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On the chaotic behaviour of a *Saccharomyces Cerevisiae* culture in a turbidostat

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A turbidostat is a continuous bioreactor in which the biomass concentration level is controlled by regulation of the dilution rate. In this paper, the behaviour of an aerobic *Saccharomyces Cerevisiae* culture in a turbidostat regulated by a PI feedback controller is discussed. In particular, it will be shown how the cybernetic model of Jones & Kompala [1], based on the competition among oxidative and fermentative metabolic pathways and capable of describing some dynamic features of the system, can predict the presence of chaotic regimes in a specific range of operating parameters of the controller. The transition to chaos is determined by period doubling and intermittency according to Feigenbaum scenario, but the shift among different metabolic routes causes some unusual behaviours of the periodic windows.

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1. Introduction

The oscillatory behaviour of yeasts population growing on glucose in continuous aerated bioreactors has been detected since 1969 [2]. The reactor type in which this phenomenon is observed is the chemostat. It consists in a perfectly mixed vessel in which a yeast population is fed by a constant inlet stream with a constant nutrient concentration. The reaction volume is fixed, so the entering flow rate is equal to the exiting one. It was noticed that, for a special range of dilution rate (defined as the ratio between inlet flow rate and reactor volume) and carbon source concentration in the inlet stream, the system produces autonomous periodic oscillations: they concern some important state variables such as biomass and nutrient concentration, oxygen uptake rate (OUR), respiratory quotient (RQ) and fraction of budded cells ([3], [4]).

Despite the rich bibliography about the topic, there is not a definitive explanation of these phenomena. The most successful approaches are the following two:

1. The oscillation are produced by the periodic modifications in metabolic pathways [1]. This explanation is mainly motivated by the change in the RQ between values greater than 1 (which indicate the prevalence of fermentation) and less than 1 (prevalence of respiration);

2. The oscillations depend on the synchronisation of yeast cell budding process ([5],[6]). This interpretation is justified by the observation of the fluctuation of percentage of budded cells in the microbial population.

Each of these analyses is focused only on a part of the experimental evidences observed in this physical system, but an unique theoretical model which is able to describe adequately all the main aspects of this phenomenon does not exist. [7]. Anyway the first approach is constructed on less restrictive hypotheses and requires a simpler model and, hence, it will be adopted also here.

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In the case of chemostat, only periodic regimes have been observed, but, as reported by Davey [8], also some more complex asymptotic regimes can exist in the case of turbidostat. Turbidostat is a perfectly-mixed constant-volume bioreactor in which the biomass concentration is regulated by a feedback controller through the manipulation of the flow rate. The aim of this controller is to keep the yeast concentration constant and equal to a set point value imposed by the operator. Whilst in a chemostat the microbial growth is limited by the poor concentration of substrates, in the case of turbidostat the population is mainly controlled by the amount of cells expelled by the reactor.

In their paper, Davey et al.[8] individuated an operating condition of the turbidostat for which a non-periodic behaviour is detected: the calculation of the first Lyapunov exponent on the experimental time series of the dilution rate values confirmed that it was effectively chaotic. Anyway, in that paper, the control algorithm is not clearly defined by the authors and it is impossible to identify its role exactly in this phenomenon. Moreover, some spurious effects connected with the windup of the controller seem to affect this result significantly.

The aim of this paper is to describe the behaviour of a yeast population in a turbidostat adopting the kinetic model developed by [1] analysed the presence of chaotic regimes, the routes which bring to chaos, some special features of the chaotic attractors and their physical meaning.

2. Description of the model

In order to verify the existence of such chaotic behaviours and describe their effects on system state variables, a model which is able to predict its dynamic evolution must be chosen: the cybernetic one by Jones & Kompala [1] is suitable for this purpose. In its several applications (e.g., [9],[10]), the cybernetic approach has proved its efficiency in describing the main features of the competition between different metabolic pathways which contribute to microbial growth through a reduced number of equations. The basic hypothesis of this model is that each cell can be considered as a biological system able to allocate the available feeding and energetic resources in order to optimise some objective functions connected with biomass proliferation. In other words, when competing metabolic pathways are possible, the cells tend to privilege the one which allows a higher product generation rate by the massive synthesis and activation of its key enzymes. This corresponds to assume that the cell is capable to shift its own resources toward the most efficient metabolic route for its growth and reproduction.

