A Comparison Between Different Modeling Techniques for the Production of Bio-ethanol from Dairy Industry Wastes

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In the present work, the fermentation process aimed at obtaining bio-ethanol starting from ricotta cheese whey (RCW), a waste biomass rich in lactose, was simulated by both a pure neural network model (NM) and a multiple hybrid neural model (HNM). The simulation results showed that the developed HNM was capable of providing an accurate representation of the actual time evolution of lactose, ethanol and biomass concentrations even in conditions never exploited during model development. HNM predictions indeed exhibited an average percentage error lower than 10 %, as compared to the experimental data collected during RCW fermentation runs. The proposed methodology, leading to the formulation of a hybrid paradigm, may allow overcoming some of the inherent difficulties accompanying the development of reliable models that are called to describe the true behavior of biotechnological processes.

Key words:
Whey, fermentation, ethanol, grey-box models, artificial neural networks, modelling

Introduction

Fermentations are inherently un-steady state processes, usually performed in batch and fed-batch mode of operation. Significant variations of raw material properties and problems during bioreactor start-up are commonly observed and have to be tackled by proper control systems, which, therefore, are called to suppress the influence of external disturbances, to ensure the process stability and to optimize the process performance. The starting point for the implementation of any kind of automatic control is definitely represented by the availability of a predictive model of the process under study.

Most of the available models aimed at describing the fermentation process are based on the experimental measurement of extracellular metabolites concentrations, i.e. substrate(s), product(s) and biomass.[1] This modeling approach, however, is highly unstructured and precludes any interpretation of the actual cell physiology. The formulation of structured model, conversely, takes into account the microorganisms' metabolism and, therefore, the control and the regulatory mechanisms taking place in living cells, thus leading to a very complex reaction network, which requires a considerable computational effort.[1,2] Moreover, with the aim of validating a rigorous structured model, advanced analytical techniques are to be exploited to gain experimental evidence of the actual intracellular metabolism.[1] Lei *et al.*[4] developed a structured model for *Saccharomyces cerevisiae* focusing on both oxidative and oxido-reductive metabolism; on the basis of several assumptions regarding the abiotic and the biotic factors involved in the reaction, the authors defined a set of 12 reaction steps. For each of the steps, a Michaelis Menten kinetics was assumed and, finally, 36 kinetics parameters were experimentally estimated. Garcia-Ochoa *et al.*[5] developed a semi-structured model for xanthan production; the biomass growth was actually calculated by a non-structured model, namely a logistic function, but the product formation was described considering the cells metabolism, thus leading to a final reaction mechanism that accounted also for intracellular species. In order to avoid any measurement of intracellular species, the assumption of a pseudo-steady state order for both ATP and Cofactor within the cell was formulated to identify three key-components, corresponding to each of the extracellular metabolites. From an accurate analysis of the available papers, it is evident that fully mechanistic models aimed at describing the fermentation process resulted in a series of complex reactions whose resolution either necessitates a set of simplifying hypotheses, which may not be applicable in several cases, or is too onerous and time consuming for practical purposes. An alternative approach to theoretical modeling was actually represented by black-box models (BBMs), which, however, do not make use of any transport equation that might help determining, on the basis of fundamental principles, the mutual relationships existing between the inputs

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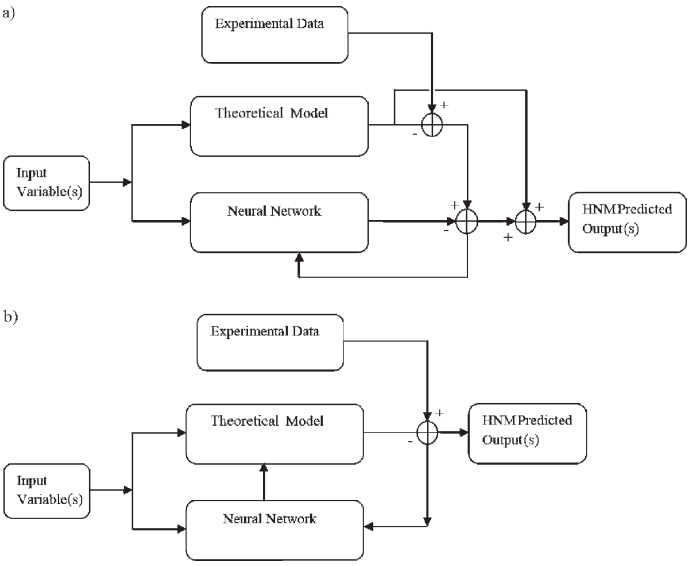
and the outputs. Among BBMs, Artificial Neural Networks (ANNs) are noteworthy. ANNs are a data-driven method capable of learning from examples; no *a priori* knowledge of the process is therefore necessary for their definition. ANNs are composed of interconnected computational elements, called neurons; each neuron receives input signals from the related units, elaborates these stimuli by an activation function and, eventually, generates an output signal, which is transferred to other neurons.

