Comparison of Artificial Neural Networks (ANN) and Adaptive Neuro-Fuzzy Inference System (ANFIS) Models in Simulating Polygalacturonase Production

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The artificial neural network (ANN) method was used in comparison with the adaptive neuro-fuzzy inference system (ANFIS) to describe polygalacturonase (PG) production by *Bacillus subtilis* in submerged fermentation. ANN was evaluated with five neurons in the input layer, one hidden layer with 7 neurons, and one neuron in the output layer. Five fermentation variables (pH, temperature, time, yeast extract concentration, and K_2HPO_4 concentration) served as the input of the ANN and ANFIS models, and the polygalacturonase activity was the output. Coefficient of determination (R^2) and root mean square values (RMSE) were calculated as 0.978 and 0.060, respectively for the best ANFIS structure obtained in this study. The R^2 and RMSE values were computed as 1.00 and 0.030, respectively for the best ANN model. The results showed that the ANN and ANFIS models performed similarly in terms of prediction accuracy.

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INTRODUCTION

Enzyme production can be affected by numerous process variables and culture conditions such as pH, temperature, time, minerals, and other fermentation medium components. Thus, fermentation is a complex process due to the possible interaction of these variables. In addition, fermentation medium is crucial for cost-effective enzyme production. To reduce the number of extensive experimentation and learn about complex interactive effects, modeling and optimization is inevitable (Bas and Boyaci 2007; Mingzhi *et al*. 2009). Predictive models are informative tools for the rapid and cost-effective study of microbial growth, product development, risk assessment, and scientific purposes (Ross 1999). Response surface methodology (RSM) is the most frequently used statistical method in the optimization of fermentation medium parameters. However, RSM does not adequately explain nonlinear models (Ross 1999). To overcome this problem, artificial intelligence-based models such as artificial neural networks (ANN) and adaptive neuro-fuzzy inference system (ANFIS) need to be considered.

ANN is a mathematical algorithm and modeling method that correlates inputs and outputs (Yılmaz 2012). ANFIS is a combination of ANN and fuzzy inference system (FIS). To obtain a better modeling system, ANN can be combined with FIS to improve speed, fault tolerance, and adaptiveness (Jang 1993).

ANN and ANFIS have been successfully applied to model biological systems (Jang and Sun 1995; Bas *et al*. 2007). ANN is also a popular artificial learning tool in

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biotechnology and has been used for the optimization of bioprocesses (Monahar and Divakar 2005) and polygalacturonase, protease, laccase, and hydantoinase enzyme production from microorganisms (Nagata and Chu 2003; Dutta *et al*. 2004; Rekha *et al*. 2013; Tokatli *et al*. 2009; Chiranjeevi *et al*. 2014). Tokatli *et al*. (2009) compared RSM and ANN models for polygalacturonase activity and biomass production by *Aspergillus sojae* ATCC 20235, concluding that the ANN model predicted polygalacturonase activity better than the RSM model. In another study, Rekha *et al*. (2013) successfully used ANN for modeling polygalacturonase production from a newly isolated *Thalassopira frigidphilosprofundus*. Edupuganti *et al*. (2014) reported the optimization of alpha-galactosidase production in submerged fermentation by *Acinetobacter sp*. using feed-forward neural networks. The effect of six different variables including pH, temperature, agitation speed, carbon source (raffinose), nitrogen source (tryptone), and K_2HPO_4 on enzyme yield was evaluated, and a topology of 6-10-1 for a feed-forward neural network was constructed to determine the interactions between fermentation variables and enzyme yield. Rani *et al*. (2014) compared RSM and ANN models to enhance the production of phytase by *Rhizopus oryzae*. The ANN models were also applied to predict and enhance the production of alkaline protease from *Bacillus circulans* and glutaminase from *Bacillus subtilis* (Rao *et al*. 2008; Sathish and Prakasham 2010).

