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MODELING OF AN INDUSTRIAL PROCESS OF PLEUROMUTILIN FERMENTATION USING FEED-FORWARD NEURAL NETWORKS

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Abstract - This work investigates the use of artificial neural networks in modeling an industrial fermentation process of Pleuromutilin produced by *Pleurotus mutilus* in a fed-batch mode. Three feed-forward neural network models characterized by a similar structure (five neurons in the input layer, one hidden layer and one neuron in the output layer) are constructed and optimized with the aim to predict the evolution of three main bioprocess variables: biomass, substrate and product. Results show a good fit between the predicted and experimental values for each model (the root mean squared errors were 0.4624% - 0.1234 g/L and 0.0016 mg/g respectively). Furthermore, the comparison between the optimized models and the unstructured kinetic models in terms of simulation results shows that neural network models gave more significant results. These results encourage further studies to integrate the mathematical formulae extracted from these models into an industrial control loop of the process.
Keywords: Modeling; Pleuromutilin; Fermentation; Feed-forward neural networks.

INTRODUCTION

In an increasingly competitive worldwide antibiotic market faced with economic, environmental and safety constraints, the industrialists have to increase their antibiotic production quantitatively and qualitatively, and reduce their costs and their energy consumption. Moreover, due to the complexity and non-linearity of the phenomena (Potocnik and Grabec, 1999), a modeling of antibiotic production, which remains a challenging problem, is required.

Kinetic models, known as a white box strategy, provide an analytical expression relating the key characteristics of the physical system to its dynamic behavior. These models have been and are still extensively applied in fermentation processes (Benkortbi *et al*., 2007; Cruz *et al*., 1999; Paul and Thomas, 1996; Zangirolami *et al*., 1997). However, in some cases, those models do not apply, due to the inherent non-linearity of the system, lack of experimental information, experimental inaccuracy, or deviations from ideal conditions (Feyo de Azevedo *et al*., 1997; Silva *et al*., 2008; Saraceno *et al*., 2009).

Artificial neural networks (ANN) have gained a great popularity in the last decade as an attractive alternative to the previous approach due to their high parallelism, robustness (Basheer and Hajmeer, 2000) and, more interestingly, their inherent ability to extract from experimental data the highly non-linear and complex relationships between the variables of the problem (Si-Moussa *et al*., 2008) without any detailed knowledge of the system (Bryjak *et al*., 2004).

Several authors have investigated the use of ANN as a black or white box in fermentation. Di Massimo *et al*. (1992) built ANN-based biomass and product estimators for on-line application to industrial

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penicillin fermentation. In the work of Shene *et al*. (1999), two different neural networks (a feed-forward black box neural network and a hybrid gray box neural network) were designed to predict the state variables in ethanol batch fermentation taking into account the effect of medium composition and temperature. Kovarova-Kovar *et al*. (2000) presented the use of a combination of predictive control and an ANN-model to optimize the industrial fed-batch process for commercial production of riboflavin (vitamin B_2). However, in the paper of Huang *et al*. (2002), an auto-associative neural network model was presented for on-line fault detection in Virginiamycin production. Saraceno *et al*. (2009) simulated the fermentation of ricotta cheese whey for the production of ethanol by means of a multiple hybrid neural model (HNM), obtained by coupling a multilayer perceptron neural network to mass balance equations for lactose, ethanol and biomass.

This work is a part of research on modeling the diterpene antibiotic Pleuromutilin, which is a natural product, obtained by fermentation of *Pleurotus mutilus,* an edible mushroom. This compound is active against a variety of gram-positive bacteria and *mycoplasmas* and is an inhibitor of bacterial protein synthesis (Kavanagh *et al*., 1951; Egger and Reinshagen, 1976).

On the other hand, Pleuromutilin served as a lead structure in the development of several commercial antibiotics such as tiamulin and valnemulin used in veterinary medicine (Hannan *et al*., 1997) and retapamulin approved for the treatment of human skin infections (Hirokawa *et al*., 2008).

In this paper, we developed three feed-forward ANN models, in order to assess the difficult-to-measure quantities, such as concentrations of biomass, substrate and product, from easily measurable variables in a fed-batch mode. This would be very interesting for further applications to digital control of the process, or measurement devices, and thus improve the reproducibility of the process and increase the Pleuromutilin productivity.

MATERIALS AND METHODS

Data Collection, Pretreatment and Analysis

Nineteen complete sets of data were provided by Antibiotical, a subsidiary of the Algerian pharmaceutical company Saidal, containing data on: biomass concentration (X), glucose consumption (S), Pleuromutilin concentration (P), pH evolution, and refractive index of the fermentation broth (RI).

