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Aerobic Bioprocess Control Using Artificial Intelligence Techniques

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***Abstract*—**This paper deals with the design of an intelligent control structure for a bioprocess of *Hansenula polymorpha* yeast cultivation. The objective of the process control is to produce biomass in a desired physiological state. The work demonstrates that the designed Hybrid Control Techniques (HCT) are able to recognize specific evolution bioprocess trajectories using neural networks trained specifically for this purpose, in order to estimate the model parameters and to adjust the overall bioprocess evolution through an expert system and a fuzzy structure. The design of the control algorithm as well as its tuning through realistic simulations is presented. Taking into consideration the synergism of different paradigms like fuzzy logic, neural network, and symbolic artificial intelligence (AI), in this paper we present a real and fulfilled intelligent control architecture with application in bioprocess control.

***Keywords*—**Bioprocess, intelligent control, neural nets, fuzzy structure, hybrid techniques.

I. Introduction

BIOPROCESSES are seen as difficult to control as their dynamic behavior is highly nonlinear and time-varying, especially when operating in fed batch mode. Therefore, several testing solutions may be defined regarding nonlinear and intelligent control techniques. Numerous solutions have been recommended [1]-[4] and tested by simulation, but only few have been applied on real bioprocesses. There are two main motivations:

(i) It is difficult to obtain a "good" model of a bioprocess which is experimentally validated in order to design the control algorithm;
(ii) The instrumentation available online is generally inadequate, i.e. it is only a short list of sensors to deliver on line measurements necessary for control design & execution [5].

For this kind of bioprocess, where the mathematical model contains many structured and unstructured uncertainties, we try to combine different intelligent techniques like fuzzy logic, neural networks and symbolic AI based on natural syllogism of these techniques. In our application, neural networks are used for identification of operation regimes of bioprocess and model parameters. At the same time, the fuzzy logic structure is used for the substrate control. Therefore, we have to combine the neural networks and fuzzy logic controller for this application.

We appreciate that a model-based design control algorithm combined with heuristic control strategy could be more efficient. In this case, the modeling, identification and control strategies are developed taking into consideration of the parameters and the model structure changes in real bioprocess operation mode. The high level of uncertainties in mathematical modeling recommends considering the fuzzy modeling and intelligent techniques for control.

The state of the art in the bioprocess control systems comprises three main control strategies:

- A classic control strategy based on *a priori* model that is able to describe the overall bioprocess development [6];
- An adaptive control strategy: The control strategy is based on adaptive techniques without a global optimization approach (i.e. the bioprocess is optimized only during a simple time period)
- An intelligent control strategy: The control strategy is based on intelligent techniques that use the human expertise [7], [8].

In order to obtain a high bioprocess productivity [9], it is essential to ensure the benefits of classical control strategy (i.e. the analytical determination of optimum) with bioprocess subjective characterization (due to human expert) in order to reduce the on line information scarcity This is the basic conception for the HCT design, a new approach to the bioprocess intelligent control systems.

The main objective of this paper is to analyze the development of a control method suitable for a specific bioprocess and to implement this method on a laboratory plant. Although different types of control strategies have been implemented, the control of the fed-batch cultivation of *Hansenula polymorpha* yeast for *alcoholoxydase* obtaining has not been successfully completed due to the following reasons:

- The lack of accurate mathematical models which can describe the bioprocess behavior;
- The very complex interaction between the cells and environment;
- The lack of reliable online sensors which can monitor the process variables;
- The slow responses of the fermentation process.

To overcome these problems, various approaches including classical control structures or intelligent techniques have been applied [10]-[12]. The control structure performances, however, heavily rely on the aspects described above. Hence, a control strategy based on HCT will be developed: The process is explained, a mathematical model is designed and a control strategy based on intelligent control structure is proposed – in order to be tested with real data.

