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Non-Linear Control of Baker's Yeas Bioreactor Based on a Kinetic and Mass Transfer Model

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Abstract

A structured unsegregated cybernetic model able to describe a diauxic growth phenomena of cells colony in aerobic condition. In this paper, the model has been proven in the simulation of the behavior of a batch and fed-batch bioreactors achieving satisfactory results. For simulating fed batch system, at first, the optimal substrate feeding in to bioreactor has been obtained by solving optimal control problem. Applying this profile to process model shows that ethanol concentration is much less in comparison with a constant feed rate. Also, because in many fermentation processes, oxygen transfer is the rate limiting step, so for preventing from oxygen starvation that causes ethanol production, oxygen mass transfer coefficient is simulated on the function of impeller speed and air flow rate and then, control of oxygen concentration by PI and GLC controller have been considered. Results show that two controllers have a same performance, but because of simpler structure in PI controllers, PI controller is better than GLC for this process. Also cell concentration without using controller have been brought and shown that productivity is smaller in comparison with using controllers.

Keywords: cybernetic model; fed-batch bioreactor; optimal control; *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 from a small quantity of *S. cerevisiae* added to a liquid solution of essential nutrients, at suitable temperature and pH. The effect of variables, such as pH and temperature, is well known and their optimal set-points can be easily defined. On the contrary, yield and productiveness can be largely 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 Straight and Ramkrishna [2] and Varner and Ramkrishn [3,4]. More recently, Jones and Kompala [5,6], Di Serio et al. [7,8] have extended the use of this model for describing the growth of *S. cerevisiae* in bioreactors. In this paper, at first, the result of simulation in batch bioreactor has been brought and shown that agreements are quite satisfactory whit experimental data. As we said Also, the feeding rate of the molasses plays a very important role in the batch progression as well as the final product concentration that is obtained at the end of the batch. Since each reactor run is followed by a personnel intensive, cleaning and sterilization operation, determination of the best possible profile may be economically expensive. A process model in such a scenario, could be very useful. Using tools from control theory, optimum substrate profiles could be determined in much less time compared to experimental determination, thereby resulted in economic savings. So in this paper, for simulating feed batch system, the optimal substrate feeding in to bioreactor has been obtained by solving optimal control problem [9,10]. Although cybernetic model empowers microorganisms to allocate cellular resources for the uptake of those substrates that best fit the cellular requirements, but in this paper, for preventing from oxygen starvation that causes ethanol production, oxygen mass transfer coefficient is simulated on the function of impeller speed and air flow rate and then, control of oxygen concentration by PI and GLC controller and effect of using controllers on biomass concentration have been considered.

2. The simulation model

During the aerobic growth of *S. cerevisiae*, sugars and ethanol can be used as carbon and energy sources, whereas

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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. Biomass yields on glucose are strongly related to the prevailing metabolic pathway, being maximal only when sugar is oxidized. 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. Under oxygen starvation conditions, the fermentative metabolic pathway always predominates; at a low sugar concentration, ethanol is produced, too. Ethanol produced during the fermentative metabolic pathway in a batch culture is consumed when glucose is no longer available in the medium. This phenomena is named diauxic behavior of *S. cerevisiae*. On the basis of above considerations, it is evident that *S. cerevisiae* has intenal regulating mechanisms which direct the micro-organism towards the most convenient metabolic pathway able to optimize the use of available resources. 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 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. The variable which controls 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 [7]. Therefore,

$$u_i = \frac{r_i}{\sum r_i} \tag{1}$$

$$n_i = \frac{r_i}{\max(r_j)} \tag{2}$$

Sugar can be metabolized via two different energy producing pathways, fermentation or oxidation, depending on the sugar concentration in the medium. So, 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. In this model, 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 $\left(\frac{e_i}{e_{i_{\max}}}\right)$, the relative intracellular proper key enzyme concentration.

