v. 3, n. 32, 2023

Journal of Engineering Research

SIMPLIFIED MODEL FOR OBTAINING TACROLIMUS VIA FERMENTATION BY *STREPTOMYCES TSUKUBAENSIS*

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This work presents a simplified mathematical model for obtaining tacrolimus via fermentation by *Streptomyces tsukubaensis*. It is considered that bacterial biomass growth is described by a logistic curve associated with first-order kinetics for substrate consumption and production of total proteins and tacrolimus. The results obtained are compared with those found in the literature, demonstrating good agreement between the analyzed data.
Keywords: Tacrolimus; Fermentation; Modeling; Logistic curve; *Streptomyces tsukubaensis*.

INTRODUCTION

Tacrolimus, known as fujimycin, is a macrolide drug indicated for the prophylaxis of organ rejection in patients undergoing allogeneic liver, kidney and heart transplants, being one hundred times more potent than cyclosporine A in terms of inhibiting the proliferation of T lymphocytes. (Curvello Neto et al., 2000), that is, more effective in preventing graft rejection in transplants.

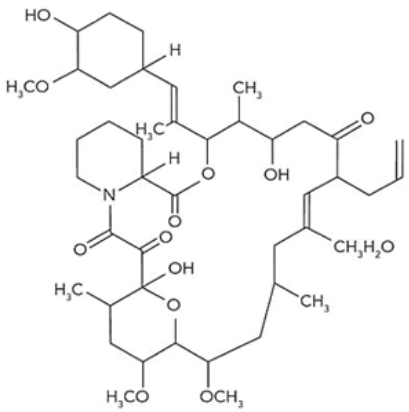

Figure 1. Representation of tacrolimus (Bertan et al., 2021).

In addition to being widely used in immunosuppressive therapeutic protocols (Garcia et al., 2004), tacrolimus is indicated for the treatment of autoimmune diseases, such as rheumatoid arthritis, lichen planus (Sanchez et al., 2004) as well as in the treatment of retinoblastomas (Eckstein et al., 2005), bronchial asthma and various dermatological disorders such as vitiligo (Tamler et al., 2011), psoriasis and atopic dermatitis (Chaudhari et al., 2012).

Tacrolimus is obtained via fermentation, classically by *Streptomyces tsukubaensis,* this drug being a secondary metabolite. In the early stages of any fermentation process, bacterial growth follows an autocatalytic pattern up to a certain stage (Kolter et al., 1993), which, in batch mode, inevitably reaches a peak and then a stage where it ceases to function. grows and begins to decay, indicating the stationary phase. Figure 2 illustrates a standard microbial growth cycle.

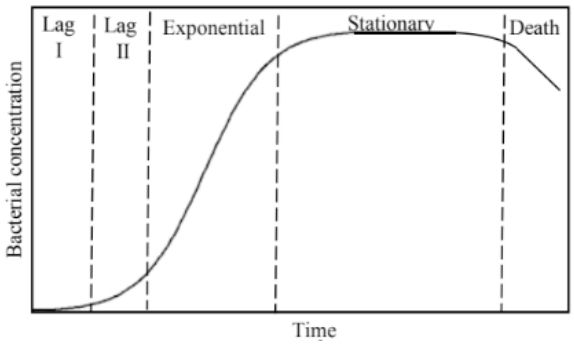

Figure 2. – Bacterial growth cycle.

The lag phase, illustrated in Figure 2, corresponds to the delay of the exponential phase, where cells divide at a constant rate. In the lag phase, a fundamental biological process occurs, the physiological adaptation of the bacteria to the inserted medium. The growth acceleration phase, also known as lag II, is the process where cell generation begins (Rolfe et al., 2012; Madar et al., 2013). During the lag phase there is virtually no increase in the microbial population, however intense metabolic activity is observed. In the exponential phase of growth, the cells grow in size and, if the condition remains favorable, each cell divides in two, grows, divides

again and so the cycle continues, so that in this phase the transformation of sources is identified primaries of carbon in biosynthetic precursors, which are channeled in several biosynthetic routes for the production of monomers (amino acids, fatty acids and sugars), subsequently polymerized (El-Mansi et al., 2012). Finally, the cell death phase is reached, which has an exponential characteristic, although the rate of cell death is relatively slower than the exponential growth phase.

