# A Hybrid Neural Model of Ethanol Production by *Zymomonas mobilis*

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#### **Abstract**

A hybrid neural model was developed for the alcoholic fermentation by *Zymomonas mobilis*. This model is composed by the mass-balance equations of the process and neural networks, which describe the kinetic rates. Strategies that combines scarce experimental data with approximate models of the process were used to generate new data for the training of the networks, minimizing the number of experiments required. The proposed hybrid neural methodology uses all the information available about the process to deal with the difficulties in the development of the model.

**Index Entries:** Alcoholic fermentation; *Zymomonas mobilis*; hybrid modeling; functional link networks.

#### Introduction

One of the most severe problems in the control and optimization of biotechnological processes is the construction of reliable models of the system. This difficulty is associated with the complex nature of microbial metabolism and the highly nonlinear nature of its kinetics. For these processes, the development of detailed models based on fundamental principles and intense kinetic studies is frequently expensive and time-consuming. Thus, it would be of great advantage to find some simple and rapid way of describing them, accurately enough for optimization and control.

Many methods have been proposed in recent years to achieve this goal. One of them is the use of neural networks (1,2), which offers a tool for direct use of process data to generate input-output relationships. The training of a neural network, however, requires a large number of experimental data. Furthermore, the interpretation of such models is difficult.

Another alternative is the use of hybrid neural models. As noted by Psichogios and Ungar (3), it is straightforward to derive an approximate

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model of a bioreactor from simple first principles considerations, such as mass balances on the process variables. A critical factor in determining the dynamic behavior of the process is the unknown kinetics. Thus, in the hybrid neural model, the aspects of the problem whose quantitative behavior is well understood are described by deterministic mathematical equations, whereas neural networks describe the kinetics. These models are expected to perform better than "black-box" neural network models, because generalization and extrapolation are confined only to the uncertain parts of the process and the basic model is always consistent with first principles. Besides, as noted in (3), owing to the process uncertainty reduction when first principles information is used, significantly less data are required for their training.

However, even when hybrid neural models are used, the required experimental data set could still be large for certain situations where data collection is too difficult and/or too expensive. Tsen et al. (4) proposed a technique to deal with this problem. It combines scarce experimental data with prior knowledge from an approximate model.

In this work, a hybrid neural model is developed for the fed-batch alcoholic fermentation by *Zymomonas mobilis*. This is a typical example of a process to which the use of the hybrid neural modeling procedure is beneficial. Although there are some models available in the literature for this process (5–7), they are different from each other and valid only for specific conditions. Changes on medium composition or the use of different strains nullify the modeling predictions.

The proposed hybrid neuronal model combines the mass-balance equations with functional link networks (FLNs) (8), whose great advantage is that the weights appear linearly, which simplifies the training procedure. Modifications are applied to the FLNs to increase their nonlinear approximation ability and to reduce size and complexity.

Two approaches are proposed to minimize the number of experiments required for the development of the hybrid neural model: the technique proposed by Tsen et al. (4) and the use of a model available from the literature (modified to describe the obtained experimental data) to augment the experimental data set.

#### Methods

#### Materials and Methods

Batch and fed-batch experiments were conducted in a two liters bioreactor, with the temperature controlled at 30°C and the pH at 6.0. *Zymomonas mobilis* strain CP4 (ATCC31821) was used. The fermentation medium consisted of glucose (the concentration varied in the experiments),  $5 \, \text{g/L}$  of yeast extract,  $1.5 \, \text{g/L}$  of  $(\text{NH}_4)_2 \text{SO}_4$ ,  $2 \, \text{g/L}$  of  $(\text{KH}_2 \text{PO}_4)$ , and  $1 \, \text{g/L}$  of MgSO<sub>4</sub>. The sterilization in autoclave was made with the glucose inside the fermenter and the other nutrients separately. During fed-batch operation, a solution containing glucose and nutrients was fed into the fermenter with

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a peristaltic pump. The experiments were conducted using a 10% (v/v) inoculum with the same composition as the fermentation medium. Samples were assayed for dry cell weight (DCW), glucose, and ethanol. After measurement of the sample volume, it was centrifuged. The supernate was removed for glucose and ethanol analyses with Glucose GOD-PAD method (Merck system) and a Varian 3350 gas chromatographer. The residue was washed and centrifuged again, dried at  $100^{\circ}$ C for 24 h, and weighed for the determination of DCW.

