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A kinetic and mass transfer model to simulate the growth of baker's yeast in industrial bioreactors

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Abstract

A structured unsegregated cybernetic model, able to simulate the growth of baker's yeast in any possible condition in multistage industrial production has been developed. The model has first been proven in the simulation of the behavior of a laboratory batch bioreactor, describing the evolution with time of the biomass growth rate, the glucose and oxygen consumption as well as the production of ethanol and carbon dioxide. The same model with the same parameters has then been used to simulate both laboratory and industrial size fed-batch bioreactors achieving satisfactory results. The effect of the oxygen mass transfer limitation in fed-batch bioreactors has also been described and discussed. The model developed allows to find and keep the optimal conditions of biomass growth in industrial fed-batch bioreactors.

Keywords: Industrial fed-batch bioreactors; Glucose fermentation; *Saccharomyces cerevisiae*

1. Introduction

The *Saccharomyces cerevisiae* biomass, mainly in the form of baker's yeast, represents the largest bulk production of any single-cell microorganism in the world. Several million tons of fresh baker's yeast cells are produced yearly for human food use [1].

The production of baker's yeast involves the multi-stage propagation of the selected yeast strain on sugar as carbon source. Baker's yeast is usually produced starting from a small quantity of *S. cerevisiae* added to a liquid solution of essential nutrients, at suitable temperature and pH. Once the cell population is grown enough, it is transferred into a larger bioreactor for a new growth stage; four or five stages are usually performed to reach a satisfactory production quantity. Therefore, the bioreactors volume changes from 1–10 dm^3 to 100–150 dm^3.

The smaller bioreactors used for the former stages operate in batch conditions. On the contrary, the larger bioreactors used for the latter stages adopt the fed-batch technique, i.e. the nutrients are fed at a variable rate to the culture broth.

In the production of yeast, it is important to maximize both the yield in comparison with the substrate conversion (g cells/g sugar) and the volumetric productiveness (g cells/l h). The effect of variables, such as pH and temperature, is well known and their optimal set-points can easily be defined. On the contrary, yield and productiveness can largely be affected from the concentration of biomass, sugar, oxygen and ethanol formation, if any. The optimal conditions giving maximum yield and productiveness change along with time together with the biomass growth: consequently, the feed rate of nutrients in the fed-batch bioreactor must be changed too. Therefore, the feeding rate of the molasses is the most critical variable and the problem is to individuate the best feeding rate sequence.

This problem could be solved by developing a structured unsegregated model to describe a growth rate able to provide information about the metabolic routes prevailing at any moment of the cells colony life and about how the growth is influenced from operation conditions.

Such a model, named cybernetic model, has been proposed for the first time by Kompala et al. [2] for the diauxic growth of *Klebsiella oxytoca*. More recently, Di Serio [3], Di Serio et al. [4,5] and Jones and Kompala [6] have extended the use of this model to the description of the growth of *S. cerevisiae* in both batch and continuous bioreactors.

In the present paper, we have modified and improved this model and extended its use to fed-batch bioreactors of both laboratory and industrial size, in the perspective of developing the process optimization.

The improved model has been tested by simulating literature batch and fed-batch growths. At the end of the paper,

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Nomenclature

$e_i/e_{i,\max}$	relative concentration of the key enzyme of the i metabolic pathway (1: glucose fermentation, 2: ethanol respiration, 3: glucose respiration)
F_{in}	liquid feed (dm^3/h)
K_i	saturation constant of the metabolic way i
K_1	saturation constant of sugar fermentation (g/dm^3)
K_2	saturation constant of ethanol oxidation (g/dm^3)
K_3	saturation constant of sugar oxidation (g/dm^3)
k_La	oxygen mass transfer coefficient (h^{-1})
Ox	oxygen concentration in liquid bulk (g/dm^3)
Ox*	oxygen solubility (g/dm^3)
S_1	sugar mass (g)
S_2	ethanol mass (g)
s_1^0	sugar feed concentration (g/dm^3)
V_L	liquid volume (dm^3)
X	yeast mass (g)
Y_1	sugar fermentation yeast yield (g of biomass/g of sugar consumed)
Y_2	ethanol oxidation yeast yield (g of biomass/g of sugar consumed)
Y_3	sugar oxidation yeast yield (g of biomass/g of sugar consumed)
β	constant of the key enzyme degradation (h^{-1})
$\mu_{1,\max}$	maximum specific rate of growth of fermentation (h^{-1})
$\mu_{2,\max}$	maximum specific rate of growth of ethanol oxidation (h^{-1})
$\mu_{3,\max}$	maximum specific rate of growth of sugar oxidation (h^{-1})

we have shown how the proposed model can successfully be used in the simulation of industrial bioreactors.