The fermentation process can also be described by mathematical models, considering its characteristic steps, such as those illustrated in Figure 2. Bacterial growth is generally marked by a sigmoid curve, in which the dependent variable is the viable cell concentration. The bacterial growth curve can be fitted by the Monod equation, which describes the dependence of the microbial growth rate on the concentration of a limiting substrate (Zentou et al., 2012) or by the Gompertz function, widely used and discussed in the literature (Zwietering et al., 1990; Baranyi et al., 1993), as well as its variants, in the form of the logistic equation, such as those found in the Verhulst and Baranyi-Roberts models (Peleg and Corradini, 2021). Associated with microbial growth, the fermentation process is described by the consumption of substrate and production of metabolites, generally based on yield coefficients, in the form of ordinary differential equations (ODE).

In this work, the objective is to propose a simplified mathematical model, directed to the description of fermentation, via *Streptomyces tsukubaensis,* to obtain tacrolimus, assuming that the classic expression for the cumulative distribution function for the growth of bacterial biomass comes from a logistic curve, associated with first-order kinetics for substrate consumption and production of total proteins and tacrolimus.

MATERIALS AND METHODS

MATERIALS

The experimental results, to evaluate the performance of the mathematical model proposed in this work, can be found in Bertan et al. (2021). The authors used a strain of *Streptomyces tsukubaensis* (DSM 42081), acquired by the Laboratory of Processes and Mass Transfer (LPTM), Department of Process Engineering (DEPro), Faculty of Chemical Engineering (FEQ) of ``Universidade Estadual de Campinas` (UNICAMP) at the Leibniz DSMZ Institute - German Collection of Microorganisms and Cell Cultures. Two fermentation assays via *S. tsukubaensis were analyzed*, aiming to obtain tacrolimus, in which the culture media are shown in Table 1.

compounds	Concentration (g/L)
Glucose or coconut oil	30
soy peptone	30
steep corn liquor	10
$MgSO_4 7H_2O$	0.5
K_2HPO_4	4
KH_2PO_4	two
$CaCO_3$	3

Table 1. Composition of culture media.

The difference between the tests is in the main carbon source: glucose for test #1, and coconut oil for test #2. Both fermentations were carried out, in batch, in a shaker maintained at 28°C and 130 rpm. In this article, the first 96 h are analyzed, considering that the maximum concentration of tacrolimus was reached at the end of that time.

METHOD

The mathematical model proposed for the description of fermentation is based on the characteristic stages of cell growth. In the lag time phase (lag phase) there is no growth, with substrate consumption; in the

logarithmic phase there is rapid growth, in which the maximum specific growth rate $(\mu max)_{\text{is observed}}$; after this stage, there is the stationary stage, reached when the nutrients are practically exhausted (Tonner et al., 2017). With these characteristics, the experimental data can be mathematically described using a logistic curve (Konopacki et al., 2020). In the present work, the classical expression for the cumulative distribution function for the growth of bacterial biomass, X, is assumed by

$$X = \frac{X_{máx}}{1+\exp(-z)} \qquad (1)$$

where Xmax is $_{\text{the}}$ maximum value of X reached in the stationary phase; z is dimensionless time, obtained by

$$z = \frac{t-(t_A - t_{máxx})}{t_{máx} - (t_A - t_{máx})} \qquad \text{(two)}$$

where t_A is the time when $X = X_{max}$; $t_{max,}$ is the time at the maximum specific growth rate, μ_{max} (Longhi et al., 2017). Substrate consumption, S, identified as fermentable sugars, protein production (P), associated with total proteins, and tacrolimus production (T) follow first-order kinetics in the form

$$\frac{dS}{dt} = -\beta_S X \qquad (3)$$

$$\frac{dP}{dt} = \beta_P X \qquad (4)$$

$$\frac{dT}{dt} = \beta_T X \qquad (5)$$

The parameters β_i are associated both with the conversion of substrate into cells (for i = S) and products (i = P or T) and with the maximum rates of consumption (i = S) or production (i = P or T). With the exception of the values of $\beta_{i,}$ which are adjusted, all parameters (values of $X_{max,}$ $t_{A,}$ t_{max}) are obtained from the experimental analysis of the data.

