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FUZZY CONTROL APPLICATION FOR THE BIOPROCESS CONTROL OF A THERAPEUTIC PRODUCT PREPARATION

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Cercetarea descrisă în această lucrare face parte dintr-un proiect complex, care studiază optimizarea bioprocesului de preparare a unui imunomodulator obținut din celule bacteriene. Bioprocesul este realizat într-un bioreactor aerob de 100 L Bioengineering, cu 42 L mediu de cultură, echipat cu senzori pentru pH, temperatură, oxigen dizolvat şi turație.

Principalul obiectiv al acestei lucrări este de a prezenta un studiu de caz, prin care să se demonstreze că o structură de control inteligent, care descrie complexitatea procesului biologic într-o manieră calitativă şi subiectivă, aşa cum este percepută de către operatorul uman, este o strategie de control eficientă pentru acest tip de bioprocese. Pentru a simula evoluția bioprocesului a fost proiectată o structură de control inteligent, bazată pe logica fuzzy. BIOSIM, o aplicație software originală, pune în aplicare o astfel de structură de control. Rezultatele obținute prin simulare au demonstrat că tehnica fuzzy este destul de potrivită pentru acest sistem non-linear, variabil în timp vs metoda clasică de control.

The research described in this paper is a part of a larger experimental project dealing with the bioprocess optimization for an immunomodulator product preparation extracted from the harvested cells. The bioprocess is performed in 100 L Bioengineering bioreactor with 42 L cultivation medium, equipped with pH, temperature, dissolved oxygen, and agitation controllers.

The main objective of this paper is to present a case study to demonstrate that intelligent control, describing the complexity of the biological process in a qualitative and subjective manner as perceived by human operator, is an efficient control strategy for this kind of bioprocesses. In order to simulate the bioprocess evolution, an intelligent control structure, based on fuzzy logic has been designed. BIOSIM, an original developed software package, implements such a control structure. The simulation study has showed that the fuzzy technique is quite appropriate for this non-linear, time varying system vs. the classical control method.

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1. Introduction

The research described in this paper is a part of a larger experimental project dealing with the bioprocess optimization for an immunomodulator product preparation extracted from the harvested cells. The production of immunomodulator product is associated with cell growth rate. Hence, the main research objective was to obtain large biomass quantities. The specific objective is to present a fuzzy control approach, based on human expert' rules vs. a modeling approach of the cells growth based on bioprocess experimental data. The kinetic modeling may represent only a small number of bioprocesses for overall biosystem behavior while fuzzy control system (FCS) can manipulate incomplete and uncertain information about the process assuring high control performance and provides an alternative solution to non-linear control as it is closer to the real world [1-2]

The bioprocesses are appreciated as difficult to control because their dynamic behavior is highly nonlinear and time varying. In order to be able to control such bioprocess the artificial intelligence techniques can be applied, such as the fuzzy modeling. Fuzzy logic provides an inference morphology that enables approximate human reasoning capabilities to be applied to knowledge-based systems [3]. The decisional matrix of the control system is determined by the transition from the objective level of information to the subjective one (i.e. the information version level). Thus, the interest is focused on human expert experience (outlined through fuzzy rules) rather than information algorithmic process [4].

2. Paper approach

2.1. Methodology

The optimization of the aerobic bioprocess with *Pseudomonas aeruginosa sp*. is to be performed in case of a bacterial immunomodulator preparation. As the formation of the immunomodulator product is growth-associated the main research objective was to get big cellular concentration. The experiments were done in a bottom driven and aerated 100 L Bioengineering® bioreactor with 42 L aqueous Organotech® peptone solution as main culture substrate. The reactor was equipped with pH, temperature, dissolved oxygen, air flow, foam, and agitation controllers. The controlled parameters of the bioprocess are the followings: temperature: 37 ^{o}C; impeller speed: 250-300 rpm; air flow rate: 20-40 L/min; pH: 7.3. The cellular growth is determined by a standard dry-weight method (usual procedure at drying at 105 ^{o}C) and by off-line determining of the Optical Density

(OD at λ = 570 nm. The substrate consumption was determined by analyzing the aminic nitrogen (Sörensen method) [5].