There are a number of studies in which models that are developed based on RSM and ANN use the same experimental design to optimize the fermentation medium. In recent years, a limited number of studies have investigated the possibility of using nonlinear modeling techniques in fermentation processes, including ANN and ANFIS. However, there has not been a comparative study of ANN and ANFIS models for predicting polygalacturonase activity. This is the first report on the prediction of enzyme production using an ANFIS model in comparison with an ANN model. This work focused on predicting the production of polygalacturonase from *Bacillus subtilis* by ANN and ANFIS models. Several ANN and ANFIS models were developed to predict polygalacturonase activity on the basis of inputs. The accuracy of the estimated data was defined, and the overall prediction ability of these two techniques was compared.

EXPERIMENTAL

Fermentation System

The data used in this study were obtained from submerged fermentation studies with *Bacillus subtilis,* which was kindly provided by the ARS culture collection, Northern Regional Research Laboratory (NRRL), Peoria, IL, USA. The details concerning the inoculation, fermentation, and measurement of enzyme activity steps are given elsewhere (Uzuner and Cekmecelioglu 2015).

Artificial Neural Network (ANN) Modeling

The input variables used in the ANN models were pH (X_1), temperature (X_2), time (X_3), yeast extract concentration (X_4), and K_2HPO_4 concentration (X_5). The ANN model was generated for output variable polygalacturonase (PG) activity (Y_1). Several ANNs with different numbers of hidden layer neurons were developed for describing PG production. Input data were randomized into three sets: learning, validation, and testing. Usually, 30% of data are used for testing, and the remaining 70% for training and validation (Mehdizadeh and Movagharnejad 2011). The experimental data included 92 data points. For ANN

modeling, the collected experimental data were divided into training (50%), checking (cross-validation) (20%), and testing (validation) (30%) datasets. ANNs were developed using MATLAB R2011a. The first step in neural network modeling is to select the topology of the network. In this network, there are input layer with five inputs, a hidden layer consisting of seven neurons, scalar weights, biases and sigmoid transfer function (logsig, Eq.1) and an output layer including one neuron, scalar weights, a bias, and a linear transfer function (purelin, Eq.2) (Fig 1a).

$$\text{logsig(x)} = \frac{1}{1 + e^{-x}} \tag{1}$$

$$\text{purelin(x)} = x \tag{2}$$

The network training was performed with the function of "trainlm", which updates weight and bias values according to Levenberg-Marquardt optimization. Trainlm employs the Levenberg-Marquardt backpropagation algorithm that can be expressed as the sum of squares of non-linear real-valued functions. "learngd" was used as the adaption learning function in the learning of the network. learngd is the gradient descent weight and bias learning function. The maximum training epochs were 1000, and the mean square error was 0.0001. The other parameters of the neural network were taken as defaults of the neural network toolbox, MATLAB R2011a.

ANFIS Modeling

Fuzzy systems are classified into three types: Mamdani's system, Tsukamoto's system, and Sugeno's system. Mamdani's system is the most utilized, whereas Sugeno's system is more compact and efficient. Moreover, it is the most popular tool for fuzzy modeling (Takagi and Sugeno 1985; Cobaner *et al*. 2009). Therefore, the Sugeno-type fuzzy system was used in this study. In the ANFIS modeling, the data sets were divided into three groups for training, testing, and validating. For ANFIS modeling, the collected experimental data were divided into training (50%), checking (cross-validation) (20%) and testing (validation) (30%) datasets. The fuzzy logic toolbox of MATLAB R2011a was used for the non-linear modeling of PG activity. The ANFIS model was optimized with a backpropagation algorithm. For selection of the best fit fuzzy model, 3 different ANFIS models that possessed the same number of membership functions (3), different types of membership function for input (Gaussian, triangular shaped, trapezoidal shaped), and similar membership functions for output (linear) were constructed. The maximum learning epochs were 1000, and the mean square error was 0.001. The ANFIS structure of polygalacturonase activity consisted of five layers (Fig. 1).