$$r_1 = m_{1_{\max}} \frac{e_1}{e_{1_{\max}}} \cdot \frac{S_1}{k_1 V_L + S_1} \qquad \text{sugar fermentetion (3)}$$

$$r_2 = m_{2_{\max}} \frac{e_2}{e_{2_{\max}}} \cdot \frac{S_2}{k_2 V_L + S_2} \cdot \frac{ox}{k_{ox} + ox} \qquad \text{ethanol oxidation(4)}$$

$$r_3 = m_{3_{\max}} \frac{e_3}{e_{3_{\max}}} \cdot \frac{S_3}{k_3 V_L + S_3} \cdot \frac{ox}{k_{ox} + ox} \qquad \text{sugar oxidation(5)}$$

This choice introduces an advantage in managing the cybernetic model because the ratios $\left(\frac{e_i}{e_{i_{\max}}}\right)$ 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 the saturation constants for the substrate of each metabolic pathway (*i*) and KO_x represents the saturation constant for the dissolved oxygen.

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

$$\frac{dX}{dt} = \left(\sum r_i n_i\right) \qquad \text{balance on biomass(6)}$$

$$\frac{dS_1}{dt} = F_{in} s_1^0 - \left(\frac{r_1 n_1}{Y_1} + \frac{r_3 n_3}{Y_3}\right) X \qquad \text{balance on sugar(7)}$$

$$\frac{dS_2}{dt} = \left(f_1 \frac{r_1 n_1}{Y_1} - \frac{r_2 n_2}{Y_2}\right) X \qquad \text{balance on ethanol(8)}$$

$$\frac{dV_L}{dt} = F_{in} \qquad \text{balance on liquid volume(9)}$$

$$\frac{d\left(e_1/e_{1_{max}}\right)}{dt}=\left(m_{1_{max}}+b\right)\cdot\left(1-e+eu_1\frac{S_1}{k_1V_L+S_1}\right)-\left(\sum r_i n_i+b\right)\cdot\left(e_1/e_{1_{max}}\right)$$ balance on fermentation key enzyme(10)

relative concentration

$$\frac{d\left(e_2/e_{2_{max}}\right)}{dt}=\left(m_{2_{max}}+b\right)\cdot\left(1-e+eu_2\frac{S_2}{k_2V_L+S_2}\cdot\frac{ox}{k_{ox}+ox}\right)-\left(\sum r_i n_i+b\right)\cdot\left(e_2/e_{2_{max}}\right)$$ balance on ethanol oxidation key(11)

enzyme relative concentration

$$\frac{d\left(e_3/e_{3_{max}}\right)}{dt}=\left(m_{3_{max}}+b\right)\cdot\left(1-e+eu_3\frac{S_1}{k_3V_L+S_1}\cdot\frac{ox}{k_{ox}+ox}\right)-\left(\sum r_i n_i+b\right)\cdot\left(e_3/e_{3_{max}}\right)$$ balance on sugar oxidation key(12)

enzyme relative concentration

where $e=\frac{a}{a+a^*}$

$$\frac{dox}{dt}=K_La\left(ox^*-ox\right)-\left(f_2\frac{r_2n_2}{Y_2}+f_3\frac{r_3n_3}{Y_3}\right)\frac{X}{V_L}$$ balance on oxygen liquid concentration(13)

Paramete	values	Unit

$$RQ=\left(\frac{\left(f_1/46\right)\left(r_1n_1/Y_1\right)+\left(f_2/32\right)\left(r_2n_2/Y_2\right)\left(2/3\right)+\left(f_3/32\right)\left(r_3n_3/Y_3\right)}{\left(f_2/32\right)\left(r_2n_2/Y_2\right)+\left(f_3/32\right)\left(r_3n_3/Y_3\right)}\right)$$

respiratory quotient(14)