2.2. BIOSIM

The research objective of this study was to design an intelligent control structure, based on fuzzy logic and to implement it on a laboratory plant. Fuzzy logic can handle uncertainty, ambiguity and vagueness and provides a means of translating qualitative and imprecise information into quantitative (linguistic) terms. Linguistic description in the form of membership functions and rules make up the model. The rules are generated *a priori* from expert knowledge. The basis for fuzzy logic is the basis for human communication.

The basic idea of a fuzzy inference system is to incorporate human's knowledge into a set of fuzzy IF-THEN rules, which involve operations of four components: a fuzzifier, a fuzzy rule base, a fuzzy inference engine and a defuzzifier. The general inference system can be shown in Fig. 1 [6].

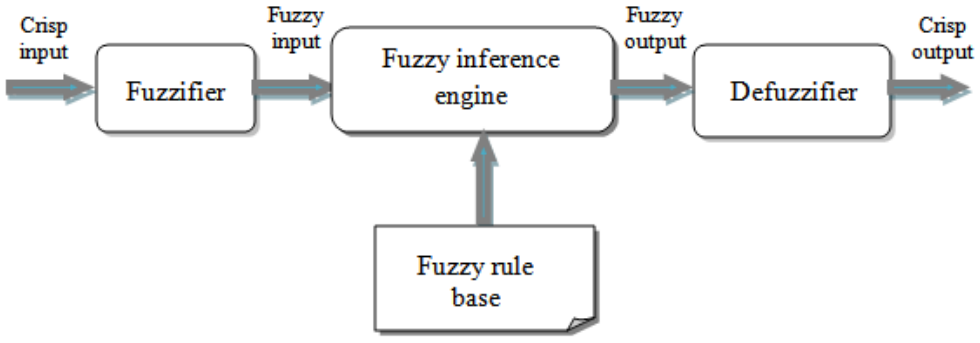

Fig.1. General structure of a fuzzy inference system

BIOSIM, an original developed software package, implements a developed control structure and allows the comparison between the performances of closed-loop fuzzy control and of the open-loop control.

2.2.1. The controller:

The BIOSIM software has a fuzzy controller at its core. The FCS characteristics are:

- For the fuzzyfier three triangular membership functions were used;
- The inferences engine uses the tables of rules to decide upon the degree of membership for the fuzzyfied values;
- The defuzzyfier applies the centroid method, a convex optimization technique.

2.2.2. The controller use:

The controller takes as crisp inputs the cellular concentrations (X) and the substrate concentrations (S). Based on the inference tables, the controller computes a value for the substrate to be added in order to get high growth rate.

2.2.3. Simulation:

The simulation routine makes use of this value to modify the current value of the substrate, which is employed in computing the new cellular concentration. The determination of the cells' concentration function of the substrate concentration is done by using different microbial growth kinetic models that define the specific growth rate (μ). Prior to decide about the values of interest, a calibration step is necessary, e.g. to determine the saturation constant (K_S) and the maximum specific growth rate (μ_{max}).

2.2.4. Theoretical background:

Several microbial growth kinetics were used to fit the experimental data: the Monod, Tessier and Moser models (without inhibition by substrate), and Andrews (considering the substrate inhibition) [7].

2.2.5. The algorithm:

The biomass increase and the substrate consumption are given by the following equations.

$$\dot{X} = \mu \cdot X \tag{1}$$

$$-\dot{S} = \frac{1}{Y} \cdot \dot{X} \tag{2}$$

The modeling of the bioprocess was done in discrete form, differential equations become difference equations.

Step 1. Compute:

$$X_k = (1 + \mu_{k-1}) X_{k-1} \tag{3}$$

Step 2. Compute:

$$S_k = S_{k-1} - \frac{1}{Y}(X_k - X_{k-1}) \tag{4}$$

Step 3. Introduce S_k and X_k into the controller and receive $A_j \geq 0$

Step 4. Compute:

$$S_k = S_{k-1} + Aj \tag{5}$$

Step 5. Compute μ_k+1 according to the chosen model

Step 6. Compute k ←k+1, go to Step 1

2.2.6. The technology:

The software uses in the background Matlab (version 2010b) application due to its several efficiency-boosting options.

