CHEMICAL ENGINEERING TRANSACTIONS
VOL. 37, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Eliseo Ranzi, Katharina Kohse- Höinghaus

ISBN 978-88-95608-28-0; **ISSN** 2283-9216
DOI: 10.3303/CET1437070

On-Line Measurements and Modelling Study in Second Generation Ethanol Production from Sugarcane

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A model based on Artificial Neural Networks (ANN) to predict the concentration of ethanol, substrate and cells from secondary measurements (pH, turbidity, CO_2 and temperature) was developed in this work. A second generation ethanol production from hydrolyzed sugarcane bagasse was considered as a study case. Experimental data were obtained from fermentation in the range of 30 to 38 °C with cell recycle. The fermentation feedstock is a mixture of molasses and hydrolyzated bagasse from the alkaline hydrogen peroxide pretreatment at 25 % of volume and 75 %, respectively. The accuracy of prediction of the ANN model is evaluated by its precision in describing experimental observations, and by the challenges involved in the use of online measurements. The model used to describe the fermentation provided a good prediction of concentration of cell, substrate and ethanol.

1. Introduction

Nowadays, there is an incentive for the development of cleaner and renewable sources of energy aiming to meet environmental requirements, to contribute with a broader social development and to reduce the political instability and rising prices tendency of fossil fuels (Sawin et al., 2012). Taking this into account, the production of biofuels and bioenergy from biomass appears to be a possible alternative that can be explored. In fact, the rational use of renewable resources may have a significant contribution in energy matrix. In this scenario, Brazil as the largest producer of ethanol from sugarcane has an interesting environment since the first generation plants (based on sugarcane molasses and broth) may be used to accommodate second generation ethanol process (based on sugarcane bagasse and straw), since the raw material is already available and the existing facilities may be shared reducing the investments.
However, the efficiency of fermentation by *Saccharomyces cerevisiae* using lignocellulosic feedstock depends on the fermentability of sugars from the hydrolyzate which may be affected by inhibitors that are by-products of the pre-treatment and hydrolysis process with impact on the kinetics, ethanol produced and hence the productivity affecting economically the whole process. This problem can be mitigated by the implementation of a better system of monitoring and control to support the robustness of the process which, in turn, contributes to the economic viability of this production process of ethanol (Herrera and Maciel Filho, 2013).

2. Experiment

Experimental data reported by Andrade (2012) were used in this work in order to train, validate and test the Artificial Neural Network (ANN). Batch fermentations were carried out in a bioreactor Bioflo III (New Brunswick Scientific Co., Inc., Edison, NJ) with 1 L of working volume, stirred by two flat blade turbines, at 300 rpm, varying the fermentation temperature from 30, 32, 34, 36 and 38 °C and keeping fixed the initial substrate concentration at 160 Kg/m^3 and initial concentration of cell of 2.8 Kg/m^3. Additionally, fermentations with cells recycle were performed at 30(2 recycles), 32(4 recycles), 34(5 recycles), 36(4 recycles) and 38 (3 recycles) °C at the same conditions. In these experiments it was used *Saccharomyces*

Please cite this article as: Herrera W., Rivera E.C., Maciel Filho R., 2014, On-line measurements and modelling study in second generation ethanol production from sugarcane, Chemical Engineering Transactions, 37, 415-420 DOI: 10.3303/CET1437070

cerevisiae grown in the laboratory of Bioprocess Engineering, at Unicamp's Faculty of Food Engineering. The fermentation feedstock is a mixture of molasses and hydrolyzated bagasse from the alkaline hydrogen peroxide pretreatment at 25 % of volume and 75 %, respectively. The sensors used for collecting online information of the variables of interest were: a turbidity transmitter (FSC 402 Metter Toledo Ingold Inc. USA) for measuring the production medium turbidity, temperature was measure by a thermocouple (N. Brusnwick Scientific), CO_2 flow rate was measured by a digital gas volumetric flow sensor and pH by a glass electrode (both from Cole-Parmer Instrument).