RESULTS AND DISCUSSION

Based on experimental data, up to 96 h, found in Bertan et al. (2021), both for the test in which glucose was used (test #1) as the main source of carbon, and for coconut oil (test # 2), the values of $t_{A=}$ 96 $_{h}$ and 72 h. It was found, for test # 1, X_{max} = 12.13 g/L and for test # 2, X_{max} = 18.60 g/L. The kinetic parameters of substrate consumption (S), protein production (P) and tacrolimus (T) are shown in Table 2.

carbon source	s	P	T
Glucose	2.80	0.62	0.15
Coconut oil	1.46	0.20	0.17

Table 2. Values of the β x10 ^{4} adjustment kinetic parameters (g/Lh).

The results displayed in Table 2 point to a faster conversion of the substrate into cells when glucose is used as a carbon source compared to the use of coconut oil, as well as favoring the production of proteins. The use of coconut oil favors the production of tacrolimus. The model proposed in Equations 1 to 4 was solved by Euler's method. Figures 3 to 10 show the results obtained in comparison to the experimental ones, demonstrating good agreement between both. In addition to inspecting the figures, the performance of the mathematical model can be monitored by analyzing the mean relative deviation, DMR, between data from the model (subscript mod) and the experimental data.

$$D.M.R(\%) = \sum_{i=1}^{n} \frac{(x_{mod} - x_{exp})_i}{(x_{exp})_i} \times 100 \qquad (6)$$

Where x refers to X, S, P or T, whose results for the DMR are presented in Table 3, corroborating the good performance of the model.

carbon source	X	s	P	T
Glucose	4.74	0.04	-0.02	8.50
Coconut oil	-2.00	-0.09	0.05	2.06

Table 3. Mean relative deviation (%).

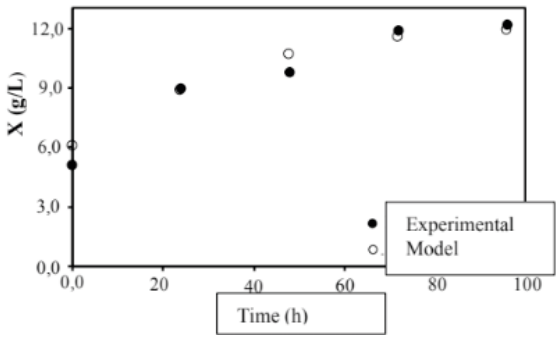

Figure 3. Biomass growth – glucose.

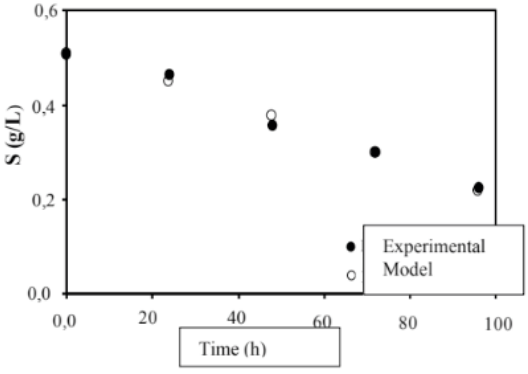

Figure 4. Substrate consumption - glucose.

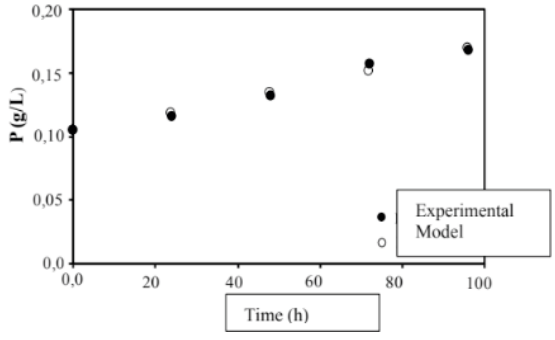

Figure 5. Protein production - glucose.

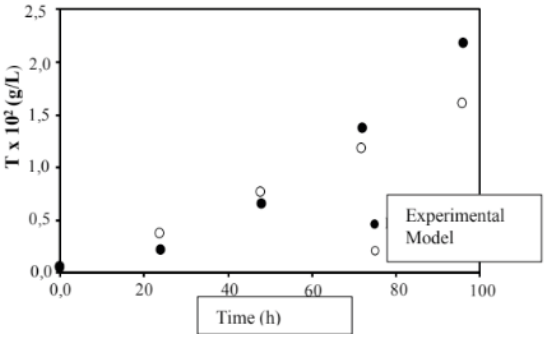

Figure 6. Production of tacrolimus - glucose.

