International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:4, No:6, 2010

Therapeutic Product Preparation Bioprocess Modeling

Mihai Caramihai[1*], Irina Severin[1], Ana Aurelia Chirvase[2], Adrian Onu[3], Cristina Tanase[1], Camelia Ungureanu[1]

[1]Politehnica University of Bucharest, 1, Polizu Street, 011061, Bucharest, Romania
[2]National Research & Development Institute for Chemistry and Petrochemistry ICECHIM, 202, Splaiul Independentei, 060021, Bucharest, Romania
[3]National Research & Development Institute for Microbiology and Immunology "Cantacuzino", 103, Splaiul Independentei, 050096, Bucharest, Romania

***Abstract*—**An immunomodulator bioproduct is prepared in a batch bioprocess with a modified bacterium *Pseudomonas aeruginosa*. The bioprocess is performed in 100 L Bioengineering bioreactor with 42 L cultivation medium made of peptone, meat extract and sodium chloride. The optimal bioprocess parameters were determined: temperature – 37 ^{0}C, agitation speed - 300 rpm, aeration rate – 40 L/min, pressure – 0.5 bar, Dow Corning Antifoam M-max. 4 % of the medium volume, duration - 6 hours. This kind of bioprocesses are appreciated as difficult to control because their dynamic behavior is highly nonlinear and time varying. The aim of the paper is to present (by comparison) different models based on experimental data.

The analysis criteria were modeling error and convergence rate. The estimated values and the modeling analysis were done by using the Table Curve 2D.

The preliminary conclusions indicate Andrews's model with a maximum specific growth rate of the bacterium in the range of 0.8 h^{-1}.

***Keywords*—**bioprocess modeling, *Pseudomonas aeruginosa*, kinetic models,

I. INTRODUCTION

THE ability to optimally control the biotechnologies and especially the productive bioprocesses is now a days of a considerable interest in the case of obtaining the therapeutic bioproducts of human use, generally expensive molecules, which must have high quality and efficiently contribute to the modern, targeted treatments of diseases. The optimal control fulfills the task to reduce the manufacturing costs, to increase the bioconversion yields, to assure the reproducibility of preparation conditions and to determine the stability of work procedures with major influence on the bioproduct quality.

Bioprocess control is mainly indented to establish a living environment close to optimal, for cell culture to grow and produce metabolites of interest. This includes achieving the necessary and uniform concentration of nutrients within the plant (source of carbon, nitrogen, phosphorus, minerals, dissolved oxygen), elimination of all toxic metabolites and control of all internal cell parameters significant for the optimal evolution of metabolism (temperature, pH and other).

Coressponding author: m.caramihai@ieee.org

II. BIOPROCESS MODELING

The knowledge about bioprocess behavior and modes of operation [1], [2], allows the metabolic routes considerations in view of bioprocess optimal control. If that knowledge is carried out in different kinetic equations, than:

The bioprocess mathematical representation can be the basis for adequate optimization and control technique applications [3], [4];

The model provides the necessary information about the characteristics of the chosen procedure [5];

A good model synthesizes the physiology and the genetic determinations of the specified microorganism. Hence, this is the best technique to predict the process efficiency [6].

The mathematical models, which describe the living cell evolution, must show the complex biosystem attributes, must be as possible extensive and non-speculative and must be based on cell's biochemistry [7]. Hence, the bioprocess model must be an acceptable compromise between the presentation of detailed internal processes (i.e. with considerable number of parameters) and the consideration of a short parameter number, easy to use and estimate [8].

In contrast to models that express mechanisms and cellular behaviors or even subcellular, only in order to control bioprocess, it can be develop models with a higher degree of empiricism, with in-line / on-line measurable variables or estimated variables obtained by using currently measured variables in conjunction with known equations (e.g. OTR – oxygen transfer rate – is estimated by using the dissolved O_2 concentration in a potential type equation) [9]. The parameters of the models are obtained quite frequently by fitting procedures based on experimental curves [10].

Based on living system specificity, the bioprocesses are characterized by *non-linearity*, *multivariability and parameter time variance* [9]-[11]. Consequently, the variables, which describe the bioprocess evolution, demonstrate a strong interdependency, which make impossible the correlative influences study [12].

The general equation presented below:

$$\dot{X} = f(X, S, O_2, pH, T, ..., t) \quad (1)$$

is only a theoretical assumption.