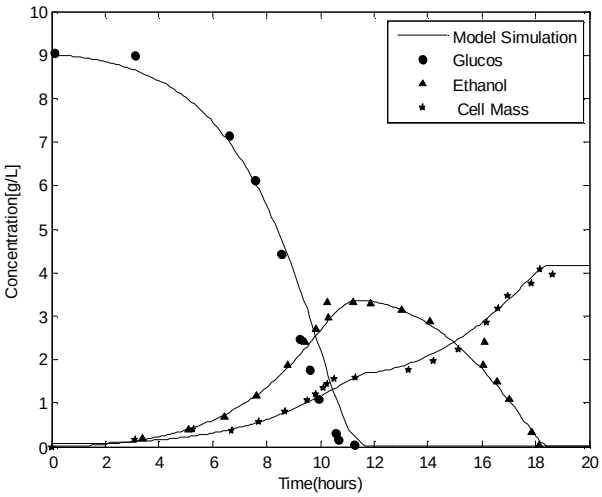
where $X, F_{in}, S_1^0, K_La, ox^*$ are, respectively, the biomass quantity in the reactor, the value of the sugar feed stream, the sugar concentration in the feed, the coefficient of gas–liquid mass transfer and the concentration of oxygen at the gas–liquid interface, and α and β are, respectively, 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 and Y_i and f_i the yields and stoichiometric coefficients for the different metabolic pathways, respectively. The respiratory quotient (RQ) is the ratio of CO2 moles produced on the oxygen moles consumed. RQ is higher than 1 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.

3. Batch Simulation results

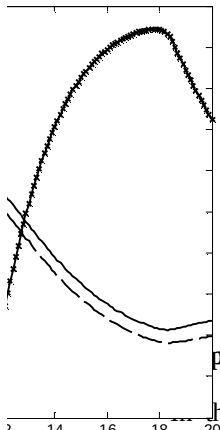
In Fig.1a, the evolutions with time of concentration of respectively biomass, glucose and ethanol are reported. In Fig. 1b, the evolution of the respiratory quotient is reported. As it can be seen in both cases agreements are quite satisfactory with experimental data.

Table 1
Model parameters values used for the simulation results

e	.909	…
b	0.2	(h^{-1})
$m_{1_{max}}$	0.45	(h^{-1})
$m_{2_{max}}$	0.20	(h^{-1})
$m_{3_{max}}$	0.33	(h^{-1})
Y_1	0.15	…
Y_2	0.74	…
Y_3	0.5	…
k_1	1.0±0.1	(g/dm^3)
k_2	0.08±0.04	(g/dm^3)
k_3	0.001	(g/dm^3)
f_1	0.41	…
f_2	1.067	…
f_3	0.52	…
Kox	4.6×10^{-5}	(g/dm^3)
(e_1/e_{1max})	.2	…
(e_2/e_{2max})	.2	…
(e_3/e_{3max})	.7	…



(a)



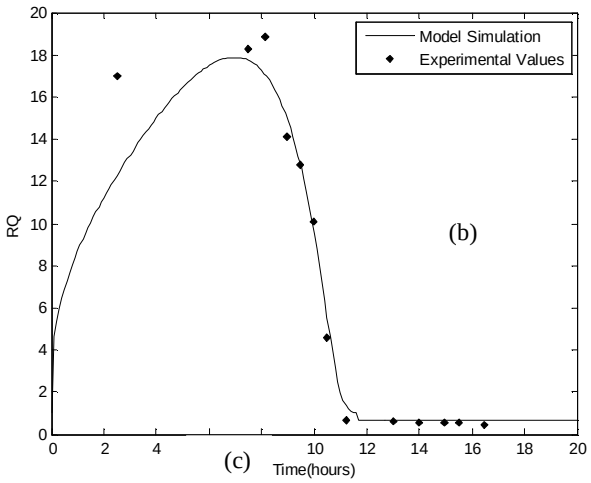


Fig. 1. Experimental data [7] and model simulation for cell mass, glucose, ethanol concentration (Fig. 1a) and respiratory quotient (Fig. 1b).
(Fig. 1c) The trends of the relative key enzyme concentration for the three metabolic pathways of *S. cerevisiae*.