In this system, the liquid feeding stream is assumed to be a sterile broth containing all the necessary substrates to the growth of yeasts in balanced quantities. The only fed carbon source is glucose. A constant sterile air flux is also supplied continuously at the bottom of the reactor. The model concerns essentially with the consumption of the carbon source: it can be glucose, fed by outside, but also ethanol, that is a product of fermentation of the cells themselves. The considered metabolic pathways are the following three (the biomass is indicated with X):

1. glucose fermentation (pathway index 1):

$$X + C_6H_{12}O_6 \rightarrow X + 2\ C_2H_5OH + 2\ CO_2 \tag{1}$$

2. ethanol oxidation (pathway index 2):

$$X + C_2H_5OH + 3\ O_2 \rightarrow X + 2\ CO_2 + 3\ H_2O \tag{2}$$

3. glucose oxidation (pathway index 3):

$$X + C_6H_{12}O_6 + 6\ O_2 \rightarrow X + 6\ CO_2 + 6\ H_2O \tag{3}$$

Each of them is supposed to be catalysed by an its own key enzyme (respectively e_1, e_2 and e_3). The effect on growth for each route is described by a Monod kinetics as indicated by the following three equations.

$$r_1 = \mu_1 e_1(t) \frac{G(t)}{G(t) + k_1} \tag{4}$$

$$r_2 = \mu_2 e_2(t) \frac{Eth(t)}{Eth(t) + k_2} \frac{Ox(t)}{Ox(t) + k_{Ox2}} \tag{5}$$

$$r_3 = \mu_3 e_3(t) \frac{G(t)}{G(t) + k_3} \frac{Ox(t)}{Ox(t) + k_{Ox3}} \tag{6}$$

The competition among the different metabolic routes is mathematically described through a couple of cybernetic variables U and V per pathway. The variable U_i is the fraction of the cell resources destined to enzyme synthesis allocated for the synthesis of i-th pathway key enzyme, while the variable V_i indicates the percentage of activated enzyme for the catalysis of the i-th pathway. These variables are defined by the following equations

$$U_i = \frac{r_i}{r_1 + r_2 + r_3} \tag{7}$$

$$V_i = \frac{r_i}{\max(r_1, r_2, r_3)} \tag{8}$$

for i=1,2,3. Two important observations should be done about this definition:

- both the synthesis and the activation of a specific key enzyme are privileged when its associated growth rate is higher than the others;
- the definition of the cybernetic variable V is a function that is continuous but only piecewise derivable.

The first element is connected with the optimization logic of the cybernetic model about the selection of the preferred metabolic pathway: in particular it can be seen how the definition of V allows to say that the key enzyme whose correspondent route j has an higher reaction rate, is fully activated (V_j=1). The corresponding pathway will be indicated as the dominant one. About the second point, it can be observed as the derivative discontinuity is located on the hypersurfaces where the system changes its dominant pathway. In [11] the authors clarified that the presence of this derivative discontinuity is an essential feature for the predictive capabilities of the model. The final model consists of nine differential equations reported in Table 1.

Equation (i) is the biomass balance, equations (ii), (iii) and (iv) concern with the consumption of the substrates (glucose, ethanol and oxygen), (v) is a balance of the intracellular storage carbohydrate mass fraction and (vi), (vii) and (viii) are the balances of the intracellular key enzyme concentrations. A physical interpretation of the single terms of the equations can be found in [1] as well as the meaning of the symbols adopted also here. The equation (ix) describes the variation of the dilution rate under the effect of the automatic control. In this case it is assumed that the controller is proportional-integral, one of the most commonly used in chemical engineering, whose operating parameters are the set point value (Xsp), the proportional gain (Kc) and the integral time (τ_I).

In the equation (ix) a further shift condition appears: it prevents the dilution rate from becoming negative if the biomass concentration is too low. The operating condition Dil=0 corresponds to the windup of the controller: in this paper, we study the operating condition range in which the windup phenomenon is absent, in order to focus our attention only on the biological effects avoiding to introduce also the "sliding" effect on this border condition. Because of the definition (8), the vector field of this system is a piecewise smooth function and so it belongs to the set of the Filippov systems. As reported by [12], the dynamical behaviour of the Filippov systems is richer than the smooth ones and their knowledge is far from being complete: in addition to the conventional bifurcations, these system can show further complications connected with the transitions through the border hypersurfaces between the domains into which the vector field is regular.