A neural model is, generally, rather complicated, since it requires many different connections and, therefore, a great number of parameters to be estimated. Moreover, it is worthwhile observing that since extrapolation based on ANNs predictions is an unreliable procedure, it is often necessary to perform many different experiments in order to train the network in an as wider as possible range of significant situations.[6] Artificial neural networks allow reliable management of large sets of data and, generally, are capable of describing the actual input-output relationships even when trained with unreliable, missing, or noisy data.[7]

A reasonable trade-off between theoretical and empirical approaches is represented by hybrid modeling, leading to a so-called "grey-box" model, which allows predicting the behavior of complex systems in a more efficient way. Hybrid model predictions are indeed given as a combination of both theoretically based and "pure" neural network models, together concurring at the obtainment of system responses. The main advantage of a hybrid system is the possibility of describing some well-assessed phenomena by as simpler as possible theoretical relationships, leaving the analysis of other aspects, generally difficult to interpret, to rather straightforward neural models.[8–11] The neural network ability to properly manage unreliable or noisy data is strongly improved when it is inserted in a hybrid structure. This is due to the theoretical part of the model that, among all the other tasks it is called to accomplish, may perform as a filtering function that limits the error propagation throughout the system even when its inputs are perturbed.

When ANNs are utilized in a hybrid model, it is first necessary to identify the respective domain of exploitation pertaining to either theoretical or empirical models in such a way as to decide which aspects of the process are to be described by a BBM and which by the fundamental relationships. Two kinds of HNMs can be generally defined depending on the interactions existing between the neural and the theoretical blocks. In a model based on a parallel architecture (Fig. 1a), the inaccuracy in the predicted value from the fundamental part is minimized by the addition of the residuals calculated by the neural network.[12] In a model based on a



F i g . 1 – *HNM structures. a) structure based on a parallel architecture; b) structure based on a serial architecture*

serial architecture (Fig. 1b), a process variable, which is difficult to measure, is estimated by a neural network and then fed as input to the theoretical block.[10] Even though hybrid neural models so far have not been so common, the serial architecture is more popular in bioreactors modeling since it allows exploiting the ANN as an estimator of kinetic parameters.[12–16]

Feyo de Azevedo *et al.*[3] compared the performance of hybrid and pure neural models in the case of Baker's yeast production achieved in a fed-batch fermenter. In their work, the theoretical part of hybrid model was represented by a mass balance equation written to describe the time evolution of biomass concentration, whereas the ANN part was appointed to determine the biomass specific growth rate on the basis of the calculated biomass concentration. James *et al.*[9] developed a grey-box soft-sensor in order to estimate biomass concentration during fed-batch fermentation of *Alcaligenes eutrophus*. Zorzetto *et al.*[17] proposed the application of two hybrid models to describe the batch fermentation of beer. The first model, based on ANN, was formulated to determine the specific growth rate of biomass from temperature and substrate concentrations; the second one, based on the Monod's equation and on the predictions provided by the already-developed neural network, was used to calculate the dependence of model parameters on temperature.

The object of the present paper was to compare different paradigms aimed at modeling the batch fermentation of ricotta cheese whey (RCW), a highly pollutant dairy waste, obtained as the main

by-product in ricotta cheese production process.[19] RCW is mainly obtained in Italy but also in other countries in the Mediterranean area. It is estimated that Italian production amounts to about 1.0 Mt of RCW per year, thus determining significant environmental problems related to its disposal.[19] Among all bio-fuels, bio-ethanol is definitely the most common. Nowadays, nearly all bio-ethanol, however, is obtained by fermentation of vegetable biomasses, essentially sugar cane and cereals, thus contributing to the observed increase of foodstuffs price. It is, therefore, necessary to identify alternative renewable and non-vegetable sources for bio-fuels production. RCW could potentially fit this requirement and may potentially represent an interesting fermentation substrate owing to its main characteristics, namely the relatively high content of lactose (w = 5 %) that could directly be fermented into ethanol, and to its low cost, as determined by the fact that it is actually a waste.[19–20] The concentration profiles of lactose, of ethanol and of biomass characterizing the time evolution of RCW fermentation process were predicted both by a pure neural model and a multiple hybrid neural model, which was formulated accounting for the transient mass balance equations referred to the main components participating in the reaction. Finally, a simple analytical model composed by two kinetic equations was proposed to infer substrate and product concentration profiles directly from biomass predictions, as provided by the developed HNM. As compared to the studies already available in the literature, the novelty of this paper is represented by the development of a reliable multiple hybrid model, which allowed predicting the true behavior of a fermentation process aiming to obtain a second-generation biofuel. It was intended, therefore, to show how the exploitation of process engineering tools and, particularly, of advanced modeling techniques represents a preliminary, fundamental step for any further investigation, e.g. process optimization, design of an efficient control system, about the process under study.