ptimal Control

he case of a fed-batch bioreactor, one goal is to maximize/minimize an appropriate performance objective. Towards achieving this goal, it is important to note that decisions made regarding the input during the course of the batch play an important role on the objective function. The system dynamics are described by $\dot{x} = f(x,u,t)$ that x(t) and u(t) are vector valued state and input respectively and t_0 is the initial time. The objective function for the optimal control problem is the minimization of ethanol concentration at the end of the batch. The general formulation for the objective function is given as,

$$J(t_0) = j\,(x(t_f), t_f) + \int_{t_0}^{t_f} L(x,u,t)dt \qquad (15)$$

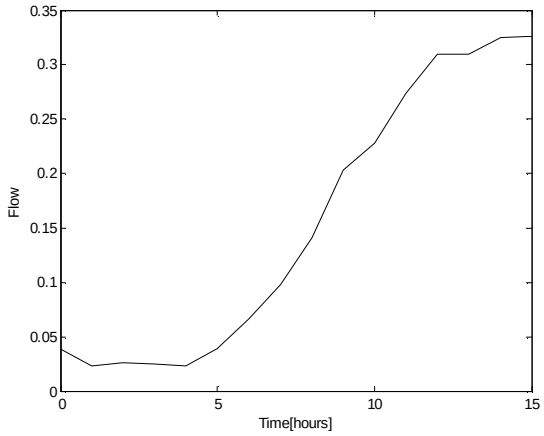
The function j accounts for the contribution of the final state, whereas *L*, accounts for the path dependence in the objective function with t_f as the final time of operation. Initially, the necessary condition for *u* to be optimal is $\frac{\partial H}{\partial u} = 0$. Hamiltonian is mathematically described as,

$$H[x(t),u(t),l(t),t] = L[x(t),u(t),t] + l^T(t) f[x(t),u(t),t] \qquad (16)$$

In this equation, l is the co-state variable and is used to incorporate the system dynamics into the objective function. The original optimal control problem is then transformed into a two-point boundary value problem, as the differential equations for the state and the new co-state variables have boundary conditions defined at $t_0(x(t_0))$ and at t_f ($l(t_f) = \left(\frac{\partial j}{\partial x}\right)^T$), respectively [11].

5. Optimal Control Results

In Fig. 2, the result of solving of optimal control problem for feed rate has been reported. Fig. 3, shows the influence of this profile on ethanol concentration and RQ in compare with constant feed rate.



$F_{in}\left(m^3/h\right)$Fig 2. Optimal feed rate profile

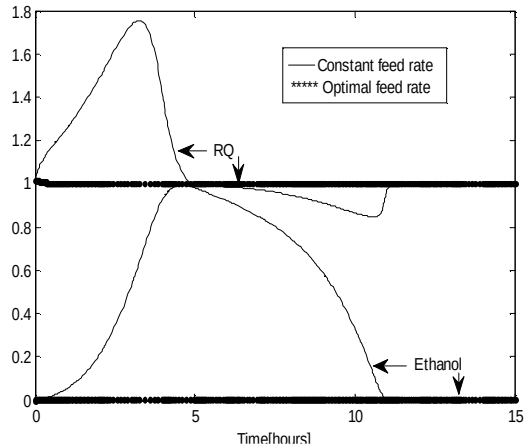


Fig 3. Ethanol concentration and RQ with constant and optimal feed rate

6. Fed-batch Simulation results

Because in many fermentation processes, oxygen transfer is the rate limiting step, correct measurement of the volumetric mass transfer coefficient is a crucial step in the design procedure of bioreactors. In order to ensure full aerobic conditions, both air flow and stirring rate are varied to keep the dissolved oxygen concentration higher than a critical value. So, by using air flow and stirring rate values, an equation for measuring oxygen mass transfer coefficient, has been proposed. For all kinds of reactors where the sole purpose is mass transfer, multiple-impeller systems are advantageous and there will be large savings on an industrial scale, especially for the bioreactors where the reaction periods are long and the power consumption cost can be a significant component to the overall production costs [12]. So, a stirred tank(D=20cm,H=40cm) with two disc turbine agitators(D=7.5cm,spacing=11cm) has been supposed. In fact yeast suspensions in the range of 25-200 kg m^{-3} are classified as Newtonian liquids. So the viscosity and density of the broth are measured during the whole period of fermentation by equations:

$$m = 0.9\times10^{-3} + 0.083\times10^{-3} X \quad (17)$$

$$r = r_w + X\left(1 - \frac{r_w}{r_x}\right) \quad (18)$$

Where ρ_W and ρ_X are the density of pure water and dry yeast and X is the yeast concentration [13]. With computing power number [14], the real gas power consumption is calculated

$$P_0 = N_P . r . N^3 . D_i^5 \quad (19)$$

By trial and error, the following equation has been proposed for computing the real gas power consumption that predicts the *K*l*a* values, well.

$$P_g = 2.448 \times \left(\frac{P_0^2 \cdot N \cdot D_i^3}{Q_g^{.56}}\right)^{.432} \quad (20)$$

It is obvious that *K*l*a* is related to gas power consumption per unit volume of broth and the superficial velocity:

$$K_L a \propto \left(\frac{P_g}{V}\right)^a (V_S)^b$$

Where the values of α and β depending on the system geometry. The following equation for *Kla* has been proposed. As it has been shown in Fig 5, the proposed equation, has a satisfactory correlation with experimental data.

$$K_L a = 1.28 * 10^{-2} \cdot \left(\frac{P_g}{V} \right)^{.425} (V_S)^{.224} \qquad (21)$$

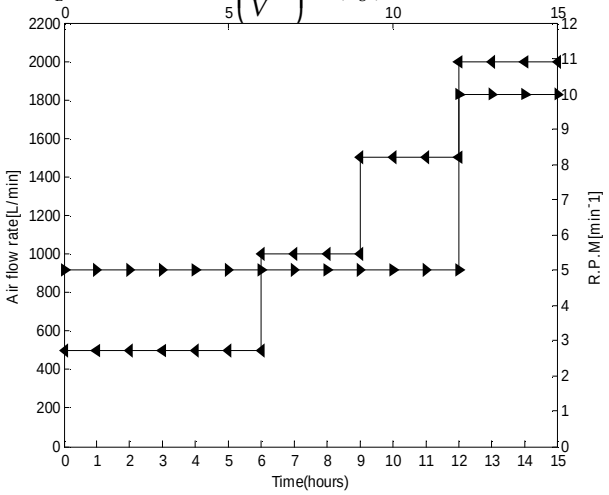


Fig 4. Experimental data of the air flow and stirrer speed rates[7]

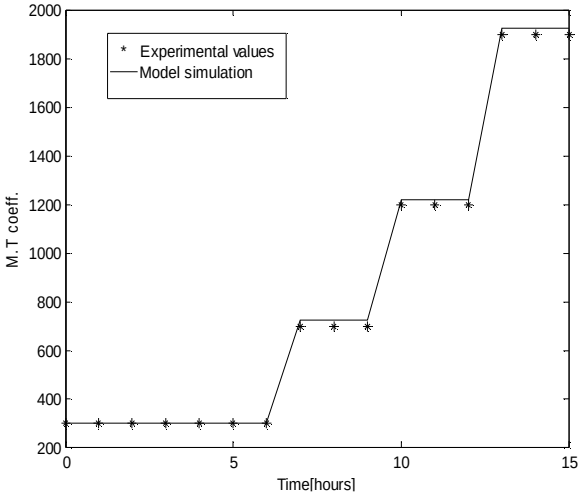


Fig 5. Experimental data [7] and model simulation for the *Kla*

By using this equation, *Kla* values are calculated and applied to process model.
In Fig. 6, the evolutions with time of concentration of biomass and dissolved oxygen have been reported.

