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ORIGINAL PAPER

Mathematical modeling of *Kluyveromyces marxianus* growth in solid-state fermentation using a packed-bed bioreactor

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Abstract This work investigated the growth of *Kluyveromyces marxianus* NRRL Y-7571 in solid-state fermentation in a medium composed of sugarcane bagasse, molasses, corn steep liquor and soybean meal within a packed-bed bioreactor. Seven experimental runs were carried out to evaluate the effects of flow rate and inlet air temperature on the following microbial rates: cell mass production, total reducing sugar and oxygen consumption, carbon dioxide and ethanol production, metabolic heat and water generation. A mathematical model based on an artificial neural network was developed to predict the above-mentioned microbial rates as a function of the fermentation time, initial total reducing sugar concentration, inlet and outlet air temperatures. The results showed that the microbial rates were temperature dependent for the range 27–50°C. The proposed model efficiently predicted the microbial rates, indicating that the neural network approach could be used to simulate the microbial growth in SSF.

Keywords *Kluyveromyces marxianus* NRRL Y-7571 · Microbial rates · Packed-bed bioreactor · Simulated annealing · Particle swarm optimization

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Introduction

Mathematical models have an important role in optimization of bioreactors used in solid-state fermentation (SSF). Bioreactor models aim to describe the overall performance of the bioreactor and consist of two sub-models: a balance/transport sub-model that describes mass and heat transfer within and between the various phases of the bioreactor and a kinetic sub-model that describes how the growth rate of the microorganism depends on the key local environmental variables [1]. The growth kinetics may be assessed by simple empirical equations or mechanistic models that attempt to describe intraparticle diffusion processes related to growth. The latter approach focuses on how growth can be limited by events that occur at the level of individual particles, instead of evaluating overall bioreactor performance [2].

More robust models of the microbial metabolism could be focused on metabolic pathways and metabolism regulation, which emulate the interactions occurring within the cell, coupling extracellular phenomena (biomass formation rate, substrate uptake and product excretion rates) with intracellular carbon flux and energy distribution by steady-state mass balances [3]. Stoichiometry models have already been used in physiological studies of submerged cultures of *Kluyveromyces marxianus* [4, 5], but there are no reports of the application of such models in SSF due to the heterogeneity of the culture medium that affects the measurements used to generate the data for the model. In addition, the packed-bed SSF is a particularly challenging batch process to model, since microbial strains must adapt to highly variable environmental conditions that involve water and nutrient gradients, oxygen limitation and mainly the high temperatures reached in the medium. It is known that the above-listed variables influence microbial metabolism and affect the process performance.

Nevertheless, developing a mechanistic model of a microorganism grown in SSF is a hard task due to the difficulty of quantifying and separating the cell and the main metabolic compounds of the solid medium because of the bed heterogeneity, which are essential to build the model [6]. Based on these difficulties, most of current bioreactor models have simple empirical kinetic sub-models, such as linear, logistic, exponential or two-phase equations [1, 2]. The greatest disadvantage of simple empirical models to predict the microbial behavior in solid-state fermentation is that they have little ability to explain the real phenomena that are occurring in the process. Consequently, bad predictions will be found when these models are applied in the simulation of the SSF data when the kinetic sub-model is to be coupled with the mass and energy balances.

The great recent advances in computers and data processing techniques encourage the use of artificial neural network algorithms [7]. An artificial neural network (ANN) can be an alternative to modeling the complex phenomena associated with the microbial growth in SSF. Although the ANN too is an empirical model like the simple kinetic equation mentioned above, it is usually very accurate if the training and validation procedures are properly carried out. So far, there are no reports in the literature regarding the use of ANN to simulate the microbial growth in solid-state fermentation.

In this context, the aim of this work was to develop a mathematical model of the growth of the yeast *Kluyveromyces marxianus* NRRL Y-7571 in solid-state fermentation using a packed-bed bioreactor configuration. An artificial neural network (ANN) was built, using the fermentation time, initial total reducing sugar concentration, and inlet and outlet air temperatures as inputs. The calculated responses were the mass of the cell, metabolic heat, carbon dioxide, ethanol and metabolic water productions, besides the total reducing sugar and oxygen consumptions.

Materials and methods

Agroindustrial residues

The medium consisted of 2 kg of sugarcane bagasse supplemented (related to the sugarcane bagasse) with pre-treated sugarcane molasses 15 wt%, corn steep liquor (CSL) 30 wt% and soybean meal 20 wt% [8]. Sugarcane bagasse was obtained from Cotrel Ltda (Erechim, RS, Brazil), CSL from Corn Products Brazil (Mogi Guaçu, SP, Brazil), soybean meal from Olfar S.A. (Erechim, RS, Brazil) and cane molasses from Éster Refinery (Campinas, SP, Brazil). The compositions of the different media are presented in Table 1.