## Hybrid Neural Model

Mass-Balance Equations

A fed-batch stirred bioreactor can be described by the following mass balance equations

$$\frac{\mathrm{dX}}{\mathrm{dt}} = (\mu - \mathrm{D})\mathrm{X} \tag{1}$$

$$\frac{dS}{dt} = -\sigma X + D(S_F - S)$$
 (2)

$$\frac{\mathrm{dP}}{\mathrm{dt}} = \pi X - \mathrm{DP} \tag{3}$$

$$\frac{dV}{dt} = DV \tag{4}$$

where X, S, and P are the biomass, substrate, and ethanol concentrations;  $S_F$  is the feed substrate concentration, D is the dilution rate, defined as D = F/V; F is the volumetric substrate feed rate; V is the volume of the reactor and  $\mu$ ,  $\sigma$ , and  $\pi$  are the specific rates of growth, substrate consumption, and ethanol formation, respectively; these are described by functional-link networks.

#### Functional-Link Networks

A neural network typically consists on many simple computational elements or nodes arranged in layers and operating in parallel. The inputs to a node are linearly weighed before the sum is passed through some nonlinear activation function, which, ultimately, gives to the network its nonlinear approximation ability. The weights, which define the strength of connection between the nodes, are estimated, through a so-called training procedure, to yield good performance The neural network nonlinearity, creates difficulties to obtain the optimal weights. If nonlinear learning rules are used, the learning rate is often unacceptably slow and local minima may cause problems (8).

One way to avoid the nonlinear learning is the use of functional-link networks. In these networks, a nonlinear functional transform or expansion of the network inputs is initially performed and the resulting terms are

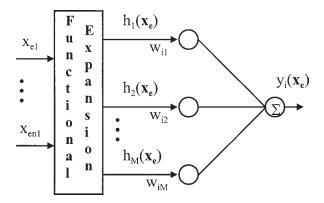


Fig. 1. Extrapolation of different experimental data points.

combined linearly. The obtained structure has a good nonlinear approximation capability and the estimation of the network weights is linear.

The general structure of an FLN is shown in Fig. 1, where  $\mathbf{x}_e$  is the input vector and  $\mathbf{y}_i(\mathbf{x}_e)$  is an output. The hidden layer performs a functional expansion on the inputs, which maps the input space, of dimension  $\mathbf{n}_1$ , onto a new space of increased dimension, M (M >  $\mathbf{n}_1$ ). The output layer consists of m nodes; each one, in fact, a linear combiner. The input-output relationship of the FLN is:

$$y_i(\mathbf{x}_e) = \sum_{j=1}^{M} w_{ij} h_j(\mathbf{x}_e) \quad , \qquad 1 \le i \le m$$
 (5)

In this work, two modifications are applied to this network to improve its performance. In the first one, the output given by Eq. 5 is transformed by an invertible nonlinear activation function. The new output is:

$$y_i(\mathbf{x_e}) = f_i(\sum_{i=1}^{M} w_{ij} h_j(\mathbf{x_e})), \quad 1 \le i \le m$$
 (6)

where  $f_i$  is an invertible nonlinear function.

The other modification is that the real inputs,  $\mathbf{x}_{e}$ , were first transformed before the functional expansion was performed.

These modifications increase the nonlinear approximation ability of the network and the estimation of the parameters still remains a linear problem.

A method based on orthogonal least-squares is used to eliminate nonsignificant nodes during the training of the network (9). This method reduces significantly the size and complexity of the network, avoiding data overfitting.

In the training of the network, the inputs  $(x_e)$  are the process state variables: biomass, substrate, and product concentrations. The outputs are

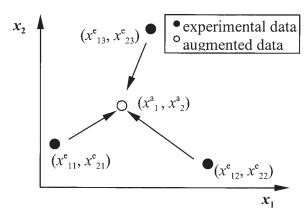


Fig. 2. General structure of an FLN.

the kinetic rates to be calculated. They are not measurable, but can be estimated from measured experimental data from a discretized version of the mass-balance equations. In this work, the discretization procedure was based on a descent difference approximation, using the experimental sample period (30 min). This period was determined by the analytical methods used and the instrumentation available. Although the obtained results have shown satisfactory behavior, any possible reduction of the sampling period should certainly improve the estimation.