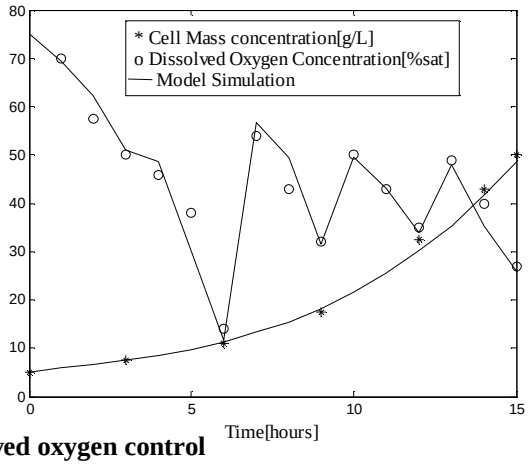


Fig 6. Experimental data [7] and model simulation for the biomass and oxygen concentration

7. Dissolved oxygen control

Oxygen transfer in aerobic bioprocesses is essential. So any shortage of oxygen drastically affects the process performance. Almost always, bioprocesses are carried out in aqueous media where the solubility of oxygen is very low owing to the presence of ionic salts and nutrients and the rate of oxygen utilization by the microorganisms is rather high. As it's shown in previous step, impeller speed and air flow rate, are two variable that affect on oxygen mass transfer coefficient. Because conventional proportional-integral (PI) has a single input-single output structure, these two variables must combine in the form of one parameter.
With substitution the values of P_g and V_s in proposed equation for Kla , this equation can be established:

$$K_L a = \frac{1.28 \times 10^{-2} \left(2 \times 1.224 \left(N_P^2 . r^2 . D_i^3 \right)^{.432} \right)^{.425}}{V^{.425} . A^{.224}} . N^{1.2825} . Q_g^{.1212} \longrightarrow \begin{cases} K_L a = c(r) . K_C \\ K_C = N^a . Q_g^b \end{cases} \qquad (22)$$

N and Q_g must be defined as a functions of K_C. Whit having minimum and maximum of N and Qg and generation some numbers between them, values for K_C get. Fitting equations to these data are the form of below:

$$\begin{cases} N = 0.93668 K_C + 3.759 \\ Q_G = 3.136 K_C + 6.7996 \times 10^{-5} \end{cases} \qquad (23)$$

Now, with having KLa values that get from control law and ρ by solving state functions and the K_C values , N and Q_g values can be found and apply to process model.

8. GLC Method

a globally linearizing control (GLC) and a conventional proportional-integral (PI) controller have been designed for controlling the total oxygen concentration, and performance of these controllers have been compared through simulation. The GLC method is a nonlinear control algorithm based on differential geometric approach. The first step in the GLC synthesis is the calculation of a state feedback, under which the closed loop input/output system is exactly linear. Then for linearized system, a controller with integral action such as PI can be designed. To implement the state feedback of the GLC, all the process state variables must be measured or estimated. Consider SISO processes with the following model:

$$\dot{x} = f(x,d) + g(x,d)u$$
$$y = h(x)$$

with a finite relative order r (the relative order is the smallest integer for which $L_g L_f^{r-1} h(x) \neq 0$). x and d are the vector of state variables and disturbances, respectively. u and y are the manipulated input and the controlled output, respectively. Under the state feedback:

$$u = \frac{n - h(x) - \sum_{i=1}^{r} b_i L_f^i h(x)}{b_r L_g L_f^{r-1} h(x)} \qquad (24)$$

where β_i's are tunable parameters, the closed loop *v-y* behavior is linear and described by the following equation:

$$b_0 y + b_1 \frac{dy}{dt} + \ldots + b_r \frac{d^r y}{dt^r} = n \qquad (25)$$

Some guidelines for tuning of β_i's parameters and other remarks for using GLC method have been described by Soroush and Kravaris [15]. The input of the linearized system (v) can be generated by a PI controller as below:

$$n = n_s + k_c\left(ox^* - ox\right) + \frac{k_c}{t_I}\int_0^t \left(ox^* - ox\right)dt \qquad (256)$$

Where ox^* is the desired profile of oxygen concentration in the bioreactor and Kc and τ_I are gain and integral time constant of PI controller, respectively.