The first layer of the model consisted of the input variable (pH, temperature, time, yeast extract, and K_2HPO_4 concentration) membership functions (MFs). The main purpose of this layer was to provide the inputs to the next layer. The second layer was a membership layer and determined the weights for each MF. The fuzzification layer (layer 2) contained 15 nodes and 45 non-linear parameters (Fig. 1). The antecedent rule layer (layer 3) with 251 nodes performed a pre-condition matching process for fuzzy rules. The consequent rule layer (layer 4) with 251 nodes and 1458 linear parameters took the inference of the rules and generated output values. The consequent rule layer (layer 4) was determined using the backpropagation algorithm. The inference rule (output) layer of polygalacturonase activity (layer 5) combined the inputs and transformed the fuzzy classification into a binary result.
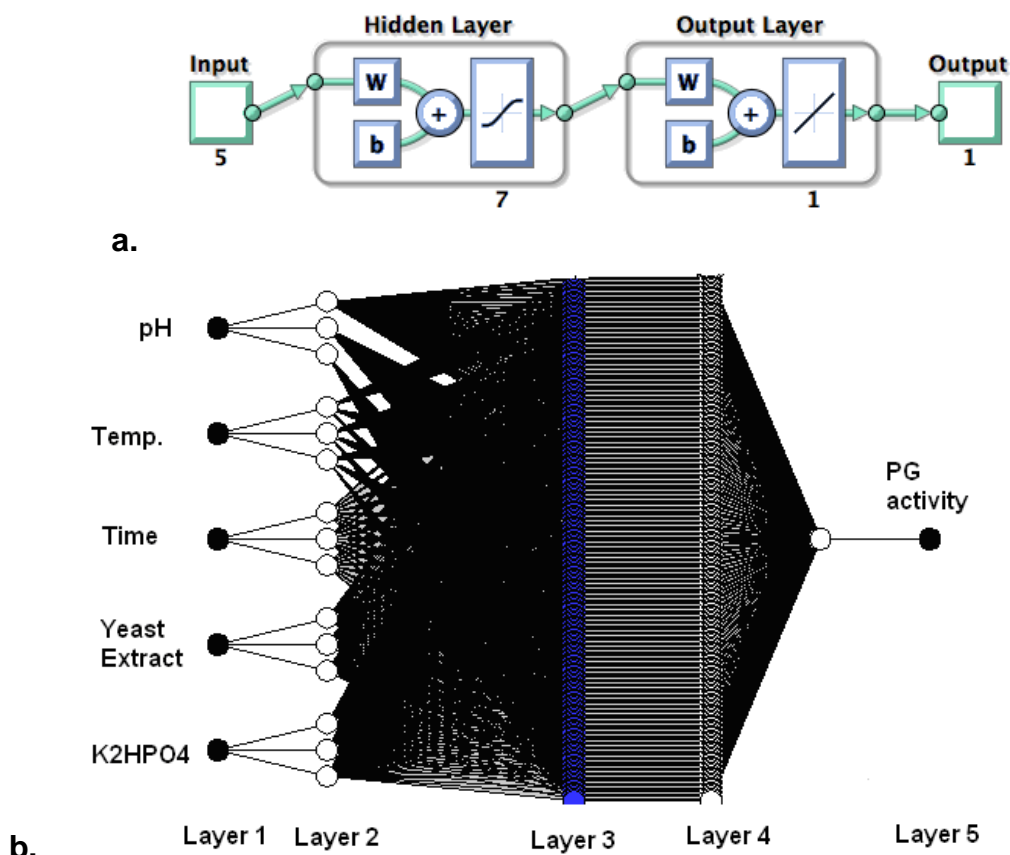
a.

b.

Fig. 1. Schematic representation of (a) ANN and (b) ANFIS to simulate polygalacturonase activity

Selection of the Best Model

To compare the constructed models and choose the best one, the performance of the models was measured using common comparison tools in modeling including R^2, root mean square error (RMSE, Eq. 3), and mean absolute error (MAE, Eq. 4),

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_p - y_e)^2} \tag{3}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|y_p - y_e\right| \tag{4}$$

where n was the number of experiments, y_p was the predicted value, and y_e was the experimental value.

Statistical Analysis

Statistical analyses were carried out to test the significance of different polygalacturonase production. Pairwise comparisons were made by Tukey's test with a significance level of 0.05.

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RESULTS AND DISCUSSION

Predictive Modeling of PG activity using ANN
Effect of architecture and topology on neural network

The selection of network topology in ANN modeling is the key issue. Several parameters such as the number of hidden layers, number of neurons, transfer function, epochs, and learning rate affected the network topology. The number of hidden neurons is one of the most important parameters of ANN modeling. A high number of neurons performs adequately for training data but may fail for testing data (over-fitting), while having too few hidden neurons may result in unsatisfactory convergence (under-fitting) (Tokatli *et al*. 2009). In this study, the number of neurons in the hidden layer was chosen by trial and error method, by varying the neurons from 3 to 12.

