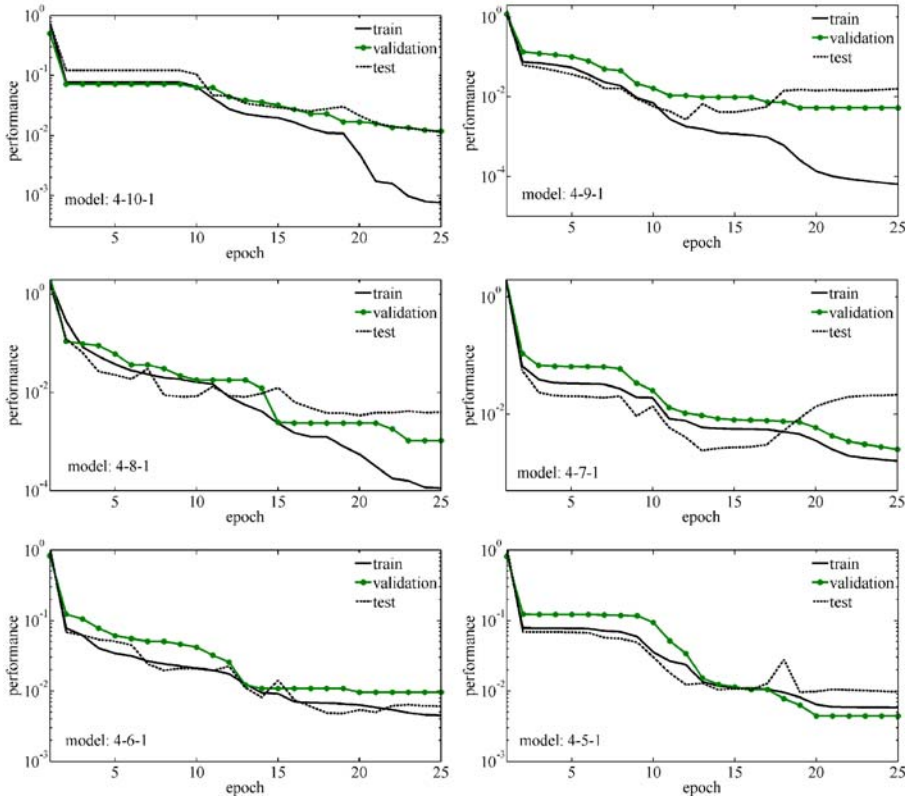


**Fig. 2** The training performance of neural networks with different number of neurons

Figure 2 illustrates the training performance of neural networks with different proposed model structure. Although the average relative error for all of the networks decreased to less than $10^{-2}$ with 25 epochs, this is not enough to evaluate the ability of the network to predict the output. In order to validate the accuracy and generalization of the models, other tests must be preformed based on different sets of data.

The acceptable values of $R^2$ and MAE mean that the model equation defines the true behavior of the system and it can be used for interpolation in the experimental domain [18].

In this regards, in order to find optimum number of neurons in the hidden layer, six different 4–*x*–1 architectures (*x* changes from five to ten) are used. It should be noted that increasing the complexity of a system causes the ability to make precise and significant statements about its behavior to be diminished (principle of incompatibility) [26]. As a result, it is observed that, by increasing the number of neurons in the hidden layer, the MAE increased. Therefore, a satisfactory degree of precision must be involved in the developed models.

In Table 2, the corresponding errors are estimated with respect to training data when the number of neurons is varied. In addition, in Fig. 3, a comparison between the correlation coefficients, $R^2$, of the network response and the corresponding target is performed in order to show the accuracy of the developed models.

As shown in Fig. 3, the model with seven neurons at hidden layer has the best performance, which validates the captured results presented in Table 2.

**Table 2** Modeling error with respect to training data.

| Model | $R^2$ | MAE |
|---|---|---|
| 4–10–1 | 0.9318 | 1.9948 |
| 4–9–1 | 0.9823 | 1.7061 |
| 4–8–1 | 0.9358 | 5.3082 |
| 4–7–1 | 0.9998 | 0.0966 |
| 4–6–1 | 0.9591 | 0.1881 |
| 4–5–1 | 0.7169 | 10.7684 |

Evaluating the accuracy of developed model should always be performed with respect to a set of data, which is not used in the training process. In Table 3, the corresponding errors are estimated with respect to validating set of data when the number of neurons is varied. In addition, in Fig. 4, a comparison between the correlation coefficients of the network response and the corresponding validating data is performed.

