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# ARE MONOD MODELS ENOUGH FOR BIOREACTOR CONTROL? PART II – SOME SIMULATION RESULTS

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***Abstract:*** In this contribution a cybernetic model and a simple Monod based model were used to simulate the control of glucose and ethanol in *Saccharomyces cerevisiae* fed-batch cultivation. The main objectives were to investigate the type of modeling, the choice of the control variable (glucose or ethanol) and the kind of control action which is more suitable to control the studied system. It was found that the cybernetic model produces better qualitative and quantitative results and also that ethanol control shows a smooth behavior when compared to glucose control. According to the results obtained, an on-off control strategy based on ethanol measurements was suggested as an easier way to control the system without using effectively a controller. *Copyright © 2007 IFAC*

***Keyword:*** Fed-batch cultivation, PI Control, Substrate control, Ethanol Control

## 1 INTRODUCTION

It is well know that the set point control of the substrate concentration during bioprocesses is a matter of particular economic and scientific interest. It plays an important role for industrial processes such as yeast fermentation or biotransformations in general (Johnston *et al.*, 2002; Miskiewicz & Kasperski, 2000; Rani & Rao, 1999). Regarding the production of yeast, the biomass yield can be raised from 20 % to approximately more than 50 % if the glucose concentration is kept below a certain level. This is due to the fact that yeast changes its metabolism from oxidative to oxidative-reductive and produces by-products like ethanol and acetate, if the substrate concentration is above the critical level (Sonnleitner & Käppeli, 1986). However several questions arise:

1. How low should the substrate (glucose) concentration be to avoid the production of byproducts?
2. How sensitive is the optimal production rate to the glucose concentration?
3. Which should be the set point concentration trajectory to attain the optimal biomass production? Is a constant low set point value the best solution?
4. It would not be better to control directly the byproduct (i.e., ethanol) concentration instead of substrate (i.e., glucose) concentration?
5. Can a simplified Monod model be used to control the system, or a more complex model (e.g., cybernetic model) must be applied?

6. If few measurements are available, is it possible to establish a simple control action profile, which could be quite close to the optimal control action?

Here, we intend to answer the above mentioned questions based on the simulation and control of two different models: cybernetic and a simplified Monod based model.

The paper is structured as follows. Section 2 describes both models used in our study. In section 3 the cybernetic model is simulated for two different control strategies: glucose and ethanol control. Low, middle, and high set point values are considered. Section 4 shows the corresponding results for the simplified model. Both models are then compared in Section 5 analyzing the control action calculated. Section 6 finalizes the paper with concluding remarks.

## 2 MODELS FOR *SACCHAROMYCES CEREVISIAE* CULTIVATION

### *2.1 Cybernetic Model (Jones & Kompala, 1999)*

When two growth substrates are available in batch culture, microorganisms preferentially consume one substrate until completion, followed by an intermediate lag phase that precedes consumption of the second substrate. It is known that *Saccharomyces cerevisiae* exhibits diauxic behavior when supplied with glucose as the only carbon and energy source. It has also been shown that the carbohydrates accumulate during the growth phase on glucose, but are quickly consumed when consumption of ethanol begins. Monod's classical model is clearly not sufficient to predict diauxic behavior, because the model is not detailed enough to include the dynamics of the intermediate lag phase and multiple metabolic pathways that are observed during the diauxic growth pattern. As a general rule, all unstructured models are too simplistic to predict these dynamics accurately, as they do not consider the intracellular regulatory mechanisms.