Several ANN models with different network topologies were trained, tested, and evaluated to select the best network topology. The R^2, RMSE, and MAE statistics from training and testing data for different ANN topologies is summarized in Table 1.

Table 1. Performance of Different Artificial Neural Network (ANN) Models in Estimation of PG Activity

Network	R^2		RMSE		MAE	
	Training	**Testing**	**Training**	**Testing**	**Training**	**Testing**
5:3:1	0.929	0.748	0.145	0.259	0.045	0.170
5:4:1	0.964	0.867	0.110	0.189	0.056	0.087
5:5:1	0.947	0.833	0.118	0.251	0.036	0.161
5:6:1	0.983	0.974	0.071	0.100	0.025	0.051
5:7:1	**0.995**	**0.968**	**0.032**	**0.095**	**0.003**	**0.031**
5:8:1	0.995	0.979	0.032	0.138	0.003	1.061
5:9:1	0.996	0.971	0.032	0.084	0.003	0.019
5:10:1	0.995	0.953	0.032	0.138	0.003	0.641
5:11:1	0.997	0.962	0.028	0.100	0.015	0.661
5:12:1	0.996	0.968	0.032	0.095	0.007	0.089

Table 1 shows that the 5-7-1 topology was the best, with minimum RMSE and MAE, and maximum R^2 values. In the case of training data set, R^2 and MAE values were 0.995 and 0.003, respectively, whereas for testing data set, R^2 was 0.968 and MAE was 0.031 (Table 1).

The effectiveness of the predictive model results was also compared using RSM for pectinase production from *B. subtilis* (Uzuner and Cekmecelioglu 2015). The obtained R^2 value (0.995) from ANN analysis was higher than the R^2 value (0.920) obtained from RSM for the same data. These results indicated that the prediction accuracy of ANN was better than the RSM model for our enzyme production process. Tokatli *et al*. (2009) performed different ANN topologies to predict polygalacturonase (PG) activity as well with a 5-2-1 network topology (R^2=0.84).

Predictive modeling of PG activity using ANFIS

The varying membership function types of input and output were used to evaluate the ANFIS model. The number of membership function (NMF), membership function type of input (MFTI), and membership function type of output (MFTO) for the ANFIS modeling of polygalacturonase activity are reported in Table 2.

Table 2. Performance of Different ANFIS Model in Estimation of PG Activity

MFTI	MFTO	NFMs	R^2		RMSE		MAE	
			Training	Testing	Training	Testing	Training	Testing
Gaussmf*	Linear	5	0.685	0.638	0.215	0.214	0.149	0.177
Gaussmf	Constant	5	0.283	0.131	0.382	0.483	0.037	0.274
Trapmf**	Linear	5	0.797	0.643	0.197	0.259	0.068	0.028
Trapmf	Constant	5	0.253	0.689	0.875	1.734	0.729	1.724
Trimf*	**Linear**	**5**	**0.911**	**0.841**	**0.127**	**0.052**	**0.041**	**0.026**

*Gaussian membership function,
**Trapezoidal-shaped membership function,
***Triangular-shaped membership function