Table 1 Characterization of the substrates employed in the fermentation medium formulation

Wt%	Sugarcane bagasse	Soybean bran	CSL	Molasses
Moisture	3.0	3.0	55.0	39.0
Protein	ND	42.5	18.8	2.2
Lipids	ND	8.5	1.0	0.7
Carbohydrate	40.0	30.0	7.5	52.2
Fiber	ND	10.0	13.0	ND
Ash	2.4	6.0	4.5	8.2

ND not determined

The cane molasses was pre-treated following the method described by Sguarezi et al. [9]. The pH of the solution of sugarcane molasses (200 g/l) was adjusted with sulfuric acid 0.5 M to 5.0. The solution was set to rest at 24°C for 24 h. The medium was then centrifuged at 5,000×g for 15 min, and the final pH was adjusted with NaOH 1.0 M until 4.0.

Microorganism and medium

The strain of *Kluyveromyces marxianus* NRRL Y-7571 obtained from NRRL (Northern Regional Research Laboratory, now the National Center for Agricultural Utilization Research, Peoria, IL) was maintained on YM agar medium (g/L): yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0 and sub-cultured every 3 weeks. Cell production for the pre-inoculum was carried out by inoculating 10 ml of liquid YM medium in a 50-ml test tube with a loopful of stock culture and incubating it at 30°C for 24 h.

The inoculum medium contained (g/l): sucrose 20.0, yeast extract 5.0, K_2HPO_4 5.0, NH_4Cl 1.5, KCl 1.15 and $MgSO_4.7H_2O$ 0.65 at an initial pH of 6.8. Each test tube with YM medium was transferred to a 500-ml Erlenmeyer flask with 100 ml of medium and incubated at 30°C and 150 rpm for 24 h [10, 11].

Solid-state fermentations

The packed-bed bioreactor consisted of a cylindrical stainless steel vessel (34 cm diameter and 50 cm height) connected to an air humidifier, which supplied air at relative humidity of 95–99%. The saturated air at the operation temperature entered in the bottom of the bioreactor and passed through the bed, reaching the exit located at the top.

The moisture content of the bagasse was corrected to 65 wt%, and it was then autoclaved at 121°C for 20 min. In a previous study we showed that the optimum initial moisture content of the substrates for inulinase production

by SSF was in the range of 60 to 75% [11]. Based on this information, the initial moisture content of the medium was then maintained at 65%. Since the sterilization process increased the moisture by approximately 3%, the moisture content of the medium was still within the optimum range. The fermentation runs were started with the inoculation of the supplemented bagasse with a volume of inoculum corresponding to a cell mass of 14 g. The supplemented solid substrate and the inoculum volume were maintained under brand agitation for 10 min in a helical homogenizer (Marconi, Brazil). The bioreactor was filled with 2 kg of sugarcane bagasse (dry basis), supplemented as described above, which corresponded to a bed height of 40 cm.

The dynamic behavior of the process was evaluated at 0, 2, 4, 8, 12, 18 and 24 h. A new fermentation was started each time, and the whole content of the bioreactor was sampled due to the impossibility of homogenization of the solids. The variation between the tests was negligible. A total of seven experimental runs were carried out at different inlet air temperatures and flow rates, as presented in Table 2.

Experimental and calculated data

The total reducing sugars (TRS) were extracted from the fermented medium by adding 100 ml of bi-distillated water in 20 g of moist fermented medium, following incubation at 40°C and 150 rpm for 60 min [11]. TRSs were quantified by the 3,5-dinitrosalicylic acid method [12]. The sugar concentration was evaluated at four different bed heights, namely 0–10, 10–20, 20–30 and 30–40 cm.

The inlet and outlet air temperatures were continuously monitored by a temperature probe (PT100, NOVUS, Brazil). In addition, the temperature of the moist substrate was monitored inside the bioreactor at 10, 20 and 30 cm from the bottom. The respiratory metabolism of the microorganism was evaluated by determining CO_2 production, assuming that all the CO_2 produced was due to respiration. The CO_2 was analyzed at the outlet air from the bioreactor by a CARBOCAP GMT220 sensor (VAISALA, Inc., Finland). The temperature probes and CO_2 sensor were connected to a data acquisition board (FIELDLOGGER NOVUS, Brazil).

Table 2 Experimental conditions investigated in this work

Run	Total reducing sugar concentration (g of TRS per kg of dry bagasse)	Volumetric air flow rate (m^3/h)	Inlet air temperature (°C)
1	42.9	2.0	27.0
2	43.1	2.0	30.0
3	39.3	2.0	33.0
4	37.4	3.0	27.0
5	44.6	3.0	30.0
6	36.0	3.0	33.0
7	42.5	2.4	30.0

The general stoichiometry for aerobic microbial growth with ethanol formation considered in this work was:

$$\alpha C_6H_{12}O_6 + \beta NH_4 + \lambda O_2 \rightarrow CH_{1,94}O_{0,76}N_{0,17} + \gamma CO_2 + \delta H_2O + \sigma C_2H_6O \quad (1)$$

where α, β, λ, γ, δ and σ are the stoichiometry coefficients (C-mol compound per C-mol biomass), which were computed by the C, H, O and N balances and the experimental measurements of CO_2 and TRS. Applying the elemental balances over Eq. (1), an uncertain algebraic system is obtained, since there are four elements (C, H, O and N) and six unknown coefficients. As there are two variables experimentally measured (TRS and CO_2), the remaining coefficients in Eq. (1) can be calculated on a carbon-mol basis (C-mol).