Table 1: Mathematical equations of the system

$$X'(t) = (r_1V_1 + r_2V_2 + +r_3V_3 - Dil(t))X(t) \quad \text{(i)}$$

$$G'(t) = Dil(t)\,(G_0 - G(t)) - \left[\frac{r_1V_1}{Y_1} + \frac{r_3V_3}{Y_3}\right] X(t) - \phi_4\left[C(t)X'(T) + C'(t)X(t)\right] \quad \text{(ii)}$$

$$Eth'(t) = \left(\frac{r_1V_1\phi_1}{Y_1} - \frac{r_2V_2}{Y_2}\right) X(t) - Dil(t)Eth(t) \quad \text{(iii)}$$

$$Ox'(t) = k_{La}\,(Ox_{ext} - Ox(t)) - \left(\frac{r_2V_2\phi_2}{Y_2} + \frac{r_3V_3\phi_3}{Y_3}\right) X(t) \quad \text{(iv)}$$

$$C'(t) = -C(t)\,(r_1V_1 + r_2V_2 + r_3V_3) - C(t)\,(r_1V_1\gamma_1 + r_2V_2\gamma_2) + r_3V_3\gamma_3 \quad \text{(v)}$$

$$e_1'(t) = \frac{\alpha G(t)U_1}{G(t) + k_1} + \alpha_{cost} - (\beta + r_1V_1 + r_2V_2 + r_3V_3)\,e_1(t) \quad \text{(vi)}$$

$$e_2'(t) = \frac{\alpha Eth(t)Ox(t)U_2}{(Eth(t) + k_2)\,(Ox(t) + k_{Ox2})} + \alpha_{cost} - (\beta + r_1V_1 + r_2V_2 + r_3V_3)\,e_2(t) \quad \text{(vii)}$$

$$e_3'(t) = \frac{\alpha G(t)Ox(t)U_3}{(G(t) + k_3)\,(Ox(t) + k_{Ox3})} + \alpha_{cost} - (\beta + r_1V_1 + r_2V_2 + r_3V_3)\,e_3(t) \quad \text{(viii)}$$

$$Dil'(t) = \begin{cases} Kp\left(X'(t) + \frac{X(t) - Xsp}{\tau_I}\right) & \text{if } (Dil(t) > 0) \text{ or } (Kp(X'(t) + \frac{X(t) - Xsp}{\tau_I}) > 0) \\ 0 & \text{if } (Dil(t) = 0) \text{ and } (Kp(X'(t) + \frac{X(t) - Xsp}{\tau_I}) \leq 0) \end{cases} \quad \text{(ix)}$$

3. Results and discussion

3.1. Setup of simulations

A extensive campaign of numerical simulations have been conducted in order to evaluate the effects of changes in the set point value of the controller on the dynamics of the system and on its stability. The parameter values adopted in this simulation are reported in Table 2 of [13]. The inlet glucose concentration is supposed to be 15 g/l. The integration of the ODE system has been performed by Mathematica5.1®: we use a code which integrates the system by means of the built in function NDSolve (BDF method for stiff differential systems). It is associated with a procedure of detection of the transition through the border hypersurfaces and a consequent shifting of the vector field in order to minimise the transition error. The simulations are performed on a PC with a 64 bit Intel Pentium IV® 3.20GHz processor.