The R^2, RMSE, and MAE values of the constructed models are presented in Table 2. The ANFIS model, including trimf input function type, linear output function type, and 5 membership function number showed the highest accuracy with a high coefficient of determination ($R^2 = 0.841$) and very low RMSE (0.052) and MAE (0.026) values (Table 2). This was higher than other models in Table 2. For the training data set, the coefficient of determination (R^2) and RMSE values were 0.911 and 0.127%, respectively. For testing the data set, R^2 was 0.841, and RMSE was 0.052% (Table 2).
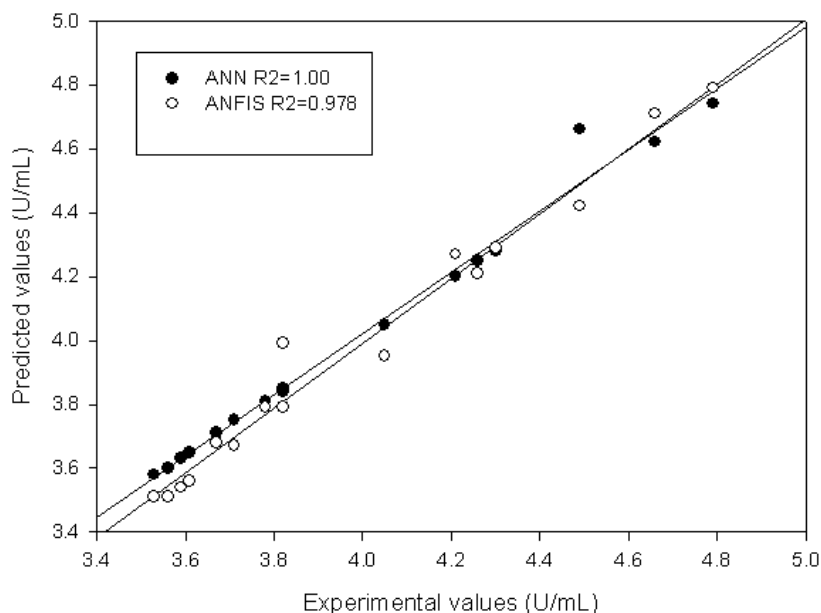

Fig. 2. Predicted versus experimental values of PG activity for ANN and ANFIS models

Comparison of ANN and ANFIS models

To evaluate the validity of ANN and ANFIS results, 16 new trials that were not involved in training were used. Figure 2 shows the plot of predicted PG activity by ANN and ANFIS against the experimental values. ANN had a correlation between predicted and actual values for PG production (r = 0.99) in comparison to ANFIS model.

The ANN model exhibited similar predictions with the ANFIS model (Fig. 2). In addition, the best-fit ANN and ANFIS models were derived from the BBD training datasets (Table 1 and 2). After the model was built, the performance of the constructed ANN and ANFIS models was also compared. The comparative statistical values of RMSE and R^2 for ANN and ANFIS models are given in Table 3.

Table 3. Summary of the Best Fit ANN and ANFIS Models for PG Activity

Parameters	Training Data		Testing Data	
	ANN	ANFIS	ANN	ANFIS
RMSE	0.032	0.127	0.095	0.052
R^2	0.995	0.911	0.968	0.841

Table 4. Validations of Measured Values According to Box-Behnken (BBD) Designs *versus* Predicted Values by ANN and ANFIS Models for Polygalacturonase Activity

PG Activity (U/mL)			
DOE	Validation Data	Predicted	
		ANN	ANFIS
BBD	3.82	3.84	3.79
	3.82	3.85	3.99
	3.67	3.71	3.68
	3.78	3.81	3.79
	4.79	4.74	4.79
	3.56	3.60	3.51
	4.49	4.46	4.42
	3.53	3.58	3.51
	3.71	3.75	3.67
	3.59	3.63	3.54
	4.26	4.25	4.21
	4.21	4.20	4.27
	4.66	4.62	4.71
	4.30	4.28	4.29
	4.05	4.05	3.95
	3.61	3.65	3.56
Mean	3.99 ± 0.41	4.00 ± 0.38	3.98± 0.42
R^2_V (%)		100.00	97.80
RMSE		0.03	0.06

Though both models performed well and offered stable responses in prediction, the ANN approach better predicted and fit the testing data. The two best-fit ANN and ANFIS models performed in terms of the independent validation-derived R^2 (R^2_V) and RMSE values for PG production (Table 4). Therefore, the accuracy of the ANN model was higher and had better fitted data than the ANFIS model.

CONCLUSIONS

1. The ANN and ANFIS methods are powerful methods to obtain correlation between input and output in complex nonlinear systems.

2. Trimf and 5-7-1 topologies yielded the best prediction polygalacturonase production using *B. subtilis* for ANFIS and ANN models, respectively.

3. The best-fit model was identified in the ANN model with the highest R^2v (0.10) and lowest RMSE (0.03) values.

4. Prediction of ANN and ANFIS models was similar. Therefore, ANN and ANFIS models can be considered as prediction tools for biological systems such as enzyme activity.

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