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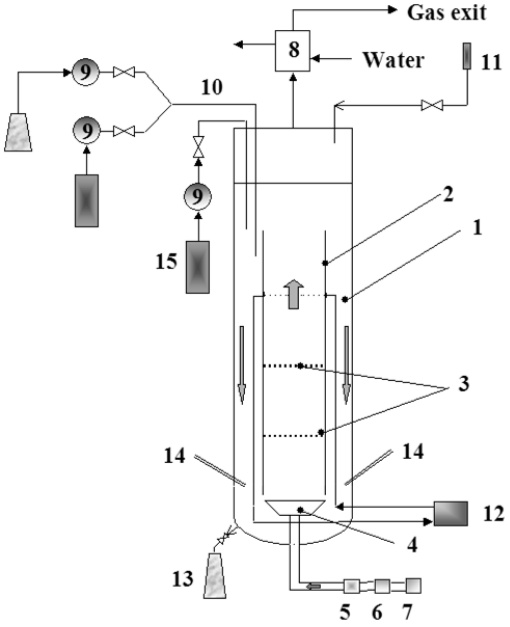


Fig. 1 Airlift bioreactor: 1 – vessel, 2 – draft tube, 3 – perforated plates against coalescence, 4 – air sparger, 5 – air filter, 6 – gas flowmeter, 7 – air compressor, 8 – gas condenser, 9 – pumps, 10 – inoculum/substrate addition, 11 – antifoam addition, 12 – water circulation jacket, 13 – medium exit, 14 – pH, pO_2 and temperature sensors, 15 – pH reagent addition

II. Bioprocess Description and Modeling

A fed-batch bioprocess for alcoholoxydase obtaining with the methylotrophic yeast *Hansenula polymorpha* CBS - 4732 was operated in an airlift lab - bioreactor (airlift with concentric tube and normal circulation – Fig. 1). The hydrodynamic behavior and the interfacial oxygen transfer rate (OTR) were studied in this airlift reactor in order to establish the optimal geometric configuration and the operation variable (air flow rate) evolution domain in accordance with the aerobic process requirements. Thus, the reactor, made from thermo-resistant glass, is characterized by the next parameters: sr=13; A_r/A_d = 0.5; V_L = 5 L; two perforated plates against coalescence placed into the riser section. Meanwhile, since a turbulent flow regime is required, the minimum level for the air flow rate is Q ≥ 900 L/h. The enzyme is used for obtaining a high-specialized kit for methanol/ethanol determination. The yeast was cultivated on a complex medium containing $(NH_4)_2SO_4$, $CaCl_2$, $MgSO_4*7H_2O$, Na_2HPO_4, KH_2PO_4, yeast extract or autolysed residual beer yeast as organic N source and microelements (Fe, B, Cu, I, Mn, Zn, Mo).

The main process parameters were:

- Continuous temperature control 37-38 ^oC;
- Continuous pH control between 4.5 and 5.0 (by addition of NH_4OH (12.5%));
- Level of pO_2 - 10% from the saturation concentration (maintained during the exponential growth);
- No foam control, whether the main parameters are optimally controlled.

The methanol was the unique C source, and introduced depending on the yeast growth rate in connection with the substrate consumption rate (in order to avoid the yeast growth inhibition by substrate concentration).

The previous studies [13], for airlift performance characterization and bioprocess optimization, demonstrated that on these conditions, a high level of alcoholoxydase was accumulated during the first part of the exponential phase growth. So that, the assumption of a growth associated product formation model becomes plausible.

According to Gaden [14] four types of product accumulation can be distinguished on the formal level based on the quantitative relationship between the product kinetic and the growth of cells:

- Type 0: The microbial cells function only as enzyme carriers;
- Type 1 includes processes in which product accumulation is directly associated with growth;
- Type 2 includes fermentation where there is no direct connection between growth and product formation;
- Type 3 includes bioprocesses with a partial association between growth and product accumulation.

Conforming to the studies presented in the experimental part, a growth associated product accumulation model (type 1) is to be considered.

Several literature [15]-[17] and original models [18], [19] of this type were designed and checked with the experimental data. Finally, the most promising proved to be the following:

$$\frac{dP}{dt} = k_1 \frac{dX}{dt} e^{k_2(X_p - X)} \quad (1)$$

where: k_1, k_2 = constants; $e^{k_2(X_P - X)}$ = autocatalysis factor (which expresses the special nature of a growth associated enzyme as bioprocess product); X = cells concentration expressed as dry weight [g/L]; Xp = cells concentration corresponding to a threshold level [g/L]; P = alcoholoxydase activity [IU/mL of extract]. Note that k_1, k_2 and X_P are *a posteriori* values. Three experiments were performed in order to establish the model parameters. The experimental results are presented in Table I. The alcoholoxydase is an intracellular enzyme, so there is a complex procedure for the preparation after the biosynthesis process (cells disruption, extraction with adequate solutions, extract concentration, etc.). The evolution of the product rate may be expressed in a first form, as the activity of the enzyme determined by a standard biochemical analysis, in the volume of extract (mL) obtained after cells' disruption and extraction.