The collected experimental data were interpolated using a cubic spline function when necessary and smoothed with a moving average digital filter. Without smoothing, the ANN tends to capture the noise in the system rather than the fundamental mechanisms of the underlying process (Laursen *et al*., 2007) which results in a lower efficiency and a longer time to train the ANN.

ANN Development Procedure

Models Description

A neural network (NN) is a computational framework that is inspired by biological neural systems. It consists of a number of interconnected simple processing units called artificial neurons. One of the most popular neural network paradigms applied to the modeling of a wide range of nonlinear systems, especially chemical and biological engineering processes, is the feed-forward back propagation neural network (FFNN) (Lee and Park, 1999; Silva *et al*., 2000), which has been used throughout this paper with forecasting horizon and supervised learning.

The proposed FFNN consists of three models that are characterized by the same general structure including an input layer, one or two hidden layers and an output layer for the prediction of biomass, glucose and Pleuromutilin concentration profiles during fermentation.

A set of input variables was identified for each model: pH, refractive index (RI), initial glucose concentration (S_0), initial inoculum concentration (X_0) and fermentation time (t). A preliminary analysis based on the improved stepwise methods used for testing the contributions of NN inputs revealed that these variables exhibited the highest influence on process performance; more details on this technique can be found in Gevrey *et al*. (2003).

The statistical analysis of input and output data is shown in Table 1.

Table 1: Statistical analysis of input and output data.

	STD	Mean
t (h)	83,7	140
pH	0,3	7
RI	0,9	2,9
X_0 (%)	1,7	4
S_0 (g/l)	2,8	24,8
X (%)	15,7	45,1
S (g/l)	4,3	15,1
P (mg of product/g of biomass)	1,3	1,7

Models Development

In this study, a procedure based on the development and optimization of the architecture of a feed-forward network is advanced. It is based, as described in Figure 1, on the design of four FFNN sub-models which differ by the type of transfer function and the type of learning algorithm commonly used in biotechnology.

