

Prediction of Ethanol Concentration in Biofuel Production Using Artificial Neural Networks

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Abstract Environmentally friendly and important enhancements in biofuel production technology are necessary in order to cut back production prices and create it as a competitive resource material. This study performed a cost-effective bioprocess to provide bio-ethanol from sugarcane molasses by chosen strains of the yeast *S. cerevisiae*, through experiments at laboratory, pilot and industrial scales. Artificial neural networks are shown to be powerful tools for system modeling. The objective of this study was to develop a straightforward, accurate and time saving prognosticative model for alcohol production. Results recommend that artificial neural networks provide a good means of effective recognizing patterns in data and accurately predicting ethanol concentration based on investigating inputs. The ethanol concentration evaluated in experiments of industrial biofuel production and this research develops a simple, accurate, nondestructive and time saving artificial neural networks model for estimation of ethanol concentration in batch ethanol fermentation from molasses based on live and dead yeast cells, sugar concentration.

Keywords: artificial neural networks, yeast, alcohol production, prediction

1. Introduction

The gradual depletion of fossil fuel and the biological environmental deterioration resulted from the over consumption of petroleum derived transportation fuels have gained great attentions and makes it imperative to develop alternatives that are renewable and environmentally friendly (Yuji et al., 2009). Ethanol is a very important industrial chemical with rising potential as a biofuel to replace vanishing fossil fuels, whose utilization could improve energy security, cut back trade deficits, decrease urban pollution and contribute very little net carbon dioxide accumulation to the atmosphere (Borjesson, 2009; Wyman, 1994). Efficient enhancements in alcohol production technology are necessary in order to cut back production prices to make grain alcohol a competitive resource material and it is a renewable energy with high potency and low environmental impact. Ethanol is a renewable energy supply because it is produced via microbial fermentation. Fermentation alcohol production is counting on traditional technologies utilizing grains (corn, wheat and sweet potato), some sugar (cane, beet sugar and molasses) and inexpensive cellulosic biomass (corn stalk, paper sludge, switchgrass) by using microorganisms such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* (Behera et al., 2010; Cardona-Alzate et al., 2006; Holzberg et al., 1967; Gnansounou et al., 2005, Yuji et al., 2009; Kopsahelis et al., 2007). The most commonly used biomasses for fermentation alcohol production are corn, sugar cane and wheat. Others include grains and waste from the beverage, brewery and wine

industries and about 12 billion gallons of ethanol were produced in 2005 (NESEA 2002). A more useful method is to model the system, which in turn requires an explicit mathematical between input and output relationship. Such explicit mathematical modeling is very difficult and is not readily tractable in poorly understood systems. Alternatively, recently soft computing methods which concern computation in an imprecise environment have gained significant attention (Bagheri et al., 2009). Artificial neural networks, which have shown great ability in solving complex nonlinear system identification and control problems and can be described either as mathematical and computational models for non-linear function approximation, data classification, clustering and non-parametric regression or as simulations of the behavior of collections of model biological neurons (Ahmadi et al., 2008). These networks are applied in many fields to model and predict the behaviors of unknown systems, very complex systems or both based on investigation of given input-output data (Ahmadian-Moghadam 2012; Nariman-Zadeh et al., 2005). This study addressed the question of whether artificial neural networks could be used to estimate ethanol concentration (outputs) based on specified variables inputs (sugar concentration, live and dead yeast cells) on a fermentation process. Such equations allow alcohol production industries and researchers to estimate ethanol production in relation to other factors like in relation to other factors such as Temperature, pH and Partial oxygen. The objective of this research was to develop a simple, accurate, nondestructive and time saving predictive model for ethanol concentration estimation in concentration in batch ethanol fermentation from molasses.

2. Material and Method

2.1. Medium Preparation

2.1.1. Pre Culture Medium

Sugarcane molasses were obtained from a Philippines Sugarcane Company Central Azucarera de Tarlac, San Miguel Tarlac, Philippines. The pre culture medium contain 5% (w/v) total sugar molasses, 0.1g $(\text{NH}_4)_2\text{SO}_4$, 0.025g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and KH_2PO_4 per 100ml that dissolved in distilled water. pH adjusted to 4.5 using 1N H_3PO_4 and H_2SO_4 or NaOH before diluting to the final volume with distilled water. Then, 5ml were transferred in test tube or 50ml E-flask, cover with cotton plug and aluminum foil. The medium was autoclaved at 121°C (1atm) for 15 min.