3. Online Measurements

Online measurements such as temperature, turbidity, pH and CO_2 flow rate were used for predicting concentration of substrate, ethanol and cell. These variables were chosen because they are related with process concentration which is difficult to measure, or they have a strong influence on productivity, yield and substrate conversion in the main final product.

3.1 Temperature

It has been shown that temperature, as a control variable, is an important factor in the study of optimization and productivity increase of microbial and fermentative process. Precise temperature control and profiling are key factors in promoting biomass growth and controlling yield (Vogel and Todaro, 1997). Cell growth rates, sugar consumption and inhibition caused by the product and substrate are affected by fermentation temperature (Siqueira, 1996). In other studies Andrade (2007) is highlighted the relevance of this variable, since to some extent, the microbial growth and the velocities of enzymatic reaction increase with temperature increase. Additionally, ethanol toxic effect increases with the temperature increase due to the higher fluidity of the cell membrane. Therefore, effect study of this variable is necessary for this process since it influences directly its performance and productivity. In Figure 1a, it is possible to observe the temperature profiles at 30, 32, 34, 36 e 38 ºC considered in this work.

3.2 Turbidity

Turbidity is the measurement of solids quantity present in the liquid. It is related to cell concentration because as the cell are growing, the free space where the light can go through is smaller, and allowing some correlation to be made. Petersen et al. (2011) concluded that online sensors for biomass measurement, including turbidity probe, have a promissory future since it is easy to calibrate, to use and it possess a higher accuracy compared to multivariable sensors. Also, it was found a good correlation between online turbidity measurement and the cell concentration; this was well fitted by a second order polynomial. In Figure 1b is observed how turbidity increases as a consequence of cell growth in a fermentation process.

3.3 pH

Metabolic processes are typically highly susceptible to even slight changes in pH, and therefore, proper control of this parameter is critical. Fermentations are performed over a broad pH range values, specifically, values between 4 and 5 are suitable for this process. Usually, pH values of industrial fermentation broth are in the range of 4.5 and 5.5 with a good buffering capacity, especially those prepared with molasses. Fermentations performed in more acidity media, around 3.5 and 4.5, results in higher ethanol yields due to a restriction in the yeast growth as well as a diminution of glycerol production, and at the same time, bacterial contamination is reduced. However, fermentations are performed well at higher levels, in high buffering capacity substrates, like the molasses with pH 5.8/5.9. The sugarcane juice is fermented without acidity correction; they are performed in natural pH ranging between 5.2 and 6.8. Taking into account these facts, the tolerance to acidity is an important characteristic for the yeast (Lima et al., 2001). A negative effect in the ethanol yield and productivity is presented at values below of 3.5. A low pH causes loss of nutrients such as nitrogen and potassium, increases the yeast sensitivity to ethanol, organic acids and SO_2, (Silverio, 2009).

Precise manipulation of pH can determinate the relative yield of the desired species over competing by-products (Vogel and Todaro, 1997). Throughout fermentation, the yeast in its metabolic route of ethanol production also produces acids which decrease the pH. In the Figure 1c, is observed all pH profiles. Many studies about pH influence on fermentation can be found in literature (Nielsen and Arneborg, 2007, Akin et al., 2008, Arroyo-López et al., 2009).

3.4 CO_2 flow rate

Much can be learned from the exchange gases in the metabolic process such as O_2, CO_2, N_2, and NH_3. In fact, most of the predictive analysis is based upon such calculations as oxygen uptake rate, carbon dioxide exchange rate or respiratory quotient (Vogel and Todaro, 1997).

In glycolysis, glucose is converted through a series of reactions to pyruvate, and energy is extracted in the form of four ATP molecules. Then, pyruvate is converted to ethanol in a two-step reaction; pyruvate is decarboxylated to form the more reactive acetaldehyde, which is reduced to ethanol. For each glucose fermented, two ethanol and two CO_2 molecules are produced, (Lehninger et al., 2005). From this information it can be concluded that CO_2 and ethanol are produced in a proportional way, by monitoring one of them, it is possible to obtain information of the other one. CO_2 production in the fermentation can be divided into three stages. In the preliminary stage occur a huge cell multiplication, small temperature increase and small CO_2 liberation. In the second stage, the CO_2 liberation occurs in an intense manner due to the large cell number presence in the medium that broken down the fermentable sugar in ethanol; the second stage is the longest duration stage. Temperature increases quickly, density is reduced, and alcohol and acidity percentages increase. In the complementary stage is observed a decrease in the intensity of CO_2 liberation until the fermentation culmination (Basso and Lima, 2001). The CO_2 flow rate profiles are shown in Figure 1d.