Equation (1) was checked with the experimental data presented in Table I. Using the DADiSP 6.7, SIGMAPlot 13 and SYSTAT 13 software packages on PC Pentium II computer, the results presented in Table II were obtained.

Fig. 2 represents the product accumulation rate evolution - model curves by comparison with the experimental data for three representative fed batch runs. The presented model can describe the enzyme activity evolution during the fed batch cultivation; by comparison with the classical form of a growth

associated product accumulation model. Introducing the concept of "autocatalysis factor" expresses the fact that the enzyme is a particular product, which is used as catalyst for culture growth sustaining. In the three experiments, two levels of alcoholoxydase activity can be reached (see Table I) based on the two enzyme systems of the cell metabolism which is used as a catalyst, and probably irrespective of the conditions of cultivation.

TABLE I
EXPERIMENTAL DATA

Time	Exp. 1		Exp. 2		Exp. 3	
[h]	D.W [g/L]	EA [IU/mL]	D.W. [g/L]	EA [IU/mL]	D.W. [g/L]	EA [IU/mL]
8	0.05	1.15				
12	1.47	2.09				
16	5.3	2.73				
19	7.68	4.41				
21			7.44	6.17	5.07	22
25			8.12	5.74		
28			9.48	5.61	7.67	26
29	15.83	4.47	11.52	5.23		
31					11.07	22.8
34	19	5.14	13.79	4.9	13.79	24
37			16.73	4.6		
39			18.54	4.43	14.24	24
42					18.55	26

TABLE II
PARAMETER VALUES

Results	Exp. 1	Exp. 2	Exp. 3
Xp	10.39	4.73	8.13
k_1	4.39	8.7	41.76
k_2	-0.03	0.04	0.06
red. χ sq.	0.06577	0.05694	0.08248

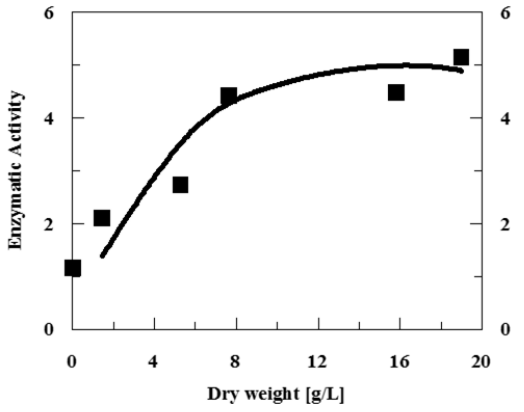
The idea of two different systems for the enzyme using in the cellular growth is sustained by the good fitting of the same model, but with two levels for the k1 value (k1 experiment 3 >> k1 experiment 1 and 2). Meanwhile the parameter X_P represents a threshold value, which defines the present model behavior in comparison with the classical Gaden model:

- if $X_P = X$, (1) corresponds to Gaden model;
- if $X_P < X$, product rate is greater than product rate obtained by Gaden model;
- if $X_P > X$, product rate is lesser than Gaden product rate.

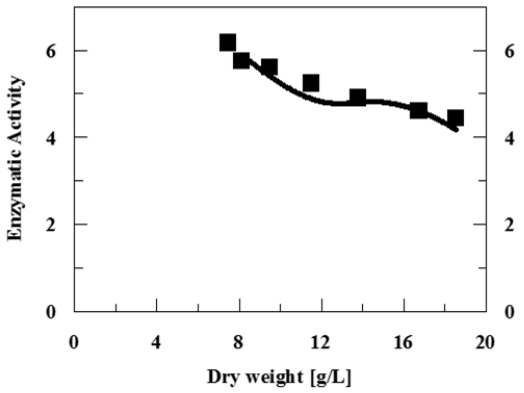
For these reasons, the control strategy for the *Hansenula polymorpha* cultivation must stop when the biomass concentration tills to the threshold value, X_P. In the other case, the bioprocess becomes sub optimal.