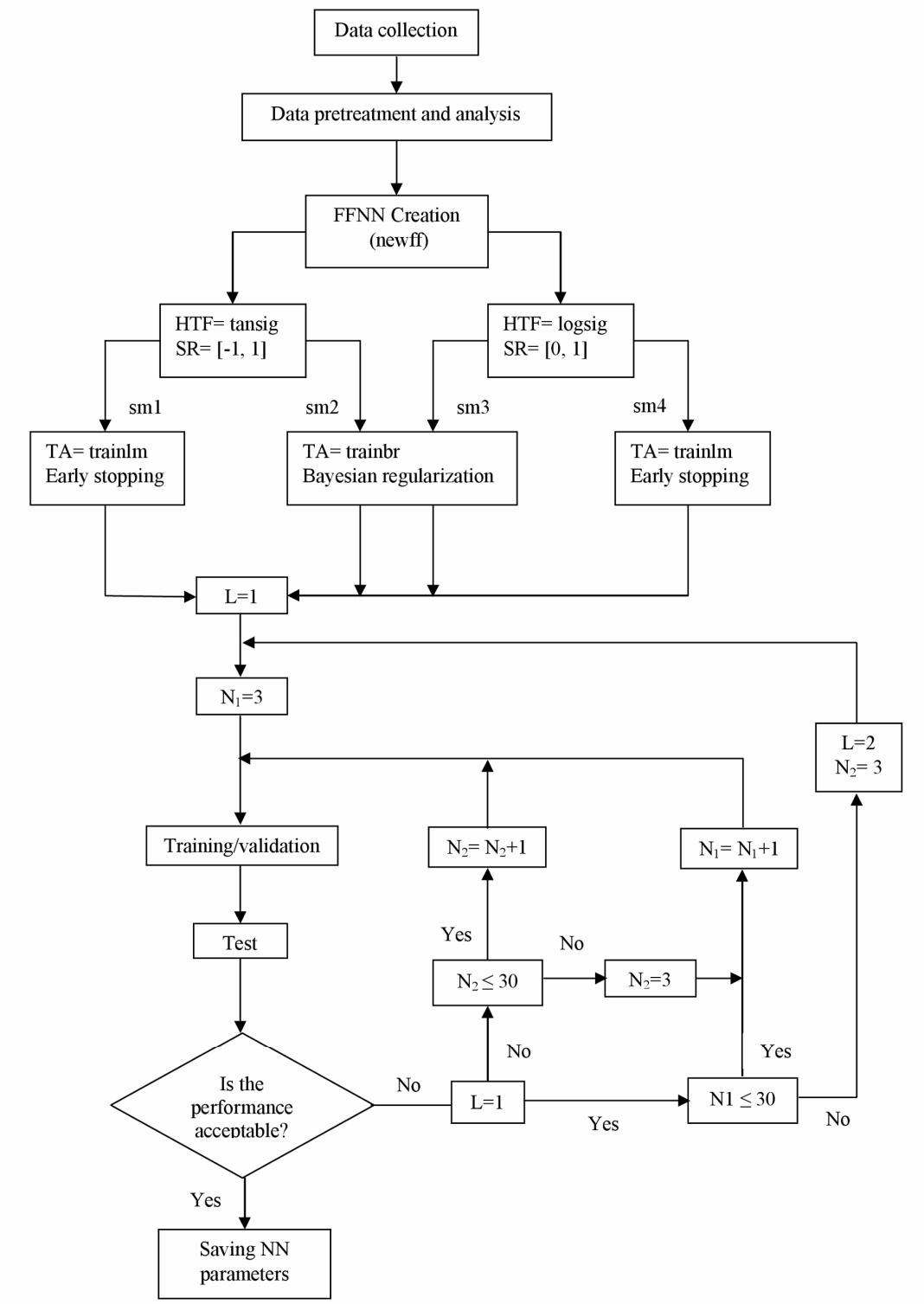


Figure 1: Flow diagram for feed-forward neural network development.

In order to make the training of the four sub-models more efficient by preventing the transfer function from becoming saturated and making the training of the networks very fast, all inputs and outputs were scaled in the range (SR) 0 to 1 and -1 to 1 depending on the hidden transfer function (HTF) used (log-sigmoid or hyperbolic tangent sigmoid, respectively), using the following scaling equation:

$$x_N = (y_{max} - y_{min}) * \left(\frac{x - x_{min}}{x_{max} - x_{min}} \right) + y_{min} \quad (1)$$

where x_N is the normalized value of the parameter x (t, pH, RI, X_0, S_0, X, S or P); x_{max} and x_{min} are the maximum and minimum values of x, respectively; y_{max} and y_{min} can take the values 0 and 1 or -1 and 1. For all sub-models, the linear transfer function was attributed to the output layer.

Depending on the most successful and commonly used back propagation training algorithm, two enhancement generalization techniques were used. Over-fitting or poor generalization capability occurs when a neural network over learns during the training period. As a result, such a too well-trained model may not perform well on an unseen data set due to its lack of generalization capability. To overcome this problem, early stopping and Bayesian regularization methods were applied with the most successful Levenberg-Marquardt (MATLAB code trainlm) and Bayesian regulation (MATLAB code trainbr) back propagation training algorithms, respectively (The Mathworks Inc., 2010).

The early stopping technique is a very common practice in neural network training and often produces networks that generalize well. This technique is based on the division of data sets into three parts: training, validation, and test sets. The network is trained using the training set to minimize the error and checked with the validation set after each iteration to prevent over learning for the training set and loss of ability to generalize (Bishop, 1995; Pigram and MacDonald, 2000). As a final check, the test set is used on the network to make sure that the network performs and generalizes well (Hirschen and Schafer, 2006; Hagan *et al*., 1996; Asensio-Cuesta *et al*., 2010).

The Bayesian regularization is a more sophisticated approach to improve generalization. It was first used by MacKay (1991). This method not only minimizes a linear combination of squared errors and weights, rather than simply the squared errors, but also modifies the linear combination so that, at the end of the training, the resulting network has good generalization qualities (MacKay, 1991; Danaher *et al*., 2004; Hagan *et al*., 1996; The Mathworks Inc., 2010).

In our work, the available data (551 samples) were randomly split into three distinct subsets, reserving 60% of the data (385 samples) for the training phase, 20% (83 samples) for the validation phase and the remaining 20% (83 samples) for the test phase.When early stopping was applied, the training and validation sets were used for the purposes described previously, whereas with Bayesian regularization the validation set was added to the training set to train the models. The test set for both techniques was used to test the generalization of the trained FFNN sub models.

In order to optimize the architecture for each sub-model, thus determining the number of hidden layers and the number of nodes in each layer, a trial-and-error procedure was implemented as described in Figure 1. The number of hidden layers (L) varied from 1 to 2 layers and the number of hidden neurons (N) in each layer varied from 3 to 30 neurons according to the forward method (Heaton, 2005).

The performances of various sub-models were evaluated in terms of the root mean squared error (RMSE) criterion. The RMSE was calculated using the following equation:

$$RMSE = \sqrt{\frac{1}{N}\left(y_{cal} - y_{exp}\right)^2} \quad (2)$$

where N is the total number of data; y_{cal} represents the predicted output from the neural network model for a given input, while y_{exp} is the experimental value.

The development procedure of the FFNN described above was carried out by elaborating a MATLAB program under MATLAB Neural Network Toolbox ver.7.10 (The Mathworks Inc., 2010). It was used to optimize the architecture of the three models utilized to predict the concentration profiles of biomass, glucose and product, respectively.