9. Theoretical results

In this section, performance of GLC and PI controllers on cell concentration, have been compared. Also, how these controllers perform on control of oxygen amount in bioreactor has been surveyed. Also cell concentration without using controller have been brought and shown that productivity is smaller than using controllers. Effects of uncertainty in process model on performance of control methods, have been brought, and ramp function as an input, has been applied to process model. The resulting control algorithm (GLC), has three parameters β , K_c and τ_I that must be tuned by trial and error.

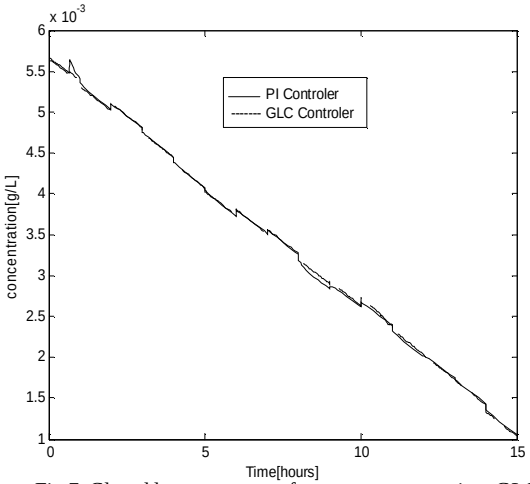


Fig 7. Closed loop response of oxygen concentration, GLC method (dash line), PI controller (line)

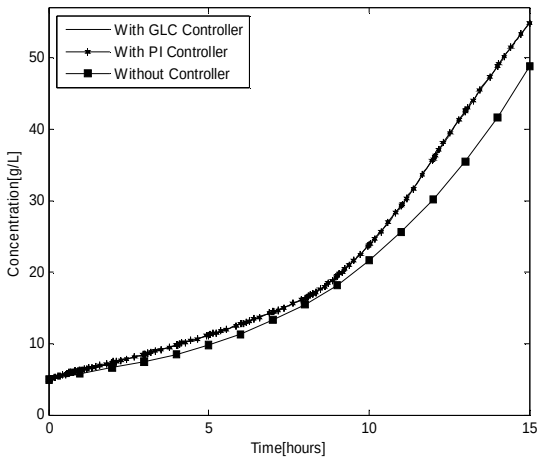


Fig 8. Closed loop response of cell concentration, GLC method (line), PI controller (line-star), without controller (line- square)

10. Conclusions

Simulation results show that agreements are quite satisfactory with experimental data. In particular, it can be seen that the cybernetic model performs well in the simulation of the lag-phases and the diauxic growth. In batch system, 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. Initially, in the presence of a high glucose concentration, the relative key enzyme concentration $\left(\frac{e_i}{e_{i_{\max}}} \right)$, promoting glucose fermentation is mainly synthesized. After the total glucose consumption during the diauxic lag-phase, the key enzyme that just promote ethanol oxidation, is synthesized and ethanol consumption starts with a different rate.
In fed-batch system, applying the optimal substrate feeding in to bioreactor cause the ethanol concentration and the respiratory quotient become much less in compare with a constant feed rate.

Results of using controllers on oxygen concentration show that although performance of GLC on the control of oxygen is more smooth than PI controller, but because of the main goal is reach to maximum of cell mass and these controllers have approximately the same performance to reach it and as a PI controller doesn't need the process model and has a simpler structure than GLC, so it seems that PI controller is better than GLC for this process. Also the effect of oxygen control on cell concentration shows that by using controller, rate of biomass production during the operation and also concentration at the end of the process is higher than natural condition. On the other hand, it is obvious that with passing the time, difference between two mode becomes higher and it's because of the bulk liquid oxygen concentration decreases with time; so the oxygen mass transfer rate becomes insufficient as a consequence of the increasing biomass concentration. Therefore, it is possible to conclude that in spite of the cybernetic model empowers microorganisms to allocate cellular resources for the uptake of those substrates that best fit the cellular requirements, for ensuring full aerobic conditions; it is necessary to use reasonable controller in order to optimize the biomass production in a bioreactor.

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