2. The simulation model

During the aerobic growth of *S. cerevisiae*, sugars and ethanol can be used as carbon and energy sources, whereas nitrogen and other minor nutrient requirements are satisfied by inorganic salts. Sugar can be metabolized via two different energy producing pathways, fermentation (1) or oxidation (2), depending on the sugar concentration in the medium.

Indeed, at a high sugar concentration, oxidation is suppressed and fermentation, only, takes place (Crabtree effect). Oxidation predominates when sugar is below 50–100 mg/dm^3.

Under oxygen starvation conditions, the fermentative metabolic pathway always predominates; at a low sugar concentration, ethanol is produced, too (Pasteur effect).

Ethanol produced during the fermentative metabolic pathway in a batch culture is consumed when glucose is no longer available in the medium after a lag-phase, i.e. *S. cerevisiae* exhibits a diauxic behavior.

Biomass yields on glucose are strongly related to the prevailing metabolic pathway, being maximal only when sugar is oxidized, i.e. when its concentration remains below 50–100 mg/l. For this reason, in fed-batch processes for yeast production, the carbon source feed must strictly be controlled to ensure a biomass yield as close as possible to the theoretical value obtainable.

On the basis of above considerations, it is evident that *S. cerevisiae* has internal regulating mechanisms which direct the micro-organism towards the most convenient metabolic pathway able to optimize the use of available resources.

The kinetic modeling of the growth behavior of *S. cerevisiae* requires a detailed knowledge of the intracellular control mechanisms and the Monod classical model is not enough. As general rule, none of the unstructured models is able to predict complicated dynamics, e.g. the diauxic growth [6].

Kompala et al. [2] have simplified these problems: invoking the cybernetic viewpoint [7], they developed a general modeling framework.

The cybernetic modeling framework is based on the hypothesis that microorganisms optimize the utilization of available substrates to maximize their growth rate at all times.

The values of the single growth rate of the different metabolic pathways are calculated applying a modified Monod equation rate, where every growth rate is proportional to the concentration of a key enzyme (e_i) controlling the single metabolic pathway.

The cybernetic modeling framework replaces the detailed modeling of regulating processes with the cybernetic variables u_i and v_i representing the optimal strategies for the synthesis and activity, respectively, of the key enzyme of the metabolic pathway, i. The value of u_i can be assessed assuming that cell resources will be allocated in such a way to obtain the maximum biomass growth rate. A law of resources allocation can be derived from the economic theory of marginal utility [2]

$$u_i = \frac{r_i}{\sum_j r_j} \tag{1}$$

The variable controlling the inhibition/activation mechanism of e_i (v_i) is determined considering the inhibition effect null when the microorganism grows on the substrate which accelerates the biomass growth rate to the utmost, whereas the inhibition effect progressively increases at a decreasing growth rate [2]. Therefore,

$$v_i = \frac{r_i}{\max_j(r_j)} \tag{2}$$

In the present work, specific growth rates for the different metabolic ways are modeled according to a modified Monod rate equation, where the modification consists in the fact that each growth rate r_i has been assumed proportional to $e_i/e_{i,\max}$, the relative intracellular proper key enzyme concentration

$$r_1 = \mu_{1,\max}\frac{e_1}{e_{1,\max}}\frac{S_1}{K_1V_L + S_1} \quad \text{sugar fermentetion} \tag{3}$$

$$r_2 = \mu_{2,\max}\frac{e_2}{e_{2,\max}}\frac{S_2}{K_2V_L + S_2}\frac{\mathrm{Ox}}{K_{\mathrm{Ox}} + \mathrm{Ox}}$$
ethanol oxidation (4)

$$r_3 = \mu_{3,\max}\frac{e_3}{e_{3,\max}}\frac{S_1}{K_3V_L + S_1}\frac{\mathrm{Ox}}{K_{\mathrm{Ox}} + \mathrm{Ox}}$$
sugar oxidation (5)

This choice introduces an advantage in managing the cybernetic model because the ratios $e_i/e_{i,\max}$ can change in the range 0–1, only. Where S_1 and S_2 represent, respectively, the quantity of sugar and ethanol in the bioreactor, Ox the concentration of dissolved oxygen, V_L the volume of the liquid in the bioreactor, K_i represents the saturation constants for the substrate of each metabolic pathway (i) and K_{Ox} the saturation constant for the dissolved oxygen which in first approximation has been assumed to be independent of any single oxidative metabolic pathway.

