Bioprocess Intelligent Control:
A case study

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Abstract—Bioprocesses are appreciated as difficult to control because their dynamic behavior is highly nonlinear and time varying, in particular, when they are operating in fed batch mode. The research objective of this study was to develop an appropriate control method for a complex bioprocess and to implement it on a laboratory plant. Hence, an intelligent control structure has been designed in order to produce biomass and to maximize the specific growth rate.

Keywords—fed batch bioprocess; mass-balance model; fuzzy control

I. INTRODUCTION

BIOPROCESSES are appreciated as difficult to control because their dynamic behavior is highly nonlinear and time varying, in particular, when they are operating in fed-batch mode. That is why they are interesting testing benches for non-linear and intelligent control techniques. Several techniques have been proposed [1-4] and tested by simulation, but only few have been implemented on real bioprocesses. There are two main reasons: at first, it is difficult to get a "good" model of the process which is experimentally validated in order to design the control algorithm; and then, the available on line instrumentation on a bioprocess is generally poor, there are only few sensors to deliver on line measurements of state variables required for control implementation.

II. PROBLEM STATEMENT BACKGROUND

The state of the art in the bioprocess control systems comprises three main control strategies [3, 5]: the classic control strategy based on *a priori* model describing the global evolution of the bioprocess, but due to the scarce bioprocess information, the model not being able to represent the whole bioprocess behavior; the control strategy based on adaptive techniques, without a global optimization ability (i.e. the bioprocess is optimized during a simple period); the control strategy based on intelligent techniques, which uses human subjective expert knowledge.

The research objective of this study was to develop an appropriate control method for a bioprocess and to implement it on a laboratory plant, namely the control of the fed batch cultivation of *Hansenula polymorpha* yeast for

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alcoholoxidase-containing biomass. At first, the process is described and a mathematical model is proposed and then the control strategy is defined and the intelligent control structure is designed. Finally, the control performances are tested through real data.

III. PAPER APPROACH

A. Methodology

A discontinuous fed-batch bioprocess for alcoholoxidase-containing biomass with the methylotrophic yeast *Hansenula polymorpha* CBS - 4732 was operated in an airlift lab - bioreactor The intracellular enzyme, to be separated further on, is used for obtaining a high-specialized kit for methanol/ethanol determination. The yeast was cultivated on a complex medium with $(NH_4)_2SO_4$, KH_2PO_4, Na_2HPO_4, $MgSO_4*7H_2O$, $CaCl_2$, yeast extract or autolysed residual beer yeast as organic N source and microelements (Fe, B, Cu, I, Mn, Zn, Mo).

$$\frac{dV}{dt} = -\frac{E_S}{\rho_S} - \frac{E_M}{\rho_M}$$

$$\frac{dX}{dt} = \frac{\mu_{max}S}{K_S + S}X + \frac{X}{V}\left(\frac{E_S}{\rho_S} + \frac{E_M}{\rho_M}\right) \qquad (1)$$

$$\frac{dS}{dt} = -\frac{\mu_{max}S}{K_S + S}\frac{X}{Y_{X/S}} - \frac{E_S\rho_S}{V} + \frac{S}{V}\left(\frac{E_S}{\rho_S} + \frac{E_M}{\rho_M}\right)$$

where: E_S and E_M are the substrate and medium loss by evaporation [g/h]; ρ_S and ρ_M are the substrate and medium densities [g/L]; $Y_{X/S}$ is the substrate conversion yield referred to the biomass [g dry matter/ g substrate]; μ is the specific growth rate [1/h]; V is the volume of the cultivation medium in the bioreactor [L]; X and S are the biomass and substrate concentrations [g/L] and *t* is the time [h], μ_{max} represents the maximum specific growth rate [1/h] and K_S is the saturation constant [g/g].