3.2. Transition to chaos

Our first analysis concerns the behaviour of the system for variations of the set point value. Supposing to fix the values of the proportional gain to Kc=0.005 l/(g h) and the one of the integral time to τ_I=1 min, we examine the asymptotic regimes obtained increasing the set point value from 7.005 to 7.045 g/l. For Xsp $<$ 6.959 g/l there is an unique non banal stable steady state. In Xsp= 6.959 g/l a supercritical Hopf bifurcation is detected, so it is likely that stable periodic or more complex regimes may be found in the investigated range of the parameter. In order to describe the behaviour of the system, a solution diagram of the Poincaré map for the Poincaré section Dil=0.13 h^{-1} is reported here (fig.1): on the vertical axis the glucose concentration is represented. As we can note from this diagram, the initial periodic stable solution undergoes a period doubling cascade bringing to chaos. This first region of the cascade until Xsp=7.0251 g/l is entirely contained in the zone of the phase space in which ethanol oxidation pathway is dominant: as a matter of fact, it is possible to show that the cybernetic variable V_2 is constant and equal to 1 for all the periodic and chaotic regimes until the value

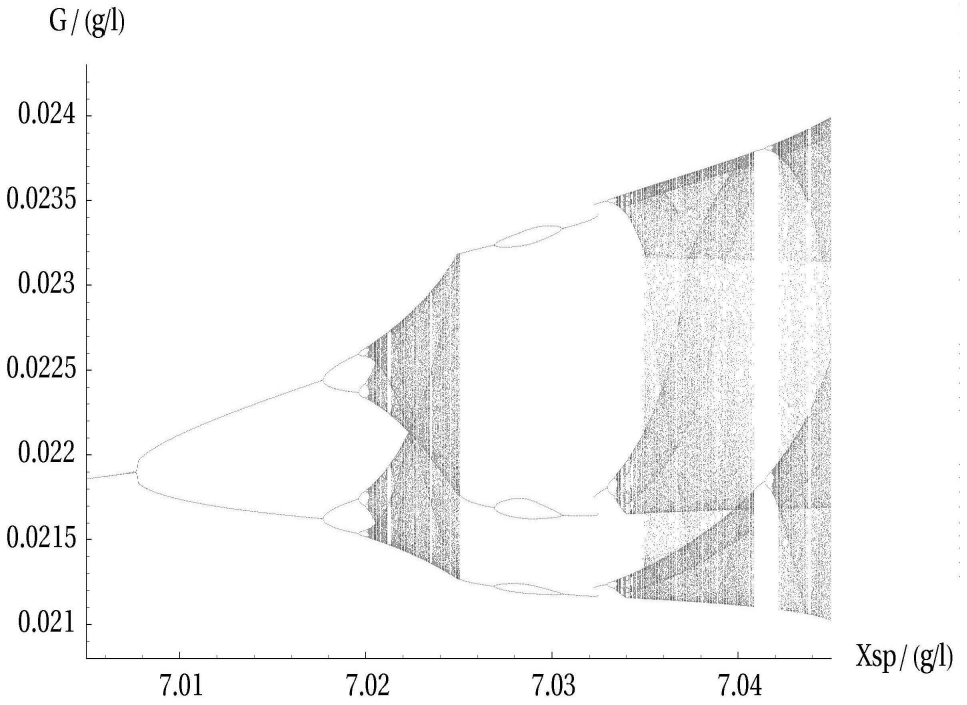


FIG. 1. Solution diagram of the Poincaré map (Poincaré section Dil=0.13 h^{-1}) for 7.005 g/l<Xsp<7.045 g/l, Kc=0.005l/(g h) and τ_I=1 min.

Xsp=7.0251 g/l.

3.3. Chaotic behaviour for Xsp<7.0251 g/l

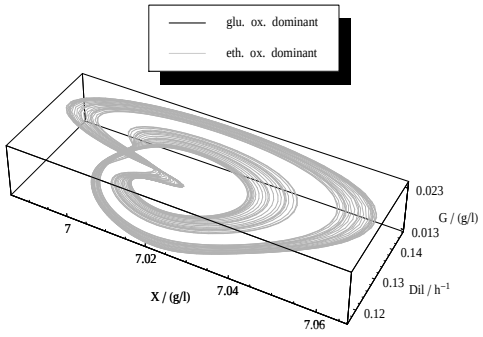
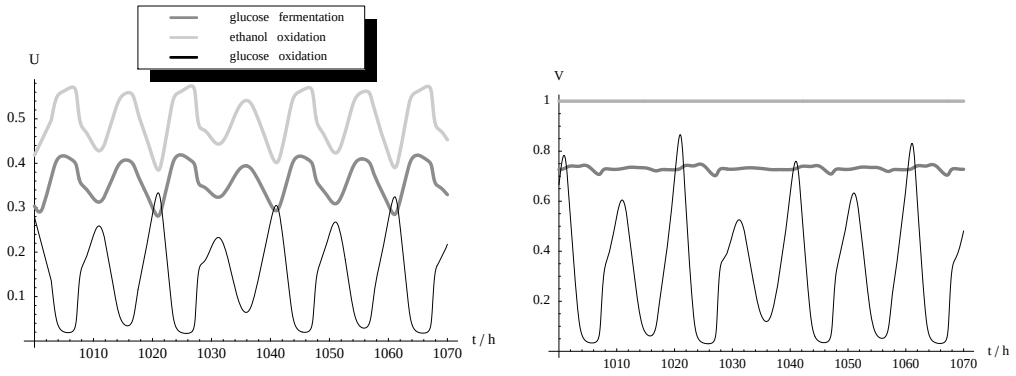