2.1.2. Seed Culture Medium

Saccharomyces cerevisiae strain HBY3 was obtained from the National Institute of Molecular Biology and Biotechnology (BIOTECH). The culture was grown at 30°C for 48 h in a medium containing 10% (w/v) total sugar (molasses or sweet sorghum syrup), 0.1g $(\text{NH}_4)_2\text{SO}_4$, 0.025g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.14g KH_2PO_4 per 100ml, dissolved in distilled water then pH was adjusted to 5.0 using 1N $\text{H}_3\text{PO}_4/\text{H}_2\text{SO}_4$ or NaOH. About 22.5ml each were transferred into 250ml E-flask, covered with cotton plug and aluminum foil containing 225ml 15% (w/v) total sugar (w/v) as above.

2.1.3. Ethanol Fermentation Medium

The ethanol fermentation medium contain 20% (w/v) total sugar (sugarcane molasses), 0.1g $(\text{NH}_4)_2\text{SO}_4$, 0.025g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.14g KH_2PO_4 per 100ml distilled water, Exactly 225ml of this medium was pH adjusted to 5.0 using 1N $\text{H}_3\text{PO}_4/\text{H}_2\text{SO}_4$ or NaOH.

2.1.4. Inoculum Build Up

S. cerevisiae strain HBY3 from the Culture Collection of the Bioprocess Engineering and Biotechnology Division, National Institute of Molecular Biology and Biotechnology of Philippines (BIOTECH), were tested for ethanol production using sugarcane molasses. Inside the laminar flow hood about 1ml from the prepared 2.5ml pre-culture medium flask was aseptically transferred to a fresh 24h yeast slant. The cells in the medium were suspended by shaking, and then the cell suspension was aseptically transferred back into the flask. The yeast strain was transferred into the seed flask containing the 15% (w/v) total sugar medium and incubated at room temperature overnight or for about 12-18h. Then the grown yeast strain was transferred in molasses medium with 20% (w/v) total sugar medium, non-sterilized, without nutrients supplementation or pH adjustment, in Erlenmeyer flasks incubated in shaker at 100 rpm, for 18 h. Afterwards, this inoculum transferred to a bioreactor.

2.1.5. Batch Fermentation of Molasses into Ethanol

The ethanol fermentation was carried out in a Sartorius Biostat A-Plus Bioreactor (Allentown, PA) with 5 Liter vessel (working volume of 3 L). After seed culture inoculation into the fermenter, temperature was maintained at 30°C and agitation speed was 100 rpm. pH

was kept at 4.5 by using automatic addition of NaOH. DO probe was polarized for 6h before calibration.

3. Analysis

Samples were obtained every 1 or 2 h for the first 8 h and every 6 h with two trial, for the remainder of the fermentation and analyzed for residual sugar, ethanol concentrations as well as the optical cell density for biomass determination. The fermented broth was discarded after fermentation and treated as a common effluent.

3.1. Yeast Population

The initial sample (0 h) was kept. Then, about 7-10ml samples were also obtained via the sampling port every 2-4 hour. Cell counting was performed immediately after every sampling. Yeast concentration in cells/mL was quantified with Haemocytometer and the viability of yeast cells was determined by methylene-blue staining

3.2. Sugar Concentration

The total sugar concentration was determined according to the phenol sulfuric acid method (Dubois 1956). 0.5ml of diluted samples or standard sugar dispense solution into a test tube and added 0.5ml with 5% phenol sulfuric acid then 2.5ml of concentrated H_2SO_4 was directly added into the solution and mixed properly. The solution was cooled and incubate the tube for about 30 minute at room temperature. A measurement of absorbance at 480 nm was recorded. A calibration curve for the spectrophotometric measurements was obtained using a sucrose standard. The optical density of glucose solutions were measured at 480 nm using distilled water as a control.

3.3. Ethanol Concentration

A gas chromatograph (Shimadzu AOC 20, with Auto injector), was used to analyze the liquid samples. Isopropanol used as standard. The standard curve was prepared using absolute ethanol. The injection sample volume was 1 ml.