The attempts to realize such global models were not successful (firstly, due to the impossibility to measure on-line the great number of bioprocess parameters, and secondly, due to the high degree of complexity, which characterizes the cell mechanisms). The deadlock was surmounted by the implementation of the models depending of few variables, or by the use of the linear models for restricted sections (periods of time). This last one is functionally taking into account that the bioprocesses are generally characterized by high time constants (hours or tens hours), hence, the bioprocess should be considered quasi-linear [11].

Mathematical models, which describe the living cells evolution, must represent the dynamic nature of the biosystem, as general as possible (and more complex as a consequence), less empirical, reflecting the biochemistry of the microorganism culture. In these conditions, the model should be set up based on a compromise between the detailed description of the bioprocess (which means the use of o great number of parameters, often undeterminable/ uncontrollable) and the use of a limited number of parameters easy to estimate and control [13], [14].

The development of a global model for the bioprocess evolution offers, besides the advantage of the analytical determination of the optimum value, the means to change the parameters during the process. In these conditions, after setting up the model, the maximizing/minimizing strategy must be established as well as the performance index [15], [16].

III. RESULTS

Several kinetic models were tested to obtain the most representative, i.e one that can express the correlation between the specific growth rate (μ) and the initial substrate concentration (S_0), for experimental data of the same bioprocess. Representative growth kinetic models from literature are presented in Table I.

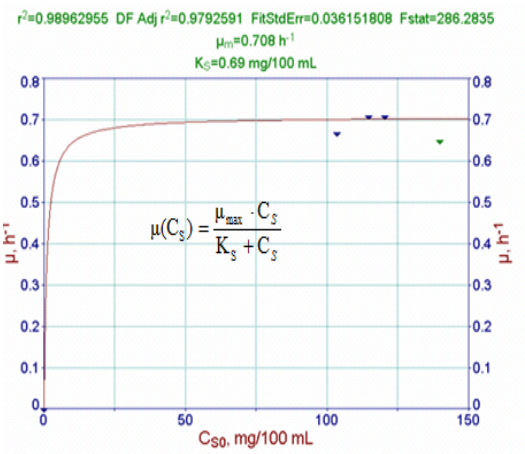
The adequacy of the proposed relationships between the specific growth rate and the initial substrate concentration for each experiment is presented in the Figure 1. The selection of the kinetic model was done by applying several analysis criteria such as: the model-data error (i.e. the model adequacy test), and convergence of the estimation rule. The estimation and the modeling analysis were realized by using the Table Curve 2D v.5.01.

Though all the four models can represent fairly enough the experimental data, one can appreciate that the Monod kinetic slowly tills to the asymptotic value, i.e. it fit not well the experimental data. Hence, the Tessier and Moser kinetics represent better the data evolution because the saturation level is faster attempted but the most representative kinetic models proposed in the literature for the approached bioprocess (i.e. the production of an imunomodulator) are not able to satisfactorily represent the whole range of experimental data, especially the substrate inhibition zone. The preliminary conclusions indicate Andrews's model with a maximum specific growth rate of the bacterium in the range of 0.8 h^{-1}.

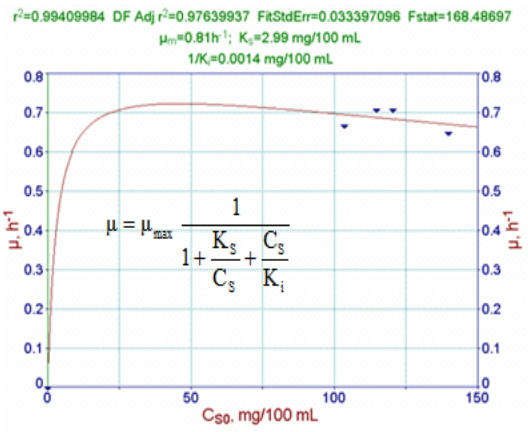
TABEL I
REPRESENTATIVE KINETIC MODELS

Model Type	*Representative equation*		*Justification*
Monod	$\mu(C_s) = \frac{\mu_{max} \cdot C_s}{K_S + C_s}$	(2)	Formal kinetic equation without inhibition by substrate C_s = substrate concentration [mg/L] K_S = Monod constant [mg/L]
Tessier	$\mu(C_s) = \mu_{max}\left(1 - e^{-\frac{C_s}{K_S}}\right)$	(3)	Without inhibition by substrate K_S = Tessier constant [mg/L]
Moser	$\mu = \mu_{max}\left[1 + K_S C_s^{-\lambda_i}\right]^{-1}$	(4)	Without inhibition by substrate λ_i = Moser constant for substrate [mg/L]
Andrews	$\mu = \mu_{max} \frac{1}{1 + \frac{K_S}{C_s} + \frac{C_s}{K_i}}$	(5)	Kinetics with substrate inhibition K_i = inhibition constant [mg/L]

