CONTINUOUS ETHANOL FERMENTATION AT HIGH GRAVITY IN THE PRESENCE OF SACCHAROMYCES CEREVISIAE: A BIFURCATION ANALYSIS.

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Abstract - In this paper, a study of the bifurcation analysis of fermentation of sugar to ethanol in presence of Saccharomyces cerivisiae at very high gravity is described. The bifurcation analysis was done for concentration, 200 gl^{-1}. Three Hopf bifurcations at 200 gl^{-1}. The system exhibits sustained oscillations between two Hopf bifurcations, which are the consequence of synchrony between the daughter and the mother yeast. The first Hopf bifurcation is located at 0.04226 h^{-1} and the second at 0.1105 h^{-1} for concentration of sugar 200 gl^{-1}. Indeed, it is better to take a dilution rate between the two Hopf bifurcations (self sustained oscillations), and the concentration at high gravity, in order to increase the ethanol productivity. At low dilution rate, the system exhibits a third Hopf bifurcation at 0.01849 $h^{-1}$and a pseudo bistability is observed in this resgion. This can be explained as switching between fermentation and respiration of the yeast, caused by the limitation of sugar, and utilization of the ethanol as a poorly substrate.

Keywords - Bifurcation; Ethanol; Fermentation; Saccharomyces Cerevisiae; High Gravity

I. INTRODUCTION

The increase of CO_2 concentration in the atmosphere, with the possibility of energy deficit, led to an orientation toward biofuels. Bioethanol, being one of the most promising biofuels, is ecologically clean, and have the potential to greatly reduce the dependence to oil. Currently, the production of the ethanol in industry is made in a predominant manner by batch fermentation. The continuous fermentations provide advantages in relation to fermentation. However, the mixture with a lower ethanol concentration imposes that the product has to be distilled [1].

Ethanol concentrations over 40 gl^{-1} exert inhibition on yeast metabolism, and turns to toxicity when ethanol concentrations in the fermentation broth exceed 90 gl^{-1} [2]. Inhibitory stresses combined with loss of cell viability result in shifting of metabolic flux away from ethanol production.
Saccharomyces cerivisiae exhibits oscillations that cannot be avoided or suppressed, in a large domain of operative conditions if the fermentation process is achieved in continuous reactor, typically between 0.09 and 0.25 h^{-1} [3]. These oscillations are joined to a simultaneous inhibition of the substrat and the ethanol. From where the necessity to develop strategies to avoid, eliminate or even taking advantage of these oscillations [4, 5, 6, 7].

For a slow process as fermentation, the dynamic simulation can be inefficient, inconclusive, and cannot be capable to localize features of the model that are responsible for certain dynamic behavior. Some dynamic features can be missed completely or can be disregarded, since one only executes a number limited of dynamic simulations. So analyzing the system stability has the advantages to make a survey retailed of the mathematical model stability representing fermentation in a reactor. This survey is generally made by method of bifurcation, which represents a fundamental change in the qualitative behavior of a nonlinear system as a parameter is varied, gives complete diagram of the stability of the system [8].

In this work, the bifurcation analysis of fermentation of sugar to ethanol in presence of Saccharomyces cerivisiae at high gravity is presented. The mathematical model for the fermentation in continuous bioreactor and the kinetic of the bioreaction, and all the constants are taken from Fengwu Bai thesis [9]. The bifurcation analysis was done for a concentration, 200 gl^{-1}. The dilution rate was taken as the parameter of bifurcation, and the results are present in the form of diagram of bifurcation for the different variables of the system.

II. MODEL DESCRIPTION

Bai has modeled the lag response of yeast cells to ethanol concentration history by introducing a weights function: a dynamic specific growth rate and a dynamic specific ethanol production rate, because ethanol production is tightly associated with the growth of yeast cells. The ethanol concentration which happened before has most significant influence on current status. The model indicated that there is a delayed response of yeast cells to ethanol inhibition effect, since the yeast cells do not respond immediately to the ethanol inhibition exerted on them, but require a period of time to adjust their intracellular metabolism to adapt such an ethanol inhibition environment.