Materials and methods

Data-driven models are actually based on experimental data and are called to provide a reliable representation of the system behavior over an as wider as possible range of operating conditions. Therefore, the utilization of well-assessed methods aimed at estimating the effects of process variables on system behavior is definitely essential when model identification is to be performed.[21] The experimental data necessary to develop the models whose performance is compared in this paper, were obtained from a set of anaerobic fermentations carried out on ricotta cheese whey. Each experimental run actually consisted of two subsequent steps: the inoculum culture preparation and the batch fermentation carried out in a stirred anaerobic bioreactor. *Kluyveromyces marxianus* (E.C. Hansen) Van der Walt *var. marxianus* (CBS 397) obtained from Centraalbureau Voor Schimmelcultures (Holland) was used in all the fermentation experiments. The inoculum culture was prepared adding a single yeast colony to 150 mL culture medium containing 50 g L^{-1} lactose, 10 g L^{-1} peptone, 5 g L^{-1} yeast extract (Fluka). The culture was kept at 37 °C for 12 hours in a 250 mL flask held in a temperature-controlled bath (OLS 200, Grant) with a roto-translational external mixing of 150 rpm.

During each fermentation test, one liter of RCW was fermented in a 2 L batch fermenter (Z611020002, Applikon) equipped with temperature, pH, and rpm controllers (ADI 1030, Applikon). Two milliliters of fermentation broth were sampled every hour to measure the time evolution of ethanol and lactose concentrations. The analyses were performed by injecting 20 μL of fermentation broth into a Jasco HPLC, equipped with a refractive index (RI 930, Jasco). The mobile phase was orto-phosphoric acid φ = 1 % (Fluka), fed at a flow rate of 1 mL min^{-1}. The column was an Alltima Amino NH_2 (Alltech).

The time evolution of biomass concentration was measured by Bactoscan (Foss).

The operating conditions and the process variables were chosen according to the factorial design method,[22] on the basis of the indications obtained from both available literature information[23–24] and the results collected after a preliminary experimental analysis on RCW fermentation. In particular, temperature (T) ranged between 32 °C and 40 °C; pH was in the range 4–6; stirring rate (rpm) was varied between 100 and 300 rpm; lactose concentration (γ^0_{lat}) was changed between 45 g L^{-1} and 90 g L^{-1}. As far as γ^0_{lat} was concerned, it is worthwhile remarking that the chosen lower bound accounted for any possible lactose degradation due to, for instance, an improper storage of RCW; whereas, the chosen upper bound accounted for a possible pre-treatment of RCW aimed at increasing, for instance by ultrafiltration, the available lactose concentration. Actually, it should be observed that a higher substrate concentration fed to the fermenter usually leads to a higher ethanol concentration in the stream flowing out of the bioreactor. This determines a lower cost for ethanol purification and, therefore, an improved downstream processing. According to the factorial design method, 16 batch runs were performed, each lasting 18 hours. With the aim of evaluating the performance of the developed models, an additional fermentation run (run

N°17) was carried out under a set of operating conditions not belonging to that chosen to perform the experimental design. Table 1 summarizes the conditions in which each experiment was performed. Considering that for each sample the concentration of lactose, of ethanol and of biomass was actually available, a total number of 969 experimental points was exploited to train, test and validate the performance of both the neural and the hybrid models developed in this paper.

Table 1 – *Batch fermentation operating conditions*

Run N°	T [°C]	pH [–]	Agitation level [rpm]	γ^0_{lat} [g L^{-1}]
1	40	6	300	90
2	40	4	100	90
3	32	6	300	45
4	40	6	100	45
5	32	6	100	90
6	32	4	300	90
7	40	4	300	45
8	32	4	100	45
9	40	4	100	45
10	40	6	300	45
11	40	4	300	90
12	32	6	100	45
13	32	4	300	45
14	32	4	100	90
15	40	6	100	90
16	32	6	300	90
17	37	5	300	50

Development of the models

Neural model development

In order to model the fermentation process of RCW, three neural networks were developed, i.e. NM1, NM2 and NM3, aimed at predicting, respectively, the time evolution of lactose, ethanol, and biomass concentration.