The elemental dry cell composition ($CH_{1.94}O_{0.76}N_{0.17}$) was obtained from Silva-Santisteban et al. [4]. The corn steep liquor (CSL) used as nitrogen source in this study was a complex mixture of free amino acids, peptides, proteins, inorganic salts, organic acids and many other compounds. A detailed stoichiometry model for the process would be very complicated, and in practice one would lump all the components of the CSL into one nitrogen-containing substrate as ammonia [13].

The above stoichiometry is valid considering that the oxygen is not limited inside the bioreactor [14] and that the yeast metabolism is essentially aerobic [5]. The production of ethanol was taken into account due to the high initial concentration of TRS in the medium, which could cause a deviation in the normal metabolic pathway to ethanol production. Based on the stoichiometry, the oxygen concentration in the outlet air stream, the global metabolic water and ethanol production in the moist solid medium were calculated considering the CO_2 measurement in the outlet air stream. It is important to emphasize that these compounds could be calculated by the TRS data, assuming that the TRS concentration is basically composed of glucose or fructose. However, the experimental error associated with the determination of the TRS concentration in the medium ($\pm 10\%$) is higher than the CO_2 measurement ($<1\%$).

The microbial growth expressed in terms of mass of cells was calculated considering the results of the oxygen uptake rate, according to the following equation [15]:

$$X_n = \frac{Y_{X/O} \cdot \Delta t \cdot \left[\frac{1}{2}\left(\frac{dO_2}{dt}_{t=0} + \frac{dO_2}{dt}_{t=n}\right) + \sum_{i=1}^{i=n-1} \frac{dO_2}{dt}_{t=i}\right] + \left(1 - \frac{a}{2}\right) \cdot X_0 - a \cdot \sum_{i=1}^{i=n-1} X_i}{1 + (a/2)} \quad (2a)$$

where

$$a = m \cdot Y_{X/O} \cdot \Delta t. \quad (2b)$$

The procedure to estimate the biomass content in a certain time (X_n) requires the knowledge of the biomass yield based on the oxygen consumption ($Y_{X/O}$) and the maintenance coefficient (m). The maintenance coefficient was set at 0.0031 gO_2/(gcell h) [15]. The oxygen in biomass ($Y_{X/O}$) yield coefficient was computed as:

$$Y_{X/O} = \frac{1}{\lambda} \quad (3)$$

The metabolic heat (Q_{gen}, kJ/h) was calculated by a suitable energy balance in the bioreactor, assuming negligible loss of heat through the bioreactor walls:

$$Q_{gen} = F \cdot \rho_{air} \cdot \left(Cp_{air} + \phi_{air,in} \cdot Cp_v\right) \cdot T_{out} - F \cdot \rho_{air} \cdot \left(Cp_{air} + \phi_{air,out} \cdot Cp_v\right) \cdot T_{in} \quad (4)$$

where F is the volumetric air flow rate (m^3/h), ρ_{air} is the density of dry air (1.2 kg/m), Cp_{air} and Cp_v are the heat capacity of the dry air and the water vapor, respectively [1,009 and 1,880 J/(kg °C), respectively], T_{in} and T_{out} are the temperature measured at the inlet and outlet air streams, respectively, $\phi_{air,in}$ and $\phi_{air,out}$ are the relative humidity of the inlet and outlet air streams, respectively. The humidity at the inlet and outlet air stream was constantly measured by a relative humidity probe (RHT-WM NOVUS, Brazil) connected to an acquisition board.

Model formulation

A feed forward neural network developed by the Process Simulation Group of DEA/URI and implemented in FORTRAN 90 language was employed with the following topology:

- Input layer:
  - The input layer was composed of four nodes related to the fermentation time, initial total reducing sugar concentration, inlet and outlet air temperatures.
- Hidden layer:
  - Only one hidden layer was used, and the number of nodes in this layer was determined so that the minimal deviation from experimental rates would be obtained.
- Transfer function:
  - The transfer function employed was the hyperbolic tangent in the output and hidden layer.
- Output layer:
  - The output layer was composed of seven nodes related to the output variables such as the microbial rates as mass of cells, metabolic heat, carbon dioxide, ethanol and metabolic water productions, besides total reducing sugar and oxygen consumptions.

The above inputs were combined to minimize the objective function F, defined according to Eq. 5.

$$F = \sum_{i=1}^{i=NR} \sum_{j=1}^{j=NPE} \left(v_{i,j}^{exp} - v_{i,j}^{calc}\right)^2 \quad (5)$$

where NR is the number of experimental rates (metabolic heat, TRS, biomass, ethanol, CO_2, O_2 and H_2O), NPE is the number of experimental points where the F was calculated, $v_{i,j}^{exp}$ and $v_{i,j}^{calc}$ are the experimental and calculated microbial rates i at point j, respectively. It is important to note that the experimental rates mentioned above are experimentally determined values (CO_2 and TRS), calculated by Eq. 1 (O_2, biomass, metabolic water and ethanol) and calculated by Eq. 4 (metabolic heat). Calculated data were used for training the ANN, assuming that the proposed stoichiometric equation is representative of the microbial phenomena that occur inside the substrate bed. This strategy was used because of the difficulty in experimental determination of the rates for O_2, biomass, metabolic water and ethanol in SSF, since the bed is a completely heterogeneous system.