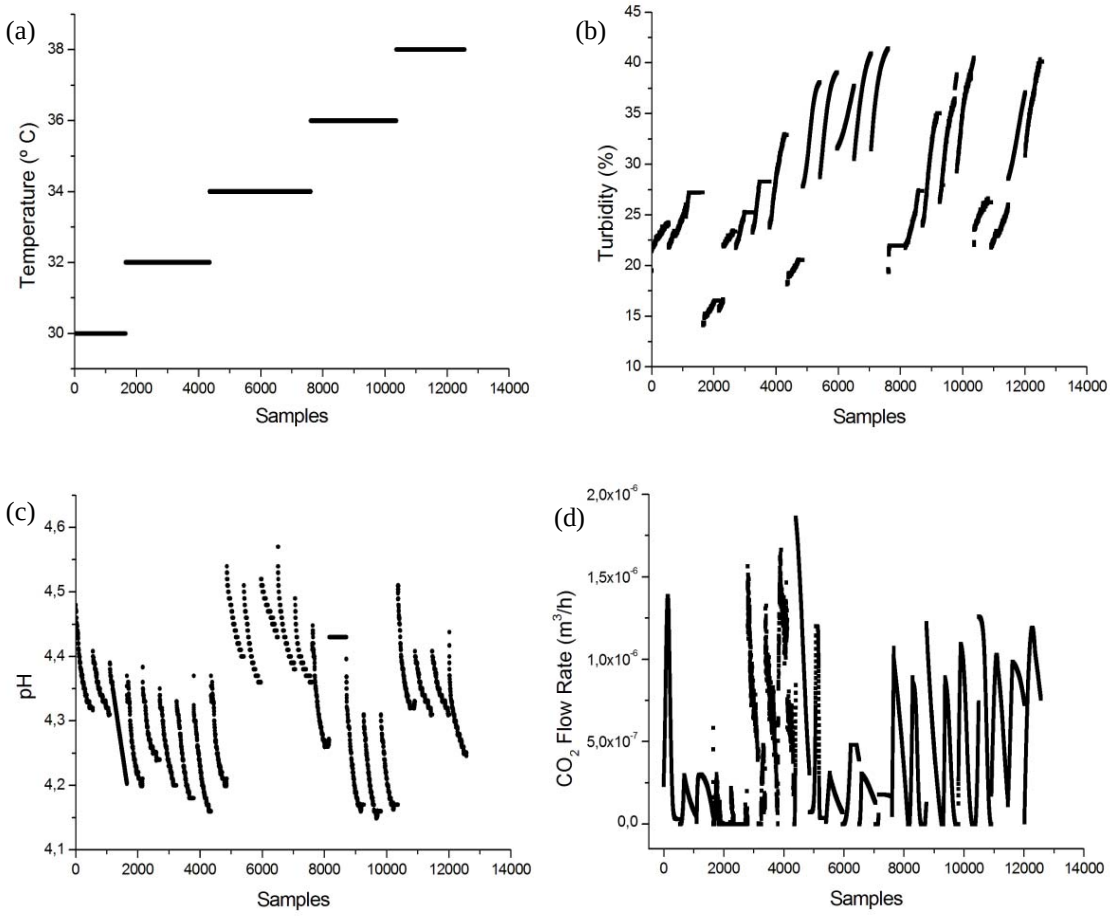

Figure 1 – a) Temperature, b) pH, c) Turbidity and d) CO_2 flow rate at 30, 32, 34, 36 and 38 ºC and all recycles

4. ANN Model Development

This section presents the considerations required to develop a modelling technique based on ANN.
This structure has nonlinear processing capabilities and universal approximation property and has already been used successfully to describe the dynamic behavior of biotechnological process, (Rivera et al., 2010).