FIG. 2. Projection of the chaotic attractor in the three dimensional phase space spanned by the state variables X, G and Dil. (controller parameters: Xsp=7.021 g/l, Kc=0.005 l/(g h) and τ_I=1 min)

An interesting point is the examination of the implications of the chaotic behaviour from a physical point of view. First of all, a three dimensional phase space projection of the chaotic attractor is reported here (fig. 2) in correspondence of the set point value X=7.021 g/l.

The first Lyapunov exponent of this attractor is 0.08, so we can effectively conclude that it is a chaotic attractor. Even if the stationary point correspondent to the imposed set point is unstable, the controller is able to contain the fluctuations of the system in a range of less than 2% of the set point value. In order to determine the physical features of this attractor, a



(a) (b)

FIG. 3. Diagram of cybernetic variables as a function of time: (a) U variables; (b) V variables (controller parameters: Xsp=7.021 g/l, Kc=0.005 l/(g h) and τ_I=1 min).

diagram of the cybernetic variables as a function of time can help to interpret how the biology of the system contribute to this condition (fig. 3).

As already said, the prevalence of the ethanol oxidation pathway is evident from the fact that V_2 is constantly equal to 1. Furthermore, the variables U_1 and U_2 tend to oscillate "in phase": in other words there is a natural synchronism between the action of the fermentation and the ethanol oxidation metabolic routes. This can be explained observing that these two pathways can be considered to be in series because the product of the first is the reactant of the second one. To enforce this explanation it can be noticed how the peaks of the curve U_2 show a little delay in comparison with the ones of the U_1 curve. The dominance of the pathway 2 over 1 is simply due to the fact that each mole of fermented glucose produces two moles of ethanol and, so the occurrence of a quite high fermentation reaction produces an higher concentration of ethanol as a consequence. Conversely, the curve of U_3 seems to be "in opposition" to the other two variables indicating the tendency to mutual exclusion of these competing mechanisms.

3.4. Period-three window

In fig. 3, in correspondence of Xsp=7.0251 g/l the Feigenbaum cascade shows a period-three window. A detailed inspection of the Poincaré map solution diagram shows that the normal mechanism of intermittency is the responsible of this transition according to the common mechanism that determines the appearance of periodic windows in Feigenbaum cascades.

Nevertheless, this window has a quite unusual structure: it shows a period doubling bifurcation in correspondence of X=7.027 g/l and an undoubling for X=7.0305, furthermore there are two fold bifurcations which give an hysteretic behaviour (only the stable solutions are indicated). A probable reason for this unusual behaviour can be the following one: for Xsp<7.0251 g/l any asymptotic regime curve is entirely included ihe region of dominance of ethanol oxidation (V_2 constantly equal to 1) such as observed in the previous subsection; instead, for Xsp>7.0251 g/l, the regime solutions (both periodic and chaotic) always cross the hypersurface which separates the region in which ethanol oxidation is dominant from the one in which glucose oxidation is. In this case there is a portion of the attractor which is characterised by V_3=1 as it will be shown in the chaotic case presented in the next subsection. The effects of such phenomenon can be also detected by the sharp edges of the Poincaré map in fig.1 just in correspondence of the onset of the period-three window.