In the training procedure, the weights and the bias were optimized using two heuristic algorithms: the Simulated Annealing combined with Nelder and Mead [16] and the Particle Swarm Optimization (PSO) [17]. In the Simulated Annealing algorithm, the initial artificial annealing temperature (T_A) and cooling rate (α) were set at 10.0 and 0.98, respectively. In the PSO algorithm, the search interval for weights and the bias were allowed to vary between −5 and 5. In addition, 40 particles were used, and the inertial

weight, the cognition and social parameters were set as 0.7, 1.0, 1.0, respectively [17].

Six experimental runs were used for training the ANN (runs 1–6 of Table 1). One additional experimental condition was used for the validation step (run 7 of Table 1). The number of experimental points used in the training and validation steps was 34,496 and 5,762, respectively. The high number of points used for training and validation is due to the large number of acquisition points of on-line variables, which were measured every 30 s.

Results and discussion

Experimental microbial rates

Figures 1 and 2 present the experimental rates obtained during the growth of the yeast *Kluyveromyces marxianus* NRRL Y-7571 in SSF for the runs 1–3 and 4–7, respectively. The first column of the two figures shows the production rates of CO_2, ethanol, metabolic water and the TRS consumption rate, while the second column presents the production rates of cells and metabolic heat and the consumption rate of oxygen. Similar behavior was verified for the seven experiments. In the first 3 h of fermentation, the yeast metabolism was slow due to the adaptation period to the new environment and the low medium temperature. This strain is characterized by an optimal growth temperature of 36°C, as already reported in the literature [9–11, 18, 19]. The highest microbial rates occurred between 7 and 9 h of fermentation, when the mean moist substrate temperature was around 36°C (average temperature from the four different measurement points), as illustrated in Fig. 3. After 9 h of fermentation the rates decreased, approaching zero. Also, the temperature of the moist substrate was around the inlet air temperature.

The influence of the inlet air temperature and volumetric air flow rate on the metabolic rates can be easily visualized in Figs. 1 and 2. The variation in the inlet air temperature caused little change in the maximum rates values, but influenced the fermentation time at which these maximum rates were observed. The fermentation time was reduced when the inlet air temperature was increased. For example, in run 1 the maximum rates were observed around 10 h, whereas in runs 2 and 3 the maximum rates were verified around 9 and 8 h, respectively. The flow rate showed little influence on the responses, since identical results were obtained with flow rates from 2.0 to 3.0 m^3/h. The same tendency was verified regarding the highest value for the outlet air temperature and mean moist solid temperature in Fig. 3.

The main cause for the decrease in the metabolic rates after 9 h of fermentation is the quick decrease of the TRS concentration in the sugarcane bagasse, which limits the growth. When the TRS concentration reaches critical values, the microbial metabolism is restricted to the maintenance, as can be verified by the low values of TRS consumption and metabolic heat generation after 15 h of fermentation.

The direct quantification of the growth in SSF is a hard task, and most studies presented in the literature used an indirect approach to quantify the cell growth. In a previous work, we used the glucosamine content to correlate the microorganism growth [11]. However, this method is usually not very accurate. In the present work, we adopted the procedure described by Brand et al. [15], where the biomass is calculated based on the oxygen consumption data. The specific growth rate for biomass production obtained in this study was lower than the rate obtained in previous works with the same strain in submerged fermentation [18, 20]. However, a direct comparison is difficult due to the different characteristics of each process. The low growth rate obtained in SSF has two opposite aspects. Little cell production decreases the product yield since most of the bioproducts are growth-associated or partially associated with growth. On the other hand, the low growth rate is interesting when associated with an elevated metabolic heat yield coefficient, since this association could reduce the temperature increase in the bioreactors to levels where no inhibitions in the growth occur. Thus, the process may become technically viable.

Mathematical modeling

It is well established that the ANN is acceptable as a modeling technique in scientific and industrial applications because of the good ability to represent multivariable systems, particularly for highly non-linear dynamic systems (such as SSF) without much knowledge of the process under consideration. However, this tool may lead to poor predictions because of conflicts with fundamental constraints represented by the conservation principles, especially outside of the training domain. Besides, an expressive amount of good quality data is required for training the neural network to obtain a good performance of prediction. This large amount of data is normally difficult to obtain in practice. In addition, the application of an artificial neural network (ANN) to model a problem usually requires five steps (network topology, transfer function, initial weight assignment, training and validation), and in each of them the researcher has to select the proper network parameters. The selection made at each step is based on experience, available guidelines and applications [19].