2.2.7. The interface:

The application interface provides to the user many working facilities, the most important are:

- definable matrix used in fuzzyfying the substrate S and the biomass X concentrations and inputs for their domains;
- definable matrix used in the functioning of the inference engine; domain names are Z (zero), PM (positive small), PME (positive medium) and PMA (positive big);
- initial substrate input;
- drop-down box, allowing the choice of the desired model to run the simulation;
- buttons for exporting and importing software configurations;
- graph windows, for the evolutions of the S and X;
- displayed set of values used in chart plotting;
- button for exporting the results to Excel files.

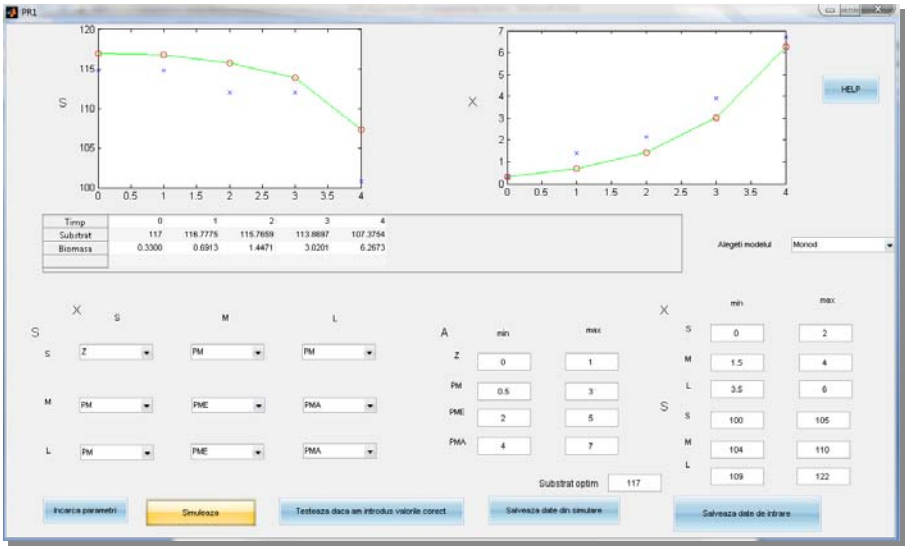

Fig. 2. The interface of Biosim software

2.3. The case study

The formation of immunomodulator product is associated with cell growth rate. Substrate (S) concentration and biomass (X) concentrations are considered the inputs of the proposed FCS. The output of the fuzzy system is the correction

[+/-] to be applied on the substrate. Fuzzy rules, presented in Table 1, were established based on the experience of human experts.

Tabel 1

The rule base

X_k / S_k	**S**	**M**	**L**
S	Z	PM	PM
M	PM	PME	PMA
L	PM	PME	PME

Further, two experimental data sets are discussed. Based on the experimental data was calculated the maximum specific growth rate (μ_{max}). In the first one, the cell growth duration was four hours with S starting at 115 mg/100mL, μ_{max}= 0.65 h^{-1}; but in the second one, the initial concentration of substrate was set at 120 mg/100mL and the cell growth was tracked over six hours, μ_{max} = 0.71 h^{-1}.

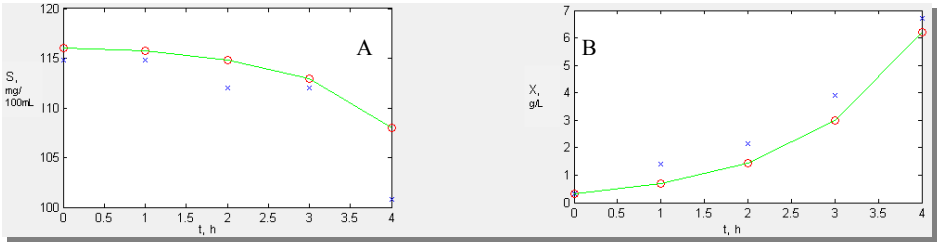

Fig. 3. Simulation results of the substrate and biomass, 1st experiment – 4 hours of growth, ("x" – experimental data, "o" – simulation), Monod model

Fig. 3 demonstrates the data obtained by simulation follow closely the experimental data, both for the substrate consumption and the biomass growth.