Comparison with Unstructured Kinetic Models

The fed-batch culture of Pleuromutilin is implemented initially in a batch mode and is then fed with a concentrated solution of the limiting substrate (undiluted form), resulting in an insignificant increase in volume. The fixed volume fed-batch

culture can be described by the following mass balance equations:

$$\frac{dX}{dt} = \mu X - \frac{F}{V}X \tag{3}$$

$$\frac{dS}{dt} = \frac{F}{V}(S_0 - S) - \frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} - m_S X \tag{4}$$

$$\frac{dP}{dt} = \pi X - \frac{F}{V}P \tag{5}$$

where X, S and P are the concentrations of biomass, substrate and Pleuromutilin respectively; t is the current fermentation time; μ is the specific growth rate of biomass; π is the specific rate of product formation; V is the industrial bioreactor (culture) volume; S_0 and F are the substrate concentration and feed rate of the medium added to the bioreactor, respectively; m_S is the maintenance coefficient; $Y_{X/S}$ is the yield of biomass per unit mass of substrate, and $Y_{P/S}$ is the yield of product per unit mass of substrate.

As the volume V of the bioreactor used in the industrial production of Pleuromutilin is very large compared to the feed F, the term F/V is neglected and therefore the equations become (Pirt, 1979; Stanbury *et al*., 1999):

$$\frac{dX}{dt} = \mu X \tag{6}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} - m_S X \tag{7}$$

$$\frac{dP}{dt} = \pi X \tag{8}$$

The specific rates of growth and Pleuromutilin formation were modeled by the following equations (Patnaik, 2001):

$$\mu = \frac{\mu_{max} S}{k_S X + S} \tag{9}$$

$$\pi = \frac{\pi_{max} S}{k_S + S + S^2/k_i} \tag{10}$$

where k_S is the Monod constant; k_i is the inhibition constant, and μ_{max} and π_{max} are the maximum values of μ and π respectively.

Kinetic simulations were carried out by developing a MATLAB program based on four stages (Cutlip and Shacham, 2008; The Mathworks Inc., 2010): (a) give initial fixed values to the kinetic parameters and then integrate the differential equations using the ode45 MATLAB function in order to obtain the calculated dependent variable values; (b) calculation of the sum of squares of the difference between the calculated and experimental values of the dependent variables; (c) application of an optimization program based on the MATLAB function "fminsearch", which modifies the kinetic parameter values so as to obtain the minimum of the sum of squares; (d) re-integrating the differential equations by using the optimal kinetic parameter values.

The calculated dependent variable values were then compared to those obtained by the optimized FFNN models.

RESULTS AND DISCUSSION

Model Performances

According to the previous discussion, three neural network models were developed with the aim of predicting biomass, glucose and Pleuromutilin concentration profiles during fed-batch fermentation. In order to optimize their structure, four sub-models depending on the training algorithms and the transfer functions used, were developed for each NN model.

Table 2 summarizes the performances of the sub-models in terms of root mean squared error (RMSE) for each NN model and their corresponding sub-models. The resulting structures of the optimized NN models are depicted in Table 3. One hidden layer was sufficient to predict with enough accuracy the biomass, substrate and product concentration profiles. It has been proven that the Bayesian regularization back propagation training algorithm coupled with its corresponding generalization enhancement technique train more successfully.

Figure 2 shows the comparison between experimental values and calculated values obtained by the simulation of the optimized NN models. It was proven that the proposed approach gives satisfactory results with agreement vector values approaching the ideal [i.e. α=1 (slope), β=0 (y intercept), R=1 (correlation coefficient)] in fitting biomass, glucose, and Pleuromutilin profiles.

Table 2: NN models and their respective sub-model performances for the test set.

Sub models	NN1		NN2		NN3	
	Hidden neurons number	RMSE*	Hidden neurons number	RMSE*	Hidden neurons number	RMSE*
Sm1	24	1.1046	21	0.2351	23	0.0054
Sm2	29	0.4826	25	0.1234	21	0.0018
Sm3	29	0.4624	18	0.1305	27	0.0016
Sm4	26	1.1572	27	0.2757	20	0.0060

*RMSE: Root Mean Squared Error

Table 3: Structure of the optimized NN models.