III. CONTROL PROBLEM STATEMENT

For this bioprocess, if the cell concentration (X) is smaller than the threshold value (X_p) the product rate is bigger than the Gaden's equation value. In this case, the set point value X_{SP} must be smaller than X_p, and the substrate concentration is the control variable.



(a)



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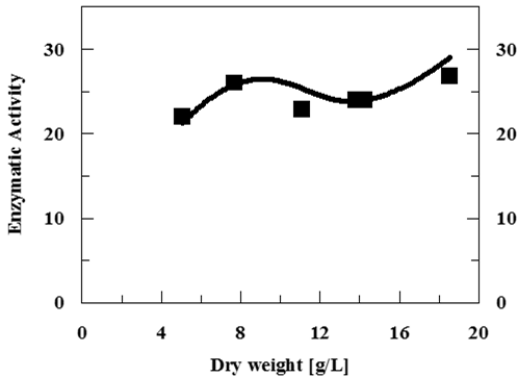


(c)

Fig. 2 Product rate evolution - comparison between experimental curve and proposed model; (a) experiment 1; (b) experiment 2; (c) experiment 3; EA [microM methanol/min/mL of extract], proposed model; EA [microM methanol/min/mL of extract], experimental data

The control objective is to maximize the alcoholoxydase production rate (dP/dt) in connection with the cells optimal physiological state. Therefore, the bioreactor is to be fed with substrate (S) so that the substrate concentration is regulated inside the bioreactor at a set point corresponding to a desired biomass specific growth rate.