During the last section of model evaluation, the model response is compared with testing data as presented in Table 4. Simulation results show that the best response is corresponding to model 4–7–1. For this model, $R^2$ and MAE values between the actual

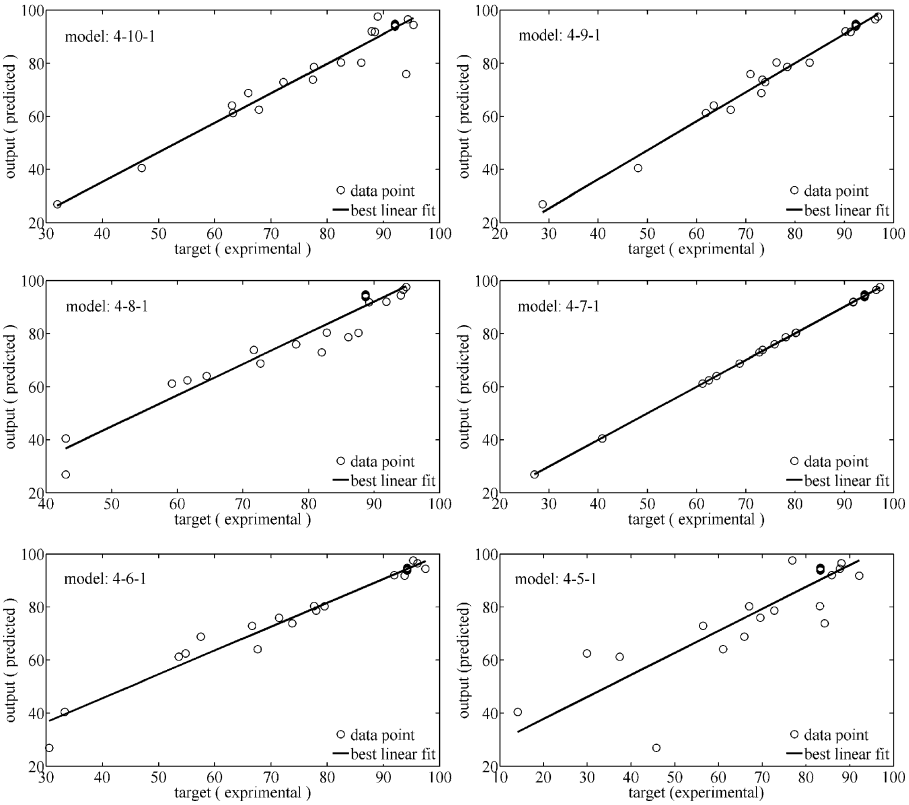


**Fig. 3** Correlation between the actual and predicted values of the ANN models with respect to training data

**Table 3** Modeling error with respect to validating data.

| Model | $R^2$ | MAE |
|---|---|---|
| 4–10–1 | 0.8084 | 8.2548 |
| 4–9–1 | 0.8024 | 2.1459 |
| 4–8–1 | 0.8707 | 6.0894 |
| 4–7–1 | 0.9241 | 1.9439 |
| 4–6–1 | 0.6790 | 6.7115 |
| 4–5–1 | 0.6079 | 10.2426 |

and predicted responses were determined as 0.9601 and 2.5569, respectively, for ANN testing set.

In this study, another popular type of feedforward networks which is radial basis function network (RBF) was also employed to predict the yield of enzymatic synthesis of adipate ester, using the same training, validating, and testing data. Simulation results with respect to training data show that the RBF model is more accurate than backpropagation network with $R^2$ 0.9999 and a smaller MAE 0.06857. In addition, the responses of these models (4–7–1 and RBF) were compared over the validating and testing datasets. However,