To determine the networks structure, it was necessary to specify: a) the number of both input and output variables; b) the number of layer(s) composing the network; c) the number of neurons composing each layer; d) the activation function of each neuron. In the present case, all the proposed neural models were characterized by the same set of input variables: temperature (T), pH, reactor stirring rate (rpm), initial lactose concentration (γ^0_{lat}) and reaction time (t). It was indeed observed that the above variables, among all the parameters that could affect the reaction progress, exhibited the highest influence on process performance. Each of the developed neural model had a single output, i.e. lactose, ethanol and biomass concentration, respectively, for NM1, NM2 and NM3. As far as the identification of neural networks architecture was concerned, both the number of hidden layers and the number of neurons comprised in each layer were determined according to a trial and error procedure, described in section 3.3.

Hybrid neural model development

The developed hybrid neural models consisted of a combination of a theoretical part, represented by a system of transient mass balance equations (eq. 1) and of a rather simple neural model.

$$\begin{cases} \dfrac{dX}{dt} = \mu X \\ -\dfrac{dS}{dt} = qX \\ \dfrac{dP}{dt} = \mu_p X \end{cases} \quad (1)$$

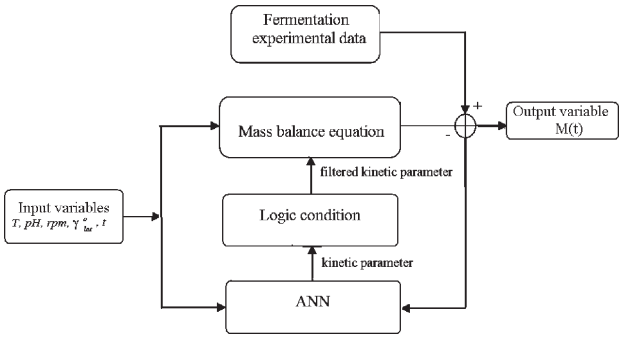
where μ was the specific growth rate of biomass, q was the substrate consumption rate function and μ_p was the specific ethanol production rate. The theoretical model was aimed at predicting the concentrations of biomass (X), lactose (S) and ethanol (P), whereas the neural model was set up to estimate the parameters, μ, q and μ_p, necessary to determine the actual reactions rates.

Eqs. (1) were then approximated by the Euler's discretization and the discretized form was used recursively to determine the biomass, lactose and ethanol concentration values at the successive time step, $t+\Delta t$, on the basis of the knowledge already achieved at time t. The values of parameters μ, q and μ_p at time t, strictly necessary to solve the discretized form of the equations, were provided by three independent neural networks, namely HNM1, HNM2 and HNM3, estimating μ, q and μ_p, respectively.

The kinetic parameters were subsequently processed by some logic conditions (eq. 2) that verified the physical reliability of networks outputs. In fact, it is expected that, during the fermentation runs, biomass and ethanol concentrations have to continuously grow, whereas lactose concentration has to monotonically decrease.

$$\begin{cases} if \quad \mu < 0 \Rightarrow \mu = 0 \\ if \quad q < 0 \Rightarrow q = 0 \\ if \quad \mu_p < 0 \Rightarrow \mu_p = 0 \end{cases} \tag{2}$$

The "filtered" values of parameters μ, q and μ_P were finally fed to the theoretical part of the hybrid model, thus allowing obtaining three "grey-box" models characterized by a serial architecture and having the general form shown in Fig. 2. The instantaneous value of biomass concentration was preliminarily estimated by HNM1. Then, the so-calculated *X(t)* was fed to HNM2 and HNM3 to determine, respectively, the time evolutions of both lactose and ethanol concentration. The choice of using the values of *X(t)* as given by the developed hybrid model, instead of the corresponding measured values, was aimed at testing the capability of the models to provide good simulation results, even if their inputs were bias affected.