In this work, the input layer was composed of fermentation time, total reducing sugar concentration, and inlet and outlet air temperatures. The inlet air temperature was

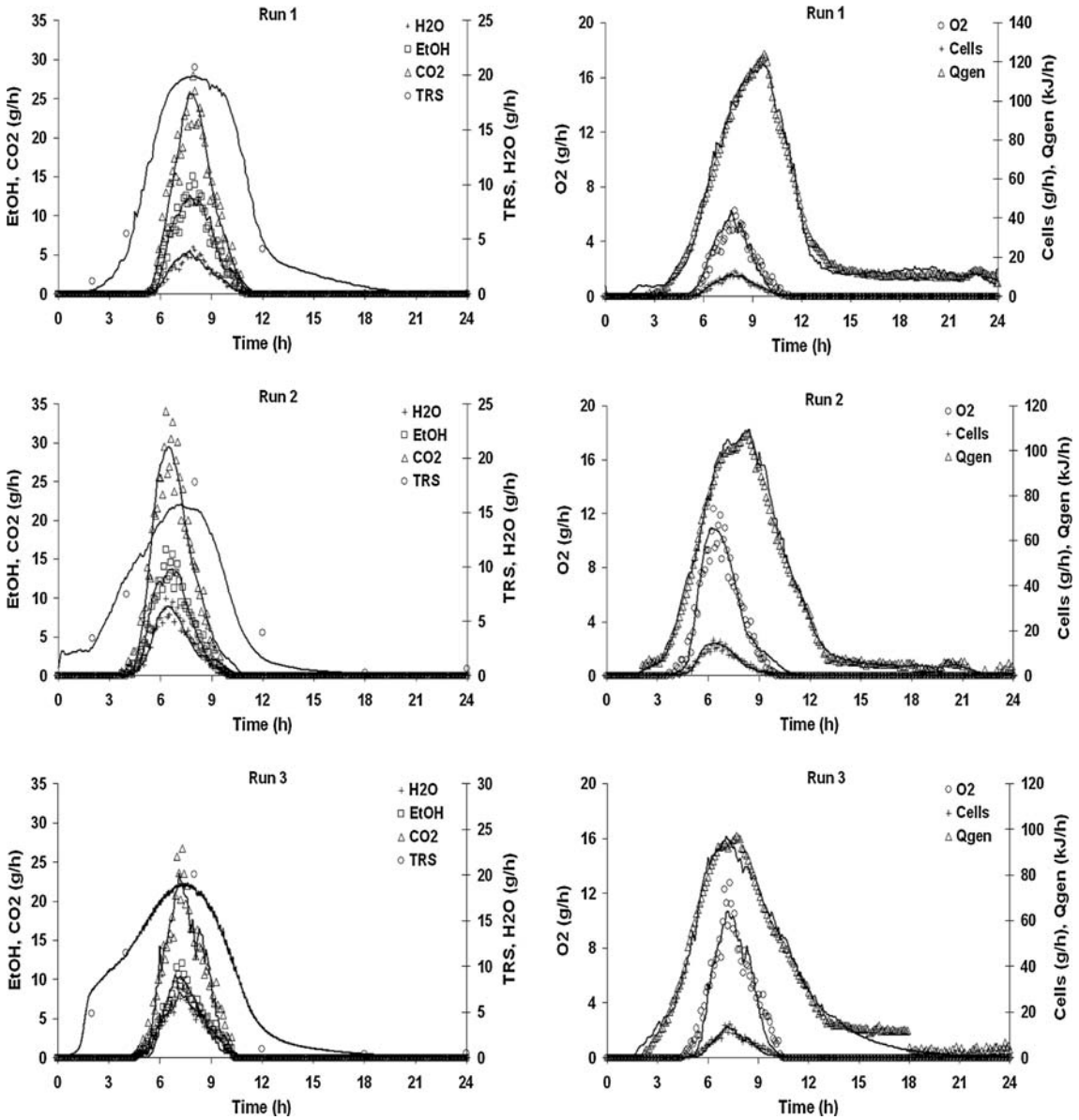

Fig. 1 Microbial rates for the runs 1–3. First column: Experimental or calculated (*symbols*) and predicted (*solid lines*) rates of the TRS, CO_2 ethanol and metabolic water; second column: experimental or calculated (*symbols*) and predicted by ANN (*solid lines*) rates of the metabolic heat, O_2 and microbial growth. The rates for O_2, ethanol, metabolic water and microbial growth were obtained by Eq. 1; the rate for metabolic heat was obtained by Eq. 4; the rates for CO_2 and TRS were experimentally measured

chosen as an input to the ANN because it influences the microbial rates and the outlet air temperature because it is a consequence of the microbial growth. In addition, these variables are easily measured. The fermentation time was chosen because the outlet air temperature curve had a Gaussian shape. In other words, at earlier fermentation times (0–9 h), the increase in the temperature had a positive effect on the rates and, from the middle to the final fermentation, the same temperature showed a negative effect. This is better visualized in Figs. 1 and 2, which present the experimental rates obtained during the growth of *Kluyveromyces marxianus* NRRL Y-7571 in SSF. The rates are expressed in terms of mass of compound per hour of fermentation. Variations in the TRS concentration among the experiments were detected due to the complex composition of the different substrates used in medium formulation. Since these variations in TRS can influence the microbial rates, the TRS concentration was included as an input to the ANN. The concentrations were expressed as grams of TRS per kg of dry sugar cane bagasse.