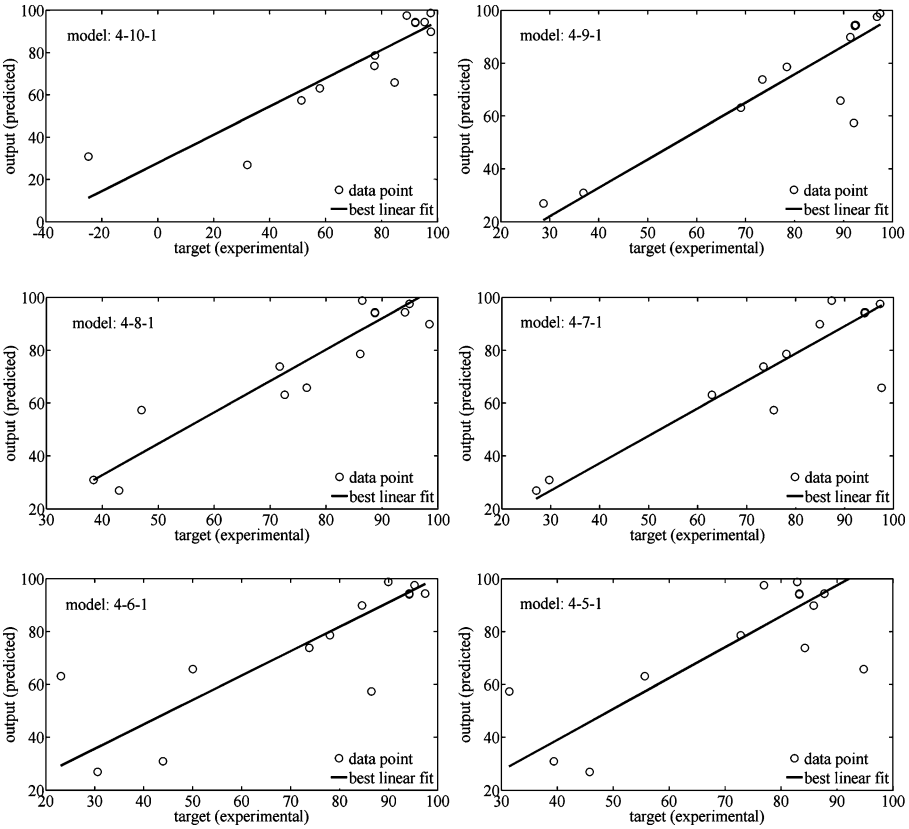


**Fig. 4** Correlation between the actual and predicted values of the ANN models with respect to validating data

**Table 4** Modeling error with respect to testing data.

| Model | $R^2$ | MAE |
|---|---|---|
| 4–10–1 | 0.8171 | 6.0656 |
| 4–9–1 | 0.7450 | 5.2847 |
| 4–8–1 | 0.9081 | 2.9873 |
| 4–7–1 | 0.9601 | 2.5569 |
| 4–6–1 | 0.8755 | 6.2612 |
| 4–5–1 | 0.8721 | 3.4647 |

the RBF model shows a poorer performance and does not have a good generalization capability in comparison with the developed models. In the case of validating dataset, the correlation coefficient ($R^2$) and MAE for RBF model were 0.7396 and 2.6737, respectively, and, for the testing dataset, $R^2$ was 0.3844 and MAE was 6.8576, respectively.

The results indicate that the produced ANN model using feedforward backpropagation neural network is properly capable of learning the relationship between the input and output parameters and hence could be employed in the further part of the study. The trained ANN model is used then to predict the esterification yield with various parametric values. Figure 5 shows the effects of reaction temperature on dioctyl adipate synthesis at substrate molar ratio of 5.5:1, amount of enzyme of 200.5 mg, and time of 195 min. The percentage of yields increased with increase in temperature within the given range (35–65 °C). Higher temperatures promote collisions between enzyme and substrate molecules to result in accelerated rates of the reaction [27]. An increase in temperature also improves solubility of the substrates and reduces viscosity; and mass transfer limitations resulted in enhancement of the reaction yield. Due to the evaporation of the solvent (boiling point of *n*-hexane=68 °C),

