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

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Abstract

In this paper a general cybernetic model has been developed to describe the growth of baker's yeast in every type of reactor (batch, fed-batch, continuous). The model, which takes into account also the mass-transfer oxygen limitation, has been tested on literature continuous runs performed at different aerating gas composition. The results obtained show that the model can describe the growth of *S. cerevisiae* also under conditions of inefficient aeration, so it should be useful to optimise and modeling industrial bioreactors. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Baker's yeast; *Saccharomyces cerevisiae*; Bioreactors

1. Introduction

The metabolism of the facultative anaerobe yeast *Saccharomyces cerevisiae* is strongly affected by culture conditions. In aerobic stirred batch cultures at high glucose concentrations, *S. cerevisiae* grows rapidly in a first exponential growth phase. The production of ethanol and the low biomass yield (about 0.15 g dry mass per g glucose consumed) are indicative of predominantly fermentative metabolism. Once glucose drops below a concentration of 50–100 mg/l, a lag in growth occurs as the culture adapts itself to the new environment which is poor in glucose but rich in ethanol. Under these conditions, the respiratory enzymatic system of *S. cerevisiae* is induced/activated and both residual glucose and ethanol are oxidatively metabolized. A second exponential growth phase then starts and biomass yield reaches a maximum value [1]. The sequential utilization of the two carbon sources with an intermediate lag phase between the two exponential phases is an example of the well known diauxic phenomenon.

The *S. cerevisiae* biomass, mainly in the form of baker's yeast, represents the largest bulk production of any single-cell microorganism throughout the world. Several million tons of fresh baker's yeast cells are produced yearly for human food use [2]. The production of baker's yeast involves a multi-stage propagation of the selected yeast strain on sugar as carbon source. The former production stages are usually performed in batch conditions and in not aerated vessels. The latter production stages, on the contrary, are highly aerobic processes occurring in a fed-batch manner in order to maintain the sugar concentration at a low level aiming at maximizing respiratory growth. In this case, it is essential to maintain the feed of sugar under strict control according to the actual metabolic cell conditions, which also depend on the development of the previous stages.

The development of a model of yeast growth, able to predict the metabolic pathway prevailing at any time

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Nomenclature

c	biomass concentration (g/dm^3)
C	yeast mass (g)
$C^{b}_{O_2}$	oxygen concentration in liquid bulk (g/dm^3)
$C^{0}_{O_2}$	oxygen solubility (g/dm^3)
e_i/e_i^{max}	relative concentration of the key enzyme of the i metabolic pathway (1: glucose fermentation, 2: glucose respiration, 3: ethanol respiration)
e_i^0/e_i^{max}	initial relative concentration of the key enzyme of the i metabolic pathway (1: glucose fermentation, 2: glucose respiration, 3: ethanol respiration)
$F_{in}(t)$	liquid feed (dm^3/h)
$F_{out}(t)$	output liquid current (dm^3/h)
J_{O_2}	oxygen transfer rate (g/h)
K_i	saturation nutrient constant of the metabolic way i
K_i^{ox}	saturation oxygen respiration constant of the metabolic way i (g/dm^3)
K_1	saturation sugar fermentation constant (g/dm^3)
K_2	saturation sugar respiration constant (g/dm^3)
K_3	saturation ethanol respiration constant (g/dm^3)
PM_x	molecular weight of x (g/mol)
s_i	ith substrate concentration (g/dm^3) (1: sugar, 2: ethanol)
s_1^F	sugar feed concentration (g/dm^3)
S_1	sugar mass (g)
S_2	ethanol mass (g)
V_l	liquid volume
W_{CO_2}	carbon dioxide produced (g)
Y_1	sugar fermentation yield (g of biomass/g of consumed sugar)
Y_2	sugar respiration yield (g of biomass/g of consumed sugar)
Y_3	ethanol respiration yield (g of biomass/g of consumed ethanol)

Greek letters

β	constant of the key enzyme degradation (h^{-1})
β_l	oxygen mass transfer coefficient (h^{-1})
$\mu_{max,1}$	maximum sugar fermentation specific rate of growth (h^{-1})
$\mu_{max,2}$	maximum sugar respiration specific rate of growth (h^{-1})
$\mu_{max,3}$	maximum ethanor respiration specific rate of growth (h^{-1})

during the process would be of major importance in carrying out the entire production.