The main process parameters were: continuous temperature control $37^{\circ}C$; a minimal level of pO_2 - 10% from the saturation concentration was maintained during the exponential growth; continuous pH control between 4.5 - 5.0 by addition of NH_4OH (12.5%); no foam control, if the main parameters are optimally controlled. The unique C source, the methanol was introduced function of the yeast growth rate in connection with the substrate consumption rate for avoiding the growth inhibition by substrate concentration. The developed model (1) is based on the mass-balance principle and on the hypothesis of a non-inhibitive substrate effect (i.e.
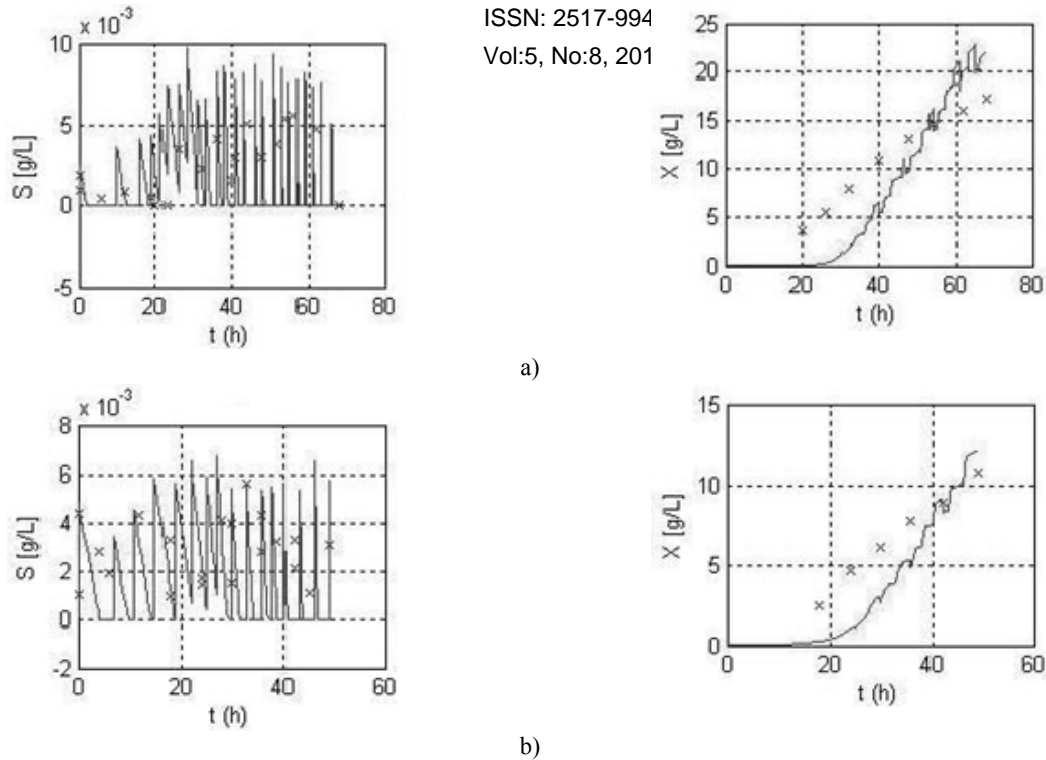
Fig. 1. Simulation results of the substrate and medium concentrations a) first experiment; b) second experiment; ('--' – simulation, 'x' – experimental data)

the specific growth rate is defined by the Monod equation). In line with the operation mode (fed-batch with discontinuous substrate feeding), there are discontinuous variations of the main variables due to: substrate feeding, medium feeding (to overcome the loss by evaporation or sample collection) or samples withdraws. That is why the following mass-balance equations are to be added to express each discontinuous modification for volume, and substrate or biomass concentrations:

$$V_k + A_{Sk} + A_{Mk} = P_{Mk} + V_{k+1}$$
$$S_k \rho_M V_k + A_{Sk} \rho_S = P_{Mk} \rho_M S_k + S_{k+1} \rho_M V_{k+1} \qquad (2)$$
$$X_k V_k = P_{Mk} X_k + X_{k+1} V_{k+1}$$

where: V_k, V_{k+1}=volume before / after modification [L]; A_{Sk}, A_{Mk}=substrate volume and respectively medium volume adding [L]; P_{Mk}=sample withdraw [L]. The same notations are used for S_k, S_{k+1} and X_k, X_{k+1}. We use: $\rho_S = 800$[g/L]., respectively $\rho_M = 1000$[g/L].

The identification of the model parameters was carried out based on measured values in order to minimize the modeling error. The identification procedure (i.e. Nelder-Mead algorithm) determines the optimum values for the following process parameters: E_S, E_M, μ_{max}, K_S and $Y_{X/S}$.

B. Experimental arrangements

The separate identification of the model parameters (decoupling of the model equations) was possible because the equation that describes the variation of the cultivation medium depends only on two parameters, the medium and substrate loss by evaporation. Based on the experimental data, in the first step, the identification procedure was able to determine the optimal values of the medium and substrate loss by evaporation. In the second identification step, the procedure determines the optimal values of the other remaining model parameters. The simulation results of the last two equations from (1) are presented in Figure 1, in comparison with the two experimental data sets.