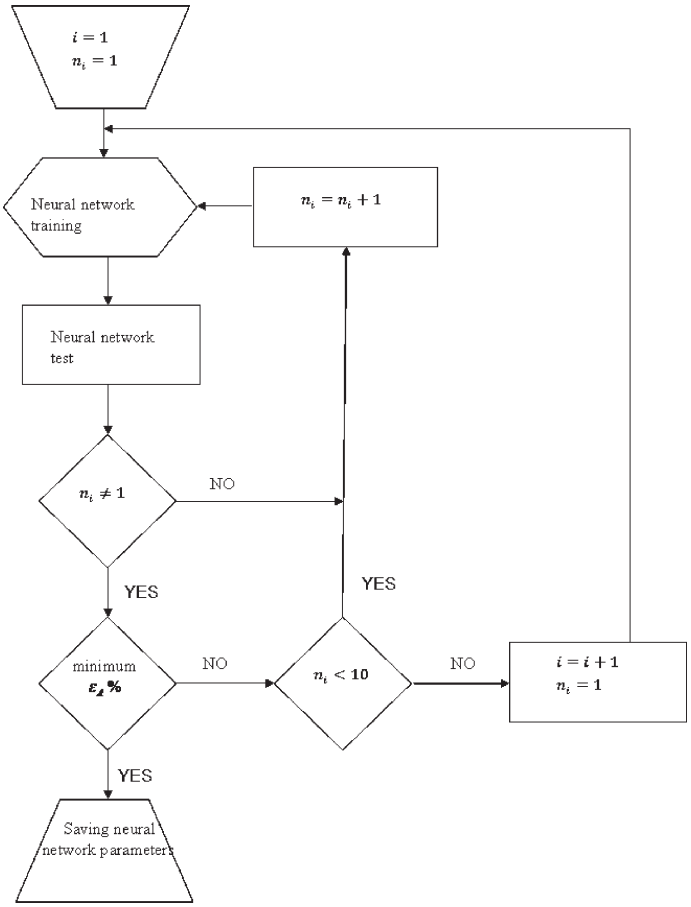
F i g . 2 – *HNM general structure*

Neural networks development

To identify the final architecture of both the pure neural models and the neural part of each HNM, an iterative trial-and-error procedure was implemented in Matlab Neural Network Toolbox Ver. 4.0.1. The procedure was based on the definition of a performance index that allowed estimating the reliability of the simulation results. In the present paper, the percentage error, ε/%, between each value of concentration, γ_p, as predicted by the model, and the corresponding measured value, γ_m, was considered:

$$\varepsilon/\% = \frac{|\gamma_p - \gamma_m|}{\min(\gamma_p, \gamma_m)} \cdot 100 \tag{3}$$

In particular, the convergence was considered to be achieved as soon as in a whole batch run the average value of ε/% was lower than 10 %. The implemented iterative procedure is schematically reported in Fig. 3. As far as the pure neural model was concerned, only the concentration values collected during the batch fermentation experiments were actually exploited to train the networks; whereas for HNM development it was necessary to preliminarily determine the values of μ, q, μ_p by interpolating the collected experimental data. Of the considered 17 batch runs, 15 experiments were used to train and test the developed networks. The experimental data were randomly split into two groups, reserving 2/3 of data (570 points) to the training phase and the remaining 1/3 (285 points) to test neural networks predictions during their development. A multi-layer perceptron (MLP) feed-forward architecture was exploited to develop all the networks;[8] the networks weights and bias were estimated by the Levenberg-Marquardt algorithm with Bayesian regularization to avoid lengthy cross validation.[25] The neuron transfer function was chosen as the hyperbolic tangent for all the layers, except for the output layer where a linear transfer function was used; the choice of these transfer functions was actually supported by preliminary training tests.



F i g . 3 – *Neural network realization procedure*

On the basis of the above-described methodology, 6 neural networks were eventually obtained: 3 networks, actually represented the pure black-box models and 3 networks constituted the neural part of the developed HNMs. The predictions of each

model was finally validated using two complete experiments (run N° 8 and run N° 17), corresponding to a total number of 114 points, which were never exploited either during the training or during the test phases. It is worth noting that run N° 17 was performed under a set of operating conditions not belonging to those defined by the experimental design.

Constant yields model

With the aim of strengthening the theoretical part of the proposed hybrid neural models, the possibility of inferring the concentration values of lactose and ethanol directly from the amount of biomass, as predicted by the hybrid model HNM1, was also evaluated. In particular, the substrate consumption rate, q, and the product growth rate, μ_p, of eqs. 1 were calculated by defining two yield coefficients, which were assumed constant throughout the fermentation progress. Actually, the assumption of constant yield factors was widely used to model biochemical reactors[26–29] and allowed obtaining two additional relationships having the following form:

$$\begin{cases} -\dfrac{\mathrm{d}S}{\mathrm{d}t} = \dfrac{1}{Y_{x/s}} \cdot \dfrac{\mathrm{d}X}{\mathrm{d}t} \\ \dfrac{\mathrm{d}P}{\mathrm{d}t} = Y_{p/s} \cdot \dfrac{1}{Y_{x/s}} \cdot \dfrac{\mathrm{d}X}{\mathrm{d}t} \end{cases}$$

with

$$\begin{cases} Y_{x/s} = \dfrac{\Delta X}{\Delta S} = \dfrac{X_{t=t_F} - X_{t=t_0}}{S_{t=t_0} - S_{t=t_F}} \\ Y_{p/s} = \dfrac{\Delta P}{\Delta S} = \dfrac{P_{t=t_F} - P_{t=t_0}}{S_{t=t_0} - S_{t=t_F}} \end{cases} \quad (4)$$

where $Y_{x/s}$ was the yield factor of biomass toward lactose, $Y_{p/s}$ was the yield factor of ethanol toward lactose, t_0 and t_F were the initial and the final time of biomass exponential growth rate, respectively. For each reaction run $Y_{x/s}$ and $Y_{p/s}$ were calculated as the arithmetic means of the experimental yield factors, as measured during the course of reaction.

This alternative approach to model substrate and product concentration profiles allowed replacing HNM2 and HNM3 with eqs. 4.

It is worth observing that the proposed theoretical structure, although very common, is simpler than other available models, which allow a more precise description of the actual phenomena involved in fermentation at the expense, however, of a higher computational effort. The substitution of HNM2 and HNM3 with eqs. 4 has to be considered, according to authors' intention, as a way to reduce the black-box nature of the developed hybrid neural model, without significantly increasing the required computational effort. Any exploitation of more complicated theoretical model was far beyond the scopes of the present paper, which, instead, was aimed at proving that rather simple hybrid neural structures were capable of overcoming some of the inherent difficulties accompanying the rigorous modeling of biotechnological processes.

Results and discussion

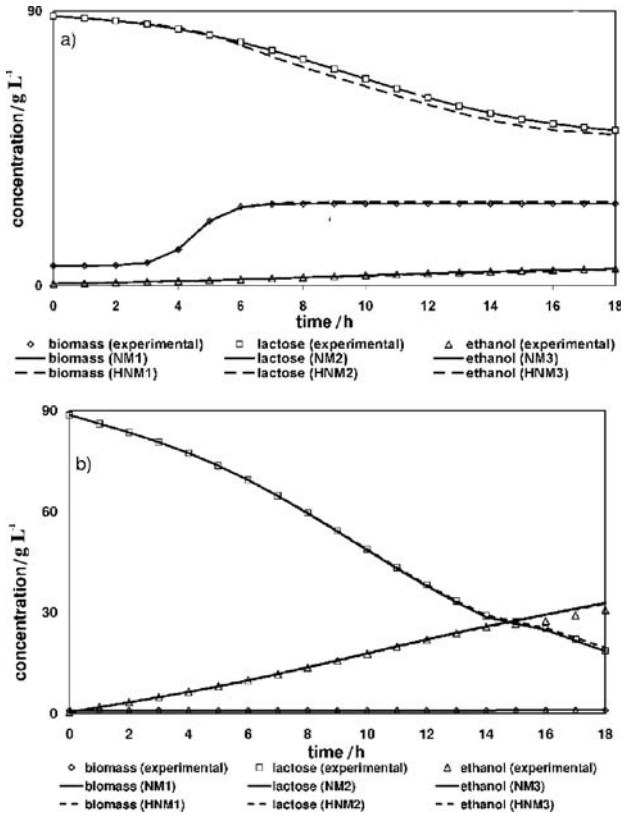
Table 2 summarizes the architecture of the networks developed according to the previously-described trial-and-error procedure. It can be observed that, due to the presence of the theoretical relationships as expressed by eq. 1, the architecture of the neural part of HNMs is simpler than that of the corresponding pure neural models.