In this concern, unstructured models have no such capabilities because the reaction rates are only related to biomass concentration and to the environment. On the contrary, structured models, incorporating any of genetic, morphological, or biochemical attributes which collectively determine the physiological state of the biomass, have a great potential to describe growth phenomena since trends and responses can be recognized which depend on changes in the biomass composition. In this way, the influence of the past history of cultivation on the present physiological state of the biomass is taken into account [1].

Recently Di Serio et al. [3] have developed a kinetic structured model using the cybernetic approach, developed by Ramkrishna and co-workers [5–7], able to simulate the performance of the baker's yeast growth in both batch and fed-batch well mixed reactors.

However, the oxygen mass transfer can have a key role in these bioreactors. It is well known, in fact, that below a threshold oxygen concentration the metabolism of *S. cerevisiae* changes favoring glucose fermentation instead of respiration. In laboratory bioreactors operative conditions are normally chosen to avoid mass transfer limitations, as, e.g., we have done in previous paper to study the kinetics of *S. cerevisiae* growth [3]. However for industrial reactors, in some cases, mass transfer limitation cannot be avoided, so in this paper we have attempted to generalize our previous model to interpret also these situations.

2. Theoretical aspects

2.1. Fundamentals of the cybernetic simulation model

The *S. cerevisiae* has internal regulatory mechanisms which direct the microorganism towards the

most convenient metabolic pathway able to optimise the use of the available resources. As a matter of fact, in *S. cerevisiae*, glucose suppression of respiration in the Crabtree effect is thought to be due to glucose repressing respiratory enzyme synthesis and/or inactivating respiratory enzymes and sugar transport activity. Only when the yeast growth conditions are conductive (i.e., presence of oxygen, absence of repression when the glucose concentration drops in the medium) glucose and successively ethanol are oxidatively metabolized. The oxidation of ethanol occurs after a lag phase and at a lower growth rate. The occurrence of a lag phase depends on the time required for the de novo synthesis of respirating ethanol enzymes [4].

Experimental runs concerning the growth of the *S. cerevisiae* (baker's yeast) performed in our laboratory and by other authors have been described in a previous paper using a cybernetic model simulating the consumption of sugars, ethanol, and oxygen as well as the production of ethanol and carbon dioxide. The model is based on the reasonable assumption that the growth of *S. cerevisiae* can occur following three different metabolic pathways: sugar fermentation, sugar respiration and ethanol respiration. Three specialized sets of enzymes promote the metabolic pathways. The model requires an initial distribution of the three sets of enzymes in the inoculum, the value of biomass yields and the values of the Monod kinetic parameters for a single metabolic pathway growth. The model well describes lag time and diauxic growth on the two carbon sources (sugar and ethanol) as well as the effects of both the composition in the culture medium and the origin of the inoculum.

The employed structured model is based on the cybernetic approach, as developed by Ramkrishna and co-workers [5–7]. The model is able to interpret the response of a microorganism to a multiple substrate because the cybernetic view of the cell assumes the existence of (a) an adaptive machinery responsible for the manufacture of the key proteins and their precursors, (b) a permanent machinery consisting of the metabolic reactions occurring constitutively, and (c) a regulator which is the decision-making apparatus of the cell and decides the policy for allocation of the critical resources depending on the environmental conditions.

According to the cybernetic model [5–7], when a single carbon source is present and a single metabolic pathway is possible, the biomass growth rate depends on both the concentration of the *i*th substrate (s_i) and that of the key enzyme (E_i), the latter representing the whole set of enzymes catalyzing the metabolic pathway of growth on s_i; a relation corresponding to a modification of the Monod equation can then be written:

$$r_i = r_i' = \frac{\mu_{\max,i}(e_i/e_i^{\max})s_i c}{K_i + s_i} \tag{1}$$

Under these conditions, the rate of change of the non-dimensionalized values of the key enzyme formation depends on the substrate concentration, as in the following relation:

$$\frac{\mathrm{d}(e_i/e_i^{\max})}{\mathrm{d}t} = \frac{(\mu_{\max,i} + \beta)s_i}{K_i + s_i} - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_i}{e_i^{\max}} - \beta\frac{e_i}{e_i^{\max}} \tag{2}$$

The value of the rate constant of the key enzyme degradation (β) is $0.05\,\mathrm{h}^{-1}$, as estimated from the mean value of the maximum enzyme concentrations measured and the rate of protein degradation [5].