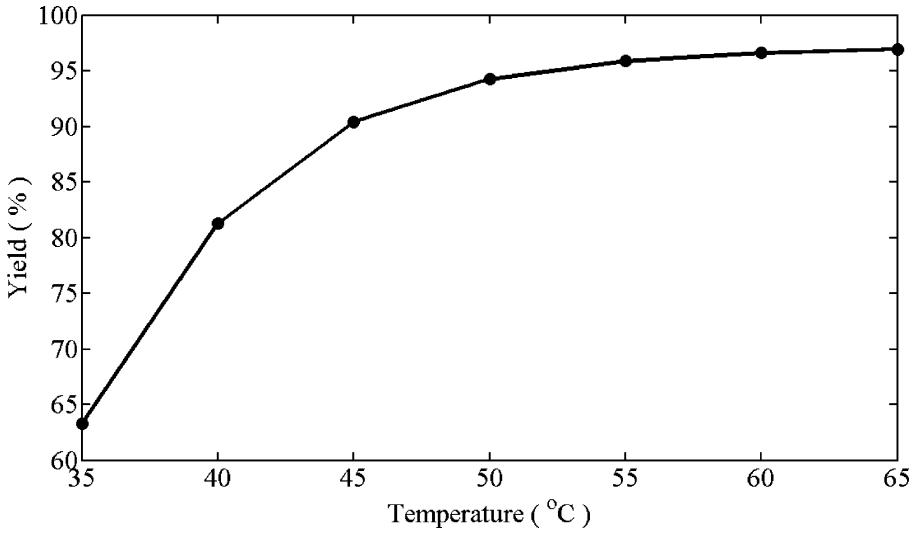


**Fig. 5** Effect of temperature on the synthesis of adipate ester. Other variables are constant; substrate molar ratio 5.5:1, amount of enzyme 200.5 mg, and time 195 min

higher temperatures were not considered for the reaction. Furthermore, high temperatures may also cause enzyme inactivation due to denaturation process.

The effect of varying amount of enzyme on esterification reaction at 195 min, 50 °C, and molar ratio of 5.5:1 is shown in Fig. 6. The percentage of yields increased with increase in amount of enzyme up to 194 mg. At this point, all substrates are bound to the enzyme and addition of any enzyme molecule may cause a decrease in the yield due to the substrate limitations [28]. However, high amount of enzyme may cause diffusion which relates to mass transfer limitation [29].

Figure 7 represents the effect of varying molar ratio (octyl alcohol/adipic acid) on the synthesis of dioctyl adipate at 50 °C, 195 min, and 200.5-mg enzyme. Increasing of the yield with increase in substrate molar ratio may be attributed to better solubility of the solid acid in higher amount of alcohol, reduction of viscosity, and also more availability of the alcohol for the enzyme.

Figure 8 illustrates the reaction time profile for the synthesis of dioctyl adipate at 50 °C, 200.5-mg enzyme, and molar ratio of 5.5:1. The percentage of yields increased with increase in incubation time up to 195 min and subsequently started to decrease. Prolonging the reaction time will increase the volume of water produced by the reaction and thus hydrolysis of ester will occur [30].

## Conclusion

In this study, feedforward backpropagation neural network models were designed, trained, and validated for yield prediction of enzymatic synthesis of dioctyl adipate ester. The optimal number of hidden neurons was determined by comparing the performance of the ANN models using correlation coefficient ($R^2$) and MAE. The final selected model, 4–7–1, successfully represented real relationship between the response and reaction parameters

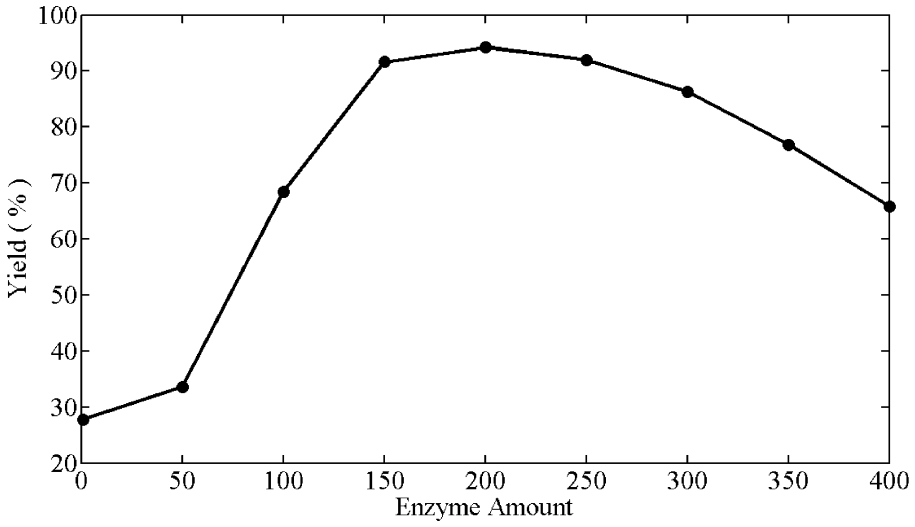


**Fig. 6** Effect of enzyme amount on the synthesis of adipate ester. Other variables are constant; reaction time, 195 min, temperature, 50 °C, and molar ratio 5.5:1