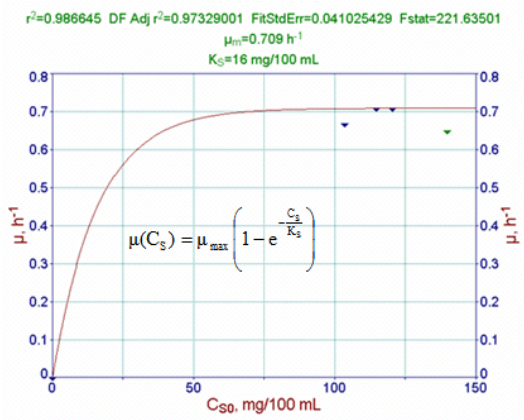
Monod model



Andrews model



Tessier model



Moser model

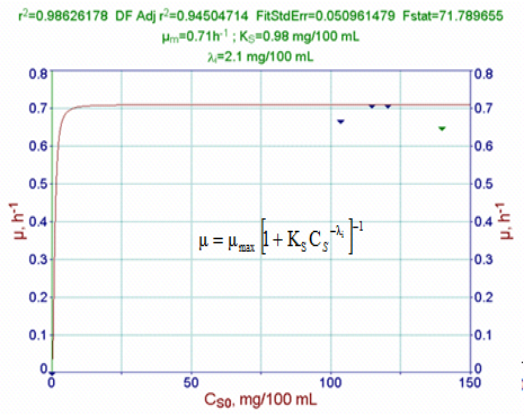


Fig. 1 The modeling of the correlation between the specific growth rate (μ) and the initial substrate concentration

After setting up the model, the maximizing/minimizing strategy must be established as well as the performance parameter.

The development of a model for the bioprocess evolution is important for the further analytical determination of optimum value and the means to change the parameters during the process.

IV. Conclusion

The modeling experiments developed in this study can conclude the following:

The kinetic modeling of the batch bioprocess was studied to design a solution for the preparation of a therapeutic product with the formation associated with growth.

The preliminary conclusions indicate Andrews's model with a maximum specific growth rate of the bacterium in the range of 0.8 h^{-1}.

Acknowledgment

This material is based on a work supported by the National Agency for Scientific Research, Romania (NASR) under Grant Number 62-051 (ATHENA Project)

Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore NASR do not accept any liability in regard thereto.

References

[1] P. M. Doran, "Biotechnol. Bioeng.", 28, p. 73, 1986.
[2] T. B. Young, H. R. Bungay, "Biotechnol. Bioeng.", 15, p. 377, 1973.
[3] A. Moser, "EFB Bioreac. Eng. Course", p.137, 1992.
[4] H. Wucherer, H. Heiler, E. Egerer, "BioEng.", 2, p. 45, 1992.
[5] L. Chen, G. Bastin, V. van Breusegem, "Adaptive Nonlinear Regulation of Fed-Batch Biological Reactors", Tech. Rep., 1991.
[6] R. Nosrati, C. Fonteix, Marc, "Recents progress en genie des procedees", Ed. Lavoisier, France, 5, p. 275, 1991
[7] S. A. Freyer, T. V. Kurten, C. Wandrey, "Adv. Biochem. Eng./ Biotechnol, A. Fiechter, (Ed.), 30, p. 780 , 1989

[8] V. Ljubenova, M. Ignatova, "Bioproc. Eng., 11, p. 107, 1994
[9] H. Eiki, T. Osono, "J. Ferm. Bioeng.", 69, p. 313 1990
[10] J. M. Cushing, "Math. Biosc.", 107, p.47, 1991
[11] P. F. Stanbury, A. Whitaker, "Principles of Fermentation Technology", Pergamon Press, Oxford, 1994
[12] B. G. Turner, D. Ramkrishna, N. B. Jansen, "Biotechnol. Bioeng.", 32, p. 46, 1998
[13] K. Shimizu, "A tutorial review on bioprocess systems engineering", Computers chem. Engng. 20, p. 915-941, 1996
[14] H. W. Ryu, M. Kim, J. N. Kim, J. S. Zun, "Appl. Biochem. Biotechnology", 10, 129-132, 2006.