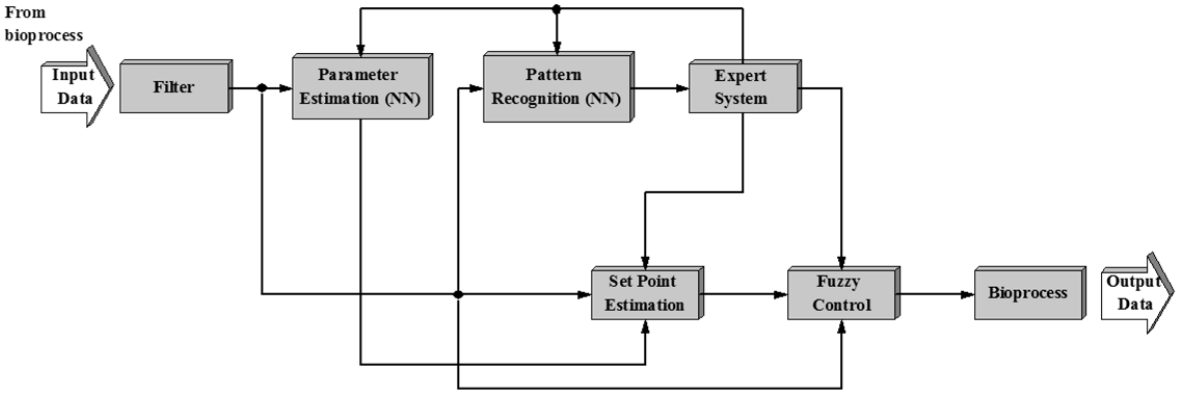


Fig. 3 Intelligent structure for bioprocess control

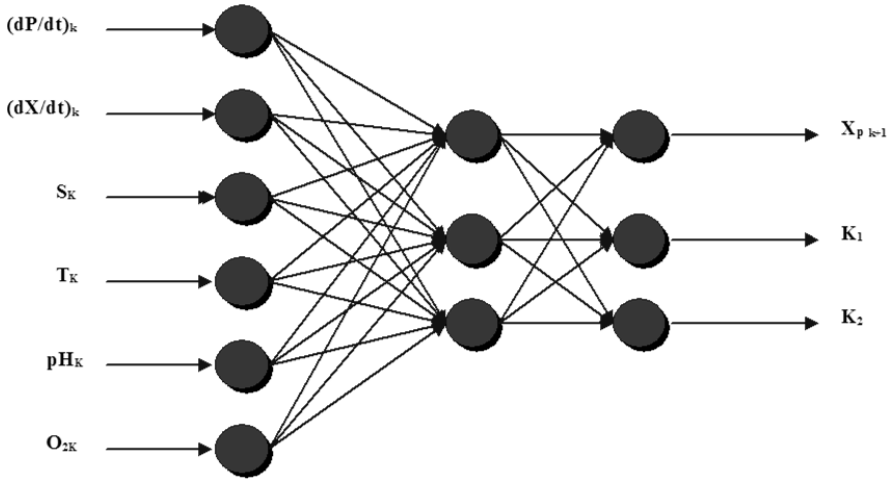


Fig. 4 The neural net for parameter estimation

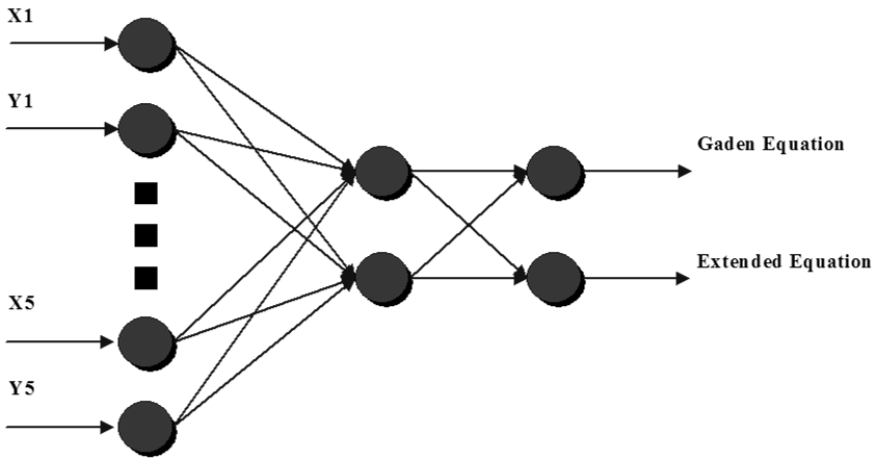


Fig. 5 Neural net for pattern recognition

A. Design of the Intelligent Control Structure

According to the previous model, the aim is to control a non-linear, time varying system with a delay time and constrained variables. To solve this control problem, a Hybrid Control Structure (HCS) is proposed [20]. Fig. 3 gives its principle. The main objective of the control strategy is to maximize the alcoholoxydase production rate. This objective can be realized by control of the substrate concentration (S) in such a way that the set point variable X_{SP} converges towards X_P in order to maximizes the alcoholoxydase production rate. The subsequent blocks compose the control system:

- Filter: The input data filtration is indispensable in any bioprocess control system. Through the input data system, the measurement noise influences the decision of the control structure and the real data are modified. Hence,

the filter must eliminate the non-consistent data values. The filter has been tested in comparison with both implemented algorithms (the smoother algorithm and TUKEY algorithm) for all the input data sets. Both procedures validate all input data. Hence, interpolated values are not mandatory. From this point of view, the interpolated data influence upon the HCS was not being tested. Moreover, this influence can be considerable and it is an important condition to be analyzed in the future

- Neural Net for Parameter Estimation (NNPE): The design of the intelligent control system involves the on line estimation of bioprocess model parameters, i.e. the set point value estimation can be built in connection with the possibility to check the bioprocess evolution. The training datasets are randomly generated within the framework of imposed domain values. The validation of the estimated values was achieved by distinguishing between the real and simulated bioprocess variable evolution (the last one was obtained using the parameter estimated values). In this case the on line measured parameters are: temperature (T), pH, the oxygen concentration (pO_2) and off line: the substrate (S), the biomass concentration (X) and product rate (dP/dt). The NNPE must estimate the biomass threshold value (X_P) and the model parameters (k_1, k_2,). The neural net structure is shown in Fig. 4.

It must be noted that the neural network is fully interconnected.

- Neural Net for Pattern Recognition (NNPR): In concordance with (1), there are two bioprocesses types; the first one is in accordance with the Gaden equation (where X = Xp), and the second one is in accordance with (1). The neural net comprises 25 inputs and 2 outputs and is shown in Fig. 5.