3.4. Model Development

Sixty one (61) data lines were used in this study and to train and calibrate the artificial neural network 60% of data lines used for training set, 15% of data lines used for cross validation set and 25% of data lines used for testing set and data were randomly extracted from the database (Table 1). Samples as well as ranges of data patterns (input output) collected from alcohol fermentation process to develop the Artificial neural networks model for an alcohol concentration. Such a neural network identification process, in turn, needs some optimization methods to find the best network architecture. In this way, genetic algorithms were deployed in a new approach to design the whole architecture of the artificial neural network, that is the number of neurons in each hidden layer and their configuration of connectivities in combination with singular value decomposition to find the optimal set of appropriate coefficients of quadratic expressions to model an ethanol concentration model (Nariman-Zadeh et al.,

2005). The parameters of interest in this multi-input, single output system that affect the alcohol concentration are live and dead yeast cells and sugar concentration. The validation sets consisted of unpredictable input-output data lines during the training process used for validation to show the prediction ability of such evolved neural networks during the training process. For artificial neural network training, databases were imported into Neuro Solution 6.0 that generates polynomial neural networks to model either simulation or experimental data of any kind. One hidden layers was considered for each model. All procedures were applied for ethanol concentration as separate models and all result were recorded.

A quantitative examination of the fit of the predictive models was made by using error measurement indices which are commonly used to evaluate forecasting models (Ahmadi et al., 2007). The accuracy of the models was determined by using by equation 1, 2, 3:

Equation 1: mean absolute deviation (MAD), computed as:

$$MAD = \frac{\sum_{i=1}^n |y_i - \hat{y}_i|}{n}$$

Equation 2: the mean absolute percentage error (MAPE), computed as:

$$MAE = \frac{\sum_{i=1}^n \left| \frac{y_i - \hat{y}_i}{y_i} \right|}{n} \times 100$$

Equation 3: the MS error (MSE), computed as:

$$MSE = \frac{\sum_{i=1}^n |y_i - \hat{y}_i|^2}{n}$$

Where y_i equals the actual value, \hat{y}_i equals the predicted value, and n equals the number of observations.

Table 1. Samples (8 sets) of data used to develop artificial neural network model

Number	input			Output	
	Sugar Concentration (mm)	Live Yeast Cells	Dead Yeast Cells	Actual Ethanol Concentration	Predicted Ethanol Concentration
1	507.286698	10170	20	2.80163	2.9831731
2	389.242183	10320	900	5.51658	5.6827545
3	507.286698	10170	20	2.80163	3.6071252
4	598.039216	8140	110	1.40117	1.1710903
5	681.505034	592	36	0.03235	0.3224345
6	645.601484	2701	50	0.27923	0.6669019
7	681.505034	592	36	0.03235	0.0154456
8	648.251192	6218	45	0.62613	0.5029117

Table 2. Model statistics and information for the artificial neural network model

Performance	Ethanol
MSE	3.947453635
NMSE	0.730304204
MAE	1.090996446
MAD	1.1823147
Min Abs Error	0.070719135
Max Abs Error	4.220244682
R ²	0.927902398
Best Networks	
Epoch No.	799
Minimum MSE	2.47E-28
Final MSE	2.47E-28
Cross Validation	
Epoch No.	766
Minimum MSE	0.001502409
Final MSE	0.002113991

MSE = MS error (standard deviation); MAE = mean absolute error; MAD = mean absolute deviation; MAPE = mean absolute percentage error

4. Results and Discussion

All models constructed from this data set were characterized by a great response for all input variables from the learning set. These equations revealed the quantitative relation between input [sugar concentration (SC), live yeast cells (LY), dead yeast cells (DY)] and output [ethanol concentration (EC)] variables under investigation. The corresponding polynomial equation representations of such a model for ethanol concentration obtained as follows:

$$\begin{aligned} & ((\tanh(((\tanh((SC \times (3.7562620958421626e - 003) \\ & + (-1.6599115288423292e + 000)) \times (2.9209031392583995e \\ & + 000)) + (LY \times (1.6531961792799413e - 004) \\ & + (-9.9786921381337257e - 001)) \times (1.8154986157776352e \\ & + 000)) + (DY \times (2.0454545454545456e - 003) \\ & + (-9.4090909090909103e - 001)) \times (-1.1742638723060640e \\ & - 001)) + (-4.4307331567432562e - 001)) \\ & \times (-4.7012114089997006e + 000))) + \\ & (((\tanh((SC \times (3.7562620958421626e - 003) + (-1.6599115288423292e \\ & + 000)) \times (-1.3042425971276653e - 002)) + (LY \\ & \times (1.6531961792799413e - 004) + (-9.9786921381337257e \\ & - 001)) \times (1.4584542840705769e - 001)) + (DY \\ & \times (2.0454545454545456e - 003) + (-9.4090909090909103e \\ & - 001)) \times (2.9141125289122682e - 001)) \\ & + (-7.8113873347145923e - 001))) \times (6.6265989486903454e \\ & + 000))) + \\ & (((\tanh((SC \times (3.7562620958421626e - 003) + (-1.6599115288423292e \\ & + 000)) \times (-6.6286370813574162e + 000)) + (LY \\ & \times (1.6531961792799413e + 000)) + (-9.9786921381337257e \\ & - 001)) \times (4.2463041922575711e + 000)) + (DY \\ & \times (2.0454545454545456e - 003) + (-9.4090909090909103e \\ & - 001)) \times (-1.1597779202392722e + 000)) \\ & + (8.3977025026179286e - 001))) \times (-3.1459022564724783e \\ & + 000))) + \\ & (((\tanh((SC \times (3.7562620958421626e - 003) + (-1.6599115288423292e \\ & + 000)) \times (2.8723813548212918e - 001)) + (LY \\ & \times (1.6531961792799413e - 004) + (-9.9786921381337257e \\ & - 001)) \times (1.4584542840705769e - 001)) + (DY \\ & \times (2.0454545454545456e - 003) + (-9.4090909090909103e \\ & - 001)) \times (-4.2423892504105010e - 001)) \\ & + (1.1884328475986905e + 000))) \times (3.8349252000126275e \\ & + 000))) + (1.8463629607556291e + 000)) \\ & - (-9.0587811912301641e - 001)) / (1.8170383687840883e \\ & - 001) \end{aligned}$$

As described earlier, the validation of results was tested by using 19 sets of data (validation sets) that were extracted from the database. The neural networks were trained with only 30 sets, and no sets were omitted. After the training process, the predicted values of neural networks were compared with those of actual values. The findings are demonstrated in (Figure 1) Results (training and validation values) showed superb agreement with actual and predicted ethanol concentration by using artificial neural network. Comparisons showed the behavior of such neural network models in predicting alcohol concentration. Equations using live and dead yeast cells and sugar concentration or their product had strong relationships with alcohol concentration, manifested in high coefficients of determination (R^2) of the equations and low standard error of estimates (Table 2). Also Table 2, summarizes the statistical results for the training and validation sets of artificial neural network models. These results indicate forecasting error measurements based on differences between the model and actual values. By considering these training data, the lowest standard deviation, mean absolute deviation, mean absolute percentage error, and the highest R^2 were calculated for ethanol concentration. For validation data, however the lowest standard deviation, mean absolute deviation, mean absolute percentage error and the highest R^2 were observed for alcohol concentration.

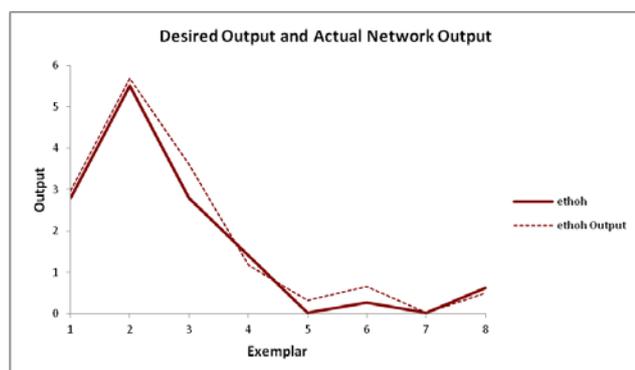


Figure 1. Desired output and actual network base on randomized date

Calculated statistics indicate that artificial neural network provides a desirable means of efficiently recognizing the patterns in data and predicting ethanol concentration based on investigating of inputs (Table 1, Table 2 and Figure 1). The genetic approach could be used to provide optimal neural networks in terms of hidden layers, number of neurons and their configuration of connectivities, so consequently a polynomial expression for dependent variables of the process can be achieved. The prediction by an artificial neural network is usually much faster than the conventional simulation programs as no lengthy iterative calculations are needed to solve differential equations using numerical methods, although the selection of an appropriate neural network topology is important in terms of model accuracy and simplicity (Gnansounou et al., 2005; Nariman-Zadeh et al., 2005; Kiani Deh Kiani 2010; Ahmadian-Moghadam 2012). The ethanol concentration evaluated in experiments of industrial Biofuel production and this research develops a simple, accurate, nondestructive and time saving, artificial neural network model for estimation of alcohol concentration in batch ethanol fermentation from molasses.

Result of this study may use in large scale biofuel production process by using artificial neural network model to predict final ethanol concentration (EC) through investigation of quantitative relationship between sugar concentration (SC), live yeast cells (LY), dead yeast cells (DY)

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