The cybernetic modeling framework is based on the hypothesis that microorganisms optimize utilization of available substrates to *maximize their growth rate at all times*. The cybernetic modeling framework replaces the detailed modeling of regulatory processes with cybernetic variables $u_i$ and $v_i$ representing the optimal strategies for enzyme synthesis and activity, respectively. For the instantaneous growth rate $r_i$ values along the three available pathways, the optimal strategies for $u_i$ and $v_i$ have been shown (Kompala et al., 1986) as:

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

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

The growth rates $r_i$ values along each pathway are modeled according to a modified Monod rate equation, with the simple modification that each growth rate is proportional to the intracellular concentration of $e_i$ for a key enzyme controlling each pathway:

$$r_1 = \mu_1 e_1 \frac{G}{K_1 + G} \qquad \textit{Glucose Fermentation} \tag{3}$$

$$r_2 = \mu_2 e_2 \frac{E}{K_2 + E} \frac{O}{K_{O_2} + O} \qquad \textit{Ethanol Oxidation} \tag{4}$$

$$r_3 = \mu_3 e_3 \frac{G}{K_3 + G} \frac{O}{K_{O_3} + O} \qquad \textit{Glucose Oxidation} \tag{5}$$

where $G$, $E$, and $O$ represent the concentrations of glucose, ethanol, and dissolved oxygen, respectively. $\mu_i$ represents the modified growth rate constant, $K_i$ and $K_{oi}$ values represent the saturation constants for the carbon substrate and dissolved oxygen for each metabolic pathway. With these growth rate equations, the common balance equations for batch (dilution rate, $D = 0$) and fed batch ($D \neq 0$) cultures can be written as follows:

$$\begin{aligned}
\frac{dX}{dt} &= \left(\sum_i r_i v_i - D\right) X \\
\frac{dC}{dt} &= \gamma_3 r_3 v_3 - \left(\gamma_1 r_1 v_1 + \gamma_2 r_2 v_2\right) C - \sum_i \left(r_i v_i\right) C \\
\frac{dG}{dt} &= D(G_0 - G) - \left(\frac{r_1 v_1}{Y_1} + \frac{r_3 v_3}{Y_3}\right) X - \phi_4 \left(C \frac{dX}{dt} + X \frac{dC}{dt}\right) \\
\frac{dE}{dt} &= -DE + \left(\phi_1 \frac{r_1 v_1}{Y_1} - \frac{r_2 v_2}{Y_2}\right) X \\
\frac{dO}{dt} &= k_{La}\left(O^* - O\right) - \left(\phi_2 \frac{r_2 v_2}{Y_2} + \phi_3 \frac{r_3 v_3}{Y_3}\right) X \\
\frac{de_1}{dt} &= \alpha u_1 \frac{G}{K_1 + G} - \left(\sum_i r_i v_i + \beta\right) e_1 + \alpha^* \\
\frac{de_2}{dt} &= \alpha u_2 \frac{G}{K_2 + G} - \left(\sum_i r_i v_i + \beta\right) e_2 + \alpha^* \\
\frac{de_3}{dt} &= \alpha u_3 \frac{G}{K_3 + G} - \left(\sum_i r_i v_i + \beta\right) e_3 + \alpha^* \\
\frac{dV}{dt} &= \dot{V}_f(t) - \dot{V}_{sam}(t)
\end{aligned} \tag{6}$$

The new state variables $X$ and $C$ represent the cell mass concentration and intracellular storage carbohydrate mass fraction, respectively. $G_o$, $k_La$, $Y$, $\phi_i$, $\gamma_i$, $\alpha$ and $\beta$ are glucose feed concentration, dissolved oxygen mass transfer coefficient, yield coefficients, stoichiometric coefficients for different substrates, stoichiometric coefficients for intracellular storage carbohydrate synthesis and consumption, the enzyme synthesis and decay rate constants, respectively. $\dot{V}_f$ and $\dot{V}_{sam}$ are the feed rate and the sample rate.

Turner and Ramkrishna (1988) have shown that including a small constitutive synthesis term for all enzymes $\alpha^*$ is important in predicting the induction of enzymes that have been repressed for long periods of time. With the inclusion of the intracellular key

enzyme concentration in each rate Eqs. (3) – (5), the rate constant $\mu_i$ in these equations is related to the experimentally observed maximum specific growth rate $\mu_{i,max}$ according to:

$$\mu_i = \mu_{i,\max} \frac{\mu_{i,\max} + \beta}{\alpha + \alpha^*} \tag{7}$$

The parameters were taken from Zhang & Henson (2001) and are shown in Table 1.