Table 2 – *ANNs architecture*

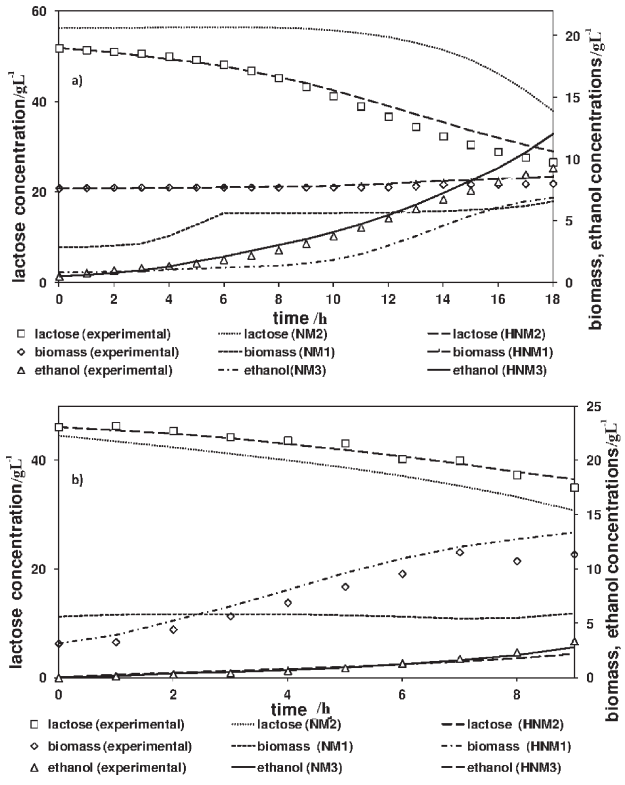
Neural Network	Number of neurons 1st hidden layer	Number of neurons 2nd hidden layer	Number of neurons output layer
NM1	8	5	1
NM2	10	10	1
NM3	10	4	1
HNM1	6	5	1
HNM2	10	3	1
HNM3	10	2	1

Figs. 4a-4b show a comparison between the predictions provided by both pure neural model and hybrid neural model when the experimental points belong to the training/test dataset. In both cases, a remarkable agreement is actually observed throughout the considered time horizon. The average percentage error is much lower than 10 % and both the models reproduce very well the actual time evolution of substrate, product and biomass concentrations measured during lab-scale fermentation performed on RCW. The performance of both the proposed models is very similar and the corresponding predictions tend to overlap.

Figs. 5a-5b show the behavior of both NMs and HNMs when they are called to reproduce the system behavior under a set of operating conditions never exploited before, thus performing the so-called model validation, e.g. a verification of the generalization capability of the models. Actually, the predictions provided by NMs and HNMs are



F i g . 4 – *Predictions during training/test. a) run conditions: T = 32 °C, pH = 6, rpm = 100, γ^0_{lat} = 90 g L^{-1}; b) run conditions: T = 40 °C, pH = 4, rpm = 100, γ^0_{lat} = 90 g L^{-1}.*



F i g . 5 – *Models validation. a) run conditions: T = 32 °C, pH = 4, rpm = 100, γ^0_{lat} = 45 g L^{-1} ; b) run conditions: T = 37 °C, pH = 5, rpm = 300, γ^0_{lat} = 50 g L^{-1}.*

rather different; the hybrid models, in fact, performed very well in both the validation tests since the calculated values of percentage errors are comparable to those obtained during the training/test phases. These results indicate that hybrid models not only do recognize the training points, but the level of knowledge they have learnt during training allows them to predict the system behavior even when they are operated in unexploited conditions.

On the other hand, pure neural models fail to a large extent since their predictions, given as the time evolutions of biomass, lactose and ethanol concentrations, are characterized by an average percentage error of about 30 %, with peak values, referred to lactose concentration (Fig. 5a), as high as 60 %. This result confirms that pure black-box models may provide reliable predictions strictly within their definition domain, whereas they become far less accurate when they are called to extrapolate.

Therefore, for the process under study, the combination of simple theoretical equations with straightforward neural models fairly widens the applicability of pure neural models even outside the training range, thus strengthening their performance. The theoretical part of HNMs, indeed, plays the role of filtering function with respect to the predictions of the neural part of HNMs, thus limiting the introduction and the propagation of errors, typical of a black-box model, and determining a significant improvement of model accuracy. This improvement is to be ascribed to the fact that the predicted kinetic constants are subsequently processed by the theoretical part of the model. This sequential transfer of signals, together with the chosen architecture, iteratively refines the estimation of the actual reaction rates. Therefore, also in conditions never exploited before, HNMs exhibit a higher reliability, as compared to a pure black-box models for which any prediction outside the training range definitely represents a mere extrapolation. Moreover, it is worth noting that the reported concentrations of both lactose and ethanol have been obtained using the estimation of biomass concentration, as provided by HNM1. The choice to feed HNM2 and HNM3 directly with the results provided by HNM1 instead with the actual experimental data, was actually aimed at testing the consistency of hybrid models predictions even if their inputs were bias affected.