4.1 ANN structure selection.

In this work, it was developed a model based on ANN to predict the concentration of ethanol, substrate and cell from secondary measurements (pH, turbidity, CO_2 and temperature) in a second generation ethanol production.

An ANN network with three layers was used: the input layer regards the data that are inserted (secondary measurements), the second is the hidden layer and it can comprise different numbers of neurons leading to different prediction performances, and the third, is the output layer. Log-Sigmoid and Linear Transfer Function are used to the hidden layer, and output layer, respectively. The relationship that represents an ANN is given mathematically as, equation (1):

$$g_k = F\left(\sum_{J=1}^{M} W_{kj}\, f\left(\sum^{N} w_{ji} x_i + \theta_j\right) + b_k\right) \quad (1)$$

$$\left(j = 1, ..., M\right); \left(k = 1, ...K\right)$$

where *N*, *M* and *K* are input, hidden and output layer, respectively. The w_{ji} is the weight of the connection of the *i*th neuron in the input layer and the *j*th neuron in the hidden layer; θ_j is the *j*th neuron bias in the hidden layer; W_{kj} is the weight of the connection between the *j*th neuron to the *k*th neuron in the output layer; b_k is the *k*th neuron bias in the output layer; the neurons activation are F() and f() functions in the hidden layer and output layer, respectively. Neural network toolbox under Matlab® software was used for training the models.

There were performed three ANN models each for every output with 12750 lines of data. The input variables are pH, turbidity, CO_2 flow rate and temperature and the outputs are concentration of ethanol, substrate and cell. The samples were randomly divided in 70% for the training set, 20 % for the validation set and 10 % for the testing set. 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 (Rivera et al., 2010), (Herrera and Maciel Filho, 2013). The accuracy of the models was determined by the quality of prediction and it is evaluated in terms of R^2, Mean Square Error (MSE), Eq.1, and Residual Standard Deviation (RSD), Eq. 2.

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\left(e_i\right)^2 = \frac{1}{N}\sum_{i=1}^{N}\left(Y_i - y_i\right)^2 \quad (2)$$

$$RSD = \frac{\left(\frac{1}{n}\sum_{k=1}^{n}\left(Y_k - y_k\right)^2\right)^{0.5}}{\overline{Y_k}} \times 100 \quad (3)$$

where Y is the predicted value, y is the experimental value and n is the number of observations.

5. Result and discussion

ANN model performance was compared with experimental data. The main objective was the prediction of the concentration of ethanol, substrate and cells from secondary measurements (pH, turbidity, CO_2 and temperature). These ANN models have been improved by selecting the network architecture considering the number of neurons in the hidden layer; this has been evaluated with R2, MSE and RDS. In the Table 1, based on these criteria it can see the best performance for the ANN prediction.

It can be seen from Figure 2, that the performance of the ANN models for the testing set showed a very good prediction when compared with the actual value for the three variables: concentration of ethanol, substrate and cell.

Besides, results from Table 1, suggest that ANN models provide an efficient resource of recognize parameters, as well as prediction of concentration of ethanol, substrate and cell. It can be said that this work developed a simple, accurate and time saving models for the estimation of critical variables second generation ethanol production.

Table 1. Performance of ANN models for prediction of concentration of ethanol, substrate and cell

ANN Output	Ethanol	Substrate	Cell
R^2	0.997	0.995	0.997
MSE	27.182	17.340	0.066
RSD	2.380	6.312	3.403
Time (min:sec)	2:24	21:34	09:15
Epochs	251	1000	257
Hidden Neurons	100	100	60