## Augmented Experimental Data Set

In this work, two approaches were used to augment the experimental data set and minimize the number of experiments required to develop the hybrid neural model.

One is the use of the technique of Tsen et al. (4), which is based on the fact that, although a qualitatively sound first-principles model may not produce predictions that are accurate in absolute terms, it is usually able to predict the relative trends in process behavior. Mathematically this means that the model can capture the gradients with respect to the process variables at least quantitatively. This technique can be used to produce hundreds of new data from a relatively small original data set.

The basic idea is illustrated in Fig. 2. In this figure  $x_1 \dots x_M$  are the M independent variables of the process model, y is the output variable, ( $x^e_{11}$ ,  $x^e_{21}$ , ...), ( $x^e_{12}$ ,  $x^e_{22}$ , ...), and ( $x^e_{13}$ ,  $x^e_{23}$ , ...) are three experimental data points. The extrapolation equation from N experimental data points to an augmented data point ( $x^a_{11}$ ,  $x^a_{22}$ , ...), is given by:

$$y_{i}^{a} = \sum_{k=1}^{N} W_{ik} \left[ y^{e} \left( x_{1k}^{e}, x_{2k}^{e}, \Lambda \right) + \sum_{j=1}^{M} \frac{\partial y}{\partial x_{j}} \Big|_{x_{j}^{e}, j' \neq j}^{\text{model}} \left( x_{j}^{a} - x_{jk}^{e} \right) \right]$$
(7)

where  $W_{ik}$  are weighting factors that are inversely proportional to the distance of the augmented data point from each experimental data points. It is described by the following equation:

$$W_{ik} = \frac{\left(\sqrt{\sum_{j=1}^{M} \left(x_{j}^{a} - x_{jk}^{e}\right)^{2}}\right)^{-1}}{\sum_{i'=1}^{N} \left(\sqrt{\sum_{j=1}^{M} \left(x_{j}^{a} - x_{ji'}^{e}\right)^{2}}\right)^{-1}}$$
(8)

If the data points are widely scattered, it is more appropriate to use only the closest experimental points for the interpolation (nearest neighbor policy).

Besides this technique, the model of Veeramallu and Agrawal (6) was used as a source of knowledge of the process. Because this model, with some modifications and new parameters, described well the obtained experimental data, it was used to generate new data.

#### Results

The experimental data obtained from three batch and two fed-batch experiments were used to develop the hybrid neuronal model for the alcoholic fermentation by *Z. mobilis*. The technique proposed by Tsen et al. (4) and the modified model of Veeramallu and Agrawal (6) were used to augment the experimental data set.

In the technique of Tsen et al. (4), the model of Garro et al. (7) was used. The kinetic rates are described by:

$$\mu = \mu_{\text{max}} \frac{S}{k_s + S} \left( 1 - \frac{P}{P_m} \right)^n \tag{9}$$

$$\sigma = \frac{\mu}{Y_{x/s}} + m \tag{10}$$

$$\pi = \alpha \cdot \mu + \beta \tag{11}$$

The model parameters are:  $\mu_{max}$  = 0.5/h,  $k_s$  = 4.64 g/L,  $P_m$  = 150 g/L,  $Y_{x/s}$  = 0.0265 g/L, m = 1.4/h,  $\alpha$  = 17.38, and  $\beta$  = 0.69/h.

It is known from the models available in the literature that the specific kinetic rates for the alcoholic fermentation by *Z. mobilis* are functions of the concentrations of substrate and product. Thus, these variables were considered as inputs to the network training.

The derivatives of the model of Garro et al. (7) with respect to the substrate and product concentrations were calculated and new data points were generated using Eq. 5. In this equation, the independent variables

 $(x_1, x_2)$  are the substrate and product concentrations and the output variables (y<sub>1</sub>) are the kinetic rates. Based on preliminary studies with simulated data, only the four experimental data points closest to the point to be generated were used in the weighting. The new data points were generated randomly and only inside the surface limited by the experimental data points. In this way, an eighty points experimental data set, obtained from five experiments, was expanded to a thousand points data set.