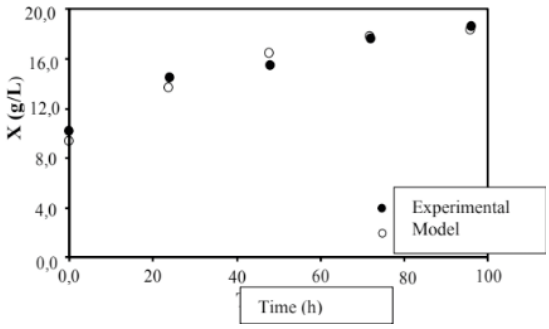

Figure 7. Biomass growth – coconut oil.

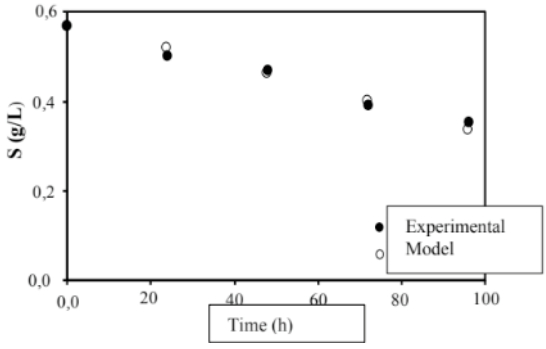

Figure 8. Consumption of substrate – coconut oil.

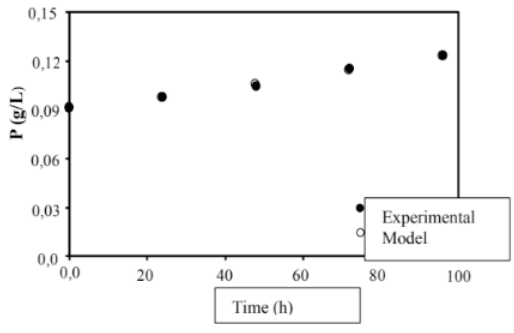

Figure 9. Protein production – coconut oil.

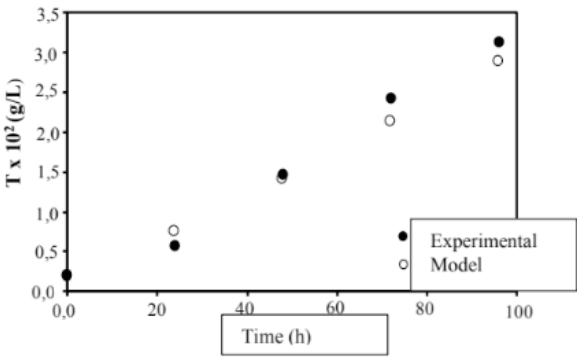

Figure 10. Production of tacrolimus – coconut oil.

CONCLUSION

The present work offers a simple mathematical modeling, based on the classic logistic curve as a cumulative distribution function, for the description of the growth of the biomass of Streptomyces tsukubaensis *in* two liquid fermentable culture media, without the need to incorporate the consumption of substrate, as the one approach found in Monod's expressions. The equation for substrate consumption and protein and tacrolimus production, based on first-order kinetics, is also simple. It must be noted, however, that the proposed model is limited to the description of fermentation until the maximum value of the drug is obtained, which is reached at the end of the exponential phase of bacterial growth. The literature (Silva et al., 2019; Bertan et al., 2021) shows that for times greater than t_A (time when $X = X_{max}$) there is a substantial decline in tacrolimus production. This phenomenon is not foreseen in the modeling proposed in this work, however, opening perspectives for the construction of new mathematical approaches, which, for sure, will allow significant physical interpretations regarding the production of secondary metabolites and their mutual interactions regarding the study aiming at the production of tacrolimus and other important macrolides, such as ascomycin.

THANKS

To CNPq for the financial support related to the doctoral scholarship, Procedure, number: -140204/2021-0.

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