The relations (2) and (3) have been modified to introduce the dependence of rates of aerobic pathways from oxygen concentration :

$$r_i = r_i' = \frac{\mu_{\max,i}(e_i/e_i^{\max})s_i c}{K_i + s_i}\gamma_i \tag{3}$$

$$\frac{\mathrm{d}(e_i/e_i^{\max})}{\mathrm{d}t} = \frac{(\mu_{\max,i} + \beta)s_i}{K_i + s_i}\gamma_i - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_i}{e_i^{\max}} - \beta\frac{e_i}{e_i^{\max}} \tag{4}$$

where the factor γ takes into account the effect of oxygen concentration:

$$\gamma_i = \frac{C_{\mathrm{O}_2}^{\mathrm{b}}}{K_i^{\mathrm{ox}} + C_{\mathrm{O}_2}^{\mathrm{b}}} \tag{5}$$

When two or more substrates and/or different environmental conditions (e.g., aerobic or anaerobic conditions) are present, regulatory mechanisms of the repression/induction of the enzyme synthesis and the

inhibition/activation of the enzyme activity occur and, consequently, different metabolic pathways become possible. The growth rate relations (3) and (4) must be rewritten consequently:

$$r_i = r'_i v_i \quad (0 \le v_i \le 1) \tag{6}$$

$$\frac{\mathrm{d}(e_i/e_i^{\max})}{\mathrm{d}t} = u_i \frac{(\mu_{\max,i} + \beta)s_i}{K_i + s_i}\gamma_i - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_i}{e_i^{\max}} - \beta\frac{e_i}{e_i^{\max}} \quad \left(0 \le u_i \le 1;\ \sum_i u_i = 1\right) \tag{7}$$

where u_i and v_i are the "cybernetic variables" of the model related to the repression/induction mechanism of the key enzyme (e_i) synthesis and to the inhibition/activation of the existing enzymes, respectively.

The value of u_i can be evaluated on the basis of the assumption that cellular resources will be allocated in such a way to obtain the maximum biomass growth rate. A law of the resources allocation can be derived from the economic theory of the marginal utility [5,7]:

$$u_i = \frac{r'_i}{\sum_j r'_j} \tag{8}$$

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 at the most, whereas the inhibition effect progressively increases at a decreasing growth rate [7]. Therefore:

$$v_i = \frac{r'_i}{\max_j(r'_j)} \tag{9}$$

As previously mentioned, the cybernetic variables u_i and v_i are related to the fractional allocation of the critical resources necessary for biomass growth.

2.2. Generalized cybernetic model to simulate the growth of S. cerevisiae

The previous proposed model [3] has been generalized to the description of the growth of *S. cerevisiae* taking into account the oxygen mass transfer. On the basis of the stoichiometric balance [3] and the rate equations (3) and (4), a mathematical model can be written to simulate *S. cerevisiae* growth in batch and fed-batch and continuous bioreactors.

Defining the biomass growth rate related to glucose fermentation:

$$r'_1 = \frac{\mu_{\max,1}(e_1/e_1^{\max})S_1}{K_1 V_1 + S_1}\frac{C}{V_1}\gamma_1 \tag{10}$$

the biomass growth rate related to glucose respiration:

$$r'_2 = \frac{\mu_{\max,2}(e_2/e_2^{\max})S_1}{K_2 V_1 + S_1}\frac{C}{V_1}\gamma_2 \tag{11}$$

and the biomass growth rate related to ethanol respiration:

$$r'_3 = \frac{\mu_{\max,3}(e_3/e_3^{\max})S_2}{K_3 V_1 + S_2}\frac{C}{V_1}\gamma_3 \tag{12}$$

we have:

Mass balance on glucose:

$$\frac{\mathrm{d}S_1}{\mathrm{d}t} = s_1^{\mathrm{F}} F_{\mathrm{in}}(t) - \left(\frac{r'_1 v_1}{Y_1} + \frac{r'_2 v_2}{Y_2}\right) V_1 - s_1 F_{\mathrm{out}}(t) \tag{13}$$