Table 1. Parameters of cybernetic model.

| Parameter | Unit | value |
|---|---|---|
| $\mu_{i,max}$ | $h^{-1}$ | 0.44, 0.19, 0.36 |
| $K_i$ | g/L | 0.05, 0.01, 0.001 |
| $Y_i$ | g/g | 0.16, 0.75, 0.60 |
| $\phi_i$ | g/g | 0.403, 2.0, 1.0, 0.95 |
| $\gamma_i$ | g/g | 10, 10, 0.8 |
| $\alpha$ | g/g-h | 0.3 |
| $\alpha^*$ | g/g-h | 0.03 |
| $\beta$ | $h^{-1}$ | 0.7 |
| $K_{O2}$ | mg/L | 0.01 |
| $K_{O3}$ | mg/L | 2.2 |
| $K_{La}$ | $h^{-1}$ | 225 |
| O* | mg/L | 7.5 |

### *2.2 Simplified Model*

This model is derived from the cybernetic model taking off the cybernetic variables and the equations of the key enzymes of each pathway. The resulting system is composed by the following equations.

$$\begin{aligned}
\frac{dX}{dt} &= \left(\sum_i r_i - D\right)X \\
\frac{dG}{dt} &= D(G_0 - G) - \left(\frac{r_1}{Y_1} + \frac{r_3}{Y_3}\right)X \\
\frac{dE}{dt} &= -DE + \left(\frac{r_1}{Y_1} - \frac{r_2}{Y_2}\right)X \\
\frac{dO}{dt} &= k_{La}(O^* - O) - \left(\frac{r_2}{Y_2} + \frac{r_3}{Y_3}\right)X \\
\frac{dV}{dt} &= \dot{V}_f(t) - \dot{V}_{sam}(t)
\end{aligned} \tag{8}$$

Growth rates:

$$r_1 = \mu_1 \frac{G}{K_1 + G} \qquad \textit{Glucose Fermentation} \ (9)$$

$$r_2 = \mu_2 \frac{E}{K_2 + E} \frac{O}{K_{O_2} + O} \qquad \textit{Ethanol Oxidation} \ (10)$$

$$r_3 = \mu_3 \frac{G}{K_3 + G} \frac{O}{K_{O_3} + O} \qquad \textit{Glucose Oxidation} \ (11)$$

The values of the parameters were maintained on the same level as for the cybernetic model.

## 3 CLOSED LOOP CONTROL OF THE CYBERNETIC MODEL

To answer the questions, which have been introduced at the beginning of this paper, two different control strategies are considered: glucose concentration control and ethanol concentration control. In both cases the dilution rate $D = \dot{V}_f(t)/V(t)$ was used as manipulated variable. The control action was calculated using standard PI controllers with anti-windup (i.e., PI=$Kp$+$Ki/s$). The controller parameters were {$Kp$=10; $Ki$=5} for glucose control and {$Kp$=2; $Ki$=2} for the ethanol control loop. In the next subsections we present the results for three different set points levels: low, middle, and high. In all simulation results, the inlet glucose concentration was $G_0$ = 100 g/L.

### *3.1 Low set point values*

Fig 1 shows the simulation results using the cybernetic model when the glucose is controlled at 0.0095 g/L. These results are compared with the case where ethanol is controlled by 0.01 g/L.

To make it easier to compare the different results, all figures in this paper are structured as follows: (i) results shown in solid lines correspond to the case where glucose is the control variable and dashed lines are for ethanol control case, (ii) the first subplot corresponds to the biomass concentration; the subplot placed at the right position to ethanol concentration; the subplot placed below in the left, the glucose concentration; and the last subplot shows the control action (i.e., dilution rate $D$).