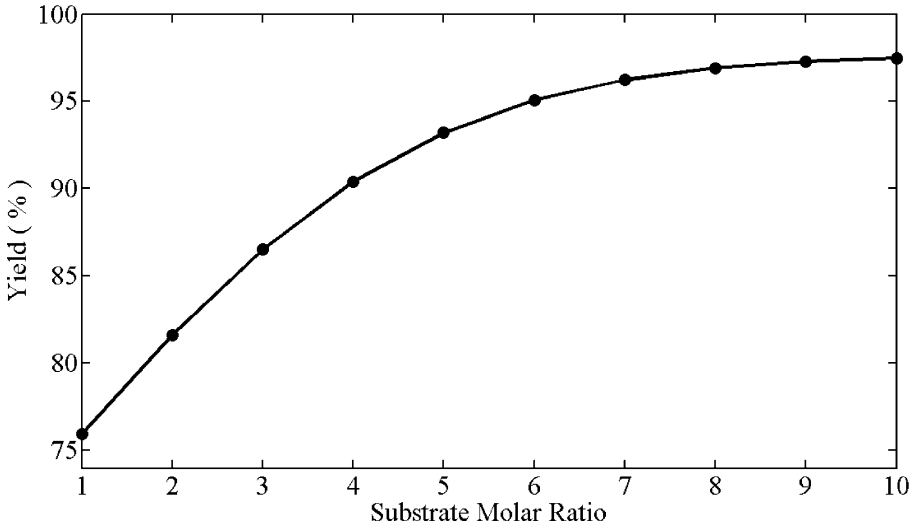


**Fig. 7** Effect of substrate molar ratio on the synthesis of adipate ester. Other variables are constant; reaction time, 195 min, temperature, 50 °C and enzyme amount, 200.5 mg

with a high correlation coefficient and a low MAE for training, validating, and testing datasets. By using the empirical model, the effect of four main reaction parameters including temperature, time, amount of enzyme, and substrate molar ratio were evaluated. The model can be used to predict the yield of adipate ester synthesis under any given conditions within the experimental range. Further modification will make the model proper to be used for yield prediction of upscale synthesis of the ester. This would help the

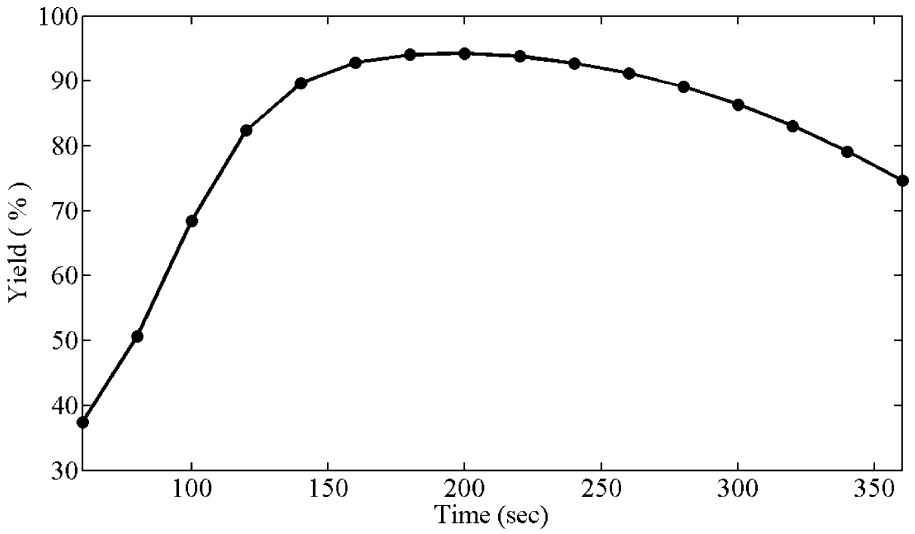


**Fig. 8** Effect of reaction time on the synthesis of adipate ester. Other variables are constant; temperature, 50 °C, 200.5 mg enzyme, and molar ratio, 5.5:1

researchers find a way to improve the reaction conditions by reducing the number of trial-and-error experimental cycles, hence also the time and cost.

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