Mass balance on ethanol:

$$\frac{\mathrm{d}S_2}{\mathrm{d}t} = \left(X_1 r'_1 v_1 - \frac{r'_3 v_3}{Y_3}\right) V_1 - s_2 F_{\mathrm{out}}(t) \tag{14}$$

where

$$X_1 = 2\frac{(1 - 0.905Y_1)}{Y_1}\frac{\mathrm{PM}_{S_2}}{\mathrm{PM}_{S_1}} \tag{15}$$

The coefficient 0.905 has been derived from stoichiometric balance based on chemical formula of dry cell material.

Mass balance on biomass:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = (r'_1 v_1 + r'_2 v_2 + r'_3 v_3) V_1 - c F_{\mathrm{out}}(t) \tag{16}$$

Mass balance on the key enzyme promoting glucose fermentation:

$$\frac{\mathrm{d}(e_1/e_1^{\max})}{\mathrm{d}t} = \frac{(\mu_{\max,1} + \beta)S_1}{K_1 V_1 + S_1}u_1\gamma_1 - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_1}{e_1^{\max}} - \beta\frac{e_1}{e_1^{\max}} \tag{17}$$

Mass balance on the key enzyme promoting glucose

respiration:

$$\frac{\mathrm{d}(e_2/e_2^{\max})}{\mathrm{d}t} = \frac{(\mu_{\max,2}+\beta)S_1}{K_2V_1+S_1}u_2\gamma_2 - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_2}{e_2^{\max}} - \beta\frac{e_2}{e_2^{\max}} \quad (18)$$

Mass balance on the key enzyme promoting ethanol respiration:

$$\frac{\mathrm{d}(e_3/e_3^{\max})}{\mathrm{d}t} = \frac{(\mu_{\max,3}+\beta)S_2}{K_3V_1+S_2}u_3\gamma_3 - \frac{\mathrm{d}(\ln C)}{\mathrm{d}t}\frac{e_3}{e_3^{\max}} - \beta\frac{e_3}{e_3^{\max}} \quad (19)$$

Change of volume in the culture broth for a generic reactor:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = F_{\mathrm{in}}(t) - F_{\mathrm{out}}(t) \quad (20)$$

Carbon dioxide production:

$$\frac{\mathrm{d}W_{\mathrm{CO_2}}}{\mathrm{d}t} = (X_2r_1'v_1 + X_4r_2'v_2 + X_7r_3'v_3)V_1 \quad (21)$$

where

$$X_2 = \frac{2(1-0.905Y_1)}{Y_1}\frac{\mathrm{PM_{CO_2}}}{\mathrm{PM}_{S_1}} \quad (22)$$

$$X_4 = \frac{6(1-0.905Y_2)}{Y_2}\frac{\mathrm{PM_{CO_2}}}{\mathrm{PM}_{S_1}} \quad (23)$$

$$X_7 = \frac{2(1-0.905Y_3)}{Y_3}\frac{\mathrm{PM_{CO_2}}}{\mathrm{PM}_{S_2}} \quad (24)$$

Assuming a valid pseudo stationary condition, the oxygen concentration can be calculated by solving the following non-linear algebraic equation before each integration step:

$$(X_3r_2'v_2 - X_6r_3'v_3)V_1 - \beta_1(C_{\mathrm{O_2}}^0 - C_{\mathrm{O_2}}^{\mathrm{b}}) = 0 \quad (25)$$

$$X_3 = \frac{6(1-0.905Y_2)}{Y_2}\frac{\mathrm{PM_{O_2}}}{\mathrm{PM}_{S_1}} \quad (26)$$

$$X_6 = \frac{3(1-0.905Y_3)}{Y_3}\frac{\mathrm{PM_{O_2}}}{\mathrm{PM}_{S_2}} \quad (27)$$

When baker's yeast growth is simulated to occur in a batch bioreactor, $F_{\mathrm{in}}(t) = F_{\mathrm{out}}(t) = 0$. For a fed-batch reactor we have $F_{\mathrm{in}}(t) \neq 0$ and $F_{\mathrm{out}} = 0$, while for a continuous reactor $F_{\mathrm{in}}(t) = F_{\mathrm{out}} \neq 0$.