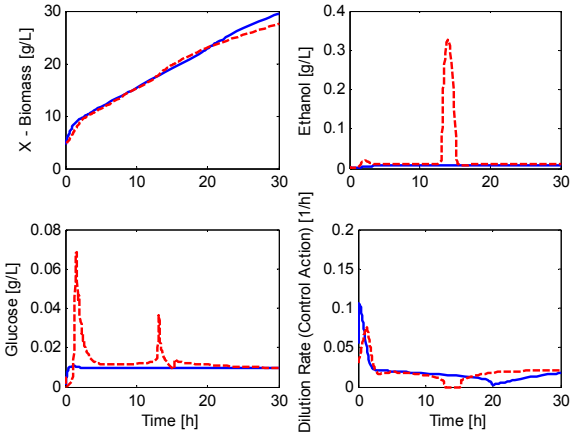


**Fig. 1:** Simulation results for glucose (solid blue lines) and ethanol (dashed red lines) control. The set points were 0.0095 g/L and 0.01 g/L for glucose and ethanol, respectively.

When ethanol was the control variable, around 12.5h the biomass curve shows a little change in inclination as if the cells were changing their metabolism. After that, ethanol starts to accumulate but it is rapidly consumed and returns to the setpoint value. Additionaly there is a reduction in the growth rate. At the beginning of the simulation, around 2h, something similar has already occurred. These facts seem to indicate the change from glucose to to ethanol as the main carbon source.

Based on Fig 1 we can conclude that for low set points, controlling the glucose concentration automatically assures low ethanol concentration and high biomass production.

### 3.2 *Middle set point values*

Fig 2 shows the simulation results using the cybernetic model when the glucose is controlled at 0.015 g/L. These results are compared with the case where ethanol is controlled at 0.1 g/L. Note that the glucose set point concentration has been increased by a factor of 1.57, while the ethanol set point was multiplied by a factor of 10.

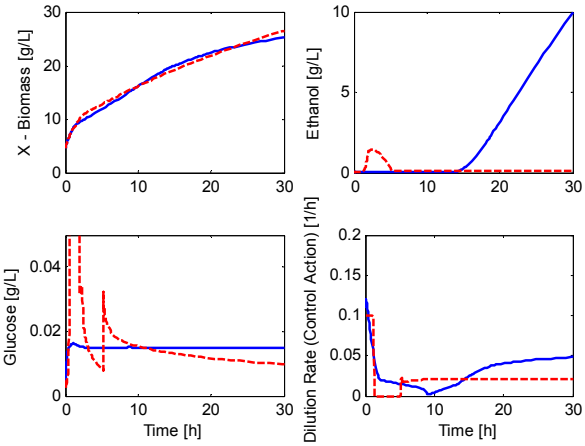


**Fig. 2:** Simulation results for glucose (solid lines) and ethanol (dashed lines) control. The set points were 0.015 g/L and 0.1 g/L for glucose and ethanol, respectively.

When glucose concentration was being controlled, at about 15h the setpoint for glucose control turned to be above the critical glucose concentration leading to a rapid increase in ethanol. On the other side, when ethanol was the control variable one can observe that the glucose concentration (even with oscillations at the beginning) after 11h fell to a value less than the setpoint for glucose. So, when ethanol is the controlled variable, the glucose concentration is maintained automatically in an interval where the oxidative-reductive metabolism is not dominant.

### 3.3 *High set point values*

To analyze the sensitivity to the set point values, both set points have been multiplied by a factor of 2 (i.e., 0.03 g/L for glucose and 0.2 g/L for ethanol). The simulation results are shown in Fig 3.