After verifying the reliability of HNMs, the possibility of inferring lactose and ethanol concentration values, directly from HNM1 predictions by a set of properly defined yield factors, was also explored. The aim of this attempt was to strengthen the developed hybrid model by introducing two ad-

ditional theoretical equations (eqs. 4) that might replace the function of both HNM2 and HNM3, which, therefore, were dropped out. In other words, it was intended to verify the actual necessity of developing HNM2 and HNM3, or, otherwise, if the prediction of lactose and ethanol concentrations could be calculated defining two yield coefficients that were actually assumed constant throughout the fermentation progress. Figs. 6a-b report a comparison, respectively for lactose and ethanol, between the predictions obtained by the present hybrid models and those calculated replacing HNM2 and HNM3 with eqs. 4. It can be observed that the assumption of constant yield factors, although widely exploited to describe the behavior of biochemical reactors, does not allow providing, in the present case, an accurate description of both lactose and ethanol concentration. This result can be interpreted observing that, actually, for any given organism in any given medium there is no *a priori* certainty that yield factors keep constant during fermentation progress. It was, in fact, observed that variations of yield factor could be due to several phenomena, e.g. the assimilation into cell mass, the provision of energy for cell synthesis, and the provision of energy for maintenance.[26]

The possibility of exploiting eq. 4, therefore, has to be preliminarily verified depending on the characteristics of the particular reaction that is to be performed. In the present case, it was proved that the constant yield factors model, even if based on the same experimental data exploited to develop the hybrid neural models, was not applicable to predict the true time evolutions of lactose and ethanol concentrations on the basis of the sole knowledge of the amount of biomass formed during the fermentation progress.

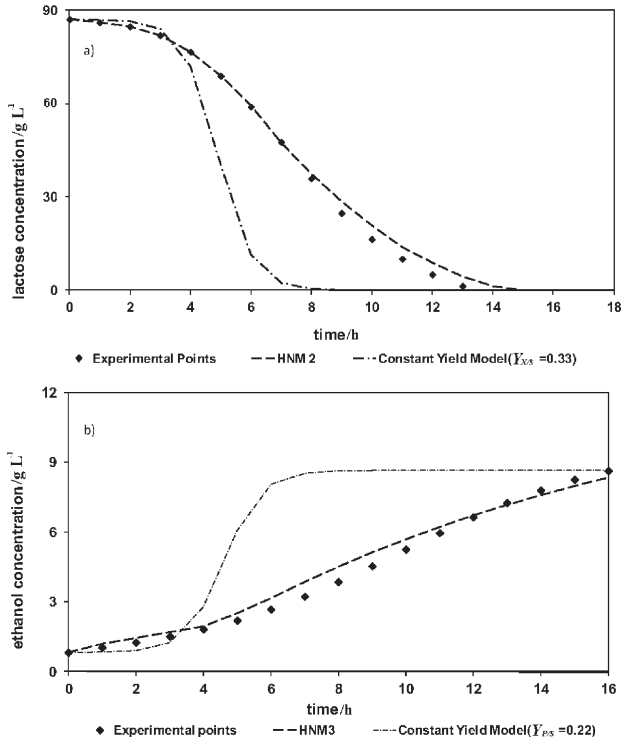


Fig. 6 – *Yield factors models: a) comparison with HNM2 results; b) comparison with HNM3 results*

Conclusions

In the present work, a comparison among different modeling approaches aimed at predicting the behavior of RCW batch fermentation process was presented. The hybrid modeling approach showed better forecasting capability than the pure neural model proving that the proper coupling of the un-steady state mass-balance equations and of a neural model led to the definition of a very effective and versatile tool for the simulation of the process under study. The proposed hybrid approach could represent the basis for the development of very robust and reliable models that might allow implementing either advanced control systems or novel optimization strategies particularly useful in biotechnological field.

List of symbols

$Y_{p/s}$ – yield factor of ethanol toward lactose, –

$Y_{X/s}$ – yield factor of biomass toward lactose, –

t_0 – initial time of biomass exponential growth rate, h

t_F – final time of biomass exponential growth rate, h

i – generic network layer, –

n_i – number of neurons in the i^{th} layer, –

P – product concentration, g L^{-1}

q – substrate consumption rate function, h^{-1}

rpm – stirring rate, rpm

S – substrate concentration, g L^{-1}

T – temperature, °C

t – batch time, h^{-1}

X – biomass concentration, g L^{-1}

γ^0_{lat} – lactose initial concentration, g L^{-1}

γ_m – concentration value experimentally measured, g L^{-1}

γ_p – concentration value predicted by the model, g L^{-1}

ε – percentage error, %

μ – biomass specific growth rate, h^{-1}

μ_p – specific ethanol production rate, h^{-1}

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