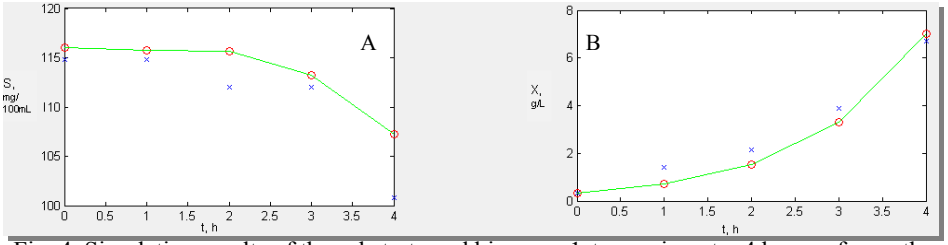

Fig. 4. Simulation results of the substrate and biomass, 1st experiment – 4 hours of growth, ("x" – experimental data, "o" – simulation), Tessier model

According to Fig.4A, substrate consumption is quite small compared with the initial concentration, indicating that one may reduce the substrate

concentration in the composition of the fermentation media. Both models represent well the experimental results. However, because it appears that the bacteria population is still growing exponentially, a four hour period is not enough for the bioprocess.

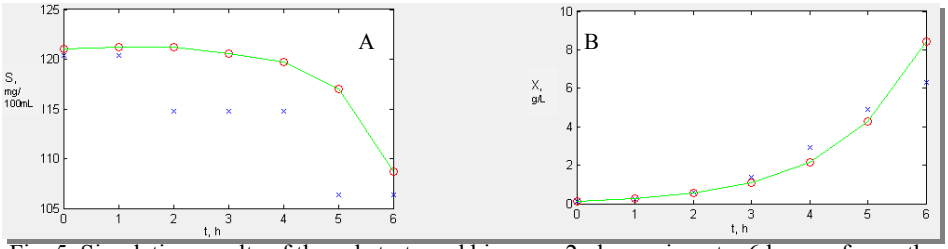

Fig. 5. Simulation results of the substrate and biomass, 2nd experiment – 6 hours of growth, ("x" – experimental data, "o" – simulation), Monod model

In Fig.5 it can be seen that substrate consumption is lower than that indicated by the simulation during the bioprocess evolution and the biomass simulation results are similar to the experimental data.

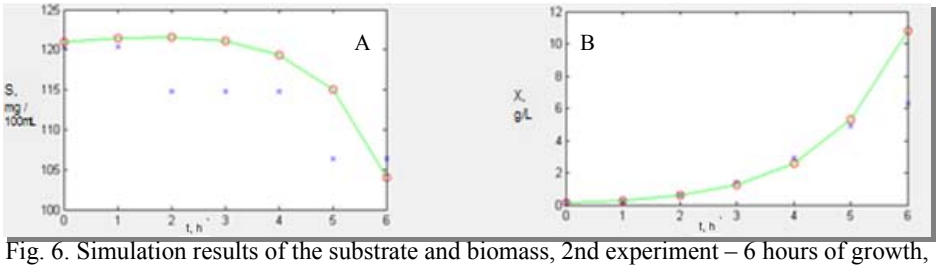

Fig. 6. Simulation results of the substrate and biomass, 2nd experiment – 6 hours of growth, ("x" – experimental data, "o" – simulation), Tessier model

Finally, as shown in Fig.6, simulation results for the cellular concentration follow closely the experimental data, but as in previous case, during entire period the substrate consumption is lower. From the simulation of substrate consumption a higher deviation is observed by comparison with the experimental decrease, because the fuzzy software BIOSIM uses a general theoretical equation probably not enough adequate to describe the real evolution. On the contrary the biomass growth is well represented by simulation.

At the same time the software can be used to demonstrate the kinetic behaviour in the discontinuous bioprocess, in the case study the adequancy of the Tessier and Monod models.

6. Conclusions

Several sets of experimental data were used to test the proposed FCS, original package BIOSIM, two sets results being put into evidence in the paper. The objective of these experiments was to reduce the bioprocess duration, but on condition to get a higher final cellular concentration. Selecting optimum initial substrate concentration and taking into account the higher growth rate, the simulation data can be applied to initiate fed batch operation.

The simulation study has showed that the fuzzy technique is quite appropriate to determine both the recommended operation conditions for this non-linear, time varying system and the cellular growth kinetics.

Acknowledgments

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R E F E R E N C E S

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