Functional-link networks were trained with the original and the augmented experimental data set. The original input vector,  $\mathbf{x}_{s} = [S \ P]$ , was transformed to:

$$\mathbf{x}_{\mathbf{e}} = \begin{bmatrix} \mathbf{S} & \mathbf{SP} \end{bmatrix} \tag{12}$$

The objective of this modification is to supply the network with inherent characteristics of the process kinetics. When the new input vector is used, the neural network is told that, if the substrate concentration is zero, the microbial growth, substrate consumption, and ethanol production are also zero. In other words, this modification *shows* to the network that this fermentation is limited by the substrate.

For the specific growth rate, the activation function used was:

$$f = \frac{1}{1 + \sum_{j=1}^{M} w_{ij} h_j(\mathbf{x_e})}$$
(13)

For the specific product formation rate, no activation function was used. The choice of the activation function was made after tests with different options.

The expressions of the FLNs that describe the specific rates of growth, glucose consumption, and ethanol formation are:

$$\mu = \frac{y_{\text{pl}}}{1 + y_{\text{pl}}} \tag{14}$$

$$\pi = y_{p2} \tag{15}$$

$$\sigma = \frac{\pi}{Y_{P/S}} \tag{16}$$

 $y_{p1}$  and  $y_{p2}$  are given in Table 1, and  $Y_{P/S} = 0.47$ . The glucose concentration experimental data are extremely noisy, owing to the experimental errors associated with the analytical technique used. Thus, the results of the calculation of the experimental substrate consumption rate (used as the target for the neural network training) are much worse, as they depend on  $\frac{ds}{dt}\Big|_{exp}$  and the differentiation amplifies

the errors. The training of the FLNs with these data leads to poor results. Because it is known from the models available in the literature that the

 $\label{eq:table 1} Table~1 \\ y_{_{p1}}~and~y_{_{p2}}~for~the~Functional-Link~Networks$ 

#### FLN trained with the experimental data set $y_{p1} = 2.5886 \cdot 10^{-2} \,\mathrm{S}$ $-2.5039 \cdot 10^{-4} SP$ $2.4587 \cdot 10^{-4} \, \mathrm{S}^2$ $+ 1.6751 \cdot 10^{-6} \, S^2P +$ $4.4613 \cdot 10^{-9} \, S^4$ $y_{p2} = 9.0938 \cdot 10^{-1} \,\mathrm{S}$ $-4.8609 \cdot 10^{-3} \text{ SP}$ $6.9052 \cdot 10^{-2} \, \mathrm{S}^2$ $+ 1.0122 \cdot 10^{-3} \,\mathrm{S}^2\mathrm{P}$ $6.5802 \cdot 10^{-6} \, S^2 P^2 + 2.3038 \cdot 10^{-3} \, S^3$ $5.9682 \cdot 10^{-5} \, \text{S}^{3}\text{P} + 8.5939 \cdot 10^{-7} \, \text{S}^{3}\text{P}^{2}$ $3.7770 \cdot 10^{-9} \, S^3 P^3 - 3.6388 \cdot 10^{-5} \, S^4$ $1.2426 \cdot 10^{-6} \, S^4 P - 1.8221 \cdot 10^{-8} \, S^4 P^2 +$ $6.2411 \cdot 10^{-13} \, S^4 P^4 + 2.6588 \cdot 10^{-7} \, S^5 P^5 1.0532 \cdot 10^{-8} \, S^5 P + 1.4014 \cdot 10^{-10} \, S^5 P^2 +$ $5.0101 \cdot 10^{-13} \, S^5 P^3 - 4.8570 \cdot 10^{-15} \, S^5 P^4 7.1562 \cdot 10^{-10} \, S^6 + 2.9846 \cdot 10^{-11} \, S^6 P 2.8948 \cdot 10^{-13} \, S^6 P^2 - 3.1309 \cdot 10^{-15} \, S^6 P^3$ FLN trained with the augmented data set $y_{p1} = 1.8745 \cdot 10^{-2} \, \text{S}$ $-1.9343 \cdot 10^{-4} SP$

substrate consumption rate can be described as a relation between the product formation ( $\pi$ ) and a yield factor ( $Y_{P/S}$ ), Eq. 16 was used to describe this rate. This equation is a function of the product concentration only, and the experimental measurements are more reliable and less noisy than glucose concentration measurements.

