MODELING THE PRODUCTION OF CLAVULANIC ACID FROM *STREPTOMYCES CLAVULIGERUS*.

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Introduction. Clavulanic acid (CA) obtained from fermentation by *Streptomyces clavuligerus* (*Sc*) is a potent inhibitor of β-lactamase enzymes that confers resistance to β-lactam antibiotics from microorganims [1]. CA production has gained increasing attention due to its importance at industrial level. Although several studies have been carried out on different operational strategies to improve CA production, surprisingly there are just very few reports on its kinetic modeling [2]. Developing kinetic studies of microbial cultures is an important task in order to understand the behavior of the bioprocess. These studies can be used as a tool for predicting, designing and optimizing the process, towards reducing the production costs [3]. The aim of this study was to propose and validate a mathematical model for describing the main dynamics in the production of CA by *Sc*.

Methods. Batch fermentations were performed in a 3L bioreactor with a working volume of 1L, 28 ° C, 1 vvm, 500 rpm and a pH of 6.8. Samples were taken every 24h [3]. *Sc* was growth in a chemically defined medium. Biomass was determined by dry weight. The glycerol and phosphate were determined by spectrophotometer. The L-Asparagine and CA were determined by HPLC. For the modeling, it was assumed that the microorganism grows according to Monod model. After obtaining the experimental data and formulating the model equations, parameter identification was carried out using a nonlinear regression analysis [4]. The criterion used for finding those kinetic parameters was the minimization of the sum of squares of the residuals [5]. The confidence range for each parameter was determined using statistics tools in order to obtain a 95% confidence in the model predictions

Results Using material balances for biomass (*X*), substrate (*S*) and product (*P*) and assuming Monod's kinetic the model is given by:

$$\frac{dX}{dt} = \left(\frac{\mu_{\max} S}{K_S + S} - k_d\right) X \qquad \text{(Eq. 1)}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\left(\frac{\mu_{\max} S}{K_S + S}\right) X \qquad \text{(Eq. 2)}$$

$$\frac{dP}{dt} = \left(\frac{1}{Y_{X/p}} \times \frac{\mu_{\max} S}{K_S + S} + \alpha\right) X \qquad \text{(Eq. 3)}$$

The experimental data and the model fit (lines) are shown in Figure1. The proposed model represents the dynamic behavior of X, S and P without the need of using step functions as it was reported by [2].

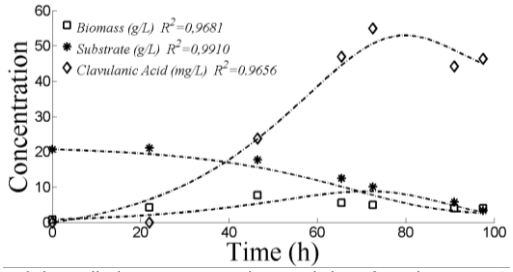

Fig. 1 Model predictions vs. experimental data for Biomass, Substrate and Clavulanic acid in bioreactor

Kinetic parameters estimated in the optimization process are shown in Table 1. In this work, it was found that kinetic parameters obtained using a chemically defined medium are very similar to those reported with complex media [2]. The high uncertainty observed for parameter α may be due to the high dispersity of the CA experimental data. However it was found that the variation of this parameter does not significantly affect the fit of the model.

Table 1. Kinetic parameters in Clavulanic Acid production, using nonlinear regression

Kinetic Parameters		Estimated values
$\mu_{\max}\left(h^{-1}\right)$	Maximum specific growth rate	0.33 ± 0.04
$K_S\left(gS/L\right)$	Saturation constant	2.58 ± 0.51
$Y_{X/S}\left(gX/gS\right)$	Yield coefficient of biomass in relate to the substrate	6.65 ± 0.33
$Y_{X/p}\left(gX/mgP\right)$	Yield coefficient of biomass in relate to the product	3.14 ± 0.29
$\alpha\left(mgP/gX\right)$	Specific f CA production rate	0.23 ± 0.11
$k_d\left(h^{-1}\right)$	Cell death constant	0.25 ± 0.09

These values show a 95% confidence level within the range.

Conclusion.

The first principles based model proposed in this work is a robust model capable of predicting the dynamic behavior of the substrate, biomass and product concentrations in the clavulanic acid production by *Streptomyces clavuligerus.*

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