Model and Kinetic Parameters Identification for Therapeutical Product Obtained According to the GMP Guidelines

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The technological solutions were elaborated to achieve the design of the production flow with respect of the Good Manufacturing Practice (GMP) guidelines. To be in line with the GMP rules a fed-batch operation mode is be designed based on the batch modelling results. As the production rate of the microbial immunomodulator is associated with the biomass growth rate, it was required to study the bacterium growth kinetics in batch process. After the selection of the kinetic model based on several batches experimental data by using the analysis criteria - modelling error and estimation rule convergence, the limiting substrate concentration to be maintained during fed-batch cells exponential growth was determined as 115 - 125 mg/L. The batch bioprocess was performed in a Bioengineering AG bioreactor with a software based control of the main variables.

Keywords: microbial immunomodulator, kinetic model, GMP

The aim of this work is to study the growth kinetics of a strain of *Pseudomonas aeruginosa* batch cultivated under aerobic conditions. The kinetic modelling is to be done as a knowledge tool needed to perform the technological bioprocess optimization for an immunomodulator production extracted from the harvested cells[1,2]. To be in line with the GMP guidelines a fed-batch operation mode will be designed based on the batch modelling results. These results must define the limiting substrate concentration to be maintained during fed-batch cells' exponential growth.

At the same time because the production of the microbial immunomodulator is associated with the cells' growth, the study of the biomass growth kinetics is also associated to the product formation.

The paper presents a modelling approach of the cells growth based on batch bioprocess experimental data and on a trial and error rule [3,4]

Problem statement and background

There are few literature studies about *Pseudomonas aeruginosa* growth kinetics and with limited significance for our work [3 - 5].

A theoretical approach in two steps was considered. Firstly μ_m (maximum specific growth rate [s^{-1}; h^{-1}]) was calculated from five representative runs in accordance with a proposed exponential model [4] of the from:

$$\frac{dX}{dt} = \mu_m X \qquad (1)$$

where: X-cell concentration [mg/L]

$$ln\frac{X}{X_o} = \mu_m t \qquad (2)$$

After the integration and the linearization, the equation (1) becomes:

In a second phase several kinetic models have been checked to obtain the most representative relationship, which express the correlation between the specific growth rate μ and the start substrate concentration (S_o), for the set of five batch experimental data. The studied models are presented in the table 1.

Model type	Representative equation	Justification
Monod	$\mu(S) = \frac{\mu_{max} \cdot S}{K_S + S}$ (3)	Formal kinetic equation with no substrate inhibition S = substrate concentration [mg/L] K_S = saturation constant [mg/L]
Tessier	$\mu(S) = \mu_{max}\left(1 - e^{-\frac{S}{K_S}}\right)$ (4)	No substrate inhibition Ks = saturation constant [mg/L]
Moser	$\mu = \mu_{max}\left[1 + K_S S^{-\lambda_i}\right]^{-1}$ (5)	No substrate inhibition, λ_i = Moser's constant for substrate [mg/L]
Andrews	$\mu = \mu_{max}\frac{1}{1 + \frac{K_S}{S} + \frac{S}{K_i}}$ (6)	Kinetic with substrate inhibition K_i = inhibition constant [mg/L]

Table 1
LIST OF THE INVESTIGATED KINETIC MODELS [5 - 7]

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Methodology

The experiments were conducted in a bottom driven and aerated 100L Bioengineering® bioreactor with 42 L aqueous Organotech® peptone solution as main culture substrate, including an inoculum of 3 L of the bacterium cells suspension.

The bioreactor includes a mechanical stirring (Rushton impeller) while the main parameters (temperature, pH, mixing speed, air flow rate) are continuously controlled. The pO2 and foam level in the reactor are only monitored.

The cell concentration was determined by a standard dry-weight method (by means of an usual procedure at 105^0C) and by means of an off line optical density method (OD) reading the signal at λ=570 nm. The substrate consumption was determined by analyzing the aminic nitrogen (derived by means of chemical titration with NaOH in the presence of formaldehyde).

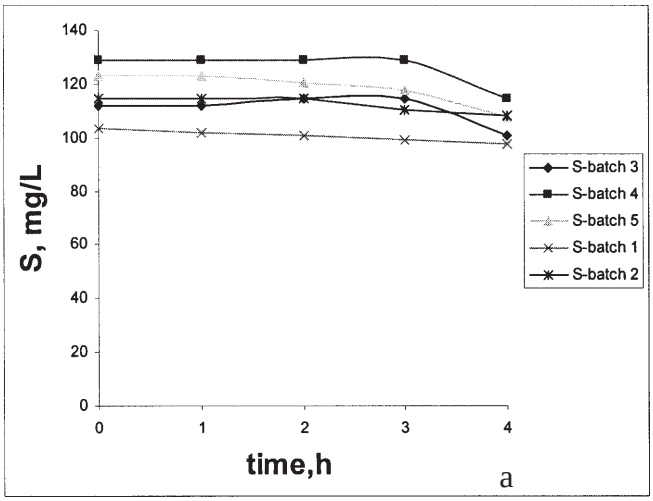
The cultivation medium mainly contains peptone and meat extract. To be in line with the EU GMP norms, these substrates prepared in the past by the manufacturer itself were replaced by ORGANOTECH (microbiological media producer accepted by these guidelines) products.