The neural net outputs are assigned to two linguistic labels: "Gaden equation" when the biomass concentration attempts the threshold value and "Extended equation" otherwise. An example for the qualitative bioprocess curves is displayed in Fig. 6

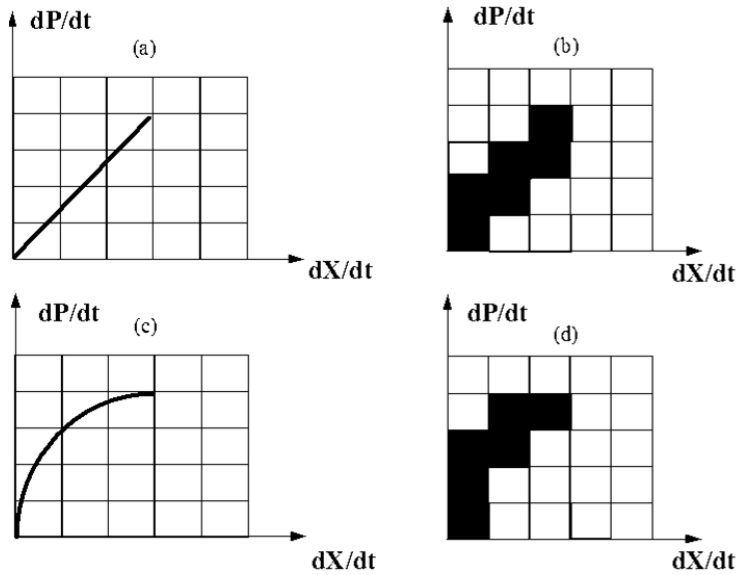


Fig. 6 The digitized evolution curves for an alcoholoxydase production bioprocess; (a), (b) classical evolution (conforming to Gaden eq.); (c) (d) non-standard bioprocess evolution (1)

Bioprocess Type	Selection			
	Set Point Estimation	Pattern Recognition (NN)	Parameter Estimation (NN)	Fuzzy Control
Alcoholoxydase production	X_{max}	• Gaden eq.; • Equation (1)	X_P, k_1, k_2	*dP/dt=f(S)*

Fig. 7 Conceptual structure of the Expert System (Decision-Making Module)

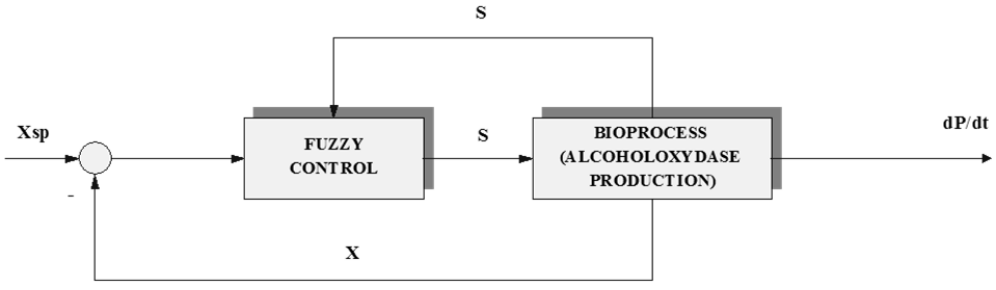


Fig. 8 Fuzzy Control Structure for alcoholoxydase production

It must be noted that the neural network is fully interconnected.

- Expert System (ES) (i.e. Decision-Making Module): The main goal of the intelligent control structure designed in this work is to maximize the performance index trough pattern curve recognition and the applicability of a suitable fuzzy algorithm. The evolution type validation is build through a large bioprocess type class, *a priori* imposed by a human expert. Hence, the human operator checks (at the start point time, $t_0 = 0$) the bioprocess class (whom the process take part) and the data filtration method. The bioprocess classes are defined within the framework of the expert system. New bioprocess classes can be added, with respect to the technological operation needs. Once the selection is performed, the expert system selects (from the knowledge database) the working modules for each component block of the control structure (Fig. 7). Now, the system is ready to work. The on line measured data affects the filter block. Hence, there are two outfitted means to "clean" the input data: The smooth method and the Tuckey method. After the filtration, the data attain the NNPR, NNPE and SPEB. Note that the input data reach these blocks (excepting the database) after the accommodation cell period (e.g. the lag period). The NNPE gives the parameter values at each sampling time. The on line measured values in addition with the estimated values become the input data for the SPEB which transfer the estimated set point values to the fuzzy control block. At last, the NNPE detects the most likely bioprocess evolution curve (if all the network outputs have identical values, the selected output is the most simple, e.g. the non-inhibitory model, the Gaden model, etc.).