3. Results and discussion

The model previously described was used to simulate a biomass production run performed by Oura [8]. The experiment was performed in a continuous reactor fed with a synthetic medium containing a glucose concentration of 50 g/dm^3. The liquid volume was kept constant at 2.7 dm^3 using a fed of 0.27 dm^3/h. Air or gas mixture with variable oxygen content was used as the aerating gas. When the oxygen content of the gas aerating the culture was changed, sample were taken daily from the suspension pumping out to ensure the achievement of steady state. This usually occurred after 3 or 4 days.

The experiment provides all the experimental data regarding glucose consumption, ethanol formation, oxygen consumption and carbon dioxide evolution at each different aerating gas composition.

From the data reported in Fig. 1 it can be seen how the anaerobic, fermentative metabolism weakens as the aerobic oxidative metabolism increases until the oxygen content of gas reaches 25–30%.

The oxidative metabolism of ethanol is inoperative under the selected conditions, because the glucose concentration was always greater than 0, so we can consider $r_3 \cong 0$.

To simulate the experiment by Oura, we need to known the values of mass-transfer coefficient and of the kinetic parameters, reported in Table 1, that are related to yeast strain and growth medium used [3].

Table 1
List of values of the model parameters obtained by mathematical regression on data reported by Oura [8]

Model parameters	
Y_1	0.105
Y_2	0.52
Y_3	0.6
$\mu_{\max,1}$	1.0 h^{-1}
$\mu_{\max,2}$	0.5 h^{-1}
$\mu_{\max,3}$	0.23 h^{-1}
K_1	0.2 g/dm^3
K_2	0.005 g/dm^3
K_3	0.02 g/dm^3
K_1^{ox}	0 g/dm^3
K_2^{ox}	1.0×10^{-6} g/dm^3
K_3^{ox}	1.0×10^{-6} g/dm^3

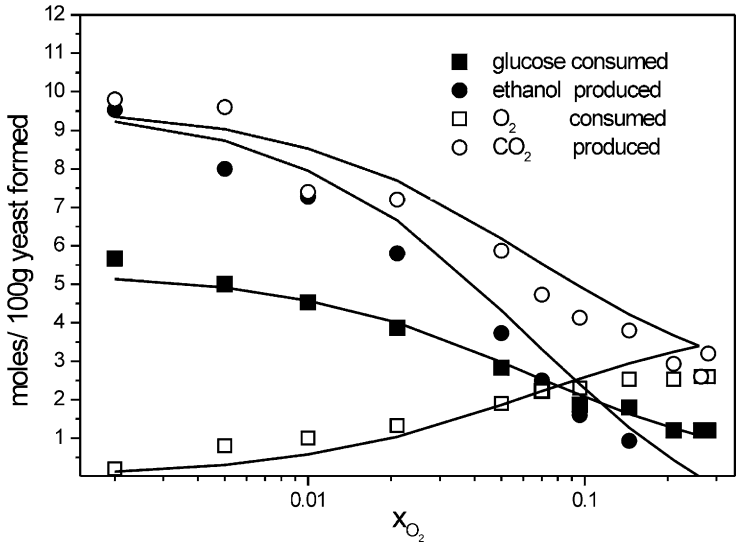

Fig. 1. Effect of the composition of aerating gas on the use of oxygen, on the consumption of glucose and on the production of carbon dioxide and ethanol during continuous culture of baker's yeast. Dots are experimental data reported by Oura [8], lines are values calculated by using the described model and the parameters reported in Table 1.

Oura measured the velocity of oxygen mass transfer by using the sulfite method at different composition of aerating gas (see Fig. 2). These data can be used to calculate the relative mass transfer parameter using the relation:

$$J_{O_2} = \beta_l(C^0_{O_2} - C^b_{O_2}) \tag{28}$$

where $C^0_{O_2}$ is the equilibrium oxygen solubility and $C^b_{O_2}$ is the bulk oxygen concentration that in the case of sulfite solution is always 0. The correspondent