The interpolation ability of the ANN was well explored based on the fermentation time and the outlet air temperature due to the wide range of these variables. The temperature range will not be extrapolated in real

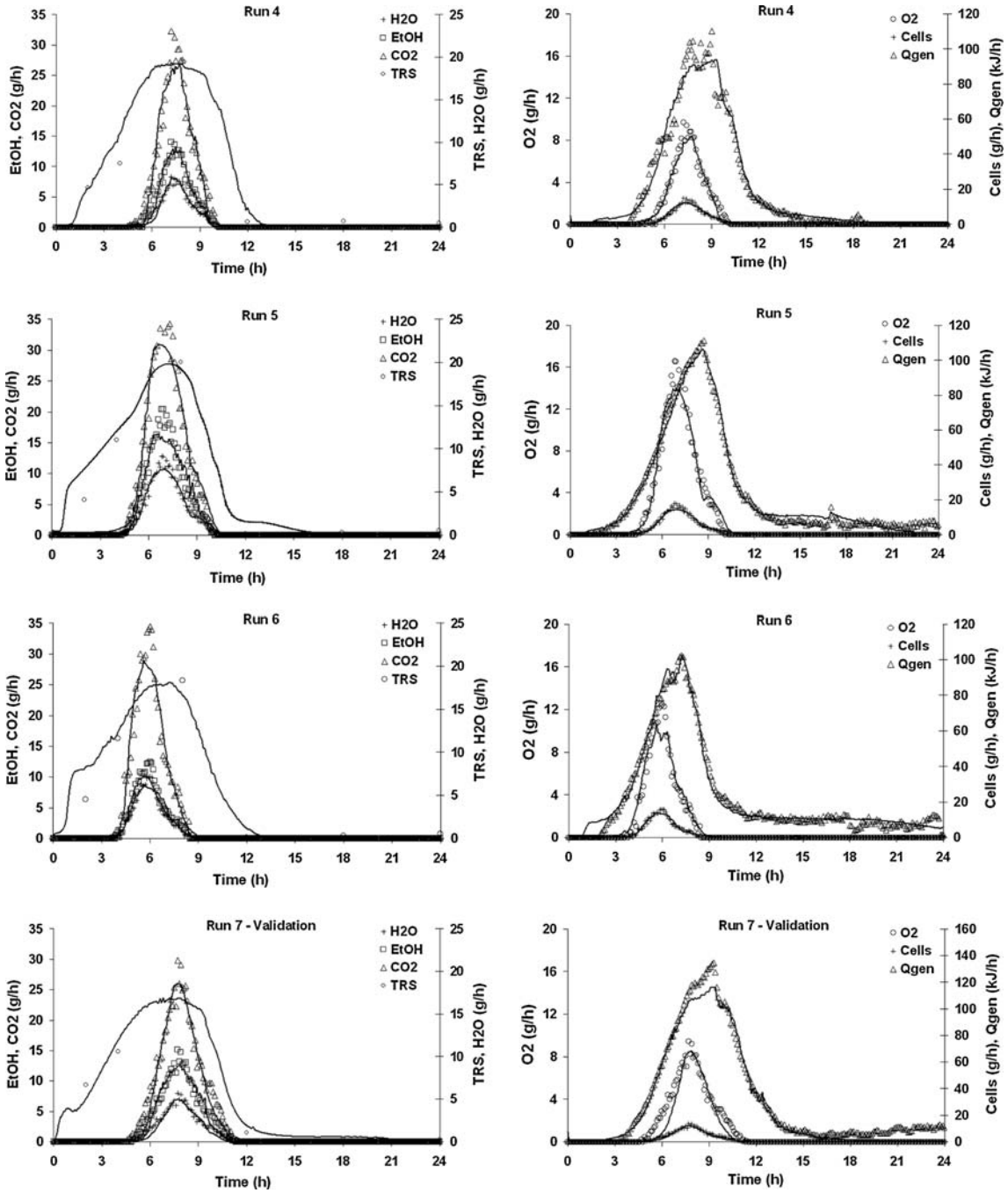

Fig. 2 Microbial rates for the runs 4–7. First column: Experimental or calculated (*symbols*) and predicted by ANN (*solid lines*) rates of the TRS, CO_2 ethanol and metabolic water; second column: experimental or calculated (*symbols*) and predicted (*solid lines*) rates of the metabolic heat, O_2 and microbial growth. The rates for O_2, ethanol, metabolic water and microbial growth were obtained by Eq. 1; the rate for metabolic heat was obtained by Eq. 4; the rates for CO_2 and TRS were experimentally measured

fermentations for two reasons. Firstly, the optimal temperature for *Kluyveromyces marxianus* growth is 36°C, and temperatures above 50°C are deleterious to the microorganism. Secondly, the microbial rates at temperatures lower than 20°C are generally slow and are not common practice. Besides, the fermentation times above 24 h are problematic in SSF, since drying of the solid medium considerably affects the process performance. Thus, any improvement in the process should be carried out within the range used to train the ANN. In addition, the amount of experimental points used to train the ANN was sufficiently large to guarantee a good modeling performance.