The controlled parameters of the bioprocess are the followings: temperature-37^0C; impeller speed-250-300 rpm; air flow rate-15-40 L/min; *p*H-7, 3.

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 modelling analysis were realized by using the Matlab v.7.0.1 framework and Table Curve 2D v.5.01 and 3D v.4.0.

Experimental part

The experimental data of five representative runs are presented in figure 1.



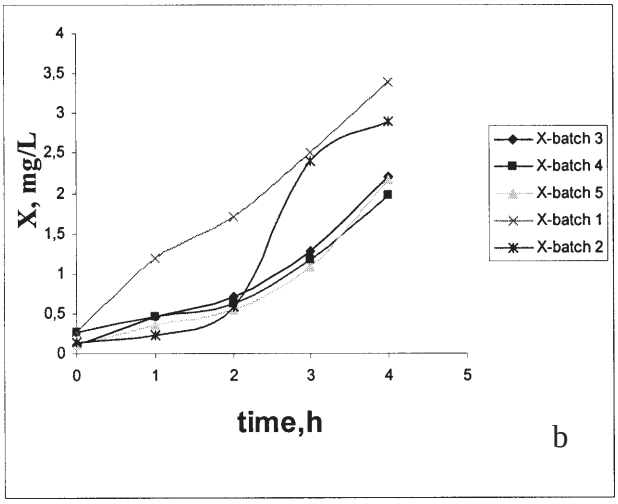


Fig. 1. Experimental data derived from a batch bioprocess: a) S=f(t) kinetic curve b) X=f(t) kinetic curve

Estimation rule

The estimated μ_m constant of the exponential model (1), by using the five runs, is presented in table 2 and figure 2.

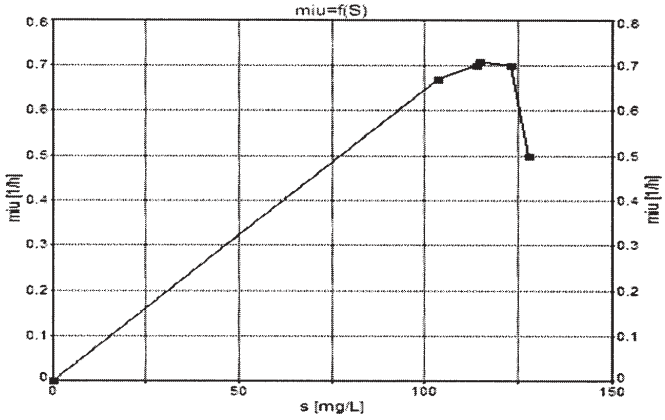


Fig. 2. Dependence of the specific growth rate upon substrate concentration

Results and discussion

For the analysed bioprocess, the limiting substrate for both cell growth and product formation is expressed by the aminic nitrogen content. The adequacy of the proposed relationships between the specific growth rate and the initial substrate concentration for each experiment is presented in the figure 3 ([8,9] for more details on model adequacy tests).

Though all the four models can represent fairly enough the experimental data, it can be appreciated that none of them, including a specially designed model to express substrate inhibition like those proposed by Andrews', is

Table 2
ESTIMATED μ_m CONSTANT

Batch	μ_m,[h^{-1}]	Representative equation	R^2
1	0.67	y=0.6717x	0.9
2	0.71	y=0.7104x	0.9433
3	0.70	y=0.7046+0.3507	0.9407
4	0.50	y=0.4974x-0.0061	0.9915
5	0.70	y=0.6972x+0.1868	0.9787

Notations: x = time, h; y = ln (X/X_0)