NN models	Training algorithm	Input layer	Hidden layer		Output layer	
		Neurons number	Neurons number	Activation function	Neurons number	Activation function
NN1	trainbr	5	29	logsig	1	purelin
NN2	trainbr	5	25	tansig	1	purelin
NN3	trainbr	5	27	logsig	1	purelin

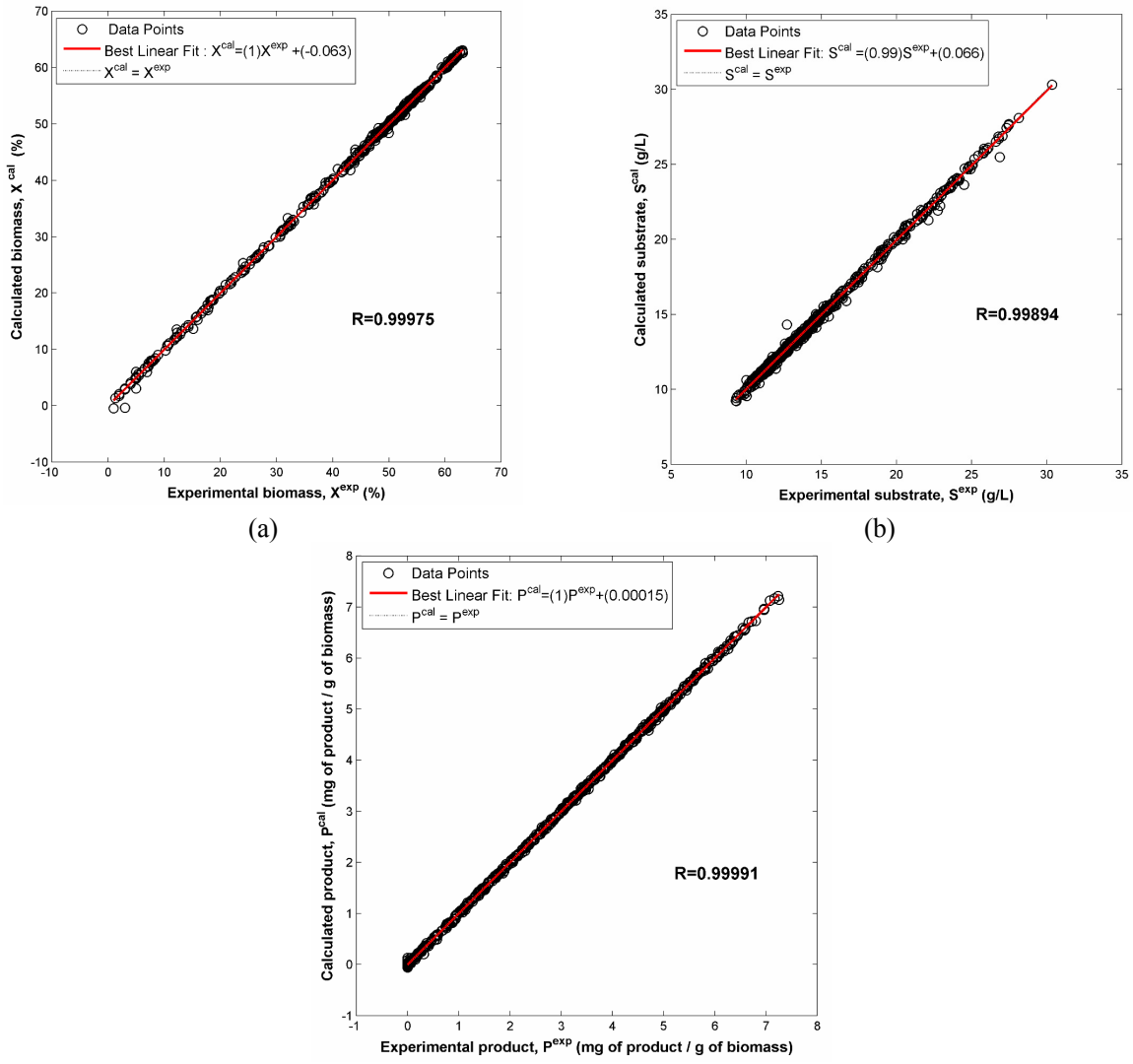


Figure 2: Comparison between experimental and calculated values for the whole data set: (a) biomass, (b) glucose, (c) product.

From the optimized NN2, represented in Figure 3, we can express substrate uptake by a mathematical model that incorporates all inputs E_i (time, pH, RI, X_0, S_0) within it as follows:

The instance outputs Z_j of the hidden layer:

$$Z_j = f_H\left[\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right] = \frac{\exp\left(\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right) - \exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)}{\exp\left(\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right) + \exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)} \quad (11)$$

j=1, 2, … , 25

The output S:

$$S = f_0\left[\sum_{j=1}^{25} w_{1j}^{H} Z_j + b_1^{O}\right] = \sum_{j=1}^{25} w_{1j}^{H} Z_j + b_1^{O} \quad (12)$$

The combination of equations 11 and 12 leads to the mathematical formula for substrate uptake taking into account all the inputs E_i (time, pH, RI, X_0, S_0):

$$S = \sum_{j=1}^{25} w_{1j}^{H}\left[\frac{\exp\left(\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right) - \exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)}{\exp\left(\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right) + \exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)}\right] + b_1^{O} \quad (13)$$