The output layer was composed of the main microbial rates as the consumption of total reducing sugar and

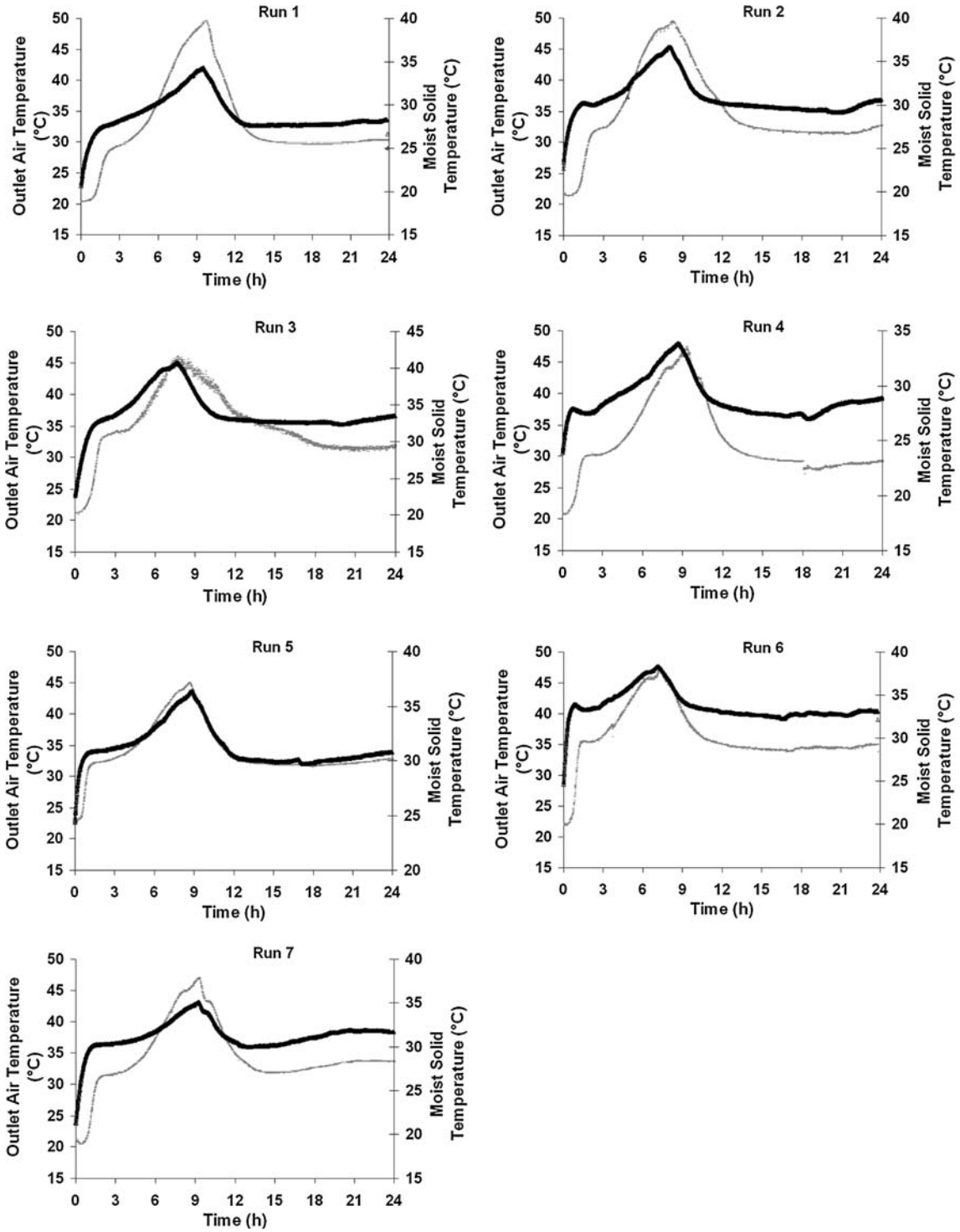

Fig. 3 Experimental outlet air temperature (*dark symbols*) and mean moist substrate temperature (*light symbols*), considered as a mean value among the measurements at 10, 20 and 30 cm inside the bed

oxygen, and production of carbon dioxide, biomass, ethanol, metabolic water and heat. One important aspect when using an artificial neural network as an alternative to modeling is the choice of the transfer function, since this affects the velocity and the performance during the training and application of the ANN. The hyperbolic-tangent transfer function increased the network learning rate and performance (data not shown), and was selected for this reason as the transfer function for the ANN used in this study. The definition of the number of hidden neurons and

the optimization algorithm will be discussed in more details in the next section.

Topology and optimization algorithm to train the ANN

The most common method to train the ANN is the back-propagation algorithm. However, we have tested two heuristic methods to estimate the weights and bias of the ANN, and their performance was compared to the several investigated topologies. In the first case, the Simulated Annealing (SA) combined to the simplex algorithm was used. The role of SA in the overall approach is to allow wrong-way movements, simultaneously providing asymptotic convergence to the global optimum. The role of the non-linear simplex is to generate system configurations. In the second case, the optimization routine was the Particle Swarm Optimization (PSO). The PSO presents interesting characteristics along the interactions. In the initial interactions, the random character of the search is high, and the particles lead to a global search over the search region. As the interactions evolve, the particles concentrate around the more promising regions found during the exploration step. This local search in the values for the weights and bias leads to improvement of the problem solution. Besides, the PSO algorithm is not very sensitive to initial guesses of model parameters, which makes its use appealing when large numbers of unknown parameters are present in the model (as is the case of the ANN) [17].