The predictions of the two hybrid neural models (using the FLNs trained with the experimental and the augmented data sets) for the batch and fed-batch experiments performed are shown in Figs. 3–7. For the batch experiments, good results were obtained from both hybrid models (Figs. 3–5). However, for at least one of the fed-batch experiments (Fig. 7), the results obtained with the hybrid neural model that uses the FLNs trained with the augmented data set are much better than those of the hybrid model using the FLNs trained with the experimental data set.

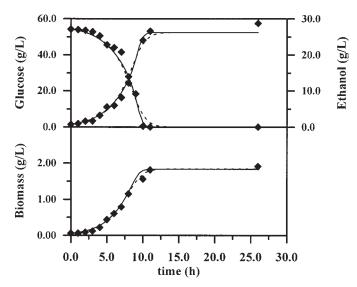


Fig. 3. Experimental results ( $\spadesuit$ ) and results of the hybrid model with experimental FLNs (—) and augmented FLNs (·····) for batch fermentation. Operational conditions: X(0) = 0.06 g/L, S(0) = 50 g/L, P(0) = 0.9 g/L and V = 2 L.

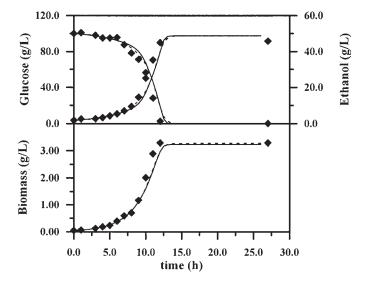


Fig. 4. Experimental results ( $\spadesuit$ ) and results of the hybrid model with experimental FLNs (—) and augmented FLNs (…) for batch fermentation. Operational conditions: X(0) = 0.04 g/L, S(0) = 100 g/L, P(0) = 0.8 g/L and V = 2 L.

Figure 8 shows the results of the model of Garro et al. (7) for one of the fed-batch experiments. It can be seen that this model does not describe properly the experimental results. Nevertheless, it could be used to describe the trends of the process behavior in the technique proposed by

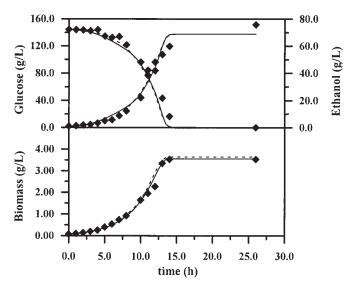


Fig. 5. Experimental results ( $\spadesuit$ ) and results of the hybrid model with experimental FLNs (—) and augmented FLNs (…) for batch fermentation. Operational conditions: X(0) = 0.07 g/L, S(0) = 150 g/L, P(0) = 0.93 g/L and V = 2 L.

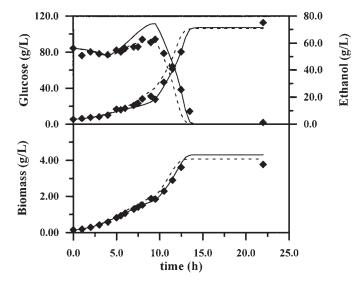


Fig. 6. Experimental results ( $\spadesuit$ ) and results of the hybrid model with experimental FLNs (—) and augmented FLNs (…) for fed-batch fermentation. Operational conditions: X(0) = 0.18 g/L, S(0) = 85 g/L, S(0) = 4 g/L and S(0) = 1 L, S(0) = 1 L, S(0) = 1 L.

Tsen et al. (4), and the hybrid neural model with the FLNs trained with the resulting augmented data set described well the experiments performed.

Another approach to minimize the experiments required for building the hybrid neural model is the use of a model available from the literature to generate new data for the neural networks training. In this work, this

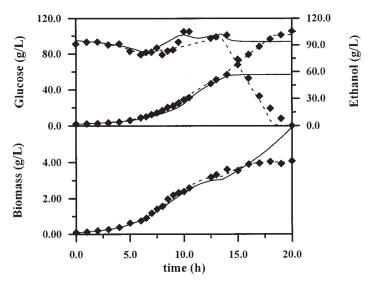


Fig. 7. Experimental results ( $\spadesuit$ ) and results of the hybrid model with experimental FLNs (—) and augmented FLNs (…) for fed-batch fermentation. Operational conditions: X(0) = 0.08 g/L, S(0) = 100 g/L, P(0) = 2 g/L and V(0) = 1 L, SF = 320 g/L and Vf = 2 L.