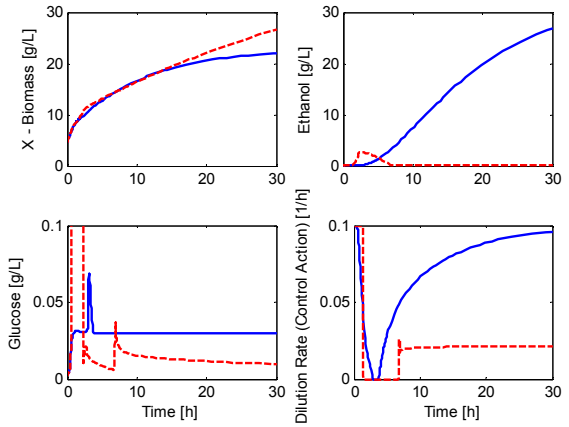


**Fig. 3:** Simulation results for glucose (solid lines) and ethanol (dashed lines) control. The set points were 0.03 g/L and 0.2 g/L for glucose and ethanol, respectively.

Again the ethanol controlled version was able to maintain the glucose concentration at a low concentration level, while for the glucose controlled loop, the ethanol concentration becomes even higher than in the previous case impacting in the final biomass production.

These results clearly show that a system using ethanol as controlled variable is much less sensitive and more effective than a system controling the glucose concentration. When glucose is used as controlled variable, small changes can lead to a shift in the metabolism, which can change the productivity of biomass considerably.

## 4 CLOSED LOOP CONTROL OF THE SIMPLIFIED MODEL

In this section results are presented for simulations employing the simplified model instead of the cybernetic model. The control action was calculated using standard PI controllers with anti-windup (i.e., PI=$Kp$+$Ki$/$s$). In the next subsections we present the results for three different set points levels: low, middle, and high. In all simulation results, the inlet glucose concentration was $G_0$ = 100 g/L.

### 4.1 *Low set point values*

The controller parameters were {$Kp$=5; $Ki$=2} for glucose control and {$Kp$=2; $Ki$=3} for the ethanol control loop. The set points are 0.0004 g/L and 0.000615 g/L for glucose and ethanol, respectively. The simulation results are shown in Fig. 4 clearly show a total equivalency between ethanol and glucose control, when the simplified model is used for low set point control simulations.

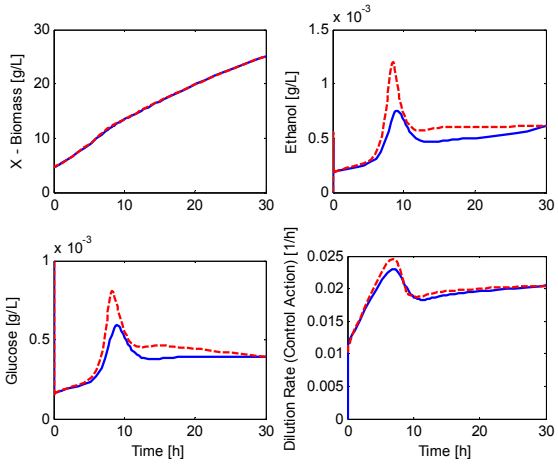


**Fig. 4:** Simulation results for glucose (solid lines) and ethanol (dashed lines) control. The set points were 0.0004 g/L and 0.000615 g/L for glucose and ethanol, respectively.

### 4.2 *Middle set point values*

Fig 5 shows the simulation results using the simplified model when the glucose is controlled at

0.0008 g/L and ethanol at 1.06 g/L. Note that the glucose set point has been increased by a factor of 2, while the ethanol set point was multiplied by a factor of 1724. The controller parameters were {*Kp*=10; *Ki*=3} for glucose control and {*Kp*=0.017; *Ki*=0.09} for the ethanol control loop.

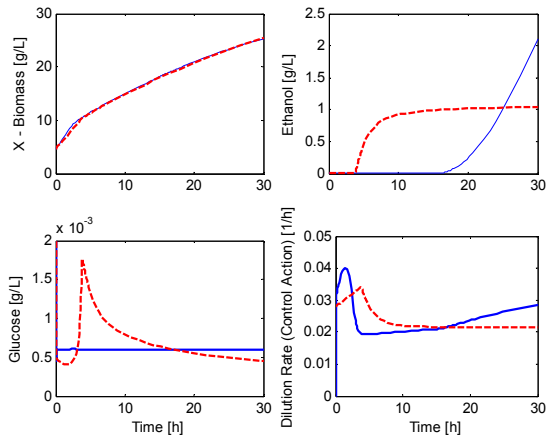


**Fig. 5:** Simulation results for glucose (solid lines) and ethanol (dashed lines) control. The set points were 0.0008 g/L and 1.06 g/L for glucose and ethanol, respectively.