The Equations of the proposed model are [9]:

$$\frac{dX}{dt} = X(Z - D)$$
$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{P/S}} Zp\, X$$
$$\frac{dP}{dt} = -D\, P + ZpX$$
$$\frac{dZ}{dt} = \omega(V_G - Z)$$
$$\frac{dV_G}{dt} = \omega(\mu - V_G)$$
$$\frac{dZp}{dt} = \omega(V_P - Zp)$$
$$\frac{dV_P}{dt} = \omega(\nu - V_G)$$

In the kinetic model the specific growth rate is expressed into two terms. One non linear term for the ethanol concentration, and another term for the substrate inhibition with the introduction of substrate constant into the kinetic expressions [9]. Bai has added an experimental parameter μ_0 that equals the dilution rate at which the limiting substrate concentration falls below a detection limit.

$$\mu = \mu_{max} \frac{S}{K_S + S + S^2/K_i} \left(1 - \frac{P}{P_{max}}\right)^{\alpha} + \mu_0$$

Ethanol production is tightly associated with the growth of yeast cells, so that a similar kinetic model for ethanol production has been developed [9].

$$\nu = \nu_{max} \frac{S}{K_{SP} + S + S^2/K_{iP}} \left(1 - \frac{P}{P_{max}}\right)^{\beta} + \nu_0$$

II. RESULTS AND DISCUSSION

Numerical results were obtained using the software XPPAUT Version 6.10 [10]. XPPAUT can solve ordinary differential equations, and make a bifurcation analysis with an incorporated bifurcation software AUTO. The dilution rate D was taken as the bifurcation parameter, and the bifurcation analysis was done for a feed concentration of sugar at CS0=200 gl^{-1}

Fig.1. represents the bifurcation diagram using the dilution rate as the bifurcation parameter. As we decrease the inlet dilution rate, we can observe different stages in the fermentation. In the first region, the system exhibits a unique stable steady-state. As the dilution rate is decreased, we observed a second region, in which a supercritical Hopf bifurcation occurs HB1 with D=0.1105 h^{-1}. In the case of a supercritical Hopf bifurcation we have a loss of stability under increase of the dilution rate, and the stable steady state is replaced by a stable limit cycle with sustained oscillations, bigger than those generated when the fermentation is conducted at very high gravity [7,9].

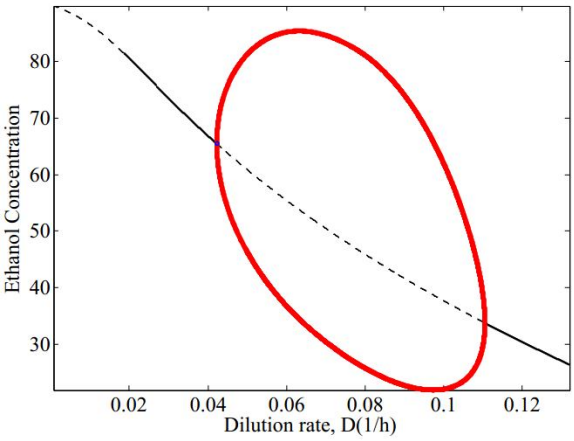


Fig.1. Bifurcation diagram for the ethanol concentration at CS0= 200gl^{-1}, periodic branch (▬), stable steady-state branch (━), unstable steady-state branch (---).

By comparison with the fermentation at very high gravity VHG, the oscillations are larger, and seen that the productivity increases when the concentration the ethanol oscillate [6], it is preferable to work in this domain of dilution.

The appearance of sustained oscillations is related to the formation, by an asymmetric process known as budding [11], of distinct subpopulations of daughters and mothers cells via a mechanism called the cell cycle, divide in two stages. The stage one G1, is growing to a critical size CSR, and second is dividing cells G2.

The size of the daughter cell is small and need to growth to a critical size to become a new mother and able to divide, while the mother cell sizes are larger and nearly equal to the critical size for a cell division. This led to an asynchrony between the daughter and the mother cell [12]. In the stage G1, the daughter cell size will be function of the growth and the time needed depends on nutrient availability, At high growth rate the cell population is synchronized, and a limit cycle appears.

It is believed that the oscillatory behavior in continuous fermentation processes is related to a delayed response of the microorganisms to changes in their environment as shown, the secreted ethanol inhibits the formation of an intracellular metabolite and the metabolite influences the specific growth rate. The inhibitory effect of the ethanol on the specific growth rate is delayed [13].

With the decrease of the dilution rate a second Hopf bifurcation HB2 occurs at D= 0.04226 h^{-1} where the limit cycle disappears, and a stable steady state is

created. The last one appears to be attained when the cell population is less synchronized.

As we continue to decrease the dilution rate from the second Hopf bifurcation HB2 a third Hopf bifurcation HB3 occurs at D=0.01849 h^{-1}. In this region, we can observe the existence of stable steady state and slightly instable limit cycle.