The agreement between real and modeling data is good enough to allow the obtainment of the optimal substrate concentration for the growth process control. The identification results show the mathematical model closely follows the experimental data.

C. A case study

For this bioprocess, the overall control objective is to obtain large biomass quantities, based on the assumption that high biomass concentration will assure the obtaining of important alcoholoxidase-active biomass.

In this paper a control system based on fuzzy logic is proposed. It is well known that fuzzy control systems can manipulate incomplete and incert information about the process assuring high control performances [4].

The proposed fuzzy control system receives information about the state of the bioprocess expressed by the biomass and substrate concentrations. Substrate concentration can be measured on-line while biomass concentration can be determined by using optic density measurements. Based on this information the fuzzy control system computes the quantity of substrate to be added into the reactor. Thus the fuzzy system must correlate the substrate to be added with the conditions existing in the cultivation medium. For avoiding high substrate additions that might inhibit the cell growth the fuzzy system can also compute 'negative' corrections that actually mean a decrease into the substrate addition.
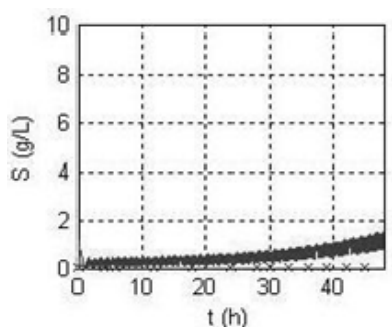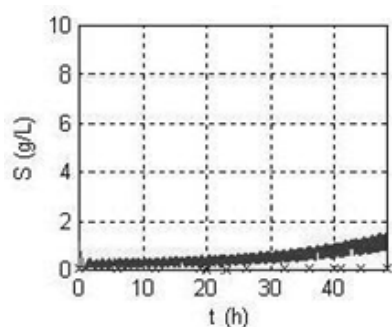
According to these observations the inputs of the fuzzy system are the biomass (X) and substrate (S) concentrations. The output of the fuzzy system is the correction to be applied on the substrate addition. This value will be computed according to the 'if-then' rules in the rule base of the system. For symbolic description of the two inputs three triangular membership functions were used that represents the small (S), medium (M) and large (L) values of the inputs in their universe of discourse. The symbolic description of the output was made using five triangular membership functions for negative big (N), negative small (NZ), zero (Z), positive small (ZP) and positive big (P) values of the output on the universe of discourse.

The production rules that are used for command computing were established based on human experts information and observations regarding the characteristics of the process under study. The rules of the fuzzy system are presented in Table 1.

Rules evaluation by the inference engine is made according to the min-max inference rule and the output defuzzyfication is made based on the centroid defuzzyficaton method.

IV. RESULTS AND DISCUSSIONS

The control loop was implemented in MATLAB, version 7.5. For control loop simulation the proposed mathematical model was used and the simulation results were compared with the experimental data.

concentration marks the substrate consumption and additions, as well as the increase of the additions along with cell growth. The biomass concentration obtained by simulation follow closely the experimental data.

V. CONCLUSIONS

An intelligent control structure has been designed for a complex bioprocess in order to maximize the producing enzyme-biomass growth rate. Several sets of experimental data were used to test and validate with good results the mathematical model and further on the control system, and two sets results are represented in the paper. Further work will deal with neuro-fuzzy control scheme. The success of the control implementation is critically dependent upon the technical operating conditions of the process.

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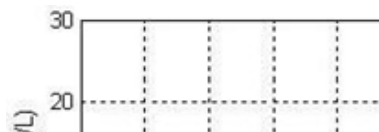[8] K. Schugerl, K. H. Bellgardt, Bioreact. Eng, Springer Verlag, Berlin, 2000

a)

TABLE I
THE RULE BASE

X_k / S_k	S	M	L
S	Z	PZ	P
M	NZ	Z	PZ
L	N	NZ	Z

b)

Fig. 2. Simulation results of the control loop: a) first experiment; b) second experiment; ('-' – simulation results; 'x' – experimental data)

The simulation results (Figure 2) show that the proposed fuzzy control system is capable of computing the substrate feedings needed for cell growth according to the biomass concentration increase. The evolution of the substrate