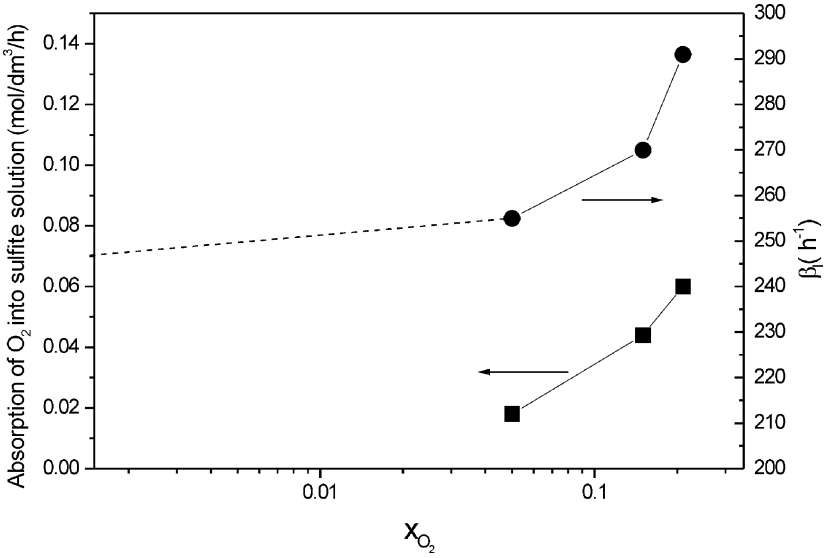

Fig. 2. Data of O_2 adsorption into sulfite solution at different composition of aerating gas reported by Oura [8] and relative calculated β_l.

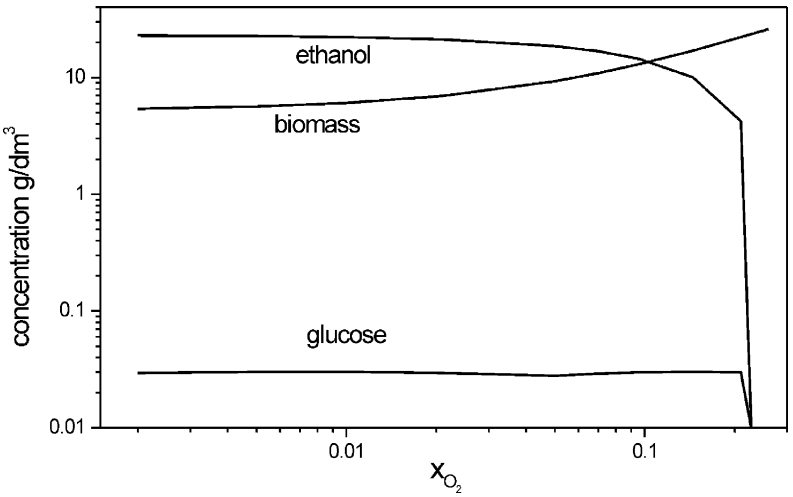

Fig. 3. Ethanol, biomass, and glucose concentration calculated as a function of different aerating gas composition.

calculated β_1 parameters have been reported in Fig. 2, too. The β_1 value correspondent to other compositions of aerating gas mixture, used in the simulation, have been determined by linear interpolation on experimental data.

The value of the constant of enzyme degradation β has been assumed to be the same as reported by Kompala et al. for *K. oxytoca*, this being reasonably independent of the kind of microorganism employed [3,5,7].

The values of parameters reported in Table 1 were obtained by regression analysis of the experimental data reported in Fig. 1 by minimization of the objective function $F = \sum[(\text{experimental} - \text{calculated})/\text{experimental}]^2$. The values of all the parameters obtained as described are reported in Table 1.

The results of regression are independent from initial enzyme concentration because the calculated values was referred to stationary systems, i.e., after