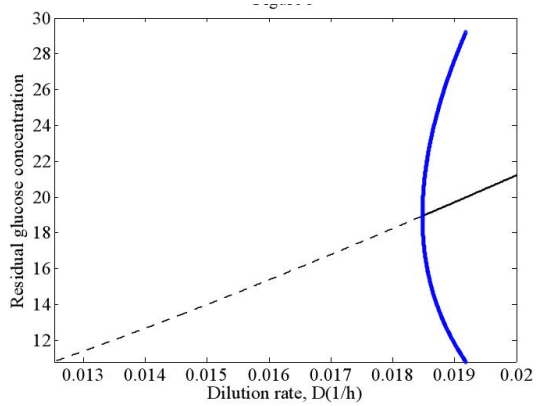


Fig.2. Bifurcation diagram for residual glucose concentration at CS0= 200gl^{-1} and at low dilution rate, unstable periodic branch ( ), stable steady-state branch ( ), unstable steady-state branch (---).

The oscillations are stable on great interval of time (up to 5000 h), and the change to a stable steady state is only observable after about 20000 h. The transition from limit cycle to a steady state corresponds to a transition from a synchronous to an asynchronous cell cycle as shown in Fig. 3. This figure confirms the existence of a 'pseudo limit cycle' in this region. The oscillations correspond to synchronous cell cycle, and since the system is stable for a long time of fermentation process**,** we can consider that in this region we have a bistability, and it is possible that this bistability is a consequence of a diauxic effect. Indeed, the diauxic effect is observed when there is a simultaneous utilization of both limiting substrates for small dilution rates. The yeast utilizes only one substrate at a time [13]. The substrate supporting the faster growth of microorganisms is the more preferred one by microorganisms than the latter.

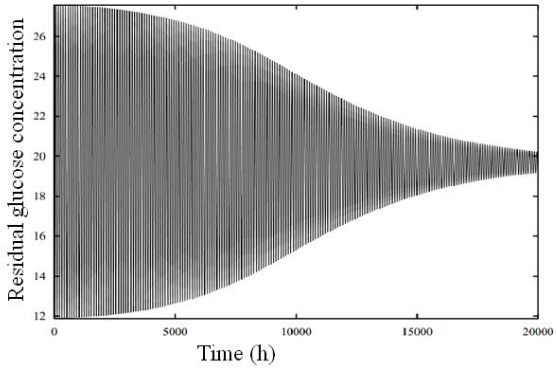


Fig.3. Concentration of residual glucose vs. time at low dilution rate D=0.019h^{-1} and CS0=200 gl^{-1}.

The cells resume growing at the same time and enter division synchronously. This may last for several generations until the synchrony is lost, and a steady state is reached which corresponds to asynchronous cell cycle.

Under limitation of sugar, both glucose and the excreted ethanol can serve as substrates for cell growth, and the following three reactions are possible [14]:

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$
$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 2\,H_2O$$
$$C_2H_5OH + 3\,O_2 \rightarrow 2\,CO_2 + 3\,H_2O$$

At low dilution rate the supply of glucose becomes insufficient to replenish its consumption, leading to a possible oxidative metabolism. Then both glucose and ethanol become limiting substrates, and Seen as consumption of glucose is shifted, its concentration rise, thereby shifting the metabolism to the fermentative pathway and the cycle is repeated [15].

CONCLUSIONS

In this paper, the bifurcation analysis of the fermentation of sugar into an ethanol in the presence of yeast saccharomyces cerivisiae was studied. It was found that the system stability is dependent on dilution rate and on the initial concentration of sugar. Indeed, at 200 gl^{-1} of sugar the system presents three Hopf bifurcations, at 0.01849 h^{-1}, 0.04226 h^{-1}, 0.1105 h^{-1}.

Between 0.04226 h^{-1} and 0.1105 h^{-1} the system presents sustained oscillations. This phenomenon is reported by several authors [13, 15, 16], and is mainly caused by ethanol concentration and the synchrony of cell cycle of the mother and daughter cells. Indeed, a steady state is caused by the asynchrony of cell cycle, and the limit cycle is caused by the synchrony of the cell cycle [14, 17].

At low dilution rate the system presents a third Hopf bifurcation at 0.01849 h^{-1}. In this region there is pseudo bistability where the steady state coexist with a limit cycle slightly instable. It is possible that this bistability is a consequence of a diauxic effect.

The oscillations are large, as found by simulation, in the present paper and by experience [18], and seen that the productivity increases when the concentration the ethanol oscillate [6], it is preferable to work in this domain of dilution. Indeed, in this case, the productivity can increase from 2.041 $gl^{-1}h^{-1}$ to 3.98 $gl^{-1}h^{-1}$, which corresponds to an increase of 95%, when we take the dilution between the two Hopf bifurcation (self sustained oscillation).

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