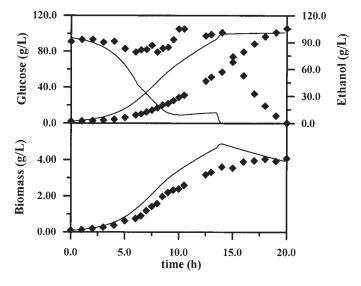


Fig. 8. Results for fed-batch fermentation. Model of Garro et al. (7) (—) and experimental data ( $\blacklozenge$ ). Operational conditions given in Fig. 7.

approach was used to generate new data for the training of a FLN for the specific product-formation rate. The objective is to obtain a network smaller than the one trained to estimate this specific rate using data generated by the technique of Tsen et al. (4). Neural networks with reduced size and complexity are important, as they enable the use of the hybrid model in an adaptive way, re-estimating on-line their weights.

The model of Veeramallu and Agrawal (6) was adopted in this work, with the equation describing the product formation rate slightly modified to account for product inhibition. New parameters were introduced and good reproduction of the experimental data were obtained. The modified model is given by the following equations:

$$\mu = \frac{\mu_{m0} \left[ 1 - \left( \frac{P}{P_{ma}} \right)^{a} \right] \left[ 1 - \left( \frac{P - P_{0b}}{P_{mb} - P_{0b}} \right)^{b} \right] \cdot S}{\left[ K_{m} + S + \frac{S(S - S_{i})}{(K_{i} - S_{i})} \right]}$$
(17)

where:

$$S - S_i = 0$$
 if  $S \le S_i$   
 $P - P_{ob} = 0$  if  $P \le P_{ob}$ 

$$\pi = \frac{q_{pm0} \cdot S}{K_m + S} \left[ 1 - \left( \frac{P - P_1'}{P_m' - P_1'} \right) \right]$$
 (18)

where:

$$\sigma = \frac{\pi}{Y_{p/s}} \tag{19}$$

The adjusted parameters values are: a = 0.50, b = 0.75,  $K_i = 200 \text{ g/L}$ ,  $\begin{array}{l} K_{m}=0.5~g/L,\,P_{ma}=217~g/L,\,P_{mb}=108~g/L,\,P_{ob}=35~g/L,\,S_{i}=80~g/L,\,\mu_{m0}=0.47/h,\,q_{pm0}=4.1/h,\,P_{i}'=52~g/L,\,P_{m}'=127~g/L_{e}\,Y_{P/S}=0.485. \end{array}$  This model was used to generate a thousand new data points for the

FLN training.

Using a trial and error procedure, it was verified that the best results were obtained for the following input vector and activation functions in the output layer:

$$\mathbf{x}_{\mathbf{e}} = \begin{bmatrix} \mathbf{S} & \mathbf{P} & \frac{1}{\mathbf{S}} & \frac{1}{\mathbf{P}} \end{bmatrix} \tag{20}$$

$$f = \frac{1}{\sum_{j=1}^{M} w_{ij} h_j(\mathbf{x_e})}$$
 (21)

The expression of the resulting FLN was:

$$\pi = \frac{1}{y_p} \tag{22}$$

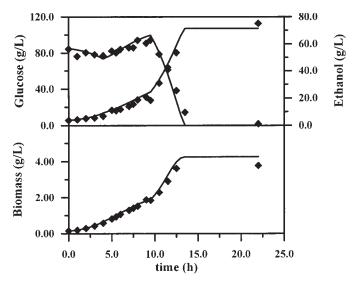


Fig. 9. Results for fed-batch fermentation. Hybrid neural model (—) and experimental data ( $\spadesuit$ ). Operational conditions given in Fig. 7.

where:

$$\begin{aligned} y_p &= 0.2426 + 0.2620 \cdot \frac{1}{S} - 0.0074 \cdot \frac{P}{S} + \\ 8.5259 \cdot 10^{-5} \cdot \frac{P^2}{S} + 4.2293 \cdot 10^{-11} \cdot P^5 + 9.6624 \cdot 10^{-5} \cdot \frac{1}{S^6} + 6.5915 \cdot 10^{-15} \cdot P^6 \end{aligned}$$

The use of a modified input vector also reduces the number of experimental data required for training and the network complexity. If the FLNs shown in this work were trained with the original input vector ( $\mathbf{x}_e = [S \ P]$ ), not only the number of experimental data required but also the number of monomials in the resulting network expressions would be much greater.