Again the ethanol controlled version was able to maintain the glucose concentration at a low concentration level, while for the glucose controlled loop, the ethanol concentration becomes higher starting from 18 h. Nevertheless, the biomass concentration is almost the same for both cases.

### *4.3 High set point values*

To analyze the sensitivity of the glucose set point value, it has been changed to 0.0012 g/L, while the ethanol set point was maintained at 1.06 g/L. Fig. 6 shows the result of these simulations. Again the ethanol control has produced a much better result.

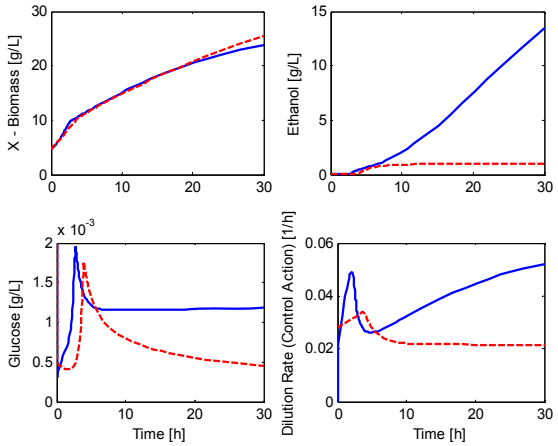


**Fig. 6:** Simulation results for glucose (solid blue lines) and ethanol (dashed red lines) control. The set points were 0.0012 g/L and 1.06 g/L for glucose and ethanol, respectively.

## 5 CONTROL ACTION COMPARISON

Now, it is time to emphasize the differences between the cybernetic and simplified Monod models concerning their application to bioreactor process control. Instead of comparing the controlled (output) variables, we will proceed the comparison on base of the corresponding calculated control action.

### *5.1 Low set point values*

The above subplot of Figure 7 compares the control actions calculated when the glucose is controlled using the simplified model (dashed line) against control action calculated on base of the cybernetic model (solid line). On the other hand, the below subplot shows the control actions calculated when ethanol is the controlled variable. These control actions are the same as shown in Figures 1 to 4, and correspond to low set point values for glucose and ethanol concentrations.

The main and the most important difference between the control actions occures not in the initial part, which is related more to the tuning, but in the middle time (20 h for glucose control and 13 h for ethanol control). The control action calculated by the cybernetic model is clearly nonmonotonic.

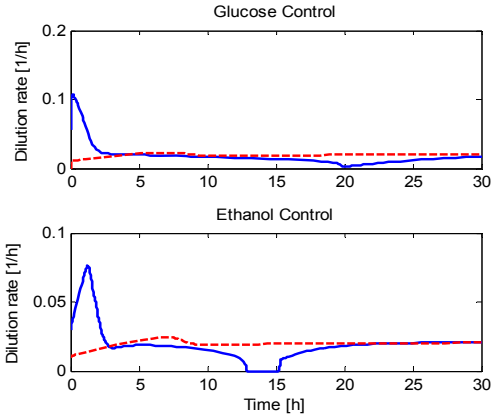


**Fig. 7:** Control actions calculated for low set point values using the simplified model (dashed line) and the cybernetic model (solid line)

### *5.2 High set point values*

For high set point values (Fig. 8), the control action pattern calculated by the cybernetic model are intensified and anticipated to occurre at 3 h and 2 h for glucose and ethanol control, respectively.