Thus, the intelligent control structure can "recognize" the bioprocess evolution curve, *ipso facto* checks the corresponding fuzzy control algorithm. The fuzzy control block draws up the control action in connection with the performance index. The control algorithms, based on human expert experience, are assumed as Fuzzy Associated Matrix (FAM). Concerning a bioprocess class, a single control algorithm is available (only the set point values are adjustable). Following these steps, a correlation between the intelligent control flexibility and the control strategy based on *a priori* model is useful. Furthermore, the bioprocess evolution curve selection implies the determination of the corresponding analytical model, i.e. the parameter values and the set point values can be obtained. Hence, these values can be modifiable in conjunction with the bioprocess evolution and the control algorithm tunes oneself (according to FAM) to the set point values.

- Set Point Estimation Block (SPEB): In the case of control systems with *a priori* model, the optimum values must be estimated through specific procedures that can exclude the off line bioprocess information. Hence, each time the on line information is lower, the optimal estimated values are uncertain, i.e. the solution can be non singular. On the other hand, the parameter values can lose their physical significance (e.g. negative values) and the model fails the meaning. Conforming to the bioprocess description, the alcoholoxydase production occurs in the first part of the growth phase, i.e. the associated model is the Gaden equation (if $X=X_P$), or (1) otherwise. Hence, the SPEB estimates the biomass concentration (X) – using a Runge-Kutta procedure in (1) – and increments this value by 1% to pass it as set point to the FC, until the X_P value is attempted.
- Fuzzy Controller (FC): The fuzzy control structure is shown in Fig. 8

The bioprocess output is dependent on substrate and biomass concentration, i.e. $\frac{dP}{dt} = f(X,S)$, but only by the substrate addition the product rate is controlled. The FAM for this bioprocess is presented in Fig. 9.

The notation significance of fuzzy labels is NB= Negative Big, NM= Negative Medium, NS= Negative Small, Z= Zero, PS= Positive Small, PM= Positive Medium, PB= Positive Big. The FAM was designed upon human experience.

The control objective is to maximize the alcoholoxydase production rate (dP/dt) in connection with the cells optimal physiological state. Therefore, the bioreactor is to be fed with substrate (S) so that the substrate concentration is regulated inside the bioreactor at a set point corresponding to a desired biomass specific growth rate ($\varepsilon = X_{SP} - X$); the set point value comes from SPEB.

Biomass Concentration Error \ Substrate Concentration	Z	PS	PM	PB
NS	PB	PS	PS	Z
NM	PB	PM	PS	Z
NB	PB	PM	PS	PS
Z	PB	PM	PS	Z
PS	PB	PB	PB	PM
PM	PB	PM	PS	PS
PB	PB	PM	PS	Z

Fig. 9 FAM for an alcoholoxydase production bioprocess

IV. Simulation Tests

The control structure has been tested through simulations, by using the previous model (1) under real data, in order to analyze the control performances in regulation and in set point tracking. The universe of discourse, U_1, for the $\varepsilon = X_{SP} - X$ variable and the control universe, V, for the substrate concentration are presented in Table III. Thus, Fig. 8 illustrates the simulation results. When $X > X_P$ (sub optimal condition) the substrate addition has a small influence and a

negative slope is shown. (see Table III). From this moment, the regulation is no more possible because the control transfers an inhibition to the biomass growth; that is to say the substrate consumption by the yeast is lower than the maximal substrate feed rate, therefore, the process is stopped. Hence, in experiment 1 the bioprocess was stopped when the biomass concentration attempts the threshold value, X_P. As one may observe (Fig. 10 (a)), the evolution curve model is above to the experimental one. The experiment 2 shows the bioprocess evolution via biomass concentration superior to the threshold value ($X > X_P$); the alcoholoxydase production rate is bigger than the experimental data, but the slope is negative. Finally, Fig. 10 (c) shows similar results (simulation curve vs. experimental data), considering the enzymatic activity determined error (± 20%).

