Quality Fed-Batch Bioprocess Control
A Case Study

Mihai Caramihai, and Irina Severin

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 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. APPROACH

A. Methodology

A discontinuous fed-batch bioprocess for alcoholoxidase-containing biomass with the methyloptrophic 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 (NH4)2SO4, KH2PO4, Na2HPO4, MgSO4*7H2O, CaCl2, yeast extract or autolysed residual beer yeast as organic N source and microelements (Fe, B, Cu, I, Mn, Zn, Mo).

\begin{equation}
\frac{dV}{dt} = \frac{E_S - E_M}{\rho_S - \rho_M} \\
\frac{dX}{dt} = \frac{\mu_{\text{max}} S}{K_S + S} X + \frac{X}{V} \left( \frac{E_S + E_M}{\rho_S - \rho_M} \right) \\
\frac{dS}{dt} = \frac{\mu_{\text{max}} S}{K_S + S} Y_{X/S} - \frac{E_S \rho_S}{V} - \frac{S}{V} \left( \frac{E_S + E_M}{\rho_S - \rho_M} \right)
\end{equation}

where: E_S and E_M are the substrate and medium loss by evaporation [g/h]; \rho_S and \rho_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]; \mu 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], \mu_{\text{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°C; a minimal level of pO2 - 10% from the saturation concentration was maintained during the exponential growth; continuous pH control between 4.5 - 5.0 by addition of NH4OH (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.
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:

\begin{align}
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} \\
X_k V_k &= P_{Mk} X_{k+1} + X_{k+1} V_{k+1}
\end{align}

(2)

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: \( \rho_S = 800 \text{[g/L]} \),
respectively \( \rho_M = 1000 \text{[g/L]} \).

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: \( E_S, E_M, \mu_{\text{max}}, K_S \) and \( Y_{X/S} \).

\[ \text{B. Experimental Arrangements} \]

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.

\[ \text{C. A Case Study} \]

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
alcohol oxidase-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.

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 I.

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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.

The simulation results (Fig. 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 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. CONCLUSION

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.

REFERENCES


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