3.5. Chaotic behaviour for Xsp >7.0251 g/l

In this subsection, the behaviour of a chaotic attractor which is not entirely contained in the ethanol oxidation dominance region is described. In fig. 5 a three dimensional projection of the chaotic attractor obtained for Xsp=7.0400 g/l, Kc=0.005 l/(g h) and τ_I=1 min) is plotted.

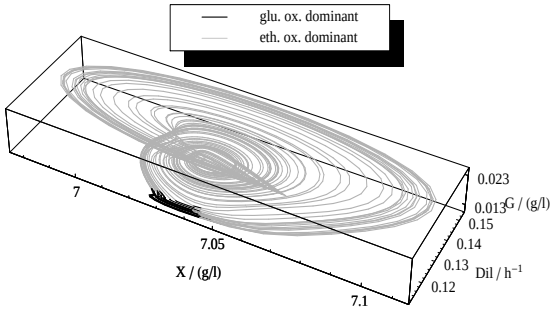
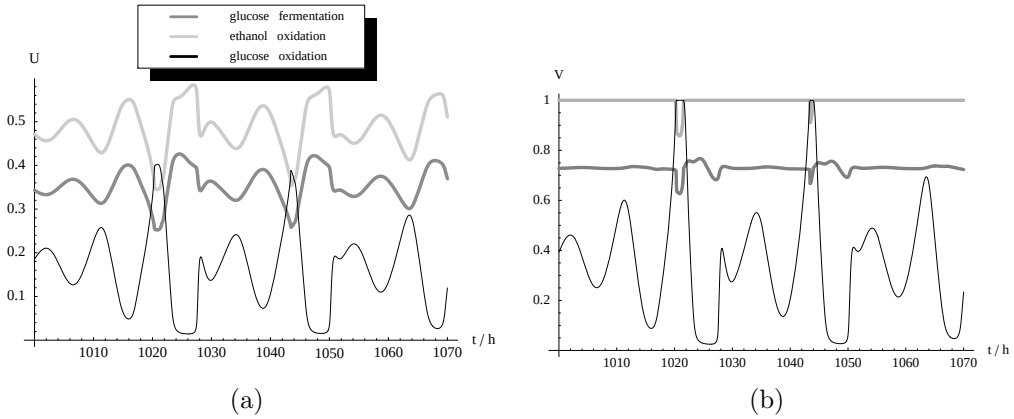


FIG. 4. Projection of the chaotic attractor in the three dimensional phase space spanned by the state variables X, G and Dil. (controller parameters: Xsp=7.04 g/l, Kc=0.005 l/(g h) and τ_I=1 min)

Also in this case, the highest Lyapunov exponent is greater than zero (0.15), confirming the chaotic nature of the attractor. As in the previous case, the diagram of cybernetic variables is reported (fig. 6)



(a) (b)

FIG. 5. Diagram of cybernetic variables as a function of time: (a) U variables; (b) V variables (controller parameters: Xsp=7.04 g/l, Kc=0.005 l/(g h) and τ_I=1 min).

In this case the cybernetic variable V_2 is not identically equal to 1: as a matter of fact, some short time intervals exist in which V_2 suddenly drops to values less than 0.9; during these intervals both glucose and ethanol concentrations are quite low and so the glucose oxidation mechanism becomes predominant. It is important to observe that this chaotic attractor shows very large fluctuations of the variable V_3 (as well as U_3): this phenomenon indicates how this dynamic regime is charachterised by the alternation between phases in which an important part of the cellular resources is employed in activate the glucose oxidation pathway and phases in which this route is almost completely repressed. As in the previous case, from the U diagram it is possible to note the synchronisation between fermentation and ethanol oxidation cybernetic variables and the substantial opposition of the glucose oxidation ones which shows its peaks in correspondence of the depression zones of the former two.

4. Conclusions

A *Saccharomyces Cerevisiae* culture in a turbidostat can show a chaotic behaviour. Although the controller succeeds in keeping the biomass amount reasonably close to the set point value, the metabolite concentrations show sustained non-periodic oscillations. These external changes require a constant modification in the microorganism metabolic regimes in order to perform a good adaptation to the environment in which they proliferate. The combination between the natural tendency to oscillation of a yeast population in connection with the dynamical effect produced by the controller provokes a chaotic competition between the inner mechanisms of feeding and reproduction of the cell population itself.

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