The influence of 5–20 hidden neurons on the value of the objective function (F) was investigated for the two optimization algorithms. Figure 4 presents the reduction of the SSR value in all the topologies investigated for the SA and PSO algorithms. The best topology was the 4-15-7 (4 inputs-15 hidden neurons-7 outputs), which leads to the lowest values of the F independently of the algorithm used. The application of too few hidden neurons limits the ability of the neural network to model the process, and the use of too many may result in learning the noise present in the database used in training.

The SA algorithm showed better results than the PSO in terms of the lowest F value, whereas the PSO was more efficient, since the number of evaluations of the objective function (Eq. 5) was smaller in all the situations. The best results obtained by the SA algorithm are due to the fact that the search region for the weights and bias is larger than that supplied to the PSO algorithm. If the search region is expanded in the PSO algorithm, the chance for concentration of some particles around false promising regions increases, resulting in a low precision of the estimated weights and bias. In this study, the search region supplied to the PSO algorithm was the same for all the weights and bias. Better results would be obtained if a different interval for each parameter was implemented. However, this is difficult in practice due to the large number of parameters.

The number of evaluations of the objective function is dependent on the selection of the parameters of each algorithm. For the SA, the artificial annealing temperature and the cooling rate determine the number of evaluations. The larger the value of the annealing temperature and the lower the cooling rate are, the more evaluations are required to optimize the parameters. Considering that the objective of this work was the development of a reliable model to predict the main microbial rates of *K. marxianus* grown in SSF, it was preferable to spend larger amounts of time to train the ANN to the detriment of computationally efficient software. Based on these results, the SA algorithm with the 4-15-7 topology was chosen.

Model simulations

The applicability of the 4-15-7 ANN to simulate the experimental microbial rates is presented in Figs. 1 and 2 for the data used during the training (runs 1–6) and for the validation step (run 7). The ANN showed good performance in predicting the experimental profiles of the microbial rates through the time domain for all seven runs. Some deviations were verified in the prediction of the maximum rate of production of CO_2 and cells and of O_2 consumption, but these deviations do not put the reliability of the ANN model at risk. The deviations between experimental values and model predictions of the TRS rate are within the experimental error associated with its measurement (±10%). It is important to note the good performance of the prediction of the rate of metabolic heat production. In practice, the rate of metabolic heat production is the most important variable in the scale-up of the bioreactors

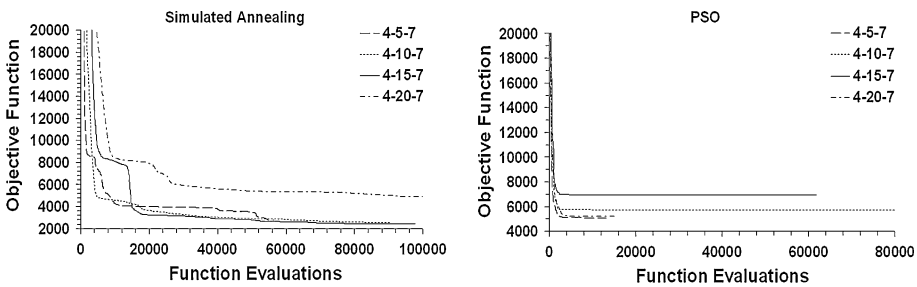

Fig. 4 Training performance of the simulated annealing and PSO algorithms

for SSF, since the greatest limitation in the industrial scale is the removal of metabolic heat. A reliable model to predict this rate is essential to simulate the global performance of the industrial bioreactor.

The success of the application of ANN to predict the main rates associated with the growth of the yeast *K. marxianus* grown in SSF is related to two aspects. Firstly, a large number of experimental points to train the network was used. Secondly, the temperature range used as input was sufficiently large to guarantee that in real application these limits could not be extrapolated. This makes the ANN a robust tool, since it is known by its good interpolation ability. In summary, the development of a mechanistic model of the microbial growth in SSF is practically impossible in practice, and the results obtained here show the possibility of using the ANN as an important tool in this sense.

Conclusions

This work evaluated the effects of the flow rate and inlet air temperature on the growth of *Kluyveromyces marxianus* NRRL Y-7571 in solid-state fermentation. Slight differences in the metabolic rate profiles were verified among the seven experimental conditions investigated. The highest metabolic rates were obtained when the mean temperature of the moist substrate reached values in the range of 30–38°C, in a fermentation time between 4 and 9 h. At the highest rates, the output air temperature reached 50°C, which is deleterious to microbial growth.

The model developed here to predict the main microbial rates of the yeast *K. marxianus* grown in solid-state fermentation showed good performance during both training and validation steps. In a general way, the approach proposed in this work was capable of correlating the complex metabolic rates involved in the cultivations of microorganisms in SSF. The proposed model structure in this work was shown to be an interesting alternative to substitute the simple empirical microbial model, which could be combined with the balance/transport sub-model to obtain a more robust and reliable tool for dynamic simulations of the SSF processes.

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