Similarly, biomass and Pleuromutilin concentrations can be expressed by the mathematical equations extracted from the optimized NN1 and NN3 as follows:

$$X = \sum_{j=1}^{29} w_{1j}^{H}\left[\frac{1}{1+\exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)}\right] + b_1^{O} \quad (14)$$

$$P = \sum_{j=1}^{27} w_{1j}^{H}\left[\frac{1}{1+\exp\left(-\sum_{i=1}^{5} w_{ji}^{I} E_i + b_j^{H}\right)}\right] + b_1^{O} \quad (15)$$

It is obvious that these FFNN mathematical equations for sugar uptake, biomass, and Pleuromutilin concentrations contain just the required degree of complexity, include the important relevant features that are operating conditions and initial conditions, and thus can readily be applied in control systems.

Comparison with Unstructured Kinetic Models

In order to establish the developed FFNN models as a plausible alternative to the unstructured kinetic models, a comparison between the two approaches was made in terms of simulation results, which is shown in Figure 4.

It is shown that the developed FFNN models outperform the unstructured kinetic models in predicting biomass, glucose, and Pleuromutilin concentration profiles.

The proposed forms of the specific rates of growth and product formation were suitable for some data sets and unsuitable for others. This is due to the behavior change of *Pleurotus mutilus* from one data set to another. This will probably require a change in the equations of the specific rates from one data set to another, thus making difficult their incorporation into a control-loop of the process.

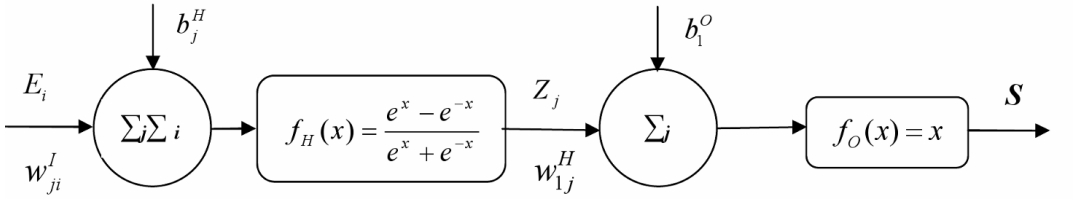
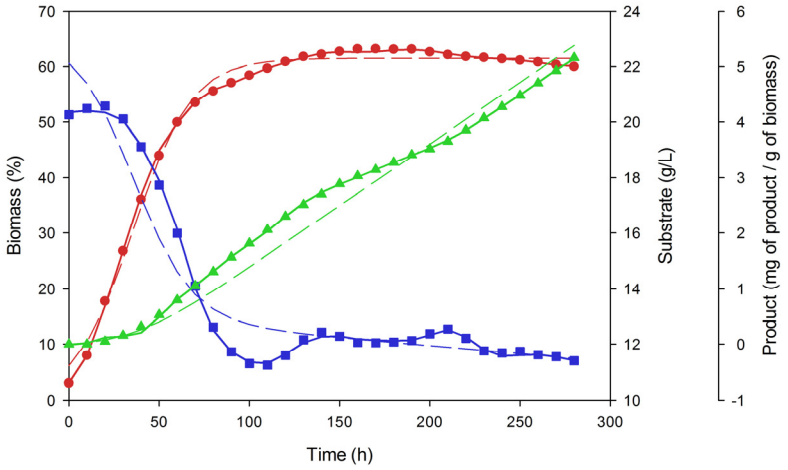
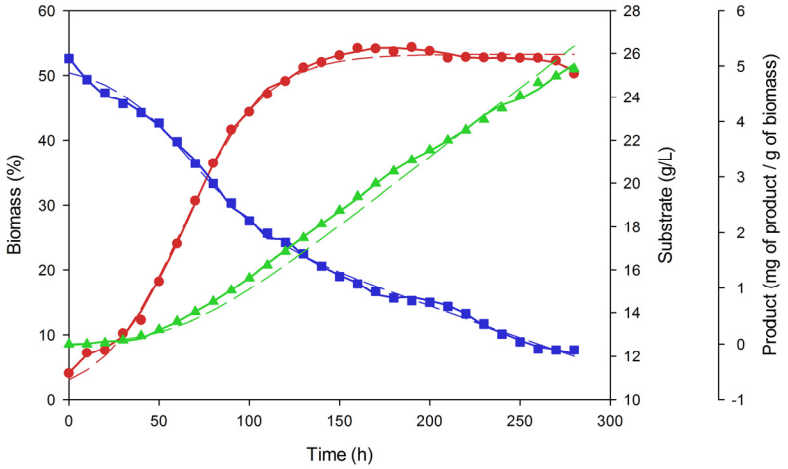