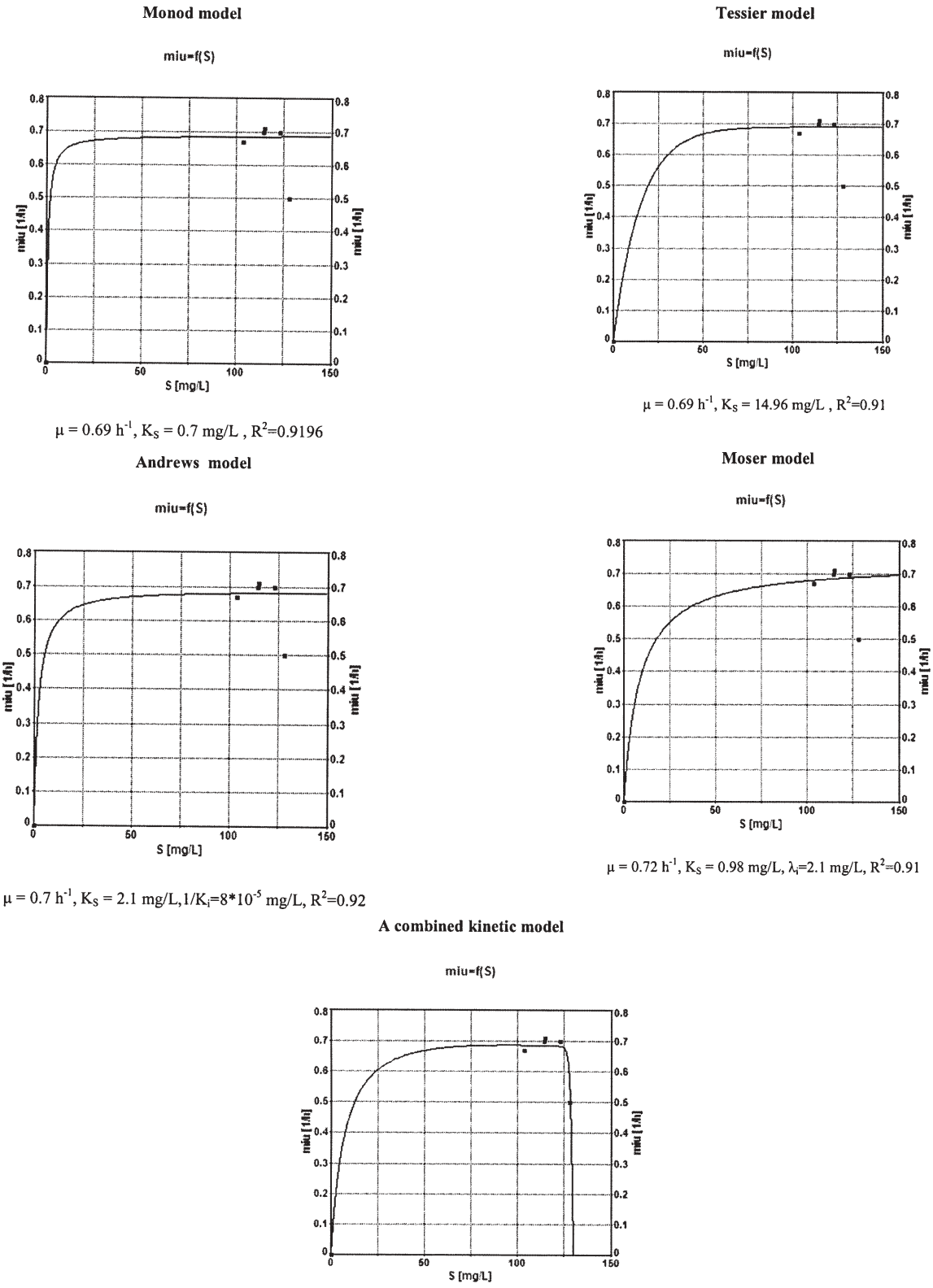


Fig. 3. The proposed relationships between specific growth rate and substrate concentration

able to realize a real good fitting, mainly for the substrate inhibition zone.

Kinetic model has been proposed as follows:

$$\mu = \frac{K_1 S}{K_S + S + \frac{S^2}{K_i}} \cdot R(S) \rightarrow \mu = \frac{K_1 S}{K_S + S + \frac{S^2}{K_i}} \left(1 - e^{(S - S_{lim})}\right) \quad (7)$$

where:

$S \in [0, Slim]$;

$R(S)$ – reduction metabolic factor;

K_1 = empirical constant;

S_{lim} = limiting substrate concentration, with $\mu(S_{lim}) = 0$;

K_S, K_i = Andrews constants.

This model is found to represent better the experimental data and it is able to account for the limiting substrate values for the situation when the specific growth rate is 0 ($\mu = 0$). Based on this model can be calculated a value of

S=115-125 mg/L i.e. the substrate concentration to be maintained during exponential growth in the fed-batch bioprocess.

This model has been suggested by the complex mechanism of inhibition and limitation with substrate of the bioreaction overall rate, pointed by the experimental data.

Conclusions

Based on the experimental data and modelling results several conclusions can be derived.

In order to design a fed-batch bioprocess for a therapeutic product preparation, the kinetics of the batch process was studied.

The most representative kinetic models proposed in the literature for the appproached 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.

A combined kinetic model was proposed and identified based on five batch runs. The model indicates that the substrate concentration to be maintained during exponential cellular growth in fed-batch bioprocess is 115-125 mg/L.

Nomenclature

X- cell concentration, [g/L]
S - substrate concentration [mg/L]
K_S - saturation constant [mg/L]
K_i - inhibition constant [mg/L]
R(S) – reduction metabolic factor
K_1- empirical constant
S_{lim}- limiting substrate concentration
t - time, h

Greeks

μ_m - maximum specific growth rate, h^{-1}
λ – wavelength, nm
λ_i - Moser's constant for substrate [mg/L]

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