With these growth rate equations, the common balance equations for batch ($F_{\mathrm{in}} = 0$) and fed-batch ($F_{\mathrm{in}} \neq 0$) bioreactors can be written as

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \left(\sum_i (r_i v_i)\right) X \quad \text{balance on biomass} \tag{6}$$

$$\frac{\mathrm{d}S_1}{\mathrm{d}t} = F_{\mathrm{in}}s_1^0 - \left(\frac{r_1v_1}{Y_1} + \frac{r_3v_3}{Y_3}\right) X \quad \text{balance on sugar} \tag{7}$$

$$\frac{\mathrm{d}S_2}{\mathrm{d}t} = \left(\phi_1\frac{r_1v_1}{Y_1} - \frac{r_2v_2}{Y_2}\right) X \quad \text{balance on ethanol} \tag{8}$$

$$\frac{\mathrm{d}V_L}{\mathrm{d}t} = F_{\mathrm{in}} \quad \text{balance on liquid volume} \tag{9}$$

$$\frac{\mathrm{d}(e_1/e_{1,\max})}{\mathrm{d}t} = (\mu_{1,\max} + \beta)\left(1 - \varepsilon + \varepsilon u_1\frac{S_1}{K_1V_L + S_1}\right) - \left(\sum_{j=1,3} r_j v_j + \beta\right)\left(\frac{e_1}{e_{1,\max}}\right) \quad \text{balance on fermentation}$$
key enzyme relative concentration (10)

$$\frac{\mathrm{d}(e_2/e_{2,\max})}{\mathrm{d}t} = (\mu_{2,\max} + \beta)\left(1 - \varepsilon + \varepsilon u_2\frac{S_2}{K_2V_L + S_2}\frac{\mathrm{Ox}}{K_{\mathrm{Ox}}+\mathrm{Ox}}\right) - \left(\sum_{j=1,3} r_j v_j + \beta\right)\left(\frac{e_2}{e_{2,\max}}\right) \quad \text{balance on ethanol}$$
oxidation key enzyme relative concentration (11)

$$\frac{\mathrm{d}(e_3/e_{3,\max})}{\mathrm{d}t} = (\mu_{3,\max} + \beta)\left(1 - \varepsilon + \varepsilon u_3\frac{S_1}{K_3V_L + S_1}\frac{\mathrm{Ox}}{K_{\mathrm{Ox}}+\mathrm{Ox}}\right) - \left(\sum_{j=1,3} r_j v_j + \beta\right)\left(\frac{e_3}{e_{3,\max}}\right) \quad \text{balance on sugar}$$
oxidation key enzyme relative concentration (12)

where

$$\varepsilon = \frac{\alpha}{\alpha + \alpha^*}$$

$$\frac{\mathrm{d}\,\mathrm{Ox}}{\mathrm{d}t} = k_L a\left(\mathrm{Ox}^* - \mathrm{Ox}\right) - \left(\phi_2\frac{r_2v_2}{Y_2} + \phi_3\frac{r_3v_3}{Y_3}\right)\frac{X}{V_L}$$
balance on oxygen liquid concentration (13)

$$\mathrm{RQ} = \frac{(\phi_1/46)(r_1v_1/Y_1) + (\phi_2/32)(r_2v_2/Y_2)(2/3) + (\phi_3/32)(r_3v_3/Y_3)}{(\phi_2/32)(r_2v_2/Y_2) + (\phi_3/32)(r_3v_3/Y_3)}$$
respiratory quotient (14)

where X, F_{in}, s_1^0, k_La, Ox^* are, respectively, the biomass quantity in the reactor, the value of the sugar feed stream, the sugar concentration in the feed, the coefficient of gas–liquid mass transfer and the concentration of oxygen at the gas–liquid interface, and β and α are, respectively, the enzyme decay and synthesis rate constant; α^* is a small constitutive synthesis term for all the enzymes and is important in predicting the induction of enzymes which have been repressed for long periods of time [6,8] and Y_i and ϕ_i the yields and stoichiometric coefficients for the different metabolic pathways, respectively. The respiratory quotient (RQ) is the ratio of CO_2 moles produced on the oxygen moles consumed. RQ is high when the fermentative glucose metabolic pathway predominates, around 1 when the oxidative glucose metabolic pathway predominates, and smaller than 1 in the case of ethanol consumption.

The model we suggest is different from that applied by Jones and Kompala [6]: as a matter of fact, we introduced the relative key enzyme concentrations and the oxidation key enzyme synthesis rates depend on the oxygen concentration in the liquid bulk. This last assumption is very important to describe the experimental situations in which the oxygen concentration decreases as consequence of mass transfer limitation (see Eqs. (11)–(13)). Besides, the model has

been simplified, without loosing in accuracy, by neglecting the intracellular carbohydrate storage.

3. Simulation results

3.1. Batch laboratory culture

The described model has been used to simulate the experimental data reported by von Meyenburg [9] in a batch run. The run is the same recently used by Jones and Kompala [6] to test their model.

All the parameters of the model can be found in the literature [6] with the exception of the values of the saturation constant, K_i. These parameters, reported in Table 1, have been used to simulate von Meyenburg data. Both K_1 and K_2 have been obtained by mathematical regression [10] on the von Meyenburg experimental data, K_{Ox} has been assumed to be equal to 1/3 of the critical oxygen concentration for the oxidative metabolic pathway [11], and ϕ_2 which been obtained from other literature source [11].

In Fig. 1a, the evolutions with time of concentration of respectively biomass, glucose and ethanol are reported. In Fig. 1b, the evolution of the respiratory quotient is reported. As it can be seen in both cases agreements are quite satisfactory. In particular, we can see that the cybernetic model well performs in the simulation of the lag-phases and the diauxic growth. Indeed, we can see that, when growth begins after an initial lag-phase, the yeast has a high growth rate mainly with a fermentative metabolic pathway with ethanol production; this is confirmed from the high values of the respiratory quotient. After the whole available glucose is consumed and after a new lag-phase, *S. cerevisiae* starts metabolizing ethanol. All these aspects of yeast growth are well simulated from the model.

To account for the long initial lag phase, low initial values of the relative enzyme concentrations have been used. As a matter of fact, the choice of the initial enzyme concentrations must consider the previous history of the inoculum which strongly affects the initial behavior of the system. However, the enzyme concentrations quickly change during the run and small errors in the estimation of the initial concentrations poorly affect the simulation. Therefore, this estimation can roughly be made with a trial and error procedure. We have proven this fact by submitting to mathematical regression analysis also the initial condition for the enzyme concentrations with the results that, as expected, the initial value of $e_2/e_{2,max}$ has no influence on the simulation, while optimal values of the other two parameters

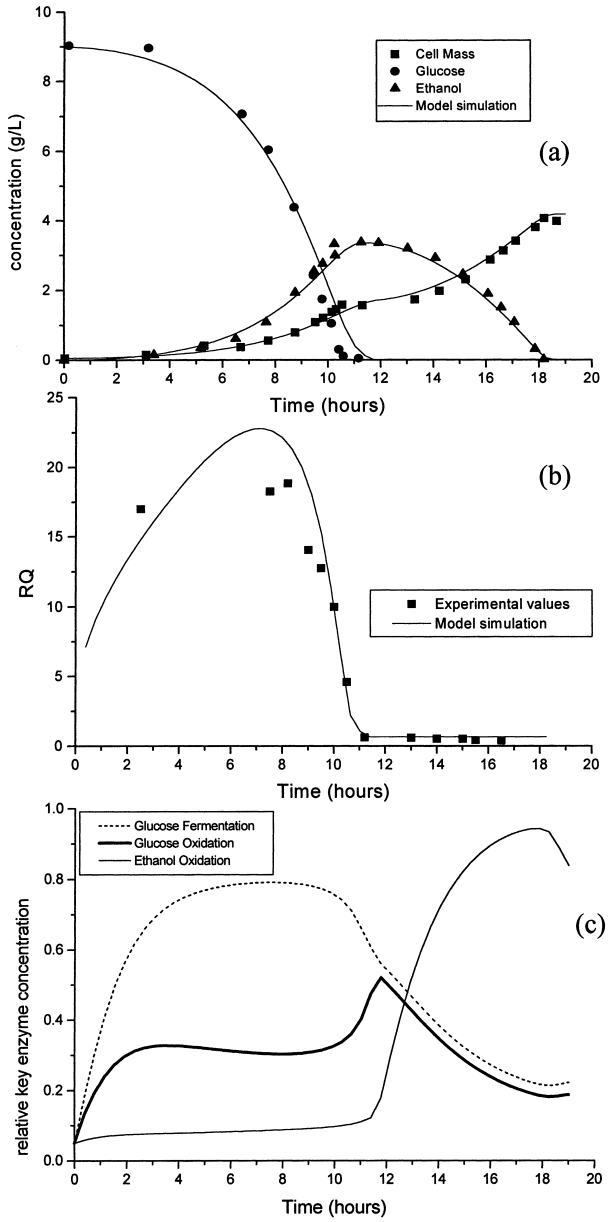

Fig. 1. Simulation of the experimental data collected from von Meyenburg [9] in an aerobic batch culture of *S. cerevisiae* for respectively cell mass, glucose, ethanol concentration (Fig. 1a) and respiratory quotient (Fig. 2a). Fig. 3a shows the trends of the relative key enzyme concentration for the three metabolic pathways of *S. cerevisiae*.

Table 1
Model parameters values used for the simulation results shown in Figs. 1–4

ε	0.909
β (h^{-1})	0.2
$\mu_{1,max}$ (h^{-1})	0.45
$\mu_{2,max}$ (h^{-1})	0.20
$\mu_{3,max}$ (h^{-1})	0.33
Y_1	0.15
Y_2	0.74
Y_3	0.5
K_1 (g/dm^3)	1.0 ± 0.1
K_2 (g/dm^3)	0.08 ± 0.04
K_3 (g/dm^3)	0.001
ϕ_1	0.41
ϕ_2	1.067
ϕ_3	0.52
K_{Ox} (g/dm^3)	4.6×10^{-5}

are in the same range found by trial and error procedure. In Fig. 1c, it is possible to appreciate the evolution with time of the relative concentration of the three key enzymes starting from the initial values assumed as parameters of the models. The behavior observed is qualitatively correct. A more strict correlation between the initial enzyme concentration and the behavior of the microorganism should be acquired by comparing many runs performed in different conditions. Initially, in the presence of a high glucose concentration, the relative key enzyme concentration $e_1/e_{1,\max}$, promoting glucose fermentation is mainly synthesized. After the total glucose consumption during the diauxic lag-phase, the key enzyme, only, promoting ethanol oxidation is synthesized and ethanol consumption starts with a different rate.

3.2. Fed-batch laboratory culture

The same model has been tested on the runs performed by Wank et al. [12] in a fed-batch reactor feeding molasses (a mixture of equal volume of cane and beet molasses containing 30 and 39.5% reducible sugar, respectively) according to a control strategy giving high yield and growth rate.

Two runs have been simulated. In the first simulated one (run A), the impeller speed and the air flow rate were fixed at 1000 rpm and 5 N l/min, respectively. In this run, the bulk liquid oxygen concentration decreases with time approaching to zero because the oxygen mass transfer rate becomes insufficient as a consequence of the increasing biomass concentration. To avoid ethanol formation, molasses feeding was limited, thus obtaining a growth speed lower than the theoretically possible one.

In order to ensure full aerobic conditions throughout the whole second run (run B), both air flow and stirring rate were varied to keep the dissolved oxygen concentration higher than a critical value given by the authors and corresponding to 15% of the saturation.

Although molasses have been used in these two runs instead of glucose, in first approximation, the same parameters as Table 1 have been applied for the simulation.

Considering that the inoculum does not show an initial lag-phase and is provided with a high oxidative activity, the initial relative concentration of the key enzyme $e_3/e_{3,\max}$ should be higher than $e_1/e_{1,\max}$ and $e_2/e_{2,\max}$. As matter of fact, the choice of the initial enzyme concentrations has to take account of the previous history of the inoculum which strongly affects the initial behavior of the system. As the enzyme concentrations quickly change, according to the microorganism growth conditions, small errors in the estimation of the initial values affect the simulation quite poorly. Therefore, this estimation can roughly be made with a trial and error procedure. The values assumed for simulating the laboratory fed-batch runs are $e_1/e_{1,\max} = 0.2$, $e_2/e_{2,\max} = 0.2$, $e_3/e_{3,\max} = 0.7$. As it has been mentioned, the introduction of a relative concentration in the model facilitates the choice of the initial conditions on the basis of the run behavior, for both batch and fed-batch bioreactors.

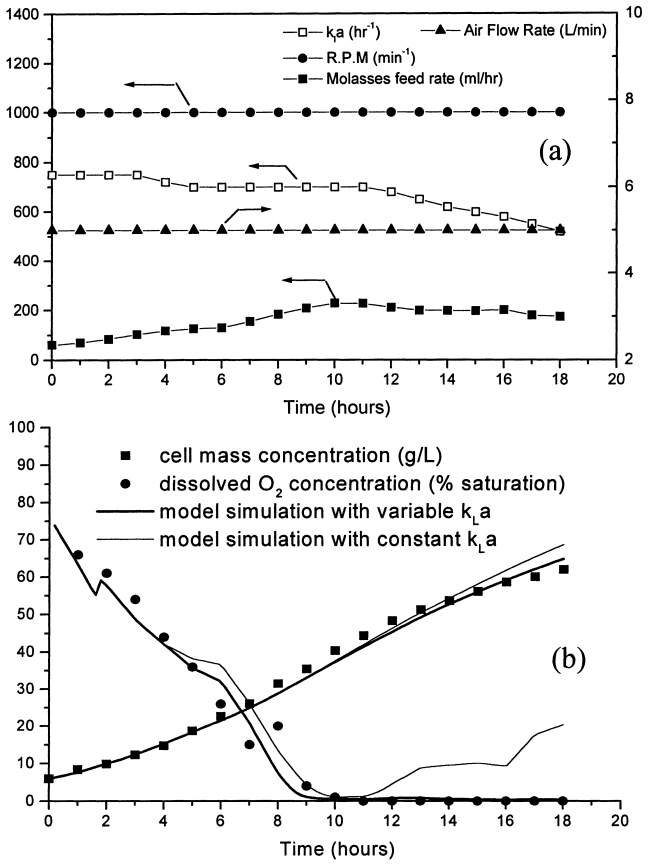

Fig. 2. (a) Experimental data of the air flow and stirrer speed rate as well as the molasses addition rate of run A (Wank et al. [12]) together with the values of $k_L a$ used in the simulation. (b) Model simulation and experimental data for the biomass and oxygen concentration.

Directly measuring the enzyme concentrations being very hard, the model validation can indirectly be achieved by examining a great number of experimental runs in different operative conditions by considering the initial enzyme concentration as model parameters.

In Fig. 2a, the experimental values of the air flow and stirrer speed rates, as well as, the molasses addition rate of run A, together with the values of $k_L a$ used in the simulation, are reported

The experimental data corresponding to the biomass and oxygen concentrations are reported in Fig. 2b. The experimental data are compared with the simulated values calculated by considering respectively $k_L a$ to be constant during the run, or considering it variable with the biomass concentration. $k_L a$ has been estimated according to a trial and error procedure by following the best fitting of the dissolved oxygen concentration. As it can be seen, in order to correctly simulate the gas–liquid mass transfer, a decrease of $k_L a$ must be taken into account at increasing biomass concentration. Besides, it can be seen that the dependence on the biomass concentration of the $k_L a$ is more stressed at high cell concentrations (for a little concentration variation, the value of $k_L a$ has a poor variation). Since high concentration yeast

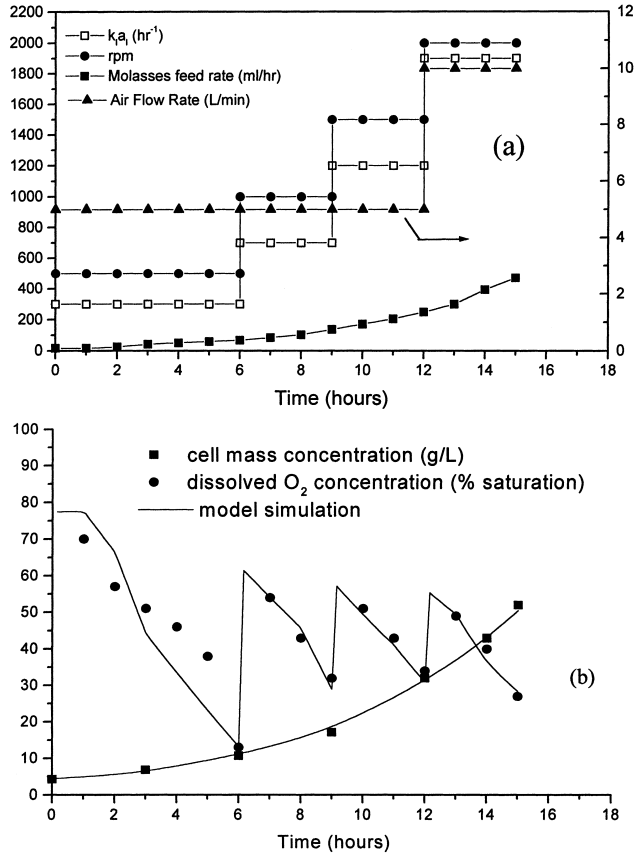

Fig. 3. (a) Experimental data of the air flow and stirrer speed rates as well as the molasses addition rate of run B (Wank et al. [12]) together with the values of k_La used in the simulation. (b) Model simulation and experimental data for the biomass and oxygen concentration.

suspensions give place to non-Newtonian systems, the dependence of k_La on the biomass concentration is not easily predictable.

Fig. 3a shows the experimental values of air flow rates, stirrer speed rates and molasses addition rates employed in run B together with the values of k_La used in the simulation. Fig. 3b presents the dynamic simulation results for the time evolution of the biomass and oxygen concentration related to run B. In this case, the system never falls under conditions in which the oxygen-transfer rate becomes growth limiting and productivity is higher than in the run of Fig. 2b.

From the observation of the obtained satisfactory agreement between the calculated values of biomass growth and the experimental data reported in Fig. 2b and 3b we can conclude that the proposed model is able to simulate fed-batch growth.

3.3. Simulation of industrial fed-batch growth

The above model has then been used to simulate the biomass growth in an industrial reactor. The reactor is a bubble column one. The biomass growth took place in fed-batch conditions and the inoculum derived from a previous batch stage.

The liquid and the gas phase are considered to be completely mixed since owing to the high flow rate of the air, the gas composition variation is small (smaller than 10%) and the calculated number of Peclet low.

The available data are: biomass concentration, ethanol concentration, air flow rate and molasses addition rates at different times.

The industrial run has been simulated still using the parameters reported in Table 1. Considering that the inoculum originates from a batch growth ended before the whole sugar consumption, the initial concentration of the enzymes has been estimated following procedure already described and turned out to be about: $e_1/e_{1,\max} = 0.8$, $e_2/e_{2,\max} = 0.2$, $e_3/e_{3,\max} = 0.2$. By submitting to mathematical regression analysis the initial enzymes concentration values we obtained values that are quite similar to ones used in the simulation. However, as shown before, small errors in the estimation of these initial concentrations poorly affect the simulation.

In order to simulate the industrial reactor, it is necessary to know the mass transfer gas–liquid parameter in the various operative conditions. For bubble columns, relations exist allowing to calculate this parameter with good reliability [13] when knowing the characteristics of the fluid, the dimensions of the column and the gas rate in the column. For yeast suspensions at high concentrations, the problem becomes more difficult because the fluid has a non-Newtonian behavior for which the classic relations mentioned in the literature cannot be used [14]. In our case, since the biomass concentration is always comparatively low (lower than 20 g/l) it has been assumed not to influence the mass transfer parameter (k_La). So, k_La has been calculated using the relation suggested by Akita–Yoshida [13], attributing to the suspension the same characteristics as water.

Fig. 4 shows the values of the percent increase of the molasses range in comparison with the initial feeding as well as

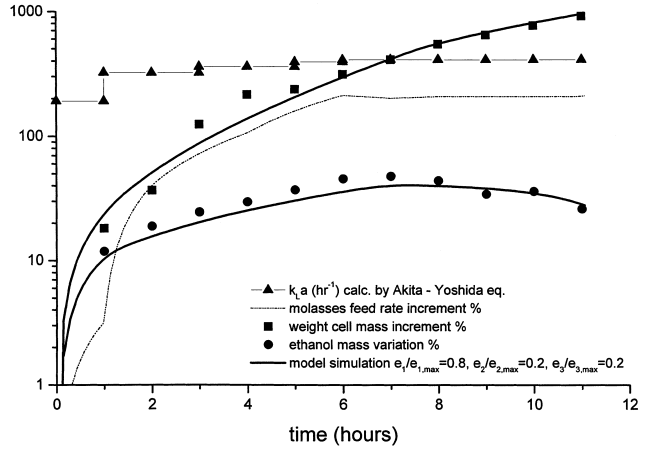

Fig. 4. Percent increase of the molasses range in comparison with the initial feeding rate [15]. Model simulation and experimental data [15] for biomass and ethanol percentage variation. Values of k_La calculated using the equation by Akita and Yoshida [13] and used in the simulations.

the values of $k_L a$ calculated applying the equation by Akita and Yoshida [13] and used in the simulations for the bubble column. The same figure also shows the calculated trends in time of the biomass and of ethanol and compares them with experimental data. From the obtained results, it can be concluded that, though the simulations have been performed using parameters derived from laboratory runs and not optimized for the system considered, the proposed simulation method supplies more than satisfactory outcomes. In Fig. 4, we used the semi-logarithmic scale to show all the available data together.

It must now be underlined that — among the parameters of the model — the initial concentrations of the key enzymes play an important role, too. As a matter of fact, for the simulated industrial run, we have imposed an initial concentration with a high concentration of fermentation key enzymes and a low concentration of oxidation key enzymes. This fact derives from the consideration that the inoculum for the industrial run originates from batch growths where sugar has not been consumed completely in conditions similar to those of Fig. 1c in the time interval from 4 to10 h.

Therefore, it is possible to conclude that in order to optimize the biomass production in a plant reactor, it is necessary to use reasonable values of key enzyme concentrations which, on the other hand, correspond to the concentration obtained at the end of the preceding step.

4. Discussion and conclusions

Cybernetic models are able to simply describe the lag-phase and diauxic growth, as it has been shown by Kompala et al. [2] in a previous work, related to the microorganism *K. Oxytoca*. More recently, the same approach has successfully been extended to the important and more complex system *S. Cerevisiae* [3–6].

In the present paper, we have introduced some modifications to the cybernetic model which are fundamental for the interpretation of fed-batch runs, especially when the oxygen concentration is quite low as a consequence of the mass transfer limitation, this condition being commonly found in the industrial fed-batch bioreactors. Moreover, in the present paper, suggestions are given for scale-up and reactor managing optimisation.

The model behavior has been tested extensively on both laboratory runs reported from the literature [4–6] and performed by us [3] and on industrial ones [15], by considering batch, continuous and fed-batch bioreactors.

Almost all the parameters of the model can be determined independently way and can be found in literature. Only two parameters, K_1 and K_2, have been estimated in this paper by regression.

The initial relative concentration of the key enzymes related to the previous conditions of the yeast can be estimated from the initial rates of the promoted processes and from the evolution with time of these rates.

Obviously, by assuming these initial relative concentrations as assigned parameters of the model gives place to correlations with K_1 and K_2. As the correct evaluation of these concentrations can hardly be made directly, qualitative considerations on the previous history of the microorganism and on the behavior shown help choosing reliable approximated values. A trial and error procedure allows then to improve their reliability. However, as the concentration of the key enzymes quickly changes during the runs, small errors in the initial values poorly affect the simulation.

The uncertainty in the initial key enzymes concentration is a weak point of this model which can be removed by interpreting a great number of runs — as we have done — and, perspectively, by finding a more direct correlation between the observed experimental data and these concentrations.

Moreover, using the cybernetic model, it is possible to scale-up the fed-batch bioreactor, provided that the same strain of yeast and the same type of substrate are used. As a matter of fact, sometimes, when a different strain or different substrate were used, different growth rates [3] were observed having a qualitatively similar but quantitatively different behavior. The model can also be used in these cases, but some of the parameters reported in Table 1 must opportunely be changed. To conclude, the model needs to be further improved for a more general application.

However, starting form batch and fed-batch laboratory data, it is now possible to scale-up and to optimize the management of the industrial bioreactor for a given type of yeast strain and substrate, which was the scope of the present work.

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