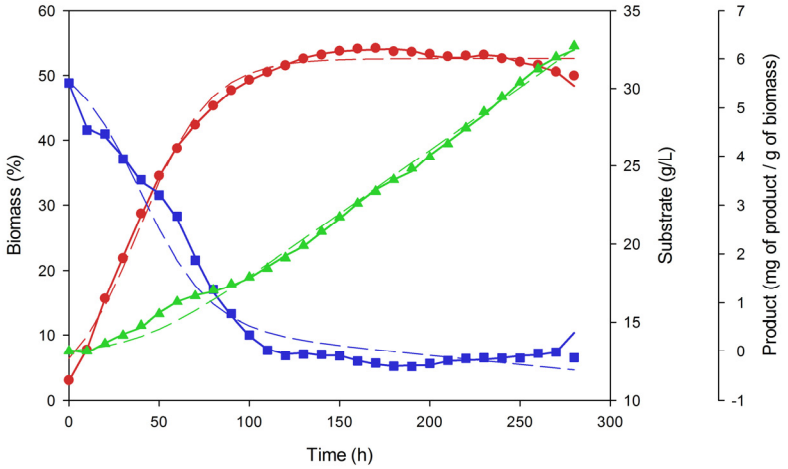
Figure 3: Schematic representation of the optimized NN2.


Biomass (%)
Substrate (g/L)
Product (mg of product / g of biomass)
Time (h)

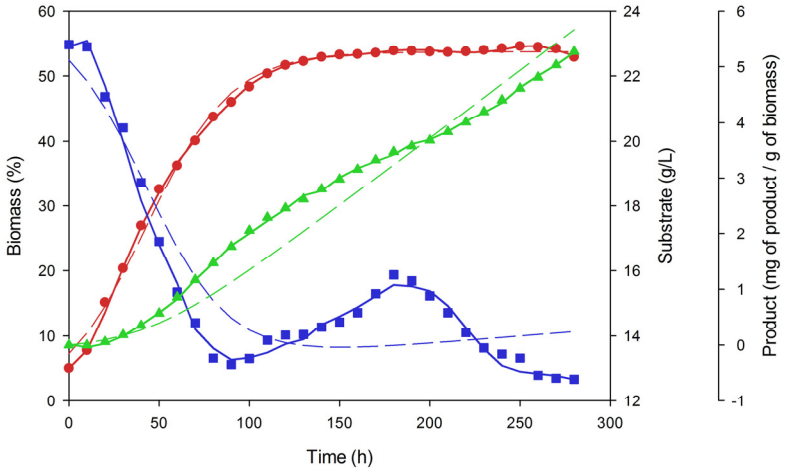
(a)


Biomass (%)
Substrate (g/L)
Product (mg of product / g of biomass)
Time (h)

(b)


Biomass (%)
Substrate (g/L)
Product (mg of product / g of biomass)
Time (h)

(c)



(d)

Figure 4: Simulation results: (a) data set 2, (b) data set 4, (c) data set 9, (d) data set 17. (The experimental data (symbols) for biomass (●), glucose (■) and Pleuromutilin (▲); FFNN modeling results are represented by solid lines and kinetic modeling results are presented by long dashed lines)

Interpolation Performances

To check the accuracy of the three FFNN models previously developed and optimized, two types of interpolation databases were used. The first database contains a set of intermediate points between the experimental points of data set number 6, which was part of the learning and testing phases (data set number 6 was chosen randomly). The second database was two complete data sets never exploited during the learning and the test phases: (a) X_0=4% and S_0=26.6g/L; (b) X_0=3% and S_0=23.5g/L. The results of interpolation performances in terms of root mean squared error (RMSE) and in terms of the correlation coefficient (R) are summarized in Table 4. The quality of fit of the first interpolation data set is depicted in Figure 5. An excellent fit to the experimental values of biomass, sugar and product can be noted.

The modeling results of the second interpolation database derived from the three optimized FFNN models are plotted in Figure 6. It can be observed that both NN1 and NN3 were able to capture the process dynamics very well; however, NN2 results fitted only fairly the experimental glucose concentrations.