To clarify the difference between the models, Fig 9 shows the simulation of the cybernetic model (solid line) when it is submitted to the control action calculated by the simplified model. To make it easier to see the difference in these Figures, it is also included the prediction obtained with the same control action when the simplified model is simulated (dashed-dot line). The dashed line is the control action calculated using the cybernetic model, while the dashed-dot line is the simulated by the simplified model with the solid line control action.

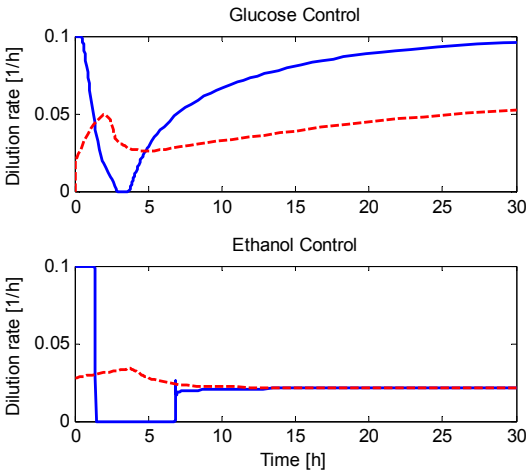


**Fig. 8:** Control actions calculated for high set points values using the simplified model (dashed red line) and the cybernetic model (solid blue line).

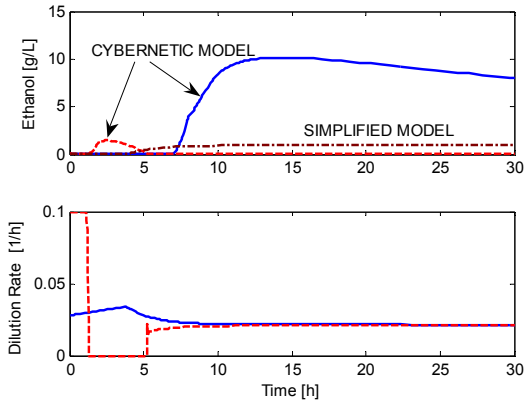


**Fig. 9:** Control action calculated using simplified model applied to the cybernetic model (solid line).

### *5.3 Optimal Control Action Design*

Based on the results presented until here, we can suggest the following easy way to implement control action profile. Fig 10 shows the typical profile.

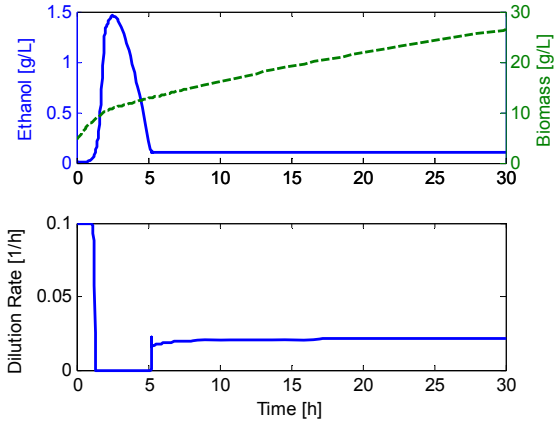


**Fig. 10:** Suggested control action profile. Solid line is ethanol concentration and dashed line, the Biomass concentration.

First a high dilution rate is used, when the ethanol concentration grows up, the dilution rate is turned to 0 and set to a final low value when the ethanol concentration decreases. This profile is very easy to be implemented and, if it works, there is no need to use a controller. However, an on-line ethanol analyzer like a biosensor must be available. The next step is to test this suggestion in a real cultivation.

## 6 CONCLUSIONS

In this contribution it was shown that glucose control has got a high sensitivity to the set point value, while ethanol control shows a smooth behavior. Therefore, we recommend controlling the ethanol concentration instead of low glucose concentration. In this paper it was also demonstrated that a simplified model is not able to produce satisfactory qualitative and quantitative results. To finalize, the paper has presented a quite simple and optimized control action. The proposed control action can be easily implemented and will be verify in our future work by experimental results.

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