In the new hybrid neural model, the FLNs which describe the specific rates of growth, product formation and substrate consumption are given by Eqs. 14, 22, and 16. It should be noted that, although the equation for the specific substrate consumption rate is the same as the one in the other model (with FLNs trained with the augmented data set obtained by the technique of Tsen et al. [4]), the resulting rate is different, as it is a function of the product formation rate.

This new approach described well the experiments performed, as can be seen from the results for the fed-batch experiments in Figs. 9 and 10.

#### Discussion

Bioreactors operating in fed-batch mode are quite difficult to model, because their operation involves microbial growth under constantly changing conditions. The use of hybrid neural models is a good solution for this modeling problem. In these models, the mass-balance equations of the

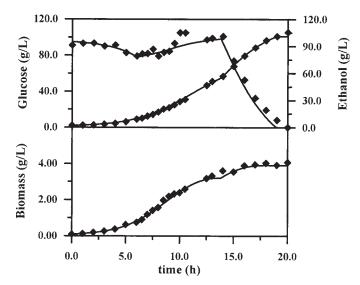


Fig. 10. Results for fed-batch fermentation. Hybrid neural model (—) and experimental data ( $\blacklozenge$ ). Operational conditions given in Fig. 6.

process are combined with neural networks, which describe the unknown kinetics.

Frequently the mass-balance equations are not the only *a priori* information available about the system dynamics. The inclusion of all the *a priori* available knowledge of the system in the modeling stage leads to a better performance of the resulting model and diminishes the time and the effort required for its development.

In this work, a hybrid neural model was built for the alcoholic fermentation by *Z. mobilis*. All the available knowledge from the process was used to solve the problems that arose in the different modeling stages.

The first challenge was the development of the hybrid model with only few experimental data. Two approaches were suggested that led to good results. The first one generates an augmented data set using a combination of the information, taking from both an available model of the process and the experimental data (4). The hybrid model whose neural networks were trained with this augmented data set described the experiments better than the hybrid model with networks trained only with the original experimental data set.

The other approach generates new data using a model available from the literature, modified to describe the experimental data. This approach was used to train a neural network for the specific product-formation rate. This network was smaller than the one trained with the data generated by the technique of Tsen et al. (4). This is an important result, because neural networks with too many parameters can offer poor generalization characteristics.

In order to obtain networks with minimal size and complexity, some modifications were made in the functional link networks structure, such as

the use of an invertible activation function and of a modified input vector, which increase the nonlinear approximation capability. A method to eliminate nonsignificant monomials during the training of the network was also used.

The simple structure of the resulting FLNs enables the use of the proposed hybrid neural model in an adaptive scheme. In this scheme, after the neural network is trained based on available data, its weights can be continuously updated during real-time application. In this way, the model is able to adapt itself to deviations from the information contained in the initial training data set.

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### References

- 1. Simutis, R., Havlik, I., and Lübbert, A. (1993), J. Biotechnol. 27, 203-215.
- 2. Teissier, P., Perret, B., Latrille, E., Barillere, J. M., and Corrieu, G. (1996), *Bioprocess Eng.* **14**, 231–235.
- 3. Psichogios, D. C. and Ungar, L. H. (1992), AIChE J. 38, 1499-1511.
- 4. Tsen, A. Y., Jang, S. S., and David, S. H. W. (1996), AIChE J. 42, 455–465.
- 5. Lee, K. J. and Rogers, P. L. (1983), Chem. Eng. J. 27, B31–B38.
- 6. Veeramallu, U. and Agrawal, P. (1990), Biotechnol. Bioeng. 36, 694–704.
- 7. Garro, O. A., Rodriguez, E., Roberto, P. U., and Callieri, D. A. S. (1995), *J. Chem. Tech. Biotechnol.* **63**, 367–373.
- 8. Chen, S. and Billings, S. A. (1992), Int. J. Control. 56, 319–346.
- 9. Billings, S. A., Chen, S., and Korenberg, M. J. (1989), Int. J. Control. 49, 2157–2189.