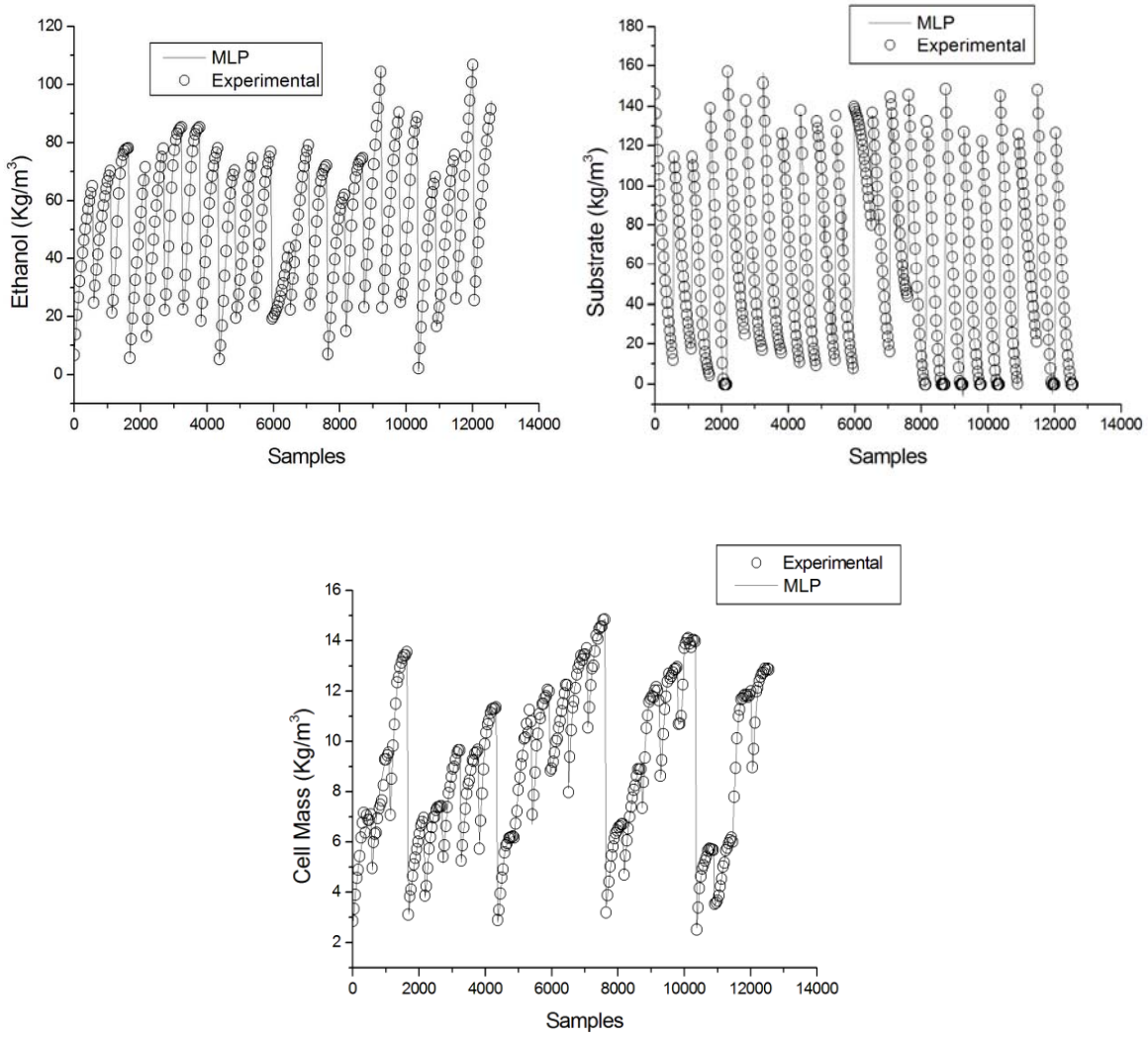

Figure 2. ANN model prediction (solid symbols) and actual values (lines) for a) Ethanol b) Substrate and c) Cell concentrations at 30, 32, 34, 36 and 38 ºC and all recycles

6. Concluding Remarks

A methodology using ANN and online data from second generation ethanol production was presented in this work. From the results, it can be said that ANN can yield efficient performance for prediction of concentration of ethanol, substrate and cell.

An appropriate choice of the architecture of the ANN model and an appropriate use of secondary measurements result in a reliable predictive model that satisfactorily describes the dynamic behavior of the process even in the presence of operational and kinetics changes of the process.

Acknowledgements

The authors thank FAPESP (Process number 2012/24326-0) and CAPES for financial support.

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