TABLE III
THE UNIVERSE OF DISCOURSE U_1 AND THE OUTPUT UNIVERSE V

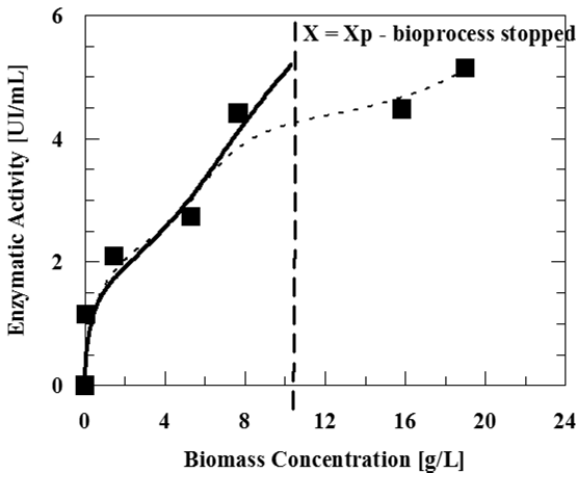
Experiment	U_1	V
Experiment 1	0 ÷ 10.39	0 ÷ 40
Experiment 2	0 ÷ 4.73	0 ÷ 40
Experiment 3	0 ÷ 8.13	0 ÷ 40

The performances in disturbance rejection have also been tested: For instance, a disturbance on the input flow rate is appropriately rejected by this control. By the same way, the robustness of this control method with respect to modeling errors (errors on the kinetic model) may be exhibited. In conclusion, these simulations under realistic conditions taking into account the process non-linearity and constraints show the good performance of the HCT.

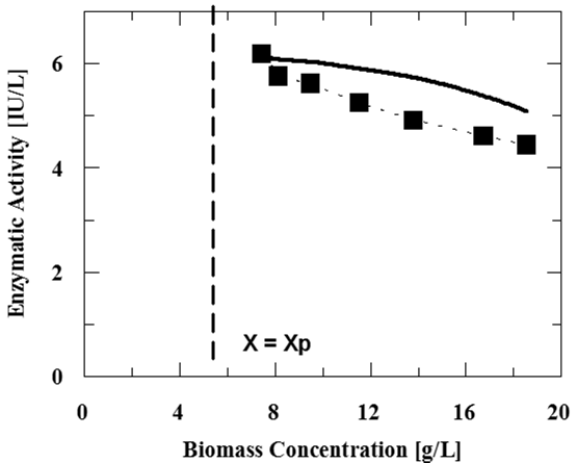
V. CONCLUSION

An intelligent control structure has been designed for a complex bioprocess in order to produce an enzyme in a desired biomass physiological state and to maximize the product formation rate. It has been tested by simulation and a first step (three experiments) has been carried out on a laboratory reactor for validation. The geometry of this reactor, airlift type with concentric tube and normal circulation, was established based on the correspondence between the aerobic bioprocess requirements and the study results on the hydrodynamic behavior and oxygen transfer capabilities.

The experiment tests show that the proposed control method responds to the objective, but, because of technical problems on the experimental process, it does not allow the quantification of the control performances. A simulation study has showed that this control method is quite appropriate for this non-linear, time varying system in spite of its constrained variables. The tuning of the design parameters is easy and the method performances are interesting in set point tracking, as well as in regulation. In particular, it seems to be less sensitive to the modeling errors that, in this application, induce deviations on the control algorithm state variables. To cope with this problem, more attention has to be paid to modeling aspects; and, it would be interesting to on line estimate the unmeasurable state variables, in particular the biomass concentration.



(a)



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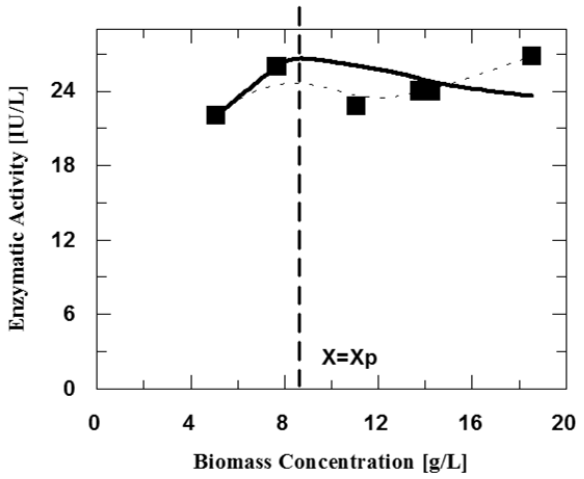

(c)

Fig. 10 *Hansenula polymorpha* cultivation for alcoholoxydase production (simulation results) – fuzzy control; (a) experiment 1; (b) experiment 2; (c) experiment 3; ▪ - real data; ——— - simulation values

Finally, it should be noted that the successful control implementation is critically dependent on the operating conditions of the technical process.

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