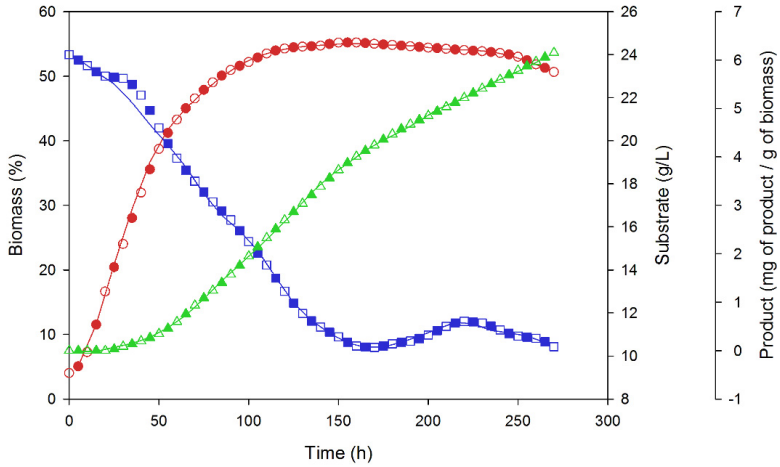
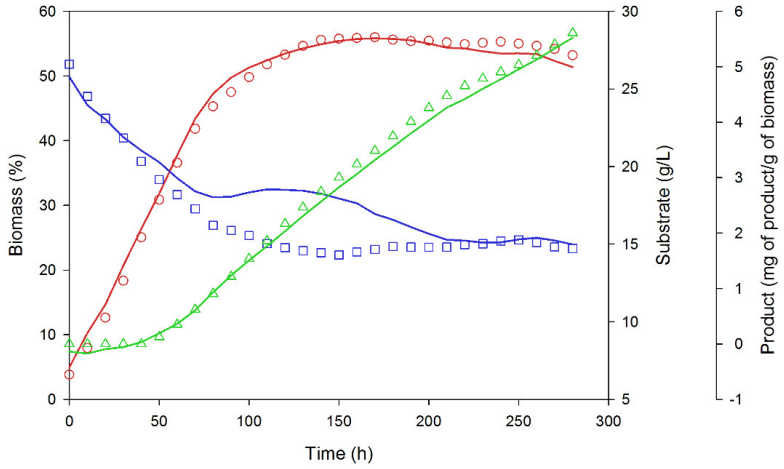


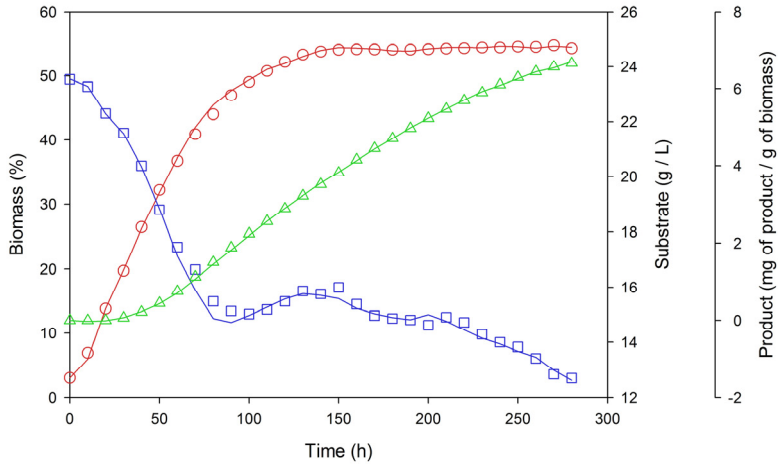
Figure 5: Simulation results of the first interpolation database (the interpolated experimental data (symbols) for biomass (○), glucose (□) and Pleuromutilin (△); FFNN modeling results are represented by solid lines)

Table 4: Interpolation results in terms of root mean squared error (RMSE) and correlation coefficient (R).

	1st interpolation database		2nd interpolation database	
	RMSE	R	RMSE	R
NN1	0.1565	0.9997	1.7100	0.9988
NN2	0.0531	0.9991	2.1810	0.9508
NN3	0.0006	0.9999	0.0797	0.9995



(a)



(b)

Figure 6: Simulation results of the second interpolation: (a) X_0=4% and S_0=26.6g/L; (b) X_0=3% and S_0=23.5g/L. (The interpolated experimental data (symbols) for biomass (○), glucose (□) and Pleuromutilin (△); FFNN modeling results are represented by solid lines)

Although the accuracy of the FFNN models was moderately satisfactory, in particular for NN2, more historical data covering the whole field of interest should be added to the training phase in order to increase more and more the predictive quality of the FFNN models.

CONCLUSION

This paper has given one more reason for the widespread use of the feed-forward artificial neural networks in modeling the fermentation of antibiotics. The purpose of the current study was to develop

three feed-forward neural network models able to assess the three key bioprocess variables (biomass, substrate consumption and product) in the industrial fermentation of Pleuromutilin produced in a fed-batch mode. One of the more significant findings to emerge from this study is that, unlike the unstructured kinetic models, the optimized FFNN models could predict with enough accuracy the profiles of concentrations for all data sets.

In addition, mathematical formulae obtained from the optimized models not only include the important elements of the process, but they are also less complex, making their integration into an industrial control of the process easier.

However, artificial neural network modeling cannot replace kinetic modeling when trying to understand the phenomenon of fermentation.

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