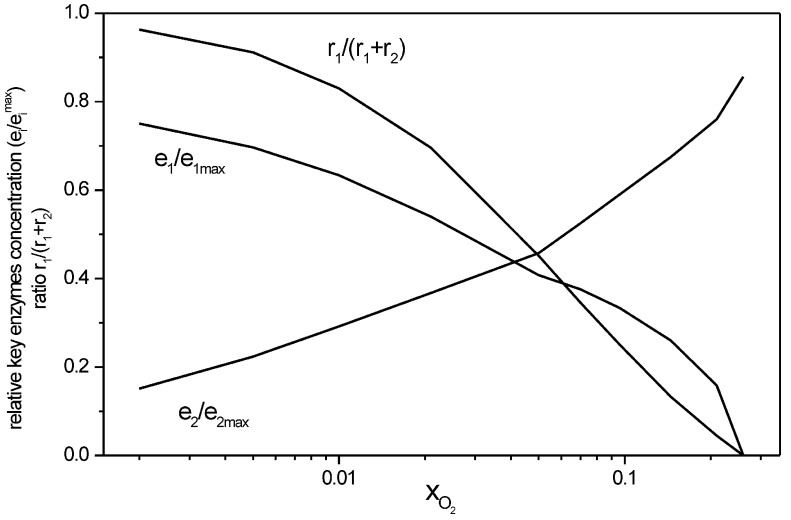

Fig. 4. Dependence of the relative concentrations of the key enzymes of fermentation and respiration and of the ratio $r_1/(r_1 + r_2)$ on the aerating gas composition.

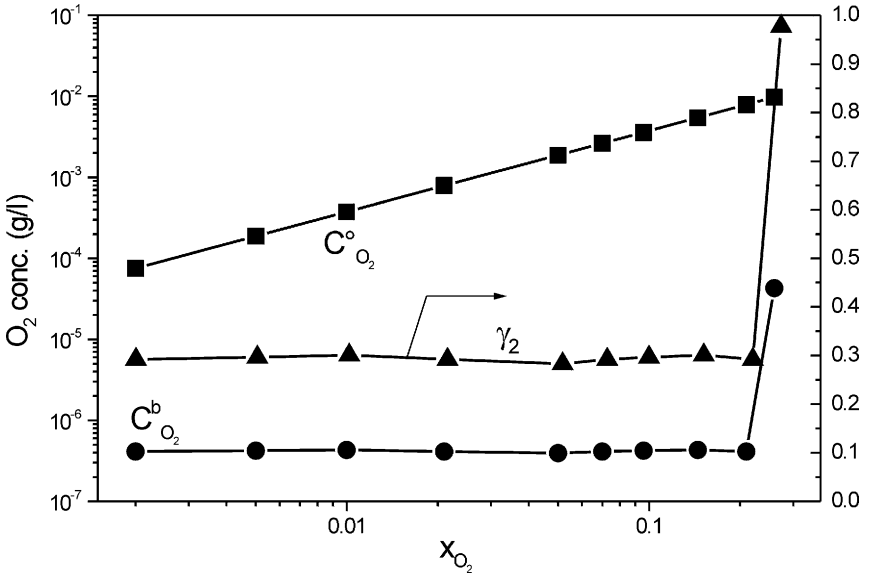

Fig. 5. Equilibrium oxygen concentration, calculated bulk oxygen concentration and relative γ_2 factor as a function of aerating gas composition.

100 h of simulated run time for each aerating gas composition.

In Fig. 1, the agreements between simulation and experimental data as regards glucose consumption, ethanol formation, oxygen consumption and carbon dioxide production as a function of composition of aerating gas have been reported.

It is possible to observe in Fig. 3 that ethanol production decreases by increasing the oxygen concentration in gaseous stream. In the meantime biomass yield increases too. The observed phenomena are the consequence of shift from glucose fermentation to respiration.

It should also pointed out that glucose concentration is always below 50 mg/dm^3, i.e., a condition where the Crabtree effect is normally absent and ethanol should not be produced. The formation of ethanol by fermentative pathway is the consequence of oxygen mass transfer limitation.

This change of the way of glucose consumption can be better evidenced in Fig. 4 where the relative concentration key enzyme of both fermentation and respiration are reported together with the ratio $r_1/(r_1 + r_2)$ as a function of the aerating gas composition.

This behavior can be explained considering the calculated value of bulk oxygen concentration and the relative γ_2 value (Eq. (5)), reported in Fig. 5.

From Figs. 4 and 5 it can be seen that only when the bulk oxygen concentration is greater than 10^{-5} g/dm^3 the respiration rate is not limited ($\gamma_2 \cong 1$), and the fermentation metabolism is completely suppressed.

From the obtained results we can conclude that the proposed model can describe the growth of *S. cerevisiae* also under conditions of inefficient aeration, so it should be useful to optimise and modeling industrial bioreactors. The application